Obesity and functional genomics-identified genes: A focus on the high-fat diet-induced gene trefoil factor 2 (Tff2) and the exercise-induced gene secreted protein acidic and rich in cysteine (Sparc) within the context of energy metabolism

Thèse

Abdelaziz Ghanemi

Doctorat en médecine moléculaire
Philosophiæ doctor (Ph. D.)

Québec, Canada

Obesity and functional genomics-identified genes: A focus on the high-fat diet-induced gene trefoil factor 2 (*Tff2*) and the exercise-induced gene secreted protein acidic and rich in cysteine (*Sparc*) within the context of energy metabolism

Thèse

Abdelaziz Ghanemi

Sous la direction de :

Jonny St-Amand, directeur de recherche

Résumé (French abstract)

L'obesité est un problème de santé en soi et c'est aussi un facteur de risque pour de nombreux autres problèmes de santé. Outre le contrôle de l'alimentation et l'activité physique, les options pharmacologiques contre l'obésité restent limitées et de nouvelles options thérapeutiques sont nécessaires. Dans ce contexte, la génomique fonctionnelle peut identifier de nouvelles options pour gérer et étudier l'obésité. Notre groupe de recherche a identifié deux gènes liés aux deux principaux facteurs ayant un impact sur le développement de l'obésité : La diète, principalement riche en gras (HFD) et l'exercice. Ces deux gènes clés sont le trefoil factor family member 2 (*Tff2*) et la secreted protein acidic and rich in cysteine (*SPARC*). Alors que *Tff2* a été identifié comme un gène induit par la HFD, *SPARC* a été caractérisé comme un gène induit par l'exercice. Notre groupe de recherche a également constaté que les souris *Tff2* knock-out (KO) sont protégées contre l'obésité induite par la HFD. Par conséquent, ma thèse explore *Tff2* et *Sparc* dans le contexte de l'obésité et le métabolisme énergétique. *Tff2* est principalement exprimé dans le système digestif où elle a une propriété de protection des muqueuses, tandis que *Sparc* est plus largement distribué et s'exprime principalement lors de remodelage tissulaire dans des situations telles que les blessures et la croissance. Les parties théoriques de cette thèse décrivent diverses propriétés de *Tff2* et *Sparc* décrites dans la littérature telles que le métabolisme et les rôles cellulaires ainsi que les implications et les applications potentielles des données que j'ai générées. Pour mes données de recherche rapportées dans cette thèse, elles sont divisées en trois publications.

La première, explore des souris *Tff2* KO pour expliquer leur protection contre l'obésité induite par la HFD. Les souris *Tff2* KO avaient des taux plus faibles de glucose, de triglycérides et de glycérol. Leurs niveaux d'expression génique et protéique indiquent moins de stockage de graisse et une dépense énergétique accrue en améliorant l'utilisation des lipides et du glucose via la phosphorylation oxydative. Nos données mettent en évidence les voies liées à *Tff2* comme potentielles cibles pour les thérapies contre l'obésité.

Via une expérimentation animale, la deuxième étude vise à identifier des implications de SPARC principalement dans le muscle dans les contextes de l'exercice. Les souris ont été divisées en huit groupes en fonction de trois variables (âge, génotype et exercice). Les effets du *Sparc* KO sur la composition corporelle, l'adiposité et le métabolisme sont vers une réduction du tissu adipeux blanc et du poids corporel, mais avec un phénotype métabolique et fonctionnel musculaire négatif. Alors que ces effets négatifs s'aggravent avec le vieillissement, ils sont relativement améliorés par l'exercice. Nos données suggèrent aussi que les changements induits par l'exercice dans le phénotype du muscle squelettique (métabolisme, force et développement), y compris les changements induits par le lactate, dépendent de SPARC.

Le troisième article à deux parties. Tout d'abord, j'explore les conséquences du *Sparc* KO et les compare aux effets du vieillissement. J'observe également les effets de l'exercice. Dans la deuxième partie, j'étudie les effets de la surexpression de *Sparc* et les compare aux avantages de l'exercice. Les mesures étaient principalement liées au poids des tissus, à l'adiposité, au métabolisme et à la force musculaire. Collectivement, ces résultats, et les données de la deuxième étude, montrent que les souris *Sparc* KO développe un phénotype semblable au vieillissement, tandis que la surexpression de SPARC et l'exercice génèrent des avantages similaires. Ces avantages visent à contrer à la fois le phénotype du vieillissement induit par le déficit en SPARC et à améliorer les changements liés à l'âge. Les applications potentielles de ces résultats sont de construire/optimiser des modèles d'animaux basés sur *Sparc* KO et, d'autre part, de développer des thérapies contre l'obésité, les troubles métaboliques ou liés à l'âge basées sur l'introduction de SPARC ou le ciblage des voies liées à SPARC pour imiter l'exercice.

L'exploration de telles voies moléculaires permettrait à la fois d'élucider certains mécanismes et de développer une nouvelle génération d'options thérapeutiques pour l'obésité et les troubles métaboliques, y compris les troubles liés à l'âge. De telles approches seraient basées sur le ciblage de TFF2, SPARC ou de leurs voies connexes.

Abstract

Obesity represents a challenge for health professionals. It is a health problem itself and it is also a risk factor for numerous health problems. Beside diet control and physical activity, the pharmacological options against obesity remain limited and novel therapeutic options are required. Within this context, functional genomics represents an emerging approach to identify novel options to manage and study obesity. Our research group has previously conducted functional genomics explorations to identify genes related to the two main factors impacting obesity development: Diet (mainly high fat) and exercise. Indeed, both diet, especially high-fat diet (HFD), and exercise are at the center of obesity management. Those functional genomics studies identified two key genes: Trefoil factor family member 2 (*Tff2*) and secreted protein acidic and rich in cysteine (*SPARC*). Whereas *Tff2* was identified as a HFD-induced gene, *SPARC* was characterized as an exercise-induced gene. Following that, our research group has also found that *Tff2* knock-out (KO) in mice protects them from the HFD-induced obesity. Therefore, my thesis explores *Tff2* and *Sparc* within the context of obesity and energy metabolism. *Tff2* is mainly expressed in the digestive system where it has mucus protection property, whereas *Sparc* is more widely distributed and is expressed mainly during tissues remodeling in situations such as injuries and growth. The theoretical parts of this thesis describe various properties of both *Tff2* and *Sparc* reported in the literatures such as metabolism and cellular roles as well as implications and potential applications of the data I generated. For the research parts reported in this thesis, they are divided into three publications.

The first one, explores *Tff2* KO-related pathways of mice at the genomic, proteinic and biochemical levels to elucidate the processes behind their protection from the HFD-induced obesity. *Tff2* KO mice had lower levels of serum glucose, triglycerides and glycerol. Western blotting and Q_RT-PCR revealed that the expression levels of selected genes and proteins are toward less fat storage and increased energy expenditure by enhancing lipid and glucose utilization via oxidative phosphorylation. The data highlight *Tff2*-related pathways as potential targets for obesity therapies.

Via an animal experiment, the second study aims to identify selected implications of SPARC mainly within the muscle in the contexts of exercise. Mice were divided into eight

groups based on three variables (age, genotype and exercise): Old or young × *Sparc* KO or wild type × sedentary or exercise. The exercised groups were trained before all mice were sacrificed. *Sparc* KO effects on body composition, adiposity and metabolic patterns are toward a reduced white adipose tissue and body weight, but with a negative metabolic and functional phenotype of the skeletal muscle. Whereas such negative effects on skeletal muscle are worsened with ageing, they are relatively improved by exercise. Importantly, our data suggest that the exercise-induced changes in the skeletal muscle phenotype, in terms of increased performance (metabolic, strength and development) including lactate-induced changes, are SPARC-dependent.

The third paper studies and compares both *Sparc* KO and *Sparc* overexpression in male and female mice. First, I explore the consequences of *Sparc* KO and compare them to the ageing phenotype. I also observe the effects of exercise. In the second part, I study the consequences of SPARC overexpression and compare them to the exercise benefits. The measurements were mainly related to tissue weights, adiposity, metabolism, and muscle strength. Collectively, these findings and data show that *Sparc* KO mice manifest an ageing-like phenotype, whereas SPARC overexpression and exercise generate similar benefits. These benefits are towards counteracting both SPARC deficiency-induced ageing-like phenotype as well as reversing the age-related changes. The potential applications of these findings are to build/optimize *Sparc* KO-based animal models of various health conditions and, on the other hand, to develop therapies based on introducing SPARC or targeting SPARC-related pathways to mimic exercise against obesity, age-related and metabolic disorders.

Exploring such molecular patterns would allow both mapping some underlying mechanisms and developing a new generation of therapeutic options for obesity and metabolic disorders, including age-related disorders. Such approaches would be based on targeting TFF2, SPARC or their related pathways.

Table of contents

List of figures

List of tables

List of abbreviations

A	Age
AbdAT	Abdominal adipose tissue
ADRB2	β-2 adrenergic receptor
Agrp/AgRP	Agouti-related protein
AICAR	5-aminoimidazole-4-carboxamide-1-β- D-ribofuranoside
AMPK	Adenosine monophosphate-activated protein kinase
AMY	Amygdala
AP	Area postrema
Apoa4/apoA-IV	Apolipoprotein A-IV
ARC	Arcuate nucleus
AT	Adipose tissue
ATP	Adenosine triphosphate
AUC	Area under the curve
BAT	Brown adipose tissue
BM-40	Basement membrane-40
BMAT	Bone marrow AT
β-AR	β-adrenergic receptor
DNAmAge	DNA methylation age
BMI	Body mass index
BW	Body weight

CAPS	N-cyclohexyl-3-aminopropanesulfonic acid
CART	Cocaine–amphetamine regulated transcript
Cd36/CD36	Fatty acids translocase
CNS	Central nervous system
COL1A1	Collagen type I alpha 1
COL1A2	Collagen type I alpha 2
COL3A1	Collagen type III alpha 1
COL4A1	Collagen type IV alpha 1
CON	Pooled sample (positive control)
COVID-19	Coronavirus disease 2019
Crh	Cart and corticotrophin releasing hormone
CVD	Cardiovascular disease
CXCR4	Chemokine (C-X-C motif) receptor 4
DA	Dopaminergic
db/db Mice	Mice lacking leptin receptor
DF	Density of each lane on the film
DGAT2	Diglyceride acyltransferase 2
DM	Density of each lane on the membrane
ECM	Extracellular matrix
EDL	Extensor digitorum longus
EE	Energy expenditure

EI	Energy intake
EpiAT	Epididymal adipose tissue
EPS	Electrical pulse stimulation
Ex	Exercise
F	Female
FABP1/2	Fatty acid-bindingprotein 1/2
FATP4	Fatty acid transport protein 4
FFA	Free fatty acids
FI	Food intake
g	Gram
G	Genotype
GC	Gastrocnemius
GLUT2	Glucose transporter 2 (solute carrier family 2)
Glut4/GLUT4	Glucose transporter type 4
GonAT	Gonadal adipose tissue
h	Hour
HF	High-fat
HFD	High-fat diet
HFHC	High-fat and high-cholesterol
IGF	Insulin-like growth factor
IL	Interleukin

IL-1	Interleukin 1
IL-13	Interleukin 13
IL-4	Interleukin 4
IL-6	Interleukin 6
IngAT	Inguinal adipose tissue
ITRs	Inverted terminal repeat sequences
KO	Knockout or knock-out
LF	Low-fat
LFD	Low-fat diet
LH	Lateral hypothalamus
LT	Lactate threshold
M	Male
m	Meter
MC4R	Melanocortin receptor 4
MesAT	Mesenteric adipose tissue
mg	Milligram
MGAT2	Monoacylglycerol acyltransferase 2
min	Minute
mM	Millimolar
MT-CO1/MtCo1	Mitochondrially encoded cytochrome c oxidase I
MT-CO2	Mitochondrially encoded cytochrome c oxidase II

MTP	Microsomal TG transport protein
MUP1/*Mup1*	Major urinary protein 1
Nac	Nucleus accumbens
NDUFB8	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8
NPY	Neuropeptide Y
NTS	Solitary tract
Nur77/NUR77	Nerve growth factor IB or NGFIB
O	Old
OGTT	Oral glucose tolerance test
OXPHOS	Oxidative phosphorylation
PC	Positive control
PFC	Prefrontal cortex
PGC1α/PPARGC1A	Peroxisome proliferator-activated receptor γ coactivator 1 α
POMC	Proopiomelanocortin
PPAR	Peroxisome proliferator-activated receptor
PPARA/PPARα	Peroxisome proliferator-activated receptor alpha
Ppargc1a	Peroxisome proliferator-activated receptor γ coactivator 1α
PVDF	Polyvinylidene fluoride
PVN	Paraventricular nuclei
PYY	Peptide YY
Q_RT-PCR	Quantitative real-time PCR

RetAT	Retroperitoneal adipose tissue
RIPA	Radio-immunoprecipitation assay
RNA	Ribonucleic acid
SAGE	Serial analysis of gene expression
SDHB	Succinate dehydrogenase iron-sulfur subunit
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sed	Sedentary
SEM	Standard error of the mean
siRNA	Small interfering RNA
SM	Skeletal muscle
SMPCs	Skeletal muscle progenitor cells
SNS	Sympathetic nervous system
Sol	Soleus
SP	Spasmolytic polypeptide
SPARC/SPARC/*Sparc*	Secreted protein acidic and rich in cysteine
STD	Molecular weight standard
T2D	Type 2 diabetes
TA	Tibialis anterior
TFF	Trefoil factor family member
Tff2/ TFF2/*TFF2*	Trefoil factor family member 2
Tg	Transgenic (*Sparc* overexpression)

TG	Triglyceride
Tg1	*Sparc* Tg line 1
Tg2	*Sparc* Tg line 2
Tg4	*Sparc* Tg line 4
Tg5	*Sparc* Tg line 5
Tg6	*Sparc* Tg line 6
TNF-α	Tumor necrosis factor alpha
Ucp1	Mitochondrial uncoupling protein1
Ucp3	Mitochondrial uncoupling protein 3
UQCRC2	Ubiquinol-cytochrome c reductase core protein II
VMN	Ventromedial nuclei
VTA	Ventral tegmental area
WAT	White adipose tissue
WC	Waist circumference
WHR	Waist-hip ratio
wk	Week
WT	Wild type or wild-type
Y	Young

This work is dedicated to my Parents, Brahim GHANEMI and Leila KARA MOSTEFA, who left this world in 2020, you inspired me and made of me what I am and what I will be !

Knowledge is power

Acknowledgments

I thank:

My supervisor Dr. Jonny St-Amand for taking me under his guidance. I appreciate his way of working and I remember that at the beginning he told me “Just follow my instructions and all will be fine” and it was as he said!

Our laboratory manager and supervisor assistant Dr. Mayumi Yoshioka, who has been a real guide for academic, scientific and laboratory tasks. Beyond her strong scientific and academic leadership, I appreciate her organisation and her human character.

The professors who accepted to give time and evaluate my thesis.

Aicha Melouane, my labmate with whom I learned a lot and who has been of an important assistance.

Octave Mucunguzi, my labmate with whom I had interesting exchanges.

Dr. Francine Durocher for her continuous assistance.

Dr. Yves Deshaies and Dr. Pierre Savard whom I knew as teachers of my courses. However, afterwards we kept in touch, and I received precious advice from them throughout my PhD studies progress.

My wife who has always been of a strong support at all the levels and in all times.

My brother, Kadour Ghanemi, who always tells me to never turn back.

All those I learned from and with (courses, animal facility staff, etc.).

The faculty of medicine, the FESP members and the administrative staff.

I finish by thanking my parents, Brahim Ghanemi and Leila Kara Mostefa, who left our world in April 2020 and to whom this thesis is dedicated. They gave me all what they had and way more what I needed. They though me precious values and principles without which I could not have done a tiny part of what I have achieved so far in my life. I will always live with the cultural and moral heritage they gave me.

Avant-propos

This thesis is by articles. Each of the 27 chapters (other than the thesis introduction and the thesis conclusion) is an inserted published article with the following details:

Chapter 1

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Broken energy homeostasis and obesity pathogenesis: The surrounding concepts. *Journal of Clinical Medicine*, 7(11): 453, 2018. doi: https://doi.org/10.3390/jcm7110453

Article Status: Published in *Journal of Clinical Medicine* (2022 impact factor: 4.964) on 20 November 2018.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 2

Abdelaziz Ghanemi and Jonny St-Amand. Redefining obesity toward classifying as a disease. *European Journal of Internal Medicine* 55: 20-22, 2018. doi: https://doi.org/10.1016/j.ejim.2018.05.025

Article Status: Published in *European Journal of Internal Medicine* (2022 impact factor: 7.749) on 26 May 2018.

Differences between the published article and the inserted version: Added french abstract, added english abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-author.

Co-author: Jonny St-Amand.

Chapter 3

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Obesity as a neuroendocrine reprogramming. *Medicina*, 2021, 57(1):66. doi: https://doi.org/10.3390/medicina57010066

Article Status: Published in *Medicina* (2022 impact factor: 2.948) on 13 January 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 4

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Regeneration during obesity: An impaired homeostasis. *Animals*, 10 (12): 2344, 2020. doi: https://doi.org/10.3390/ani10122344

Article Status: Published in *Animals* (2022 impact factor: 3.231) on 9 December 2020.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 5

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic ? *Medical Hypotheses*.144 C: 110042, 2020. doi: https://doi.org/10.1016/j.mehy.2020.110042

Article Status: Published in *Medical Hypotheses* (2022 impact factor: 4.411) on 25 June 2020.

Differences between the published article and the inserted version: Added french abstract, added english abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 6

Abdelaziz Ghanemi, Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand. Exercise and high-fat diet in obesity: Functional genomics perspectives of two energy homeostasis pillars. *Genes*. 11 (8): 875, 2020. doi: https://doi.org/10.3390/genes11080875

Article Status: Published in *Genes* (2022 impact factor: 4.141) on 31 July 2020.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand.

Chapter 7

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina*, 57 (5): 399 2021. doi: https://doi.org/10.3390/medicina57050399

Article Status: Published in *Medicina* (2022 impact factor: 2.948) on 21 April 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 8

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. High-fat diet-induced trefoil factor family member 2 (TFF2) to counteract the immune-mediated damage in mice. ***Animals***, 11 (2): 258, 2021. doi: https://doi.org/10.3390/ani11020258

Article Status: Published in *Animals* (2022 impact factor: 3.231) on 21 January 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 9

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Trefoil factor family member 2 (TFF2) as an inflammatory-induced and anti-inflammatory tissue repair factor. *Animals*,10 (9): 1646, 2020. doi: https://doi.org/10.3390/ani10091646

Article Status: Published in *Animals* (2022 impact factor: 3.231) on 14 September 2020.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 10

Abdelaziz Ghanemi, Aicha Melouane, Octave Mucunguzi, Mayumi Yoshioka and Jonny St-Amand. Energy and metabolic pathways in trefoil factor family member 2 (*Tff2*) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sciences*.215: 190-197, 2018. doi: https://doi.org/10.1016/j.lfs.2018.11.006

Article Status: Published in *Life Sciences* (2022 impact factor: 6.78) on 7 November 2018.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I did the data interpretation, hypothesized the results interpretation, designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Aicha Melouane, Octave Mucunguzi, Mayumi Yoshioka and Jonny St-Amand.

Chapter 11

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Trefoil factor family member 2: From a high-fat-induced gene to a potential obesity therapy target. *Metabolites*, 11(8):536, 2021. doi: https://doi.org/10.3390/metabo11080536

Article Status: Published in *Metabolites* (2022 impact factor: 5.581) on 12 August 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 12

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Trefoil Factor Family Member 2 expression as an indicator of the severity of the High-Fat Diet-Induced obesity. *Genes*, 12 (10): 1505, 2021. doi: https://doi.org/10.3390/genes12101505

Article Status: Published in *Genes* (2022 impact factor: 4.141) on 26 September 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 13

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Diet Impact on Obesity beyond Calories and Trefoil Factor Family 2 (TFF2) as an Illustration: Metabolic Implications and Potential Applications. *Biomolecules*, 11 (12): 1830, 2021. doi: https://doi.org/10.3390/biom11121830

Article Status: Published in *Biomolecules* (2022 impact factor: 6.064) on 4 December 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 14

Abdelaziz Ghanemi, Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *The International Journal of Biochemistry & Cell Biology*, 117: 105627, 2019. doi: https://doi.org/10.1016/j.biocel.2019.105627

Article Status: Published in *The International Journal of Biochemistry & Cell Biology* (2022 impact factor: 5.652) on 4 October 2019.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand.

Chapter 15

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Secreted protein acidic and rich in cysteine: Metabolic and homeostatic properties beyond the extracellular matrix structure, *Applied Sciences*, 10 (7): 2388, 2020. doi: https://doi.org/10.3390/app10072388

Article Status: Published in *Applied Sciences* (2022 impact factor: 2.838) on 1 April 2020.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 16

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine*, 133 C: 155179, 2020. doi: https://doi.org/10.1016/j.cyto.2020.155179

Article Status: Published in *Cytokine* (2022 impact factor: 3.926) on 30 June 2020.

Differences between the published article and the inserted version: Added french abstract, added english abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I, designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 17

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine*, 127 C: 154996, 2020. doi: https://doi.org/10.1016/j.cyto.2020.154996

Article Status: Published in *Cytokine* (2022 impact factor: 3.926) on 16 January 2020.

Differences between the published article and the inserted version: Added french abstract, added english abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 18

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Secreted protein acidic and rich in cysteine as a regeneration factor: Beyond the tissue repair. *Life*, 11 (1): 38, 2021. doi: https://doi.org/10.3390/life11010038

Article Status: Published in *Life* (2022 impact factor: 3.253) on 8 January 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 19

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Secreted protein acidic and rich in cysteine as a molecular physiological and pathological biomarker. *Biomolecules*, 11(11): 1689, 2021. doi: https://doi.org/10.3390/biom11111689

Article Status: Published in *Biomolecules* (2022 impact factor: 6.064) on 13 November 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 20

Abdelaziz Ghanemi, Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand. Exercise training of secreted protein acidic and rich in cysteine (*Sparc*) KO mice suggests that exercise-induced muscle phenotype changes are SPARC-dependent. *Applied Sciences*, 10 (24): 9108, 2020. doi: https://doi.org/10.3390/app10249108

Article Status: Published in *Applied Sciences* (2022 impact factor: 2.838) on 20 December 2020.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I partcipated in generating and analysing the data, did the data interpretation, hypothesized the results interpretation, designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand.

Chapter 21

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. *Diseases*, 9 (4): 87, 2021. doi: https://doi.org/10.3390/diseases9040087

Article Status: Published in *Diseases* (PubMed-indexed and Web of Science-Indexed) on 29 November 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 22

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Exercise, Diet and Sleeping as Regenerative Medicine Adjuvants: Obesity and Ageing as Illustrations. *Medicines*, 9 (1): 7, 2022. doi: https://doi.org/10.3390/medicines9010007

Article Status: Published in *Medicines* (PubMed-indexed) on 14 January 2022.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 23

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Impact of Adiposity and Fat Distribution, Rather than Obesity, on Antibodies as an Illustration of Weight Loss-Independent exercise benefits. *Medicines*, 8 (10): 57 , 2021. doi: https://doi.org/10.3390/medicines8100057

Article Status: Published in *Medicines* (PubMed-indexed) on 8 October 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 24

Abdelaziz Ghanemi, Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand. Secreted Protein Acidic and Rich in Cysteine (*Sparc*) knock-out Leads to an Accelerated Ageing Phenotype Which is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice. *Metabolites*, 12 (2): 125, 2022. doi: https://doi.org/10.3390/metabo12020125

Article Status: Published in *Metabolites* (2022 impact factor: 5.581) on 28 January 2022.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I partcipated in the experimental designe as well as in generating and analysing the data, did the data interpretation, hypothesized the results interpretation, designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Aicha Melouane, Mayumi Yoshioka and Jonny St-Amand.

Chapter 25

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. *Genes*, 12 (11): 1823, 2021. doi: https://doi.org/10.3390/genes12111832

Article Status: Published in *Genes* (2022 impact factor: 4.141) on 20 November 2021.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 26

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Secreted Protein Acidic and Rich in Cysteine as an Exercise-Induced Gene: Towards Novel Molecular Therapies for Immobilization-Related Musculoskeletal Atrophy in Elderly Patients. *Genes*, 13 (6): 1014, 2022. doi: https://doi.org/10.3390/genes13061014

Article Status: Published in *Genes* (2022 impact factor: 4.141) on 04 June 2022.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Chapter 27

Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand. Genetic Expression between Ageing and Exercise: Secreted Protein Acidic and Rich in Cysteine as a Potential "Exercise Substitute" Antiageing Therapy. *Genes*, 13 (6): 950, 2022. doi: https://doi.org/10.3390/genes13060950

Article Status: Published in *Genes* (2022 impact factor: 4.141) on 26 May 2022.

Differences between the published article and the inserted version: Added french abstract and renumbered the sections to fit the thesis format.

My author status: First author.

My contribution (roles): I designed the paper structure and wrote the original draft. Afterwards, I revised and edited the paper with the co-authors.

Co-authors: Mayumi Yoshioka and Jonny St-Amand.

Introduction

Imbalances in energy homeostasis and metabolic biofunctions represent the origins of diverse health problems and diseases including obesity. Obesity can result from the accumulated effects of minor imbalances between energy intake and expenditure [1]. World Health Organization defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk factor for diabetes, cardiovascular disease, cancer and other chronic diseases [2]. The prevalence of this public health burden increased in the last years in Canada [3, 4] which made its prevention and management strategies a public health concern [5, 6]. The fat accumulation is the consequence of energy imbalances related to complex factors such as food intake, genetics, and environment, resulting is an energy intake higher than energy expenditure. The modern lifestyle, characterized by both limited physical activity and increased caloric intake (due to both the quantity as well as the quality of diet), made obesity a health problem with an epidemic proportion [7] requiring developing new therapeutic approaches with improved efficacy.

As obesity management mainly focuses on diet and exercise, my thesis explores two key genes related to these two important factors. The two genes have been identified via functional genomics methods as related to both diet (high fat) and exercise. For the diet, I have focused on high-fat (HF) diet since HF food promotes weight gain through the high caloric density, low satiety effect and high palatability of HF nutrients, as well as the weak potency for fat oxidation and energy expenditure that are associated to fat ingestion [8-10]. Indeed, both limiting caloric intake and exercise are at the center of the approaches to manage obesity. Therefore, finding genes related to both diet, mainly HF diet, and exercise and exploring their metabolic and energy homeostasis implications would represent a starting point to identify novel molecular therapeutic targets for obesity in addition to deepen our knowledge about obesity-related underlying mechanisms. Functional genomics represents one of the strong tools for such genes characterization via exploring genes-related energy metabolism variations.

Functional genomics allows the study of gene expression under the influence of dynamic conditions such as HF diet and exercise. Our research team has already identified two genes specifically expressed after HF diet and exercise respectively. Trefoil factor

family member 2 (*Tff2*) has been identified as a newly found HF-specific gene [11, 12] for which its deficiency in mice leads to a protection from HF diet-induced obesity [13]. Secreted protein acidic and rich in cysteine (*SPARC*/*Sparc*) was characterized as an exercise-induced gene, both in vivo [14] and in vitro (electrical pulse stimulation; considered as the in vitro form of exercise) [15]. These represent key steps toward understanding the underlying mechanisms of obesity and identifying potential therapeutic targets. As HF diet and exercise are two key pillars of energy homeostasis and obesity research, the laboratory work of my PhD studies explored the roles of the HF diet-induced gene *Tff2* and the exercise induced gene *Sparc* in the contexts of energy balance and metabolism. For the *Tff2,* I explored changes in *Tff2* knock out (KO) mice challenged with either HF or low-fat diets with fasted mice as the control group (which is novel). The research focused on explaining how *Tff2* KO mice were protected from HF diet-induced obesity. For the *Sparc* exploration, it had two parts. One explored *Sparc* KO effects with exercise (Vs sedentary mice) and age (old Vs young mice) as variables. The second part explored the *Sparc* overexpression impacts on the metabolism of both male and female mice. An analytic interpretation that combines *Sparc* KO and *Sparc* overexpression as compared to ageing and exercise is also presented. Changes in *Tff2* KO, *Sparc* KO and *Sparc*-overexpressing mice, were mainly evaluated by mapping the metabolic profiles and the related phenotypes via selected parameters related to adiposity, metabolism and energy homeostasis in selected tissues mainly related to energy expanditure.

The thesis starts with an overview and generalities about obesity (chapters 1 to 5). After that I present a review (chapter 6) that highlights the importance of functional genomics in identifying diet-related and exercise-related genes, such as our two genes of interest, in obesity research before I move to the chapters related to both *Tff2* and *Sparc* respectively. Chapters 7 to 9 introduce animal models of obesity and TFF2. Chapter 10 is my research exploring energy and metabolic pathways in *Tff2* KO mice beyond the protection from HF diet-induced obesity. This is followed by chapters 11 to 13 that provide outcomes and potential applications of those *Tff2*-realted findings.

The remaining chapters are related to *Sparc* parts of my thesis. I start by introducing diverse aspects of SPARC (chapters 14 to 19) before presenting my research paper showing

that exercise training of *Sparc* KO mice suggests that exercise-induced muscle phenotype changes are SPARC-dependent (chapter 20). Then, I introduce relevant information about exercise and ageing (chapters 21 to 23) before presenting the second research paper about *Sparc* in which I show that *Sparc* KO leads to an accelerated ageing phenotype which is improved by exercise whereas SPARC overexpression mimics exercise effects in mice (chapter 24). I finally present perspective in terms of how to apply SPARC-related properties in biomedical research and therapeutics (chapters 25 to 27).

The key objectives and hypotheses of the research articles (chapters 10, 20 and 24) cover various aspects. For chapter 10 (*Tff2* part), since *Tff2* KO mice are protected from HF diet-induced obesity although they have an increased food intake, this chapter aims to answer the question of how *Tff2* KO mice are protected from HF diet-induced obesity. The hypothesis is that *Tff2* KO mice have increased energy expenditure and metabolic activity. In addition, *Tff2* KO mice would also have increased uptake of biochemical fuels (glucose, free fatty acids, triglyceride and glycerol) by the key metabolic tissues such as adipose tissue, skeletal muscle and liver. For chapters 20 and 24 (*Sparc* parts), since SPARC is overexpressed with exercise and declines with ageing, we aim to investigate SPARC implications (including metabolic implications) and mechanisms within the contexts of both exercise and ageing. The first hypothesis is that SPARC is required for exercise benefits which would mean that whereas *Sparc* KO would limit exercise benefits, SPARC overexpression would generate an exercise-like phenotype. The second hypothesis is that SPARC decline is involved in ageing process which would mean that whereas *Sparc* KO would speed up ageing, SPARC overexpression would counteract ageing.

The final goal of my research work is to both contribute to the elucidation of obesity-related pathways and mechanisms as well as to identify potential therapeutic targets. These targets would represent starting points towards a new generation of molecular approaches in obesity-related pharmacology. Such therapeutic advances would also be of benefits in other related diseases and health problems such as ageing, sarcopenia, diabetes and metabolic disorders. The results related to both *Tff2* and *Sparc* highlight the importance of functional genomics as a molecular tool to identify genes of interest in obesity and its related diseases.

Chapter 1. Review - Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Quebec G1V 0A6, Canada

[2] Functional Genomics Laboratory, CREMI, Québec Genome Center, CHUL-CHU de Québec Research Center, Québec, Québec G1V 4G2, Canada

Received: 25 September 2018; Accepted: 19 November 2018; Published: 20 November 2018

1.1 Résumé (French abstract)

L'obésité représente une accumulation anormale de graisse résultant de déséquilibres énergétiques. Définir et classer l'obésité est le premier défi pour comprendre ce problème de santé multifactoriel. Dans cette revue, nous rapportons des exemples illustratifs sélectionnés de mécanismes liés à l'obésité. Nous discutons également de l'axe intestin-cerveau et des hormones en tant que contrôleurs de l'homéostasie énergétique et les impacts de l'obésité sur des tissus métaboliques clés. Le concept de « équilibre énergétique brisé » est détaillé comme une étape clé dans l'obésité. Le manque de sommeil et les facteurs psychologiques ont également un impact sur le développement de l'obésité. La description de telles voies mécanistiques permettrait aux cliniciens, aux biologistes et aux chercheurs de développer et d'optimiser des approches et des méthodes pour le diagnostic, la classification, l'évaluation clinique, le traitement et le pronostic de l'obésité.

1.2 Abstract

Obesity represents an abnormal fat accumulation resulting from energy imbalances. It represents a disease with heavy consequences on population health and society economy due to its related morbidities and epidemic proportion. Defining and classifying obesity and its related parameters of evaluation is the first challenge toward understanding this multifactorial health problem. Therefore, within this review we report selected illustrative examples of the underlying mechanisms beyond the obesity pathogenesis which is systemic rather than limited to fat accumulation. We also discuss the gut-brain axis and hormones as the controllers of energy homeostasis and report selected impacts of obesity on the key metabolic tissues. The concepts of "broken energy balance" is detailed as the obesity

starting key step. Sleep shortage and psychological factors are also reported with influences on obesity development. Importantly, describing such mechanistic pathways would allow clinicians, biologists and researchers to develop and optimize approaches and methods in terms of diagnosis, classification, clinical evaluation, treatment and prognosis of obesity.

Keywords: obesity; pathogenesis; homeostatic mechanisms; energy misbalance; “broken energy balance”; therapy

1.3 Defining and Classifying Obesity: Fat Distribution Rather Than Fat Accumulation

The World Health Organization defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk factor for diabetes, cardiovascular disease, cancer and other chronic diseases [1]. The fat accumulation towards adipogenesis and adipocytes expansion is the consequence of energy imbalances related to complex factors (genetics, environment, etc.) and results from an energy intake higher than energy expenditure. The modern obesogenic environment, characterized mainly by limited physical activity and increased caloric intake, due to both the quantity (theoretically limitless availability) and the quality (high-fat and high-sugar) of diet, has significantly contributed to making obesity a health problem of epidemic proportions [2] worldwide. Therefore, there is a need to better understand, define and classify obesity according to updated parameters (biological, physiological, etc.). Body mass index (BMI) is used to evaluate the obesity status at a population level [1]. Although BMI is not accurate to characterize obesity at the individual level nor a body fat measure, it is still used in the daily clinical practice due to the fact that it is simple to measure and with almost no cost. However, the clinical and biological information about the patient profile that BMI provides remain limited.

Therefore, additional parameters have been suggested to classify obesity and, more importantly, to evaluate the associated risks depending on factors beyond simple anthropometric measures. Indeed, taking into consideration more parameters, such as the hypertriglyceridemic-waist phenotype [3], waist-hip ratio (WHR) and waist circumference (WC) [4,5], has allowed a certain categorization of obese patients. However, the limits of such parameters have been shown through the history of obesity classification [6], which has encouraged researchers to continuously develop more optimized classification systems with measures that would reflect the impact that obesity has on both individual and population health. Indeed, the addition of measures, such as lipidic blood profile and fat distribution, allowed a more optimized characterization of obese patients. The first step toward establishing an accurate obesity classification system is a precise understanding of the molecular mechanisms behind its development. This requires looking at obesity as complex metabolic misbalances in key cell types including adipocytes, hepatocytes and

myocytes with different etiologies (genetics, biological stress, unhealthy diet, etc.), leading to a variety of clinical outcomes (diabetes, hypertension, atherosclerosis, metabolic syndrome, insulin resistance, etc.) [7,8], rather than a simple fat accumulation that develops once adiposity increases beyond a "normal" level of development (Figure 1.1). Moreover, obesity-related metabolic patterns are not specific nor limited to some organs or tissues but rather impact all the biological systems such as endocrine functions as well as physiological homeostasis which makes obesity management treatment more challenging.

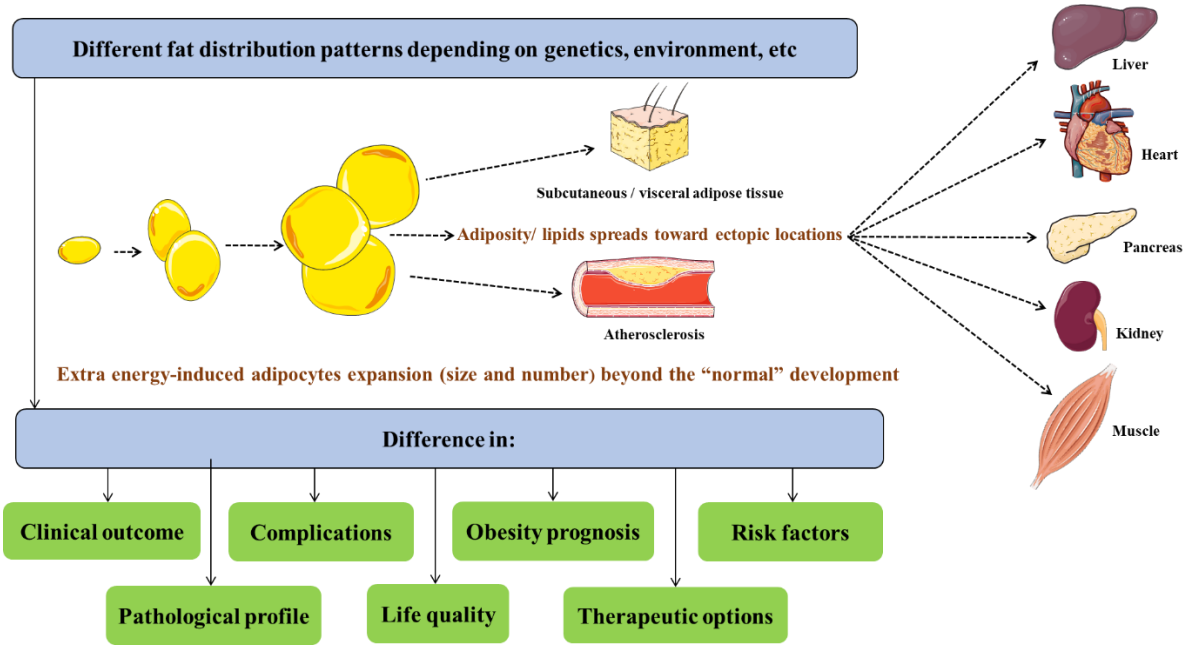


Figure 1.1. Different fat distribution patterns with diverse outcomes.

Indeed, obesity represents a systemic molecular and cellular homeostatic dysfunction. For instance, diverse illustrative examples of molecular-level impacts of obesity could be given and correlated with clinical features of obesity. Indeed, insulin resistance observed in obese patients is associated with lipid levels [9] and the palmitate lipotoxicity link with selective insulin resistance in hepatocytes put a spotlight on the lipid effects [10]. In addition, phenomena such as atherosclerosis are related to high-fat diet and levels of blood lipids [11,12]. On the other hand, reducing weight, which is mainly the loss of adiposity, leads to significant improvement in different health indicators especially cardiovascular risk factors [13,14]. Moreover, exercise and healthy diet even without weight loss still improve the cardiometabolic profile of obese individuals [15], suggesting that reducing lipid mass percentage (increasing muscle mass percentage in the case of exercise intervention) rather than the whole body weight is behind these observed beneficial effects. Therefore, the amelioration in the risk factors profile would be attributed to changes in WHR and WC, which are also two parameters that reflect both cardio-metabolic risk and fat distribution [4,5]. Thus, WHR and WC represent good parameters to optimize obesity definition, classification and the evaluation of the related risks. These

observations further suggest that the underlying causes of obesity-related health disorders are lipid accumulation and distribution, rather that body weight. This is important too, precisely because some individuals have the same BMI but different body fat mass and, especially, different forms of adiposity distributions [16], indicating the importance of fat distribution types (central, ectopic, subcutaneous, etc.) (Figure 1.1) in pathogenesis and clinical diversity [17].

Understanding obesity through the underlying pathway changes will allow us to overcome the main challenge facing obesity studies, which includes explaining the etiologies and mechanisms of obesity linking molecular and cellular modifications to the clinical outcomes. Therefore, enabling a better understanding of this health problem is the first step toward developing efficient therapies for this medical challenge.

1.4 Gut-Brain Axis and Energy Homeostasis Hormones: Is This Neuro-Endocrine System Inefficient in Obesity?

Factors leading to or protecting from obesity, such as food intake, energy expenditure, lipids storage and glucose usage, are under the control of different neuro-endocrine systems. The gut-brain axis and metabolic hormones represent the best illustrations. Gut-brain axis refers to the signals (including gastrointestinal hormones) exchanged between selected neuroanatomical structures and the digestive system to control those factors leading to or protecting from obesity [18]. Therefore, to fully map the pathways beyond the metabolic changes, we need a description of the central mechanisms controlling energy homeostasis via gastrointestinal hormones, such as ghrelin, glucagon like peptide 1, peptide YY 3–36, cholecystokinin and amylin, which have modified levels in obese patients compared to non-obese individuals [18]. These hormonal signals are messengers from the gastrointestinal tract, and provide the brain with a “report” about the ingested food so that the energy homeostasis centers can adjust signals toward balancing the energy metabolism (food intake and energy expenditure). However, these gastrointestinal hormones, in addition to insulin, are secreted mainly following the ingestion of carbohydrates, rather than lipids, showing that the brain may remain “blind” when lipids are ingested. This fact could be one of the significant causes of why lipids accumulate easily, since there might not be strong signals sent to the brain when lipid transit through the gastrointestinal system to limit the food intake. Moreover, dietary lipids, which are more available and more accessible than ever in the modern diet, have high caloric density and are associated with high palatability, but have a limited effect on satiety [19], which further contributes to increasing the energy intake toward developing obesity.

Importantly, further elucidation of the modifications at the gut-brain axis represents also a key concept to understanding obesity from a neuroendocrine perspective. The “deregulation” of the gut-brain axis might be the starting point for obesity pathogenesis, rather than an adaptive consequence. Indeed, once obesity is established, the mechanisms controlling energy balance that are governed by central neural networks (mainly with

receptors of gastrointestinal hormones), are blunted and are not able to protect from the diet-induced obesity [20,21], nor are they able to reverse it anymore (Figure 1.2). Following this, the brain sets a new increased body weight "reference" and biological regulation acts toward maintaining it. This makes losing weight, or maintaining body weight after weight loss, difficult [22] due to different mechanisms. These mechanisms include metabolic slowing [22] as an adaptation ability to compensate the decreased food energy intake and/or the increased energy expenditure, along with a modification in the reward system, creating an addiction-like situation via reward circuits involving diverse neurological structures including the brain areas of the melanocortin, opioid and endocannabinoid networks in addition to the dopamine mesolimbic system [23]. Therefore, clarifying changes seen among obese patients in these brain networks would allow us to identify the related central pathways and understand the physiopathology beyond this abnormal accumulation of stored energy. An accumulation that is caused by an inefficiency of the neuro-endocrine system supposed to control energy metabolism.

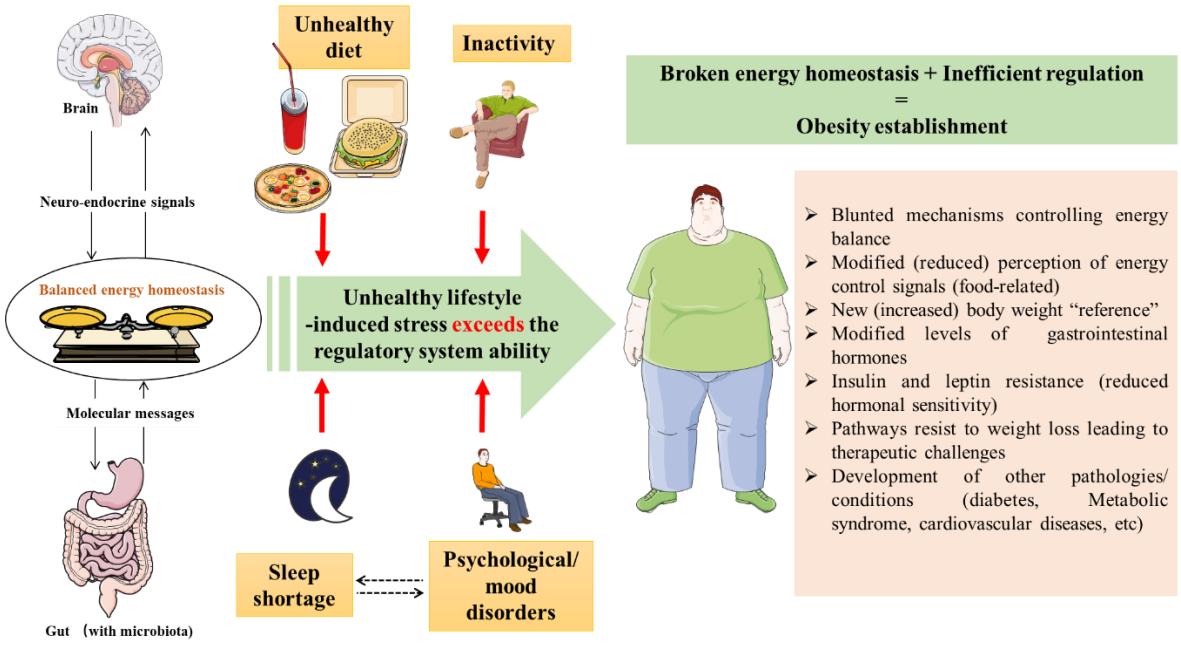


Figure 1.2. Theory of obesity establishment despite the existence of mechanisms balancing the energy homeostasis. An unhealthy lifestyle (inactivity, unhealthy diet, sleep shortage and psychological disorders) puts pressure on the energy homeostasis balance and breaks it, which leads to obesity development. Once obesity established, the mechanisms of energy balance are blunted, and the brain sets an increased body weight as a new reference, which makes correcting obesity difficult (biological pathways resist weight loss).

Following the same line of thought, elements in food such as glutamate and fatty acids stimulate signals leading to modifications in energy metabolism that are towards weight loss [18]. Therefore, we suggest that the biological perception (and therefore the response in terms of energy balance control) of such signals would be modified in obese subjects, and with limited signaling effects. In addition to the gut-brain axis, other

hormones influence the energy homeostasis. For instance, adipokines modulate various metabolic functions such as glucose and lipid metabolism, energy expenditure, insulin sensitivity and satiety [24], which makes adipokines among the key hormones in energy homeostasis. Leptin, probably the key hormone in obesity, is produced by adipose tissue [25] and controls both energy expenditure and food intake [26]. The production of this hormone correlates with the body fat mass which highlight it as modified in obesity and thus, worth exploring for a potential "molecular description" of obesity at the central level as well (central control, rather that direct peripheral metabolic effects).

Importantly, since a neuro-endocrine system that controls energy homeostasis exists, how can some individuals still develop obesity? This is probably explained by the limits of resistance of this system. Although this system tends to balance energy expenditure and intake, putting "biological pressure" on this system via an unhealthy lifestyle such as high-fat diet and inactivity will overcome the efficiency of this system and exceeds its regulatory ability. Once the energy homeostatic balance is "broken", the neuro-endocrine system will fail to establish energy balance due to the inefficiency of this system (Figure 1.2). Importantly, the role played by gut microbiota within the context of obesity and the gut-brain axis [27] makes it worth exploring, as well. However, at this stage, it seems essential to ask an important question: is this neuro-endocrine system equilibrium broken after obesity develops, or does obesity represent a consequence of the loss of the efficiency of this system? Answering this question would be a major step towards understanding obesity pathogenesis, and therefore, how it should be treated.

1.5 Metabolic Tissues Undergo the Energy Misbalance and the "Modified Paradigm" of Signaling Molecules in Obesity

Biology gave humans the ability to store energy as a tool to face situations of hunger and food shortage. From an evolutionary viewpoint, and compared to modern societies, humans who lived decades ago did not have limitless access to food, nor did they follow the modern unhealthy lifestyle [28]. Thus, the tissues and organs under the modern obesogenic environment, which represents a biological pressure, undergo changes that could be adaptive to this imbalanced energy homeostasis.

Adipose tissue has the ability to store energy, but once the balance of the gut-brain axis is broken (Figure 1.2), the expansion of adipose tissue both in size and cell number becomes important, obesity is established and adiposity spreads toward ectopic locations [16], which results in different fat distribution patterns (Figure 1.1). The starting point of metabolic consequences of obesity is mainly due to increased fat storage with the visceral adiposity as the most deleterious form. For instance, a study aiming to map the visceral adipose tissue metabolism pattern in obese subjects has shown increased oxidative stress and markers and markers of elevated glucose levels, in addition to elevated levels of plasmalogens and changes in various lipidic molecules (including glycerol phosphorylcholine, ceramides and sphingolipids) [29]. Such observations fit with and

explain some of the clinical aspects seen among obese patients or epidemiologically linked to obesity. As an illustration, the increase in oxidative stress-produced free radicals correlates with the epidemiological links between obesity and some cancers [30–32].

In addition, variations in various lipidic molecule patterns observed in obese patients show that the problems associated with lipids in obese patients is not limited to an accumulation in adipose tissue, but are rather exported to other tissues and systems (Figure 1.1) such as the liver (non-alcoholic steatohepatitis) [33], blood vessels walls (atherosclerosis) [34], and muscles (insulin resistance) [35]. This illustrates the systemic character of obesity consequences. Within this context, the known effects of hormones like insulin and leptin on the metabolism of lipids and glucose/glycogen act mainly on the muscle and adipose tissue [36]. Changes seen in these two key hormonal systems in obese subjects allow us to predict the impact of obesity on the main metabolic organs as a consequence of modified insulin sensitivity for example, especially with the interactions between leptin and insulin [37] and the distribution of leptin receptors [38]. These lead to important metabolic imbalances in lipid and glucose metabolism so that these two energy substrates become "biotoxic" rather than just biofuels. Indeed, diabetes complications illustrate the consequences of a deregulated glucose metabolism and cardiovascular disease points to those of lipid metabolism imbalances.

In obesity, we have increased adiposity with greater adipocyte size and number, leading to a higher leptin concentration, but without reduced food intake (leptin resistance [39]), which is an example of the consequences of altering the neuro-endocrine system controlling energy homeostasis. Moreover, the increased body weight of obese patients suggests an increased need for muscle to carry extra weight, which would require more energy usage that could also be affected by insulin resistance (resulting from unhealthy lifestyle [40]) and deregulation of the metabolism of both glucose and lipids. Metabolic syndrome [41] could be seen as the most significant manifestation of these metabolic disorders, where systems and tissues become metabolically unhealthy, with insulin signaling being at the center of the syndrome [7]. Importantly, the implication of both leptin and insulin in diverse homeostatic functions (not only energy metabolism) [39, 42] makes the development of insulin and leptin resistance a physiological problem beyond energy metabolism. Furthermore, since brown adipose tissue (BAT) is not only under the influence of the sympathetic nervous system but is also controlled by factors such as adipokines and metabolites [43], the balance and metabolic properties of this adipose tissue may be modified in obese patients following the hormonal deregulation as well.

These selected examples highlight some of the effects of obesity on key metabolic tissues (adipose tissue, liver and muscle), placing obesity among the top metabolic disorders and further support the need for a "metabolic classification" for obesity. Moreover, our illustrations highlight the concept of the "broken homeostatic system" (Figure 1.2), within which signaling molecules properties and metabolites affect the physiological systems differentially during obesity compared to a healthy status. Such a concept of a "modified paradigm" of hormones and signaling molecule effects on metabolic

functions of obese individuals is extremely important, since it indicates how the same hormone, for instance, could have different properties (for example insulin resistance or sensibility) depending on whether it is under physiological (healthy) or pathological (obesity) conditions, and could therefore explain possible "contradictory" results of studies comparing healthy individuals with obese patients beyond which we can find that gut-brain axis hormone levels in obese patients are different from those in non-obese individuals [18].

1.6 Beyond the Energy Balance: Sleep Shortage and Psychological Effects as Examples of "Non-Caloric Factors"

Obesity cannot be reduced to a biochemical balance or a mathematical model of energy homeostasis. It is, indeed, a complex consequence with a variety of influencing factors, including "non-caloric" representing factors other than food intake and physical activity. For instance, sleep shortage has been linked to obesity [44–46], and the literature also reports how circadian rhythms regulate diverse metabolic functions [47]. The studies of Spiegel et al. indicated lower glucose tolerance, decreased leptinemia, increased cortisol and ghrelin plasma concentrations, and higher hunger and appetite in cases of sleep deprivation [48,49]. Additionally, sleep restriction, via a variety of pathways, also increases insulin resistance with risk of diabetes and modifies appetite-related hormones such as glucagon-like peptide 1 and leptin [50]. These alterations allow us to map a metabolic link between restricted sleep duration and obesity via selected hormonal (leptin, cortisol, glucagon-like peptide 1 and ghrelin) functions and the metabolism of lipids and carbohydrates. Indeed, such biological changes toward increased food intake and energy storage lead to fat accumulation (Figure 1.3). The fact of staying awake could send "wrong signals" to the brain to indicate the need for staying active, and thus a need for energy, which leads to an increased appetite combined with energy storage (fat accumulation) as an "adaptation" to this energy need which is, in fact, due to the control mediated by hormones (cortisol, ghrelin, etc.) that are also deregulated under such sleep shortage conditions. Thus, a regulated circadian rhythm should remain a part of a healthy lifestyle, as well as an approach to obesity management and metabolism regulation [51]. Additionally, it is worth mentioning that both psychological outcomes and sleeping quality are improved following weight loss [52,53], which would reduce obesity risk and improve metabolic profile. This also indicates that not only do sleeping and psychological status affect obesity development, but obesity also has an impact on these two parameters, as well (Figure 1.3).

On the other hand, different studies have also linked psychological disorders and mental health problems such as depression and anxiety [54] to obesity [55–58] (Figure 1.3). Such problems could either be directly related to obesity or rather be a consequence of how society - including healthcare professionals - behaves toward obese patients [59], and thus could be described as a sociopsychological consequence. In both cases, assistance could be required in the form of psychotherapy, social help or even economic assistance for obese

patients. Moreover, psychotherapy [60] will not only assist the obese patients in dealing with the psychological consequences related to obesity, but it could also further stimulate them and strengthen their will to adhere to weight loss programs [61] - via sports psychology, for instance [62] - to changes behavior [63], which is key to initiating obesity therapy.

Within this context, it would seem acceptable to assume that sleep shortage and psychological factors contribute to the status of a "broken homeostatic system" described previously (Figure 1.2), leading to inefficient energy metabolism control. Moreover, sleep quality and psychological factors are two concepts that are often associated with each other. Thus, having a problem with either would affect the other and increase the probability of developing obesity or worsen the prognosis when obesity or overweight are already present. Moreover, obesity association with mood disorders (depression and anxiety risk) and cognitive deficiency (in learning and memory) [27] would create a vicious circle. Importantly, the possible interactions between the different neuronal networks involved in energy balance, sleep control and psychologic (and psychiatric) regulation would not only explain the effects of sleep shortage and psychology, as "non-caloric factors", on obesity but also require an increased pharmacovigilance for pharmacotherapies targeting any of these systems to prevent the effects on the other neuronal networks [64].

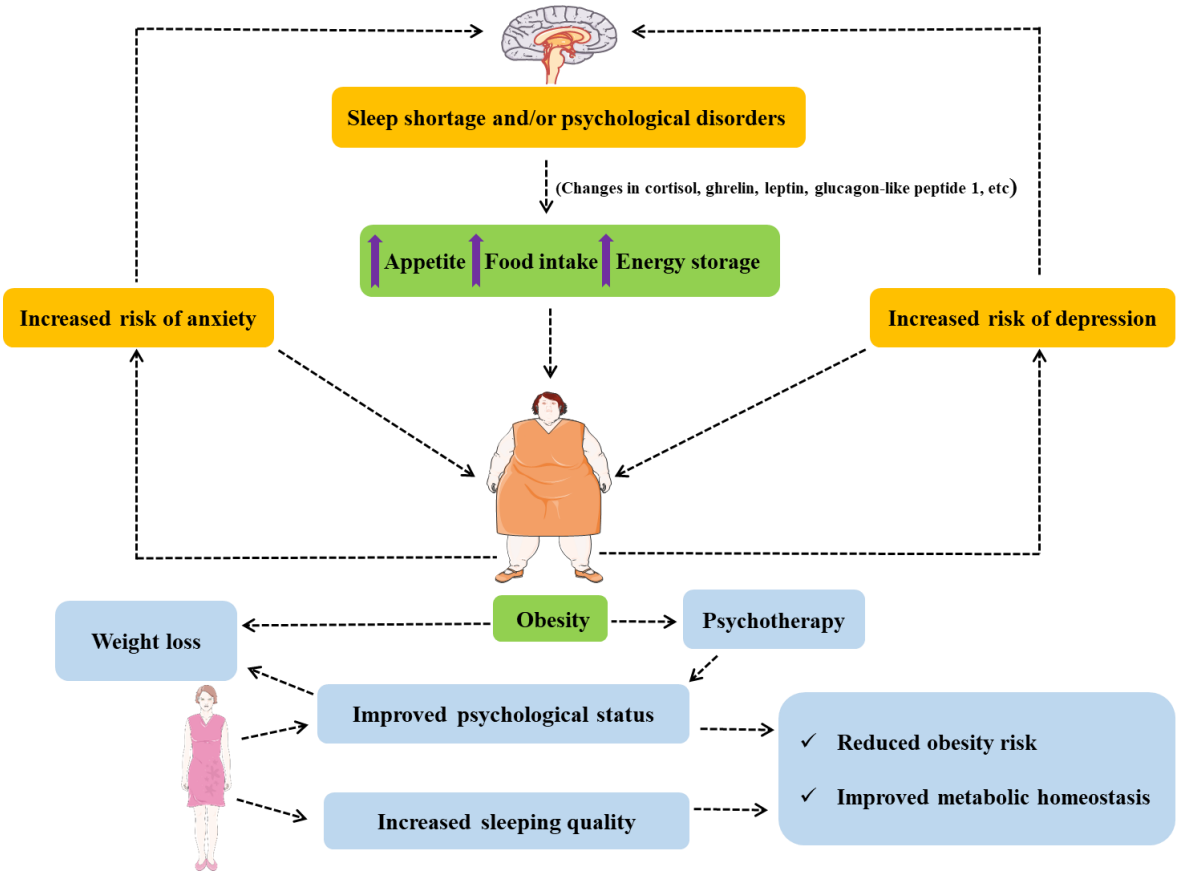


Figure 1.3. Sleeping, psychology and obesity.

1.7 Toward an Optimized Obesity Management

The BMI and different anthropometric measures represent the most commonly used parameters to classify individuals as overweight, obese or “normal”-weight. However, evaluating the biological disorders (glucose metabolism disorders, lipid accumulation, etc.) and mechanistic imbalances seen in obese patients at various levels (hormonal, metabolisms, psychology, signal resistance, etc.) should be used as supplementary elements in diagnosis and classification. This description would explain the underlying mechanistic origins of the clinical manifestations of obesity such as hyperlipidemia and hyperglycemia, rather than just report them. In addition, establishing a mechanistic link between the pathogenesis of obesity and molecular biochemical variations (hormonal levels, glycemia, triglycerides, fatty acids, etc.) could make it possible to either add or exclude biological variables from the classification parameters of obesity, diagnosis and therapeutic targets. Also, putting a spotlight on the molecular mechanisms of obesity may strengthen the available diagnostic tools by adding new biological parameters to the standard measure of BMI. Moreover, it clarifies the links between obesity and other metabolic-related diseases such as diabetes, inflammation and metabolic syndrome [41].

Importantly, these same parameters could be used as tools to establish prognosis, follow the clinical evolution and evaluate obesity therapies during laboratory research as well as clinical trials. Measuring such parameters will also represent strategic tools for prognosis and evaluation of the therapeutic approaches, although it might have a high cost. For instance, measuring different intestinal hormone levels may be a good indicator of the gut-brain axis interaction acting in terms of energy storage, food intake and appetite.

The mapping of such mechanistic pathways in metabolic and energy homeostasis in overweight and obese patients, leading to obesity-related disorders, would allow a more optimized definition and classification of obesity by taking into consideration more parameters among those described within this review. Considering these mechanistic changes and their clinical consequences in obese patients makes the pathways related to them potential therapeutic targets for obesity or at least for one of the symptoms or even other diseases that involve one or more of the metabolic changes seen in obesity. Indeed, exploring factors related to the metabolism of energy and appetite control, such as hormones of the neuro-endocrine system, represents an emerging path for the treatment of obesity [18] and the related disease, including adipokine-based strategies [65]. More importantly, the existence of an adrenergic-independent possibility to activate the BAT represents strong hope of losing weight via increased energy dissipation [43]. This approach exploits the high capacity of the BAT to uncouple the oxidative phosphorylation from the production of adenosine triphosphate (ATP) and dissipate energy in heat form rather than synthesizing ATP, which makes BAT a biological calory-burning engine. This principle could be combined with the white adipose tissue (WAT) beiging promotion [65] to both reduce WAT and increase energy expenditure.

On the other hand, the complex interactions of the homeostasis control centers and the various implicated neurotransmitters could allow us to consider obesity as a consequence of a deregulation at the nervous system and thus similar to a neurological disorder. Such an approach could be a starting point towards a more sophisticated neuropharmacological treatment of obesity that targets the neurological structures and neurotransmitters involved in energy homeostasis control. Moreover, the reward system underlying hedonic stimulation could be targeted, via an approach that mimics drug addiction therapies. However, the side effects and the related pharmacovigilance would be a challenge due to the complexity of the neuroendocrine system. Following the same line of thought, and due to the strong hormonal component in obesity, hormonal pathways (such as leptin and insulin-related) still deserve further investigations to identify novel molecular targets within the obesity-related pathways of these hormones.

Importantly, studying the metabolic effects of obesity might make some diseases considered as chronic (such as type 2 diabetes) “reversible” [66]. Indeed, since type 2 diabetes results in part from insulin resistance, treating obesity would increase insulin sensitivity and lead to a remission of type 2 diabetes [67], especially with the emerging concept of adipose tissue targeting to treat obesity-associated diabetes [65] with increased clinical hopes.

Moreover, the ability of the adipose tissue to store elements such as persistent organic pollutants and the important biological functions governed by adipose tissue (metabolic, protective, etc.) [68] reminds us about the importance of this tissue and, more importantly, increases the pharmacovigilance associated with obesity therapies and adverse effects including those related to the liberation of organic pollutants stored in the adipocytes. Such side effects demonstrate that further development of new therapeutic approaches and combining therapies with those existing is required in order to optimize them.

New research strategies, such as functional genomics and the development of novel animal models [69], would further clarify the molecular pathogenesis of obesity and map the links with the clinical aspects along with the therapeutic consequences. Importantly, the clinical consequences of developing obesity represent a bigger challenge than obesity itself. Therefore, the objective of clinicians and health care professional should not be exclusively weight loss but rather assisting the patients to establish a healthy lifestyle that balances diet, physical activity, sleep cycle and psychological status. Such an approach would not only lead to weight loss and fat mass reduction, but also improve the clinical factors related to or affected by obesity such as insulin resistance, lipid metabolism disorders, cardiorespiratory fitness, cardiometabolic conditions and chronic inflammation. Furthermore, this same approach of balancing lifestyle is beneficial even without weight loss, since many individuals who follow specific diets or exercises do not lose weight. Thus, weight loss should be seen as one single result among the benefits of a healthy life style prescribed for obese individuals rather than the unique objective especially that the risks are about the location and the distribution of the adipose tissue (mainly visceral adipose tissue) rather

that the adiposity that could be subcutaneous for example, reflecting the need to redefine obesity [70], how we assess it, treat it and establish the prognostic of the obese patients in order to further link the underlying mechanisms to the clinical outcomes and prognosis.

Funding: This research received no external funding.

Acknowledgments: All figures were created using images from: http://smart.servier.com.

Conflicts of Interest: The authors declare no conflict of interest.

1.8 References

1. Obesity. Available online: http://www.who.int/topics/obesity/en/ (accessed on 20 November 2018).
2. Obesity: Preventing and managing the global epidemic: Report of a WHO consultation. *WHO Tech. Rep. Ser.* 2000, *894*, 1–253.
3. Arsenault, B.J.; Lemieux, I.; Despres, J.P.; Wareham, N.J.; Kastelein, J.J.; Khaw, K.T.; Boekholdt, S.M. The hypertriglyceridemic-waist phenotype and the risk of coronary artery disease, results from the EPIC-Norfolk prospective population study. *CMAJ* 2010, *182*, 1427–1432.
4. Misigoj-Durakovic, M.; Soric, M.; Durakovic, Z. Anthropometry in cardio-metabolic risk assessment. *Arh. Hig. Rada. Toksikol.* 2014, *65*, 19–27.
5. Al-Domi, H.; Al-Shorman, A. Increased waist circumference is associated with subclinical atherosclerosis in schoolchildren. *Diabetes Metab. Syndr. Clin. Res. Rev.* 2019, *13*, 264–269.
6. Sharma, A.M.; Kushner, R.F. A proposed clinical staging system for obesity. *Int. J. Obes. (Lond)* 2009, *33*, 289–295.
7. American Diabetes Association. Consensus Development Conference on Insulin Resistance: 5–6 November 1997. *Diabetes Care* 1998, *21*, 310–314.
8. Saltiel, A.R.; Olefsky, J.M. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 1996, *45*, 1661–1669.
9. Kuhlmann, J.; Neumann-Haefelin, C.; Belz, U.; Kalisch, J.; Juretschke, H.P.; Stein, M.; Kleinschmidt, E.; Kramer, W.; Herling, A.W. Intramyocellular lipid and insulin resistance: A longitudinal in vivo 1H-spectroscopic study in Zucker diabetic fatty rats. *Diabetes* 2003, *52*, 138–144.
10. Li, Z.; Lai, Z.W.; Christiano, R.; Gazos-Lopes, F.; Walther, T.C.; Farese, R.V. Global Analyses of Selective Insulin Resistance in Hepatocytes due to Palmitate Lipotoxicity. *Mol. Cell. Proteomics* 2018, *17*, 836–849.
11. Siri-Tarino, P.W.; Sun, Q.; Hu, F.B.; Krauss, R.M. Saturated fatty acids and risk of coronary heart disease: Modulation by replacement nutrients. *Curr. Atheroscler. Rep.* 2010, *12*, 384–390.
12. Hur, S.J.; Min, B.; Nam, K.C.; Lee, E.J.; Ahn, D.U. Effect of dietary cholesterol and cholesterol oxides on blood cholesterol, lipids, and the development of atherosclerosis in rabbits. *Int. J. Mol. Sci.* 2013, *14*, 12593–12606.
13. Wood, P.D.; Stefanick, M.L.; Dreon, D.M.; Frey-Hewitt, B.; Garay, S.C.; Williams, P.T.; Superko, H.R.; Fortmann, S.P.; Albers, J.J.; Vranizan, K.M.; et al. Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. *N. Engl. J. Med.* 1988, *319*, 1173–1179.
14. Anderson, J.W.; Brinkman-Kaplan, V.L.; Lee, H.; Wood, C.L. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. *J. Am. Coll. Nutri.* 1994, *13*, 256–261.
15. Gaesser, G.A.; Angadi, S.S.; Sawyer, B.J. Exercise and diet, independent of weight loss, improve

cardiometabolic risk profile in overweight and obese individuals. *Phys. Sportsmed.* 2011, *39*, 87–97.
16. Despres, J.P. Body fat distribution and risk of cardiovascular disease, an update. *Circulation* 2012, *126*, 1301–1313.
17. Gastaldelli, A.; Gaggini, M. Ectopic fat: A target for cardiometabolic risk management. *Expert Rev. Cardiovasc. Ther.* 2016, *14*, 1301–1303.
18. Buhmann, H.; Le Roux, C.W.; Bueter, M. The gut-brain axis in obesity. *Best Pract. Res. Clin. Gastroenterol.* 2014, *28*, 559–571.
19. Maher, T.; Clegg, M.E. Dietary lipids with potential to affect satiety: Mechanisms and evidence. *Crit. Rev. Food Sci. Nutr.* 2018, *23*, 1–26.
20. Liu, M.; Shen, L.; Liu, Y.; Woods, S.C.; Seeley, R.J.; D'Alessio, D.; Tso, P. Obesity induced by a high-fat diet downregulates apolipoprotein A-IV gene expression in rat hypothalamus. *Am. J. Physiol. Endocrinol. Metab.* 2004, *287*, E366–E370.
21. Ziotopoulou, M.; Mantzoros, C.S.; Hileman, S.M.; Flier, J.S. Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. *Am. J. Physiol. Endocrinol. Metab.* 2000, *279*, E838–E845.
22. Fothergill, E.; Guo, J.; Howard, L.; Kerns, J.C.; Knuth, N.D.; Brychta, R.; Chen, K.Y.; Skarulis, M.C.; Walter, M.; Walter, P.J.; et al. Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. *Obesity* 2016, *24*, 1612–1619.
23. Richard, D. Cognitive and autonomic determinants of energy homeostasis in obesity. *Nat. Rev. Endocrinol.* 2015, *11*, 489–501.
24. Blüher, M.; Mantzoros, C.S. From leptin to other adipokines in health and disease: Facts and expectations at the beginning of the 21st century. *Metabolism* 2015, *64*, 131–145.
25. Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L.; Friedman, J.M. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994, *372*, 425–432.
26. Halaas, J.L.; Gajiwala, K.S.; Maffei, M.; Cohen, S.L.; Chait, B.T.; Rabinowitz, D.; Lallone, R.L.; Burley, S.K.; Friedman, J.M. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995, *269*, 543–546.
27. Agusti, A.; Garcia-Pardo, M.P.; Lopez-Almela, I.; Campillo, I.; Maes, M.; Romani-Perez, M.; Sanz, Y. Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function. *Front. Neurosci.* 2018, *12*, 155.
28. Popkin, B.M.; Adair, L.S.; Ng, S.W. Global nutrition transition and the pandemic of obesity in developing countries. *Nutr. Rev.* 2012, *70*, 3–21.
29. Candi, E.; Tesauro, M.; Cardillo, C.; Lena, A.M.; Schinzari, F.; Rodia, G.; Sica, G.; Gentileschi, P.; Rovella, V.; Annicchiarico-Petruzzelli, M.; et al. Metabolic profiling of visceral adipose tissue from obese subjects with or without metabolic syndrome. *Biochem. J.* 2018, *475*, 1019–1035.
30. Kruk, J. Overweight, obesity, oxidative stress and the risk of breast cancer. *Asian Pac. J. Cancer Prev.* 2014, *15*, 9579–9586.
31. Marseglia, L.; Manti, S.; D'Angelo, G.; Nicotera, A.; Parisi, E.; Di Rosa, G.; Gitto, E.; Arrigo, T. Oxidative stress in obesity, a critical component in human diseases. *Int. J. Mol. Sci.* 2014, *16*, 378–400.
32. Reuter, S.; Gupta, S.C.; Chaturvedi, M.M.; Aggarwal, B.B. Oxidative stress, inflammation, and cancer, how are they linked? *Free Radic. Biol. Med.* 2010, *49*, 1603–1616.
33. Anjani, K.; Lhomme, M.; Sokolovska, N.; Poitou, C.; Aron-Wisnewsky, J.; Bouillot, J.L.; Lesnik, P.; Bedossa, P.; Kontush, A.; Clement, K.; et al. Circulating phospholipid profiling identifies portal contribution to NASH signature in obesity. *J. Hepatol.* 2015, *62*, 905–912.
34. Lovren, F.; Teoh, H.; Verma, S. Obesity and atherosclerosis, mechanistic insights. *Can. J. Cardiol.* 2015, *31*, 177–183.
35. Polsky, S.; Ellis, S.L. Obesity, insulin resistance, and type 1 diabetes mellitus. *Curr. Opin. Endocrinol. Diabetes Obes.* 2015, *22*, 277–282.
36. Dimitriadis, G.; Mitrou, P.; Lambadiari, V.; Maratou, E.; Raptis, S.A. Insulin effects in muscle and adipose tissue. *Diabetes Res. Clin. Pract.* 2011, *93*, S52–S59.

37. Paz-Filho, G.; Mastronardi, C.; Wong, M.L.; Licinio, J. Leptin therapy, insulin sensitivity, and glucose homeostasis. *Indian J. Endocrinol. Metab.* 2012, *16*, S549–S555.
38. Wada, N.; Hirako, S.; Takenoya, F.; Kageyama, H.; Okabe, M.; Shioda, S. Leptin and its receptors. *J. Chem. Neuroanat.* 2014, *61–62*, 191–199.
39. Li, S.; Li, X. Leptin in normal physiology and leptin resistance. *Sci. Bull.* 2016, *61*, 1480–1488.
40. Díaz, B.B.; González, D.A.; Gannar, F.; Pérez, M.C.R.; De León, A.C. Myokines, physical activity, insulin resistance and autoimmune diseases. *Immunol. Letters* 2018, *203*, 1–5.
41. Despres, J.P.; Lemieux, I. Abdominal obesity and metabolic syndrome. *Nature* 2006, *444*, 881–887.
42. Andoh, T. Subchapter 19A—Insulin. In *Handbook of Hormones*; Takei, Y., Ando, H., Tsutsui, K., Eds.; Academic Press: San Diego, CA, USA, 2016.
43. Villarroya, F.; Vidal-Puig, A. Beyond the sympathetic tone: The new brown fat activators. *Cell. Metab.* 2013, *17*, 638–643.
44. Bayon, V.; Leger, D.; Gomez-Merino, D.; Vecchierini, M.F.; Chennaoui, M. Sleep debt and obesity. *Ann. Med.* 2014, *46*, 264–272.
45. Ogilvie, R.P.; Patel, S.R. The epidemiology of sleep and obesity. *Sleep Health* 2017, *3*, 383–388.
46. Koren, D.; Dumin, M.; Gozal, D. Role of sleep quality in the metabolic syndrome. *Diabetes Metab. Syndr. Obe. Targets Ther.* 2016, *9*, 281–310.
47. Poggiogalle, E.; Jamshed, H.; Peterson, C.M. Circadian regulation of glucose, lipid, and energy metabolism in humans. *Metabolism* 2018, *84*, 11–27.
48. Spiegel, K.; Leproult, R.; Van Cauter, E. Impact of sleep debt on metabolic and endocrine function. *Lancet* 1999, *354*, 1435–1439.
49. Spiegel, K.; Tasali, E.; Penev, P.; Van Cauter, E. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann. Intern. Med.* 2004, *141*, 846–850.
50. Reutrakul, S.; Van Cauter, E. Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. *Metabolism* 2018, *84*, 56–66.
51. Baron, K.G.; Reid, K.J. Chapter 26—Relationship between Circadian Rhythms, Feeding, and Obesity. In *Modulation of Sleep by Obesity, Diabetes, Age, and Diet*; Watson, R.R., Ed.; Academic Press: San Diego, CA, USA, 2015; pp. 243–253.
52. Xanthopoulos, M.S.; Berkowitz, R.I.; Tapia, I.E. Effects of obesity therapies on sleep disorders. *Metabolism* 2018, *84*, 109–117.
53. Lasikiewicz, N.; Myrissa, K.; Hoyland, A.; Lawton, C.L. Psychological benefits of weight loss following behavioural and/or dietary weight loss interventions: A systematic research review. *Appetite* 2014, *72*, 123–137.
54. Atlantis, E.; Goldney, R.D.; Wittert, G.A. Obesity and depression or anxiety. *BMJ* 2009, *339*, 871.
55. Sagar, R.; Gupta, T. Psychological Aspects of Obesity in Children and Adolescents. *Indian J. Pediatr.* 2018, *85*, 554–559.
56. Chu, D.-T.; Minh Nguyet, N.T.; Nga, V.T.; Thai Lien, N.V.; Vo, D.D.; Lien, N.; Nhu Ngoc, V.T.; Son, L.H.; Le, D.-H.; Nga, V.B.; et al. An update on obesity: Mental consequences and psychological interventions. *Diabetes Metab. Syndr. Clin. Res. Rev.* 2019, *13*, 155–160.
57. Bose, M.; Olivan, B.; Laferrere, B. Stress and obesity: The role of the hypothalamic-pituitary-adrenal axis in metabolic disease. *Curr. Opin. Endocrinol. Diabetes Obes.* 2009, *16*, 340–346.
58. Singh, M. Mood, food, and obesity. *Front. Psychol.* 2014, *5*, 925.
59. Soto, L.; Armendariz-Anguiano, A.L.; Bacardi-Gascon, M.; Jimenez Cruz, A. Beliefs, attitudes and phobias among Mexican medical and psychology students towards people with obesity. *Nutr. Hosp.* 2014, *30*, 37–41.
60. Pan, L.; Li, X.; Feng, Y.; Hong, L. Psychological assessment of children and adolescents with obesity. *J. Int. Med. Res.* 2018, *46*, 89–97.
61. Burgess, E.; Hassmen, P.; Welvaert, M.; Pumpa, K.L. Behavioural treatment strategies improve adherence

to lifestyle intervention programmes in adults with obesity: A systematic review and meta-analysis. *Clin. Obes.* 2017, *7*, 105–114.
62. Morelli, V.; Davis, C. The potential role of sports psychology in the obesity epidemic. *Prim. Care* 2013, *40*, 507–523.
63. Chisholm, A.; Hart, J.; Mann, K.; Perry, M.; Duthie, H.; Rezvani, L.; Peters, S. Investigating the feasibility and acceptability of health psychology-informed obesity training for medical students. *Psychol. Health Med.* 2016, *21*, 368–376.
64. Ghanemi, A. Psychiatric neural networks and neuropharmacology: Selected advances and novel implications. *Saudi Pharm. J.* 2014, *22*, 95–100.
65. Kusminski, C.M.; Bickel, P.E.; Scherer, P.E. Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat. Rev. Drug Discov.* 2016, *15*, 639–660.
66. Uusitupa, M. Remission of type 2 diabetes: Mission not impossible. *Lancet* 2018, *391*, 515–516.
67. Catoi, A.F.; Parvu, A.; Muresan, A.; Busetto, L. Metabolic Mechanisms in Obesity and Type 2 Diabetes, Insights from Bariatric/Metabolic Surgery. *Obes. Facts* 2015, *8*, 350–363.
68. Tremblay, A.; Picard-Deland, E.; Panahi, S.; Marette, A. Adipose tissue and sustainable development: A connection that needs protection. *Front. Pharmacol.* 2015, *6*, 110.
69. Fernandes, M.R.; Lima, N.V.; Rezende, K.S.; Santos, I.C.; Silva, I.S.; Guimaraes, R.C. Animal models of obesity in rodents: An integrative review. *Acta. Cir. Bras.* 2016, *31*, 840–844.
70. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.

Chapter 2. Special Article - Redefining obesity toward classifying as a disease

Abdelaziz Ghanemi [a,b], Jonny St-Amand [a,b]

[a] CREMI, CHU de Québec Research Center, Quebec, Canada

[b] Department of Molecular Medicine, Faculty of Medicine, Laval University, Quebec, Canada

Received 22 March 2018; Received in revised form 19 April 2018; Accepted 21 May 2018;
Available online 26 May 2018

2.1 Résumé (French abstract)

L'obésité peut être définie comme une accumulation anormale ou excessive de graisse qui peut nuire à la santé. Néanmoins, il existe un débat sur la classification de l'obésité en tant que maladie. Deux positions émergent, dont une qui considère l'obésité comme une maladie compte tenu des complications et des pathologies liées à l'obésité. L'autre position décrit l'obésité comme une adaptation à notre mode de vie moderne. La mise à jour de la définition de l'obésité permettra de concilier ces deux positions qui peuvent être considérées comme complémentaires plutôt que contradictoires. Une telle redéfinition de l'obésité, basée sur plus de critères, va remodeler notre vision de l'obésité et va avoir des impacts sur différents aspects tels que l'assurance maladie, les politiques de financement de la recherche scientifique et même le bien-être social et psychologique des patients souffrant d'obésité.

2.2 Abstract

Obesity can be defined as abnormal or excessive fat accumulation that may impair health. Nevertheless, there is a debate around classifying obesity as a disease. Two positions are emerging, including one which considers obesity as a disease given the obesity-related complications and pathologies. The other position describes obesity as an adaptation to our modern lifestyle. Updating the definition of obesity will make it possible to reconcile these two positions; that can be seen as complementary rather than contradictory. Such redefinition of obesity, based on more criteria, will reshape our vision of obesity and impact different aspects such as health insurance, policies of

funding scientific research and even the social and psychological well-being of patients suffering from obesity.

Keywords: Obesity; Disease

2.3 Introduction

Obesity has been pointed since the medical diagnosis and scientific observation was developed and according to the World Health Organization, it has an epidemic evolution nowadays [1]. The simple definition of obesity is the accumulation of an excess of energy stored in form of excess body fat. This excess results from the fact that the energy ingested from diet is superior to the energy expenditure, such as physical activity, resulting in an energy balance dysregulation, which is due to complex and multifactorial aetiologies [2]. Beyond body dissatisfaction and aesthetic issues for obese individuals, many studies have linked obesity to numerous health problems and comorbidities [3,4]. However, debates are still taking place among health professionals, decision makers, politicians, biological researchers and public health professionals on whether obesity should be redefined as a disease.

Such debate finds its importance in the fact that depending on its answer many decisions and policies would be affected. Indeed, whether to consider obesity as a disease would have impacts at different levels such as whether health insurance companies should cover expenses related to anti-obesity therapies for obese individuals, amounts of grants given for obesity research, how important it is to train health professionals to be specialized in obesity and provide an improved and personalized healthcare, whether considering bariatric surgery as aesthetic or therapeutic, how preventive measures against obesity should be undertaken and how far can we consider obese individuals responsible for being obese. Therefore, it is important to put obesity within an appropriate context since whether to consider it as a disease or not is not just a medical question, but it is an issue that has consequences on diverse domains including politics, finance, ethics and therapeutics. Thus, we can understand that doctors, politicians, psychologists, insurance companies, obese individuals, etc. would have different opinions and positions regarding this issue based on their knowledge, their positions or interests, their experience with obesity, and the side from which they look at and consider obesity.

2.4 Obesity as a disease

Obesity epidemic in industrialized countries brings obesity as a very critical risk factor for chronic diseases [1,5]. For instance, since the beginning of the 21st century, more than 50% of Canadians are overweight and 15% are obese [6,7]. This epidemic has a tremendous impact on public health since obesity, especially intra-abdominal fat mass, is associated with a dysregulation of lipoprotein-lipid metabolism and several pathologies including coronary heart disease, type 2 diabetes, liver

disease, cancers [8–14], sleep disordered breathing conditions (including both obesity hypoventilation syndrome and obstructive sleep apnea) [15], gastroesophageal reflux disease [16], gallstones, pancreatitis [17] and osteoarthritis [18] as well as respiratory system disorders [19]. Indeed, the visceral obesity, which could be considered as the most dangerous form of obesity, is at the center of the metabolic syndrome that both includes hyperglycemia, hypertension, and dyslipidemia and also increases the risks of developing metabolic disorders and disease such as insulin resistance leading to type 2 diabetes, in addition to cardiovascular disease [20–22]. Therefore, visceral obesity is clinically described as cardiometabolic risks enhancer which correlates with the improvement of most of cardiovascular risk factors with weight loss [23,24]. All these diseases illustrate the impact obesity has on diverse physiological systems and highlight it as a systemic disease affecting sleep, digestion, cardiovascular functions, inflammatory profile etc. rather than a simple aesthetic problem combined to a dysregulation of several metabolic functions.

Importantly, regardless of diseases risks increase in obese patients, morbid obesity represents a serious health condition where the individual has an increased mass to carry which represents a mechanical challenge affecting muscles, junctions and the respiratory system. Therefore, morbid obesity can interfere with essential physical and physiological functions such as breathing or walking which impacts the quality of life via limiting the mobility, encouraging the sedentary lifestyle and reducing the participation in diverse activities. These could lead to worsen the cardiometabolic and cardiorespiratory profiles and to a social isolation and eventually socio-psychological problems. Thus, would worsen the obesity-related disorders and the likelihood of exercise, which is among the most prescribed solutions for obese individuals, will reduce especially with the lack of social interaction that is usually a stimulus for an active lifestyle. These effects of morbid obesity on the locomotor and respiratory system make weight loss more challenging and the patient enters a vicious circle. Morbidly obese are at higher risk for illnesses including sleep apnea, high blood pressure, diabetes, heart disease, gastroesophageal reflux disease, gallstones, osteoarthritis, and several types of cancer [10,15–18,22,25,26]. Moreover, several studies have reported that obesity independently impacts mortality [8,9,27,28] indicating a heavy impact of obesity itself regardless of the pathologies or disorders caused by obesity or for which it represents a risk factor.

The strongest argument supporting that obesity should be classified as a disease [29] is the heavy impacts it has on health, life quality and life expectancy [1,30]. Indeed, the morbidity and mortality related to obesity make it a disease since obesity affects the life quality of individuals through numerous health problems and biological disorders both at the physical and psychological levels. More important, obesity increases independently the risk of mortality.

In healthy individuals, the physiological regulatory process in the body involves diverse signals, such as those controlled by hormones and nervous system, that regulate the body weight via controlling the input and the output of energy (diet, exercise, activities, body temperature control, metabolisms, etc.) and such control is governed by diverse

molecular and cellular signals. However, once the obesity is established, those mechanisms protecting from obesity are blunted [31] so that the signals that control body weight (energy and metabolism balance) are not effective anymore and disorders/dysregulations are seen among factors such as satiety feeling and biochemicals metabolic profile. Such dysregulations and disorders not only worsen the obesity but are also behind many of the morbidities and complications developed in obese individuals. Such pathological profile represents another pattern of a disease.

In addition, obesity has complex and diversified aetiologies, pathophysiology, influencing factors and mechanisms including, but not limited to, genetics and environments [2]. Therefore, it is, as many other diseases, a consequence of an unhealthy lifestyle in terms of diet, sleeping, physical activity in addition to a genetic component and the existence of an obesogenic environment. Such concepts related to obesity further support defining obesity as a disease. A supplementary argument is that obesity has a chronic character in term of persistence. In fact, it is hard for an individual who lost weight to keep the body weight for a long period since it seems that once obesity is established, the body sets a new "reference" for the body weight and the regulatory mechanisms act toward gaining an amount of their lost weight [32] in order to go closer to that reference body weight that was before the weight loss which is the one considered as reference by the brain. Therefore, more obesity is morbid, more difficult it is to "reverse" it which could require pharmacotherapeutic interventions [33] thus, is toward classifying obesity as a chronic disease with a clear pathological pattern: Obesity starts as an energy imbalance leading to lipids accumulation. These fat depots will have impact at the metabolic, mechanical, hormonal, physiological and functional levels via numerous molecular disorders, cellular dysfunctions and systemic perturbations. The reversibility of obesity depends on how morbid it is, the associated pathologies, the type of prescribed therapeutic approached such as diet, physical activity, pharmacological approaches and bariatric surgery, and the general lifestyle of the obese patient as well as their medical conditions in term of associated pathologies and syndromes.

2.5 Obesity as an adaptation

On the other hand, obesity could also be seen as an adaptation of the body to our modern lifestyle which is toward habits requiring less efforts (technology assistance) yet with an increased food, especially with high caloric density, availability and accessibility. The extra fat that the body accumulates represents energetic reserves kept for situations such as fasting or hunger thus, the biological process of accumulating fat storage in diverse parts of the body is a part of the human physiology as a function that allows the human to survive if he faces situations when he has limited access to food. Moreover, lipids are not just an element we should avoid and be afraid of, but rather elements of high biological and physiological importance for homeostatic functions. Indeed, in addition of being a form of energy storage, lipids have diverse important roles in numerous functions of the body and

at different levels, such as immunology, cell structure, transport of molecules and the remarkable beneficial functions of the adipose tissue in detoxification [34].

Nowadays, we have more available food and thus, more energy to store and therefore the ability of the individuals to store fat increased accordingly. The existence of both metabolically healthy obese [35] and the metabolically unhealthy and normal weight individuals [36] represents maybe the strongest argument of those refusing to classify obesity as a disease since such evidence shows that not all obese have health problems whereas health issues reported as related to obesity are seen among non-obese individuals. Therefore, leading to a dissociation of obesity from the health problems reported as caused or increased by obesity. However, metabolically healthy obese might still have higher risks of cardiovascular diseases [37] which indicates that debates around the metabolically healthy obese still require further discussion mainly on how to define a "metabolically healthy" especially with the known links between metabolic disorders and the cardiovascular diseases.

Furthermore, gluteal-femoral adipose tissue was even associated with cardiometabolic benefits supporting that obesity is not about storing excess fat but rather about which form of obesity and depending on which fat distribution is observed [38]. This indicates that some forms of obesity could even be beneficial rather than leading to morbidities. Such concepts could change our 'classical" approaches of understanding the nature of obesity and its consequences.

Obesity is a condition that can be prevented or corrected by behavioral habits in term of lifestyle such as regular physical activity and dietary management before a need of any pharmacological and surgical approaches [1]. This supports the understanding of obesity as an adaptation of the body to the "unusual" lifestyle because the body is "programmed" to be active and spend energy on daily tasks including searching food which is nowadays available for individuals in almost limitless amount especially in industrialized countries.

2.6 Redefined obesity: Toward reconciliating the two positions

The origin behind such debate, whether obesity is a disease, is mainly due to both the non-existence of a precise and an international scientific definition of obesity as well as the lack of a clear understanding of the obesity-related molecular pathways. Shall we define obesity according to the body mass index? According to the risk of developing disease related to obesity? Or based on other criteria? Answering such questions comes before and would be the starting point to answer the question: Is obesity a disease?

Considering the previous "contradictory" arguments, there is a need to reconciliate them and put them within suitable medical contexts. Therefore, we would support a more precise approach through categorizing the obesity and redefining it. Indeed, the option is to classify obesity as a disease but before that we need to redefine it within an appropriate precise context. Such a way of thinking fits the scientific and medical facts and also considers the arguments of both those considering obesity as a disease and those saying it is

not. This will reconciliate the two positions and what seems to be contradictory arguments would rather be complimentary justifications.

Therefore, we first need a novel innovative detailed definition of obesity that considers new research such as those mentioning that the body mass index is not enough to evaluate the health risks of obese individuals and add new parameters including the waist circumference, triglycerides and the associated metabolic abnormalities [39]. However, herein another challenge comes out, numerous biological mechanisms explaining how obesity leads to health problems or influences other diseases developments still unclear.

Furthermore, many links established between obesity and other diseases have been concluded based on epidemiologic studies and statistical observations of populations which might not be very precise because the choice of the populations studied are different in terms of ethnicity, environment and lifestyle. For instance, if we observe that many obese individuals in Europe develop certain types of cancer, this does not mean that obese individuals in Korea will also develop the same types of cancer. If we had a better understanding of obesity, a better pathophysiological classification would have been possible. Indeed, we have a poor molecular understanding of how obesity accrues and how it leads to metabolic and health disorders and we need clear descriptive pathways linking the underlying molecular causes to the clinical outcomes and on which we can build a logic for both a diagnosis and treatments to support a potential classification of obesity as a disease.

As a conclusion, the first step remains to elucidate the mechanisms of how obesity develop and affects the human health. After we have precise details of such processes we could classify obese individuals as patients for which obesity affects or generates health problems. Importantly, by "effects of obesity on the human health" we need to take into consideration the psychological effects [14] that can result from the self-vision of the obese individuals or from how the society judges or behaves toward them which would have deep impacts on their psychosocial well-being. In this latter case, although the health problem might not be caused by the obesity itself, but rather coming from the society behaviour toward the obese person, it could be considered as a factor further supporting the classification of obesity as a disease and justify a psychosocial therapy for example.

2.7 References

[1] Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894(i-xii):1–253.

[2] Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. N Engl J Med 2017; 376:254–66.

[3] Chandler M, Cunningham S, Lund EM, Khanna C, Naramore R, Patel A, et al. Obesity and associated comorbidities in people and companion animals: a one health perspective. J Comp Pathol 2017; 156:296–309.

[4] Busutil R, Espallardo O, Torres A, Martinez-Galdeano L, Zozaya N, Hidalgo-Vega A. The impact of obesity on health-related quality of life in Spain. Health Qual Life Outcomes 2017;15:197.

[5] Noor MI. Obesity epidemic in Asia: issues and challenges. J Nutr Sci Vitaminol (Tokyo) 2015;61(Suppl): S212.

[6] Katzmarzyk PT. The Canadian obesity epidemic: an historical perspective. Obes Res 2002; 10:666–74.
[7] Statistics Canada. Overweight and obese adults (self-reported). Government of Canada, in. 2016.
[8] Rabkin SW, Mathewson FA, Hsu PH. Relation of body weight to development of ischemic heart disease in a cohort of young north American men after a 26 year observation period: the Manitoba study. Am J Cardiol 1977; 39:452–8.
[9] Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham heart study. Circulation 1983; 67:968–77.
[10] Bierman EL, Bagdade JD, Porte Jr. D. Obesity and diabetes: the odd couple. Am J Clin Nutr 1968; 21:1434–7.
[11] Despres JP. Abdominal obesity and the risk of coronary artery disease. Can J Cardiol 1992; 8:561–2.
[12] Mobley D, Baum N. The obesity epidemic and its impact on urologic care. Rev Urol 2015; 17:165–70.
[13] Corey KE, Kaplan LM. Obesity and liver disease: the epidemic of the twenty-first century. Clin Liver Dis 2014; 18:1–18.
[14] McElroy SL. The epidemic of depression with obesity. J Clin Psychiatry 2015;76: e1340–2.
[15] Raveendran R, Wong J, Chung F. Morbid obesity, sleep apnea, obesity hypoventilation syndrome: are we sleepwalking into disaster? Perioperative Care and Operating Room Management 2017; 9:24–32.
[16] Camacho DR, LeMasters PE. Gastroesophageal reflux disease in the obese. Techniques in Gastrointestinal Endoscopy 2015; 17:83–8.
[17] Bonfrate L, Wang DQH, Garruti G, Portincasa P. Obesity and the risk and prognosis of gallstone disease and pancreatitis. Best Pract Res Clin Gastroenterol 2014;2 8:623–35.
[18] Kulkarni K, Karssiens T, Kumar V, Pandit H. Obesity and osteoarthritis. Maturitas 2016; 89:22–8.
[19] Banerjee A, Heiden E. Chapter 11 - Obesity and the Effects on the Respiratory System A2 - Weaver, Jolanta Urszula, in: Practical Guide to Obesity Medicine. Elsevier; 2018. p. 109–21.
[20] Hoffman EL, VonWald T, Hansen K. The metabolic syndrome. S D Med, Spec No 2015:24–8.
[21] Samson SL, Garber AJ. Metabolic syndrome. Endocrinol Metab Clin North Am 2014; 43:1–23.
[22] Silva AO, Silva MV, Pereira LKN, Feitosa WMN, Ritti-Dias RM, Diniz PRB, et al. Association between general and abdominal obesity with high blood pressure: difference between genders. J Pediatr (Rio J) 2016; 92:174–80.
[23] Wood PD, Stefanick ML, Dreon DM, Frey-Hewitt B, Garay SC, Williams PT, et al. Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. N Engl J Med 1988; 319:1173–9.
[24] Anderson JW, Brinkman-Kaplan VL, Lee H, Wood CL. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. J Am Coll Nutr 1994; 13:256–61.
[25] Ades PA, Savage PD. Obesity in coronary heart disease: an unaddressed behavioral risk factor. Prev Med 2017; 104:117–9.
[26] Druso JE, Fischbach C. Biophysical properties of extracellular matrix: linking obesity and Cancer. Trends in Cancer 2018; 4:271–3.
[27] D'Apuzzo MR, Novicoff WM, Browne JA. The John Insall award: morbid obesity independently impacts complications, mortality, and resource use after TKA. Clin Orthop Relat Res 2015; 473:57–63.
[28] Nasraway Jr. SA, Albert M, Donnelly AM, Ruthazer R, Shikora SA, Saltzman E. Morbid obesity is an independent determinant of death among surgical critically ill patients. Crit Care Med 2006; 34:964–70. [quiz 971].
[29] Upadhyay J, Farr O, Perakakis N, Ghaly W, Mantzoros C. Obesity as a disease. Med Clin North Am 2018; 102:13–33.
[30] Whitaker BN, Fisher PL, Jambhekar S, Com G, Razzaq S, Thompson JE, et al. Impact of degree of obesity on sleep, quality of life, and depression in youth. J Pediatr Health Care 2018;32: e37–44.
[31] Mucunguzi O, Melouane A, Ghanemi A, Yoshioka M, Boivin A, Calvo E-L, et al. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr

Metab 2017; 14:66.
[32] Fothergill E, Guo J, Howard L, Kerns JC, Knuth ND, Brychta R, et al. Persistent metabolic adaptation 6 years after "the biggest loser" competition. Obesity (Silver Spring) 2016; 24:1612–9.
[33] Saunders KH, Umashanker D, Igel LI, Kumar RB, Aronne LJ. Obesity pharmacotherapy. Med Clin North Am 2018; 102:135–48.
[34] Tremblay A, Picard-Deland E, Panahi S, Marette A. Adipose tissue and sustainable development: a connection that needs protection. Front Pharmacol 2015; 6:110.
[35] Stefan N, Haring HU, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. Lancet Diabetes Endocrinol 2013; 1:152–62.
[36] Wang B, Zhang M, Wang S, Wang C, Wang J, Li L, et al. Dynamic status of metabolically healthy overweight/obesity and metabolically unhealthy and normal weight and the risk of type 2 diabetes mellitus: a cohort study of a rural adult Chinese population. Obes Res Clin Pract 2018; 12:61–71.
[37] Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically healthy obese and incident cardiovascular disease events among 3.5 million men and women. J Am Coll Cardiol 2017; 70:1429–37.
[38] Lemieux I. Energy partitioning in gluteal-femoral fat: does the metabolic fate of triglycerides affect coronary heart disease risk? Arterioscler Thromb Vasc Biol 2004; 24:795–7.
[39] Arsenault BJ, Lemieux I, Despres JP, Wareham NJ, Kastelein JJ, Khaw KT, et al. The hypertriglyceridemic-waist phenotype and the risk of coronary artery disease: results from the EPIC-Norfolk prospective population study. CMAJ 2010; 182:1427–32.

Chapter 3. Opinion - Obesity as a Neuroendocrine Reprogramming

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, 2705 Boul. Laurier, Québec, QC G1V 4G2, Canada

Received: 24 December 2020; Accepted: 11 January 2021; Published: 13 January 2021

3.1 Résumé (French abstract)

L'élucidation des voies moléculaires et cellulaires derrières l'établissement de l'obésité reste le principal défi qui limite les progrès réalisés dans la compréhension de l'obésité. Ici, on présente l'obésité comme une reprogrammation neurologique et endocrinienne qui permet de s'adapter au déséquilibre métabolique que représente l'obésité. En effet, lors du développement de l'obésité, le bilan énergétique est déplacé vers un stockage accru de l'énergie. Cela nécessite des changements sous le contrôle des systèmes neuroendocriniens correspondant aux changements accompagnant le phénotype induit par l'obésité. Une telle reprogrammation neuroendocrinienne peut expliquer pourquoi il est difficile de perdre du poids une fois l'obésité établie, car cela signifierait aller à l'encontre de nouvelles « références » endogènes. L'études des concepts entourant la classification de l'obésité en tant que reprogrammation neuroendocrinienne pourraient optimiser notre compréhension des mécanismes sous-jacents et, surtout, révéler certains des mystères entourant la pathogenèse moléculaire de l'obésité.

3.2 Abstract

Obesity represents a health problem resulting from a broken balance between energy intake and energy expenditure leading to excess fat accumulation. Elucidating molecular and cellular pathways beyond the establishment of obesity remains the main challenge facing the progress in understanding obesity and developing its treatment. Within this context, this opinion presents obesity as a reprogrammer of selected neurological and endocrine patterns in order to adapt to the new metabolic imbalance represented by obesity status. Indeed, during obesity development, the energy balance is shifted towards increased energy storage, mainly but not only, in adipose tissues. These new metabolic patterns that obesity represents require changes at different cellular and metabolic levels under the control of the neuroendocrine systems through different regulatory signals. Therefore, there

are neuroendocrine changes involving diverse mechanisms, such as neuroplasticity and hormonal sensitivity, and, thus, the modifications in the neuroendocrine systems in terms of metabolic functions fit with the changes accompanying the obesity-induced metabolic phenotype. Such endocrine reprogramming can explain why it is challenging to lose weight once obesity is established, because it would mean to go against new endogenous metabolic references resulting from a new "setting" of energy metabolism-related neuroendocrine regulation. Investigating the concepts surrounding the classification of obesity as a neuroendocrine reprogrammer could optimize our understanding of the underlying mechanisms and, importantly, reveal some of the mysteries surrounding the molecular pathogenesis of obesity, as well as focusing the pharmacological search for antiobesity therapies on both neurobiology synaptic plasticity and hormonal interaction sensitivity.

Keywords: obesity; neurology; endocrinology; neuroendocrine; reprogramming

3.3 Obesity as a Neuroendocrine Reprogramming

The nervous and the endocrine systems are the main regulators of the various homeostatic functions, including digestion, energy metabolism, cellular replication, tissues renewal, and fluids circulations. However, following selected factors (diet, intoxications, etc.) or internal changes (pathogens, cancer, etc.) related to both environmental impacts and genetic factors, these regulatory properties might lose their efficiency, or their balancing pathways may be reshaped. Within this context, energy homeostatic patterns are governed by diverse signals exchanged mainly between the control centers and the metabolic tissues (mainly adipose tissues, muscles, and the liver). This results in a balance between the energy intake and energy expenditure. However, under the influence of exogenous stimuli, such as an increased caloric intake, combined with a sedentary lifestyle (reduced energy expenditure) or certain therapeutic interventions, these regulatory mechanisms lose their ability to maintain the balance, and, therefore, the energy homeostasis is broken [1]. Such broken balance results in the development of obesity, with all its consequences on health at different tissular levels [2], following the accumulation of the excessive energy storages within specific tissues and locations.

It is widely accepted that biology gave to mammals the ability to store energy as an advantage to make full usage of the available calories during the period of food abundance in order to survive the periods of food shortage and hunger. Hibernating animals (a status of deep physiological and metabolic changes [3–6]) are among the best illustrative examples of this property, as they store enough lipids within their adipose tissues prior to the hibernating period. Lipids are the nutritive elements with the highest caloric density, making them the best form of energy biostorage. This last property is confirmed by the fact that, unlike glucose, the lipids represent a weak stativity signal ("undetected" by centers sending signals to stop food intake), which increases their ratio within the food amount. Lipids are extremely important for biological functions, such as thermoregulation, energy

productions, cellular membrane structures, and caloric storages. Therefore, the negative impacts of lipids seem to start only with excessive fat accumulations or abnormal locations, such as ectopic deposits [7], leading to the known obesity consequences, including cardiovascular diseases, inflammation, and metabolic syndrome [2].

Obesity, as a health problem, is an extreme form of fat storage. This fat storage was initially an ability reflecting a biological adaptation to food availability in the surrounding environment. Obesity pathogenesis and mechanisms are full of mysteries, and most studies on obesity focus on its basic definition as excessive abnormal energy storage resulting from having an energy intake superior to energy expenditure. However, this metabolic broken balance [1] could be the outcome of obesity rather than its underlying mechanism, and the starting point could result from numerous neurological and endocrine changes. Indeed, the implications, effects, and interactions of the nervous and endocrine systems with the diverse organs and tissues involved in both obesity development and energy balance indicate a possible classification of obesity as a neurological disease combined with endocrine abnormalities.

Starting with the nervous system, the existence of brain centers that both receive signals form the digestive system (where the nutritive elements are detected) and controlling food intake support such a neuroendocrine approach. These signals include ghrelin [8], glucagon-like peptide 1 [9], and peptide YY [10], which act on centers including the hypothalamic melanocortinergic system [11]. These signals have been associated with diseases and complications linked to obesity such as diabetes [12], and they represent promising therapies for neurological diseases due to properties such as neurotrophic and neuroprotective actions [13]. This again shows the "neurological" character of these energy metabolism signals. The concept of food addiction [14,15] in the context of obesity [16], with the neurobiological mechanism similar to drug abuse with the dopaminergic rewarding system [17], further highlights the neurological reprogramming that results from a neuroplasticity [18] of the involved neurons through functional and structural adaptation [19]. This aims to meet the novel neuronal activities required to adapt to obesity status. In addition, the existence of pharmacological approaches targeting the nervous system to treat obesity, such as utilization of the glucagon-like peptide-1 receptor [9,20], go beyond the usage of the classical obesity-related pillars; exercise and diet [21] further indicate that the nervous system's involvement in obesity development. Coming back to the endocrine system, both insulin resistance [22] and leptin resistance [23] illustrate best how obesity status reprograms hormonal functions during obesity via modifying the interaction quality of energy balance-control hormones with their target tissues.

Biology provides organisms (mainly mammals) with the ability to store energy as a mechanism to face possible hunger periods or adapt to a lack of food resources. However, with an increase in food resources, this biostorage ability, initially vital, could transform into a leading cause of obesity development. This ability of organisms to store energy (fat) makes it difficult to lose weight afterwards. Indeed, once an increased body weight/fat

stores level is reached, it becomes the “new reference” toward which the metabolism and energy homeostasis are shifted in order to maintain or return to the newly set up point of fat storage level, thereby hindering weigh loss attempts. Within the context of brain involvement in energy control, this central new “reference” of body/fat weight could be explained through a neuroplasticity related to the centers controlling food intake and energy expenditure. This neuroplasticity could also occur in the peripheral neurons and, therefore, impact the innervations or the peripheral metabolic tissues. This is another illustration of the reprogramming of the neurological control of energy balance and how it contributes to obesity. For instance, the liver [24] and adipose tissues [25] are innervated by neurons that modify their metabolic activities. These properties explain the observed decrease in the basal metabolic rate seen with weight loss [26]. This decrease in the basal metabolic rate occurs to compensate for the reduced food intake or, more generally, the energy balance in order to prevent or limit weight loss, thereby predisposing individuals to weight regain. This property is a consequence of the setup of the new body/fat level reference, as described above, and is representative of the survival ability provided by biology to store energy to endure food shortage periods. Similar to neuroplasticity, the modifications of receptor sensitivity, such as that of insulin receptors, indicates endocrine reprogramming that makes receptors in need of the strongest stimulations. This may also represent the need to store more energy to adapt to the obesity status of requiring more energy storage and, therefore, more insulin (and also more leptin, for which a resistance also develops during obesity). These calorie- and hormonal-centric explanations (neuroendocrine) also extend to the regulation of metabolic diseases like obesity. Indeed, it fits, for instance, with the hypothesis linking a high-fat diet with trefoil factor 2 (TFF2) as a lipid-induced signal for which the corresponding gene (*Tff2*) is induced by the high-fat diet [27], whereas its knockout provides protection from obesity [28] by leading to an antiobesity metabolic phenotype [29].

This concept of neuroendocrine reprogramming has as a particular outcome: the establishment of a novel energy balance status for optimum energy storage rather than limitation of caloric intake. Indeed, before obesity develops, satiety signals are strong enough to limit food intake, whereas once obesity is established, the hunger becomes “chronic” (reduced/inefficient satiety signals), leading to an increased food intake and more energy storage with less energy expenditure (metabolic slowing). Therefore, this reprogramming is the process via which the organisms shape their metabolic phenotype as an adaptation to the new status that obesity represents in terms of the need for increased energy intake and storage capacity. These neuroendocrine changes could explain obesity outcomes such as regeneration impairment [30] as well as the beneficial effects of exercise [31–33] and the molecules induced by exercise (e.g., [34–36]), beyond which there is exercise-induced neuroendocrine reprograming that shapes the metabolic phenotype and explains the importance of regular exercise in obesity therapy.

Elucidating the concepts surrounding the classification of obesity as a neuroendocrine reprogrammer could optimize the understanding of its underlying

mechanisms and, importantly, reveal some of the mystery surrounding the molecular pathogenesis of obesity, as well as focusing the pharmacological search for antiobesity therapies on neurobiology synaptic plasticity and hormonal interaction sensitivity.

Author Contributions: A.G. drafted the manuscript; A.G., M.Y. and J.S.-A. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: A.G. received a Merit scholarship program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme).

Conflicts of Interest: The authors declare no conflict of interest.

3.4 References

1. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
2. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
3. Green, S.R.; Storey, K.B. Regulation of the α-ketoglutarate dehydrogenasecomplex during hibernation in a small mammal, the Richardson's ground squirrel (Urocitellus richardsonii). *Biochim. Biophys. Acta Proteins Proteom.* 2020, *1868*, 140448.
4. Ruberto, A.A.; Logan, S.M.; Storey, K.B. Temperature and serine phosphorylation regulate glycerol-3-phosphate dehydrogenase in skeletal muscle of hibernating Richardson's ground squirrels. *Biochem. Cell Biol.* 2019, *97*, 148–157.
5. Bell, R.A.V.; Storey, K.B. Purification and characterization of skeletal muscle pyruvate kinase from the hibernating ground squirrel, Urocitellus richardsonii: Potential regulation by posttranslational modification during torpor. *Mol. Cell. Biochem.* 2018, *442*, 47–58.
6. Nelson, O.L.; Robbins, C.T. Cardiovascular function in large to small hibernators: Bears to ground squirrels. *J. Comp. Physiol. B* 2015, *185*, 265–279.
7. Neeland, I.J.; Ross, R.; Després, J.P.; Matsuzawa, Y.; Yamashita, S.; Shai, I.; Seidell, J.; Magni, P.; Santos,

R.D.; Arsenault, B.; et al.Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: A position statement. *Lancet Diabetes Endocrinol.* 2019, *7*, 715–725.
8. Hyland, L.; Park, S.B.; Abdelaziz, Y.; Abizaid, A. Metabolic effects of ghrelin delivery into the hypothalamic ventral premammilary nucleus of male mice. *Physiol. Behav.* 2020, *228*, 113208.
9. Drucker, D.J. Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. *Cell Metab.* 2018, *27*, 740–756.
10. Alhadeff, A.L.; Golub, D.; Hayes, M.R.; Grill HJ: Peptide, Y.Y. signaling in the lateral parabrachial nucleus increases food intake through the Y1 receptor. *Am. J. Physiol. Endocrinol. Metab.* 2015, *309*, E759–E766.
11. Poher, A.L.; Tschöp, M.H.; Müller, T.D. Ghrelin regulation of glucose metabolism. *Peptides* 2018, *100*, 236–242.
12. Yu, Z.W.; Liu, R.; Li, X.; Wang, Y.; Fu, Y.H.; Li, H.Y.; Yuan, Y.; Gao, X.Y. Potential roles of Glucagon-like peptide-1 and its analogues in cognitive impairment associated with type 2 diabetes mellitus. *Mech. Ageing Dev.* 2020, *190*, 111294.
13. Salcedo, I.; Tweedie, D.; Li, Y.; Greig, N.H. Neuroprotective and neurotrophic actions of glucagon-like peptide-1: An emerging opportunity to treat neurodegenerative and cerebrovascular disorders. *Br. J. Pharmacol.* 2012, *166*, 1586–1599.
14. Fletcher, P.C.; Kenny, P.J. Food addiction: A valid concept? *Neuropsychopharmacology* 2018, *43*, 2506–2513.
15. Gordon, E.L.; Ariel-Donges, A.H.; Bauman, V.; Merlo, L.J. What Is the Evidence for "Food Addiction"? A Systematic Review. *Nutrients* 2018, *10*, 477.
16. Lennerz, B.; Lennerz, J.K. Food Addiction, High-Glycemic-Index Carbohydrates, and Obesity. *Clin. Chem.* 2018, *64*, 64–71.
17. Lindgren, E.; Gray, K.; Miller, G.; Tyler, R.; Wiers, C.E.; Volkow, N.D.; Wang, G.J. Food addiction: A common neurobiological mechanism with drug abuse. *Front. Biosci.* 2018, *23*, 811–836.
18. Solinas, M.; Belujon, P.; Fernagut, P.O.; Jaber, M.; Thiriet, N. Dopamine and addiction: What have we learned from 40 years of research. *J. Neural Transm.* 2019, *126*, 481–516.
19. Gulyaeva, N.V. Molecular Mechanisms of Neuroplasticity: An Expanding Universe. *Biochemistry* 2017, *82*, 237–242.
20. Lau, J.; Bloch, P.; Schäffer, L.; Pettersson, I.; Spetzler, J.; Kofoed, J.; Madsen, K.; Knudsen, L.B.; McGuire, J.; Steensgaard, D.B.; et al. Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide. *J. Med. Chem.* 2015, *58*, 7370–7380.
21. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
22. Małodobra-Mazur, M.; Alama, A.; Bednarska-Chabowska, D.; Pawelka, D.; Myszczyszyn, A.; Dobosz, T. Obesity-induced insulin resistance via changes in the DNA methylation profile of insulin pathway genes. *Adv. Clin. Exp. Med.* 2019, *28*, 1599–1607.
23. Izquierdo, A.G.; Crujeiras, A.B.; Casanueva, F.F.; Carreira, M.C. Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later? *Nutrients* 2019, *11*, 2704.
24. Shimazu, T. Innervation of the liver and glucoregulation: Roles of the hypothalamus and autonomic nerves. *Nutrition* 1996, *12*, 65–66.
25. Zeng, X.; Ye, M.; Resch, J.M.; Jedrychowski, M.P.; Hu, B.; Lowell, B.B.; Ginty, D.D.; Spiegelman, B.M. Innervation of thermogenic adipose tissue via a calsyntenin 3β-S100b axis. *Nature* 2019, *569*, 229–235.
26. Johannsen, D.L.; Knuth, N.D.; Huizenga, R.; Rood, J.C.; Ravussin, E.; Hall, K.D. Metabolic slowing with massive weight loss despite preservation of fat-free mass. *J. Clin. Endocrinol. Metab.* 2012, *97*, 2489–2496.
27. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.

28. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity 2013, 21, 1389–1395.
29. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
30. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
31. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
32. Hargreaves, M.; Spriet, L.L. Skeletal muscle energy metabolism during exercise. Nat. Metab. 2020, 2, 817–828.
33. Beer, N.J.; Dimmock, J.A.; Jackson, B.; Guelfi, K.J. Exercise-related factors that influence post-exercise energy intake: A psychological perspective. J. Sci. Med. Sport 2020, 23, 1068–1073.
34. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
35. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
36. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine (SPARC) as a regeneration factor: Beyond the tissue repair. Life 2021, 11, 38.

Chapter 4. Opinion - Regeneration during Obesity: An Impaired Homeostasis

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Quebec City, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Quebec City, QC G1V 4G2, Canada

Received: 13 November 2020; Accepted: 7 December 2020; Published: 9 December 2020

4.1 Résumé (French abstract)

La régénération représente les processus biologiques qui permettent aux cellules et aux tissus de se renouveler et de se développer. Avec l'obésité, une variété de changements et de réactions sont observés. Cela inclut l'inflammation et les troubles métaboliques. Ces changements induits par l'obésité ont un impact sur les processus de régénération. De tels impacts qu'a l'obésité sur la régénération affecteraient le développement des tissus et des organes et auraient également des conséquences sur les résultats des thérapies qui dépendent de la régénération cellulaire (comme les brûlures, la radiothérapie et la leucémie) administrées aux patients souffrant d'obésité. Par conséquent, une attention particulière doit être accordée aux patients souffrant d'obésité dans des contextes biologiques, thérapeutiques et cliniques qui dépendent de la régénération.

4.2 Simple Summary

Regeneration represents the biological processes that allow cells and tissues to renew and develop. During obesity, a variety of changes and reactions are seen. This includes inflammation and metabolic disorders. These obesity-induced changes do impact the regeneration processes. Such impacts that obesity has on regeneration would affect tissues and organs development and would also have consequences on the outcomes of therapies that depend on cells regeneration (such as burns, radiotherapy and leukemia) given to patients suffering from obesity. Therefore, a particular attention should be given to patients suffering from obesity in biological, therapeutic and clinical contexts that depend on regeneration ability.

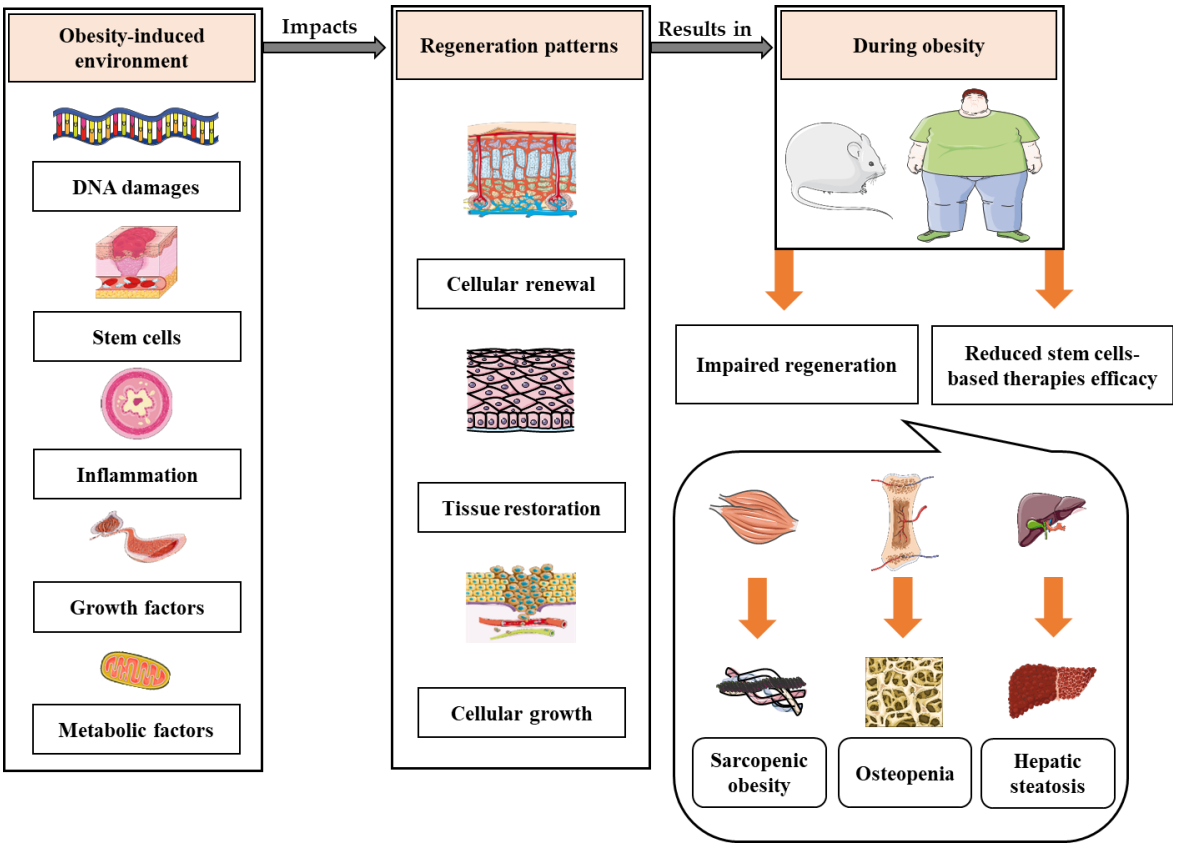
4.3 Abstract

Obesity is a health problem that, in addition to the known morbidities, induces the generation of a biological environment with negative impacts on regeneration. Indeed,

factors like DNA damages, oxidative stress and inflammation would impair the stem cell functions, in addition to some metabolic and development patterns. At the cellular and tissulaire levels, this has consequences on growth, renewal and restoration which results into an impaired regeneration. This impaired homeostasis concerns also key metabolic tissues including muscles and liver which would worsen the energy balance outcome towards further development of obesity. Such impacts of obesity on regeneration shows the need of a specific care given to obese patients recovering from diseases or conditions requiring regeneration such as burns, radiotherapy and leukemia. On the other hand, since stem cells are suggested to manage obesity, this impaired regeneration homeostasis needs to be considered towards more optimized stem cells-based obesity therapies within the context of precision medicine.

Keywords: obesity; regeneration; homeostasis

4.4 Graphical abstract



4.5 Regeneration during Obesity: An Impaired Homeostasis

Endogenous processes and biological homeostasis require a proper regeneration ability summarized in the biological capacity to restore, renew and grow different cells and

tissues. This regeneration ability (governed by complex cellular functions and molecular processes) requires optimum conditions defined by biological environments in terms of pH, cytokines, growth factors and diverse signals and messenger activities.

Within this context, obesity, as a pathological status of broken homeostasis [1,2], induces the generation of a negative regeneration environment, which, biologically, means conditions that limit or reduce the ability of cellular and tissular restoration, renewal and growth. Indeed, diverse works throughout the literature report direct and indirect impacts of obesity on regeneration in different cells and tissues. Stem cells are key players for regeneration and these cells are impacted by obesity. For instance, data indicate that stem cells-based hematopoietic and osteogenic regeneration is impaired during obesity [3], probably as a consequence of bone marrow adipose tissue expansion that leads to a deterioration within the skeleton [4], the obesity-induced bone marrow microenvironment modifications [5] and the increased endoplasmic reticulum stress in bone marrow mesenchymal stem cells [6]. In addition, proliferative and migratory abilities of adipose derived mesenchymal stem cells derived from obese subjects are reduced [7]. Obesity also alters the stem cells' differentiation potential [8]. These obesity impacts on stem cells might result from different mechanisms, including the reshape of stem cell extracellular vesicles [9], inflammation promotion [5] and the resulting surrounding microenvironment modifications resulting from obesity [10,11]. Within this context, it is worth mentioning that during obesity, inflammatory-related balance is impaired. Indeed, M1 macrophages (promote pro-inflammation) are enhanced and M2 macrophages (anti-inflammatory factor) are down regulated [12]. Such macrophage polarization supports the classification of obesity as a chronic pro-inflammatory disease [12] leading to metabolic dysfunctions, including insulin resistance [13]. To further understand the critical balanced roles of inflammation, it is essential to state that although obesity-induced inflammation might impair regeneration, inflammatory-related processes such as those mediated by macrophages are required for damaged tissue elimination as a step preparing the regeneration as explored by Varga T et al. [14–16].

Regarding skeletal muscles, which form the locomotor system with the skeleton, their regeneration is also impaired by obesity [17]. Importantly, satellite cells (muscle stem cells) are reduced in obese mice (post trauma [18]) with a diminished fusion capacity [19], showing the deep impacts of obesity on muscle regeneration starting at the stem cell level. Moreover, this obesity senescence has also been said to affect satellite cells and skeletal muscle in the context of obesity [20], which indicates the worsening effect ageing has on regeneration.

Within the metabolics of skeletal muscle, the expression of the mitochondrial glycerol 3-phosphate dehydrogenase (which promotes skeletal muscle regeneration) is reduced in animal models of obesity [21]. Such mitochondrial implications are in correlation with the suggested regulatory role mitochondrial biogenesis plays during muscle regeneration [22,23]. These illustrations of the close links between obesity, mitochondria and skeletal muscle provide a piece, among others [24–26], of the mechanistic puzzle of

obesity impacts on skeletal muscle regeneration from a metabolic perspective. Furthermore, the deficiency of vitamin D, which plays a role in skeletal muscle regeneration [27], is associated with obesity [28] as well.

The other important element linking obesity to the generation of an environment leading to reduced regeneration ability is inflammation detected within different tissues, including skeletal muscle [29], adipose tissue [30,31] and liver [30], which are the key metabolic tissues governing most of the energy balance homeostasis. Inflammation impairs the regeneration of the skeletal muscle [32], and these impacts, among others, that obesity has on muscle regeneration are of particular importance since muscle is the key tissue for energy expenditure. Therefore, reduced metabolic performance of the muscle would reduce the energy expenditure and worsen obesity by shifting the metabolic balance towards further energy storage. Importantly, the impact obesity has on skeletal muscle in terms of regeneration could worsen sarcopenic obesity and explain a part of its pathogenesis [33], including that shared with obesity and leading to commune outcomes such insulin resistance and cardiometabolic impacts [34], through reducing muscle regeneration in an obesity developing environment. Exploring an example from liver's regenerative properties, the other key metabolic organ, indicates that hepatic steatosis (which develops during obesity [35,36]) impairs liver regeneration through diverse mechanisms, including oxidative stress [37], which could worsen the existent energy balance status as well.

Selected growth and metabolic factors are also modified during obesity. For instance, insulin [38] and leptin [39] increase during obesity. Since such factors are related to cellular growth and metabolism [40–44], these changes could impact regeneration ability. Indeed, insulin roles within both adipocytes and bone stromal stem cells of the bone marrow [45] suggest an impact of increased insulin on both regeneration and metabolism, as illustrated by the obesity-associated hypermetabolism leading to bone fragility [46], especially with the important roles that marrow adipocytes play in bone homeostasis [47] and remodeling [48]. This fits with the findings that obese db/db (mice lacking leptin receptor) have their postnatal regenerative osteogenesis compromised [49]. More extensive investigations linking growth and homeostatic factors (hormones, neurotransmitters, metabolic mediators, etc.) changes during obesity to regeneration impairment would provide molecular links allowing better clarification of the mechanistic pathways and identify molecular targets to correct these effects on regeneration homeostasis.

In addition, obesity represents a risk factor for cancer [50], which represents a pathological cellular replication and therefore indicates a "wrong" direction of renewal and growth of cells. This could be the result of a loss of the regeneration balance resulting from obesity-induced environment impacting the regeneration or the cancer, depending on the situation, as illustrated by the links between the liver cancer progression and an abnormal microenvironment [51] or the effect of neurostimulation in both cancer and regeneration contexts, respectively [52]. Thus, understanding key differences between the physiological regeneration process and obesity-induced regeneration impairments combined with an

understanding of obesity-associated cancer growth could contribute to a better understanding of carcinogenesis pathways.

Looking into subcellular and molecular pathways allows one to identify more mechanistic links between obesity and its negative impacts on regeneration. These include a reduced level of growth factors [53], altered DNA repair [54], induced DNA damage [55] and epigenetic changes [56]. This could explain the other important facts supporting the negative impacts obesity has on regeneration: reduced healing ability associated with obesity, as illustrated by the example of the slow bone fracture healing in obese mice [53], and increased risk of neurodegenerative diseases with obesity [57]. This shows the need to take particular care of obese patients recovering from diseases or conditions requiring regeneration, such as burns, radiotherapy, and leukemia, because of compromised regeneration ability.

Since stem cells are suggested to manage obesity [6,58,59], the negative environment generated by obesity could limit the efficacy of such an approach and therefore would first, as a preliminary step, reduce obesity (by diet, exercise or pharmacological tools) prior to stem cell administration, similarly to patients being prepared for bariatric surgery, in order to improve the biological environment receiving the stem cells and therefore optimize the therapeutic outcomes. In addition, since the negative impacts obesity has on regeneration are worsened by factors such as age [3], stem cell application to manage obesity should consider such factors towards more personalized stem cells-based therapies. Moreover, it is important to highlight the need for further studies to understand molecular players and allow one to define personalized treatments. The diversity of the available animal models of obesity could lead to differences in the results of obesity–regeneration interaction investigations. For instance, the use of diet-induced obesity animal models [60] would mimic obesity's general effects, whereas the use of more specific models such as db/db or ob/ob animal models [61] would generate data closer to the effects of leptin in the obesity context.

The effects that an obesity-induced environment has on diverse regeneration patterns at different levels impact the development of different tissues as well as related metabolic, biochemical and physiological functions. Such impaired regeneration will have clinical, therapeutic and research applications and implications.

Author Contributions: A.G. drafted the manuscript; A.G., M.Y. and J.S.-A. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Abdelaziz Ghanemi received a merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the

program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme).

Conflicts of Interest: The authors declare no conflict of interest.

4.6 References

1. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
2. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
3. Ambrosi, T.H.; Scialdone, A.; Graja, A.; Gohlke, S.; Jank, A.-M.; Bocian, C.; Woelk, L.; Fan, H.; Logan, D.W.; Schürmann, A.; et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. *Cell Stem Cell* 2017, *20*, 771–784.e6.
4. Tencerova, M.; Figeac, F.; Ditzel, N.; Taipaleenmäki, H.; Nielsen, T.K.; Kassem, M. High-Fat Diet-Induced Obesity Promotes Expansion of Bone Marrow Adipose Tissue and Impairs Skeletal Stem Cell Functions in Mice. *J. Bone Miner. Res.* 2018, *33*, 1154–1165.
5. Da Silva, S.V.; Renovato-Martins, M.; Ribeiro-Pereira, C.; Citelli, M.; Barja-Fidalgo, C. Obesity modifies bone marrow microenvironment and directs bone marrow mesenchymal cells to adipogenesis. *Obeity* 2016, *24*, 2522–2532.
6. Ulum, B.; Teker, H.T.; Sarikaya, A.; Balta, G.; Ku¸skonmaz, B.; Uckan, D.; Aerts-Kaya, F. Bone marrow mesenchymal stem cell donors with a high body mass index display elevated endoplasmic reticulum stress and are functionally impaired. *J. Cell. Physiol.* 2018, *233*, 8429–8436.
7. Pérez, L.M.; Bernal, A.; Martín, N.S.; Gálvez, B.G. Obese-derived ASCs show impaired migration and angiogenesis properties. *Arch. Physiol. Biochem.* 2013, *119*, 195–201.
8. Wu, C.-L.; Diekman, B.O.; Jain, D.; Guilak, F. Diet-induced obesity alters the differentiation potential of stem cells isolated from bone marrow, adipose tissue and infrapatellar fat pad: The effects of free fatty acids. *Int. J. Obes.* 2012, *37*, 1079–1087.
9. Ma, S.; Wong, W.T.; Wang, D.H. Obesity reshapes stem cell extracellular vesicles. *Cytom. Part A* 2018, *93*, 177–179.
10. Fuster, J.J.; Ouchi, N.; Gokce, N.; Walsh, K. Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease. *Circ. Res.* 2016, *118*, 1786–1807.
11. Iyengar, N.M.; Gucalp, A.; Dannenberg, A.J.; Hudis, C.A. Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. *J. Clin. Oncol.* 2016, *34*, 4270–4276.
12. Kraakman, M.J.; Murphy, A.J.; Jandeleit-Dahm, K.; Kammoun, H.L. Macrophage Polarization in Obesity and Type 2 Diabetes: Weighing Down Our Understanding of Macrophage Function? *Front. Immunol.* 2014, *5*, 470.
13. Esser, N.; Legrand-Poels, S.; Piette, J.; Scheen, A.; Paquot, N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res. Clin. Pract.* 2014, *105*, 141–150.
14. Varga, T.; Mounier, R.; Patsalos, A.; Gogolák, P.; Peloquin, M.; Horvath, A.; Pap, A.; Daniel, B.; Nagy, G.; Pintye, E.; et al. Macrophage PPARγ, a Lipid Activated Transcription Factor Controls the Growth Factor GDF3 and Skeletal Muscle Regeneration. *Immunity* 2016, *45*, 1038–1051.
15. Varga, T.; Mounier, R.; Gogolak, P.; Poliska, S.; Chazaud, B.; Nagy, L. Tissue LyC6- Macrophages Are Generated in the Absence of Circulating LyC6- Monocytes and Nur77 in a Model of Muscle Regeneration. *J. Immunol.* 2013, *191*, 5695–5701.
16. Varga, T.; Mounier, R.; Horvath, A.; Cuvellier, S.; Dumont, F.; Póliska, S.; Ardjoune, H.; Juban, G.; Nagy, L.; Chazaud, B. Highly Dynamic Transcriptional Signature of Distinct Macrophage Subsets during

Sterile Inflammation, Resolution, and Tissue Repair. *J. Immunol.* 2016, *196*, 4771–4782.
17. Fu, X.; Zhu, M.; Zhang, S.; Foretz, M.; Viollet, B.; Du, M. Obesity impairs skeletal muscle regeneration via inhibition of AMP-activated protein kinase. *Diabetes* 2015, *65*, 188–200.
18. Xu, P.; Werner, J.-U.; Milerski, S.; Hamp, C.M.; Kuzenko, T.; Jähnert, M.; Gottmann, P.; De Roy, L.; Warnecke, D.; Abaei, A.; et al. Diet-Induced Obesity Affects Muscle Regeneration After Murine Blunt Muscle Trauma—A Broad Spectrum Analysis. *Front. Physiol.* 2018, *9*, 674.
19. Geiger, A.E.; Daughtry, M.R.; Yen, C.; Kirkpatrick, L.T.; Shi, H.; Gerrard, D.E. Dual effects of obesity on satellite cells and muscle regeneration. *Physiol. Rep.* 2020, *8*, e14511.
20. Dungan, C.M.; Peck, B.D.; Walton, R.G.; Huang, Z.; Bamman, M.M.; Kern, P.A.; Peterson, C.A. In vivo analysis of γH2AX+ cells in skeletal muscle from aged and obese humans. *FASEB J.* 2020, *34*, 7018–7035.
21. Liu, X.; Qu, H.; Zheng, Y.; Liao, Q.; Zhang, L.; Liao, X.; Xiong, X.; Wang, Y.; Zhang, R.; Wang, H.; et al. Mitochondrial glycerol 3-phosphate dehydrogenase promotes skeletal muscle regeneration. *EMBO Mol. Med.* 2018, *10*, e9390.
22. Duguez, S.; Féasson, L.; Denis, C.; Freyssenet,D.Mitochondrial biogenesis during skeletal muscle regeneration. *Am. J. Physiol. Metab.* 2002, *282*, E802–E809.
23. Wagatsuma, A.; Kotake, N.; Yamada, S. Muscle regeneration occurs to coincide with mitochondrial biogenesis. *Mol. Cell. Biochem.* 2010, *349*, 139–147.
24. Wagatsuma, A.; Kotake, N.; Kawachi, T.; Shiozuka, M.; Yamada, S.; Matsuda, R. Mitochondrial adaptations in skeletal muscle to hindlimb unloading. *Mol. Cell. Biochem.* 2010, *350*, 1–11.
25. Hood, D.A. Invited Review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J. Appl. Physiol.* 2001, *90*, 1137–1157.
26. Hood, D.A.; Irrcher, I.; Ljubicic, V.; Joseph, A.-M. Coordination of metabolic plasticity in skeletal muscle. *J. Exp. Biol.* 2006, *209*, 2265–2275.
27. Owens, D.J.; Sharples, A.P.; Polydorou, I.; Alwan, N.; Donovan, T.F.; Tang, J.; Fraser, W.D.; Cooper, R.G.; Morton, J.P.; E Stewart, C.; et al. A systems-based investigation into vitamin D and skeletal muscle repair, regeneration, and hypertrophy. *Am. J. Physiol. Metab.* 2015, *309*, E1019–E1031.
28. Pereira-Santos, M.; Costa, P.R.F.; Assis, A.M.O.; Santos, C.A.S.T.; Santos, D.B. Obesity and vitamin D deficiency: A systematic review and meta-analysis. *Obes. Rev.* 2015, *16*, 341–349.
29. Farrell, N.J.; Norris, G.H.; Ryan, J.; Porter, C.M.; Jiang, C.; Blesso, C.N. Black elderberry extract attenuates inflammation and metabolic dysfunction in diet-induced obese mice. *Br. J. Nutr.* 2015, *114*, 1123–1131.
30. Van Der Heijden, R.A.; Sheedfar, F.; Morrison, M.C.; Hommelberg, P.P.H.; Kor, D.; Kloosterhuis, N.J.; Gruben, N.; A Youssef, S.; De Bruin, A.; Hofker, M.H.; et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. *Aging* 2015, *7*, 256–268.
31. Vieira-Potter, V.J.; Valentine, R.J.; Wilund, K.R.; Antao, N.; Baynard, T.; Woods, J.A. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. *Am. J. Physiol. Metab.* 2009, *296*, E1164–E1171.
32. Perandini, L.A.; Chimin, P.; Lutkemeyer, D.D.S.; Câmara, N.O.S. Chronic inflammation in skeletal muscle impairs satellite cells function during regeneration: Can physical exercise restore the satellite cell niche? *FEBS J.* 2018, *285*, 1973–1984.
33. Polyzos, S.A.; Margioris, A.N. Sarcopenic obesity. *Hormones* 2018, *17*, 321–331.
34. Hong, S.-H.; Choi, K.M. Sarcopenic Obesity, Insulin Resistance, and Their Implications in Cardiovascular and Metabolic Consequences. *Int. J. Mol. Sci.* 2020, *21*, 494.
35. Hurr, C.; Simonyan, H.; Morgan, D.A.; Rahmouni, K.; Young, C.N. Liver sympathetic denervation reverses obesity-induced hepatic steatosis. *J. Physiol.* 2019, *597*, 4565–4580.
36. Khoo, N.K.; Fazzari, M.; Chartoumpekis, D.V.; Li, L.; Guimaraes, D.A.; Arteel, G.E.; Shiva, S.; Freeman, B.A. Electrophilic nitro-oleic acid reverses obesity-induced hepatic steatosis. *Redox Biol.* 2019, *22*, 101132.
37. Allaire, M.; Gilgenkrantz, H. The impact of steatosis on liver regeneration. *Horm. Mol. Biol. Clin. Investig.* 2018, *41*.

38. Lee, Y.; Berglund, E.D.; Yu, X.; Wang, M.-Y.; Evans, M.R.; Scherer, P.E.; Holland, W.L.; Charron, M.J.; Roth, M.G.; Unger, R.H. Hyperglycemia in rodent models of type 2 diabetes requires insulin-resistant alpha cells. *Proc. Natl. Acad. Sci. USA* 2014, *111*, 13217–13222.
39. Sáinz, N.; Barrenetxe, J.; Moreno-Aliaga, M.J.; Martínez, J.A. Leptin resistance and diet-induced obesity: Central and peripheral actions of leptin. *Metabolism* 2015, *64*, 35–46.
40. Raut, P.K.; Kim, S.-H.; Choi, D.Y.; Jeong, G.-S.; Park, P.-H. Growth of breast cancer cells by leptin is mediated via activation of the inflammasome: Critical roles of estrogen receptor signaling and reactive oxygen species production. *Biochem. Pharmacol.* 2019, *161*, 73–88.
41. Barrios-Correa, A.A.; Estrada, J.A.; Contreras, I. Leptin Signaling in the Control of Metabolism and Appetite: Lessons from Animal Models. *J. Mol. Neurosci.* 2018, *66*, 390–402.
42. Pérez-Pérez, A.; Vilariño-García, T.; Fernández-Riejos, P.; Martín-González, J.; Segura-Egea, J.J.; Sánchez-Margalet, V. Role of leptin as a link between metabolism and the immune system. *Cytokine Growth Factor Rev.* 2017, *35*, 71–84.
43. Titchenell, P.M.; Lazar, M.A.; Birnbaum, M.J. Unraveling the Regulation of Hepatic Metabolism by Insulin. *Trends Endocrinol. Metab.* 2017, *28*, 497–505.
44. Laron, Z.; Werner, H. Insulin: A Growth Hormone and Potential Oncogene. *Pediatr. Endocrinol. Rev.* 2020, *17*, 191–197.
45. Tencerova, M.; Okla, M.; Kassem, M. Insulin Signaling in Bone Marrow Adipocytes. *Curr. Osteoporos. Rep.* 2019, *17*, 446–454.
46. Proietto, J.; Tencerova, M.; Frost, M.; Figeac, F.; Nielsen, T.K.; Ali, D.; Lauterlein, J.L.; Andersen, T.L.; Haakonsson, A.K.; Rauch, A.; et al. Faculty Opinions recommendation of Obesity-Associated Hypermetabolism and Accelerated Senescence of Bone Marrow Stromal Stem Cells Suggest a Potential Mechanism for Bone Fragility. *Fac. Opin. Post-Publ. Peer Rev. Biomed. Lit.* 2020, *27*, 2050–2062.
47. Cornish, J.; Wang, T.; Lin, J.-M. Role of Marrow Adipocytes in Regulation of Energy Metabolism and Bone Homeostasis. *Curr. Osteoporos. Rep.* 2018, *16*, 116–122.
48. Muruganandan, S.; Govindarajan, R.; Sinal, C.J.; Shanmugam, M.; Rajgopal, G. Bone Marrow Adipose Tissue and Skeletal Health. *Curr. Osteoporos. Rep.* 2018, *16*, 434–442.
49. Röszer, T.; Józsa, T.; Kiss-Tóth, E.D.; De Clerck, N.; Balogh, L. Leptin receptor deficient diabetic (db/db) mice are compromised in postnatal bone regeneration. *Cell Tissue Res.* 2013, *356*, 195–206.
50. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metabolism* 2019, *92*, 121–135.
51. Li, H.-M.; Ye, Z.-H. Microenvironment of liver regeneration in liver cancer. *Chin. J. Integr. Med.* 2017, *23*, 555–560.
52. Boilly, B.; Faulkner, S.; Jobling, P.; Hondermarck, H. Nerve Dependence: From Regeneration to Cancer. *Cancer Cell* 2017, *31*, 342–354.
53. Gao, F.; Lv, T.-R.; Zhou, J.-C.; Qin, X.-D. Effects of obesity on the healing of bone fracture in mice. *J. Orthop. Surg. Res.* 2018, *13*, 145.
54. Yu, H.; Harrison, F.E.; Xia, F. Altered DNA repair; an early pathogenic pathway in Alzheimer's disease and obesity. *Sci. Rep.* 2018, *8*, 1–6.
55. Włodarczyk, M.; Nowicka, G. Obesity, DNA Damage, and Development of Obesity-Related Diseases. *Int. J. Mol. Sci.* 2019, *20*, 1146.
56. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. *Cell Metab.* 2019, *29*, 1028–1044.
57. Mazon, J.N.; De Mello, A.H.; Ferreira, G.K.; Rezin, G.T. The impact of obesity on neurodegenerative diseases. *Life Sci.* 2017, *182*, 22–28.
58. Payab, M.; Goodarzi, P.; Heravani, N.F.; Hadavandkhani, M.; Zarei, Z.; Falahzadeh, K.; Larijani, B.; Rahim, F.; Arjmand, B. Stem Cell and Obesity: Current State and Future Perspective. *Atherosclerosis* 2018, *1089*, 1–22.
59. Matsushita, K.; Dzau, V.J. Mesenchymal stem cells in obesity: Insights for translational applications. *Lab. Investig.* 2017, *97*, 1158–1166.

60. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
61. Ahmad, A.; Ali, T.; Kim, M.W.; Khan, A.; Jo, M.H.; Rehman, S.U.; Khan, M.S.; Bin Abid, N.; Khan, M.; Ullah, R.; et al. Adiponectin homolog novel osmotin protects obesity/diabetes-induced NAFLD by upregulating AdipoRs/PPARα signaling in ob/ob and db/db transgenic mouse models. *Metabolism* 2019, *90*, 31–43.

Chapter 5. Letter to Editors - Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic?

Abdelaziz Ghanemi [a,b], Mayumi Yoshioka [b], Jonny St-Amand [a,b]

[a] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, Québec G1V 4G2, Canada

[b] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

Received 17 June 2020; Accepted 23 June 2020

5.1 Résumé (French abstract)

Avec la pandémie de la maladie à coronavirus 2019 (COVID-19), les gouvernements ont imposé des mesures qui peuvent aggraver le problème de l'obésité. Le confinement réduit l'activité physique, est susceptible d'augmenter l'apport alimentaire et entraîne des troubles du sommeil, des problèmes de santé mentale et même de l'alcoolisme, qui contribuent tous au développement de l'obésité. La crise économique réduit le pouvoir d'achat et incite à acheter des aliments à haute densité calorique (peu couteux). D'autre part, la COVID-19 pourrait avoir un impact sur les fonctions pulmonaires, ce qui limiterait la capacité du patient à faire de l'activité physique. Ces effets conduisent au développement de l'obésité qui, avec ces complications, rend la personne plus vulnérable à la COVID-19 (cercle vicieux). Par conséquent, nous nous demandons si les mesures prises pour freiner la propagation de la COVID-19 feront que la pandémie de COVID-19 sera remplacée par celle de l'obésité.

5.2 Abstract

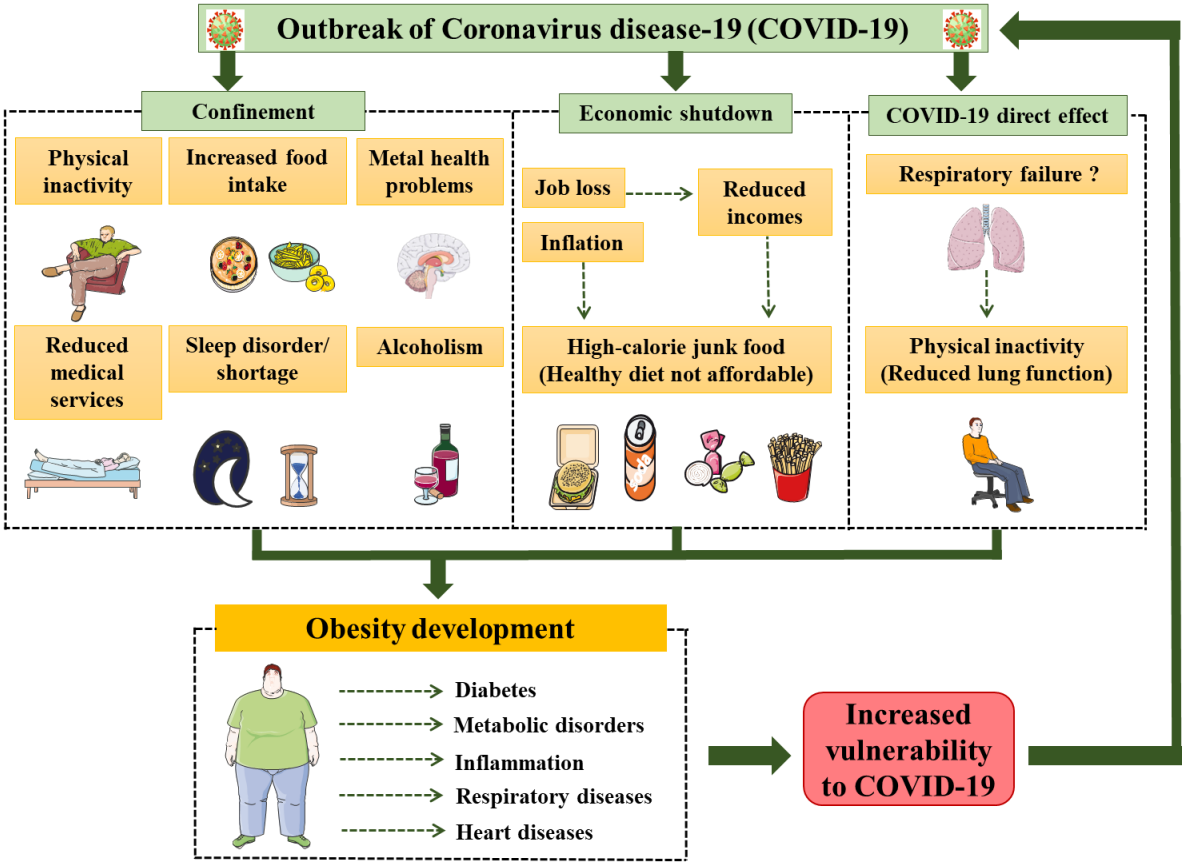
With the coronavirus disease-2019 (COVID-19) pandemic, governments have imposed measures that can worsen the obesity problem. First, the confinement of individuals reduces their physical activity (sedentary lifestyle) and is likely to increase their food intake. Confinement can also lead to sleep disorders, mental health problems and even alcoholism, all of which contribute to the development of obesity. In addition, accessibility to healthcare is reduced because health system is saturated with COVID-19 patients.

Furthermore, the economic crisis that accompanies COVID-19 crisis reduces the purchasing power of individuals who switch to the cheapest available options which are high-caloric density diets. On the other hand, COVID-19 might impact lungs functions which would limit the patient ability to perform physical activity and thus, accentuates the sedentary lifestyle.

These effects of the confinement, the economic crisis and COVID-19 (pathology) lead to the development of obesity. Moreover, obesity and the complications associated with it (diabetes, cardiovascular diseases, etc.) will make the persons more vulnerable to COVID-19 (vicious circle). Therefore, we ask ourselves whether the measures taken to curb the spread of COVID-19 will increase the portion of the population that suffers from obesity and therefore we could be replacing COVID-19 pandemic with that of obesity.

Keywords: Coronavirus disease-2019 (COVID-19); Obesity

5.3 Graphical abstract



5.4 Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic?

Dear editors,

With the current outbreak of coronavirus disease-2019 (COVID-19) [1], debates are taking place around the consequences this pandemic would have on different aspects of the human health including obesity. This virus has the ability to spread in a way, so far, too fast to be easily controlled. Thus, in order to avoid the overload of the health system, many governments and public health authorities worldwide have imposed (among other implemented measures) home confinement and general lockdown that led to a variety of consequences. Among these consequences, we put a spotlight on those well-known to be related to obesity epidemiology through selected illustrations.

During this critical period of COVID-19 pandemic, the mental health is among what health professionals are most concerned about. Mental health complications including depression, anxiety, stress, and diverse psychological problems can result from isolation and reduced social activities, human connection and physical interaction [2–4] due to home confinement, closed parks and gymnasiums, etc. Such mental problems can also result from, lead to or be associated with disturbed sleeping cycle and sleep shortage (that can also result from home confinement [5]) and vice versa [6–12]. Both psychological status and sleeping disorders could lead to increased food intake and obesity risk [13,14]. In addition, mental health and sleep disorders may require prescribing medicines that impact the energy balance and increase weight [15], which would further contribute the development of obesity. Indeed, obesity is basically defined as resulting from an increased energy intake compared to the energy expenditure, the physical activity and the food intake are two important pillars in obesity-related energy balance [13]. Within this context, home confinement combined to the related mental problems (like anxiety and depression) would lead to increased food intake, especially that many individuals have important food storages at home cumulated prior and during the lockdown. Following the same logic, confinement and its impact on mood could incite to higher alcohol consumption which can further contribute to weigh gain [16,17]. For the energy expenditure part, the home confinement (absence of work out equipment) and the disturbed mental health (lack of sufficient motivation) would lead to a physical inactivity (sedentary lifestyle). Therefore, these result in an energy balance towards an obesity establishment.

Within the context of diet choice, the socioeconomic crisis expected to develop during and after the current COVID-19 crisis [18,19] would lead to inflation of different products prices. Therefore, less individuals will afford to buy healthy food (expensive) especially knowing that millions lost their jobs and have seen their incomes significantly decreased. Therefore, the consumption of junk food (unhealthy and with a high caloric density), which is more affordable, more available and easy to store, will increase and lead to increased risk of obesity among other health problems [20]. Moreover, patients who get the respiratory illness of COVID-19 might have reduced lung function [21,22] (possibly even after they recover), which would limit their ability to perform physical activity due to the respiratory failure. These would further switch the energy balance towards developing obesity following the decrease of physical activity-related energy expenditure.

Importantly, individuals avoiding public places or keeping away from health care facilities (to protect themselves from contracting COVID-19), via limiting the number of times they leave home, may neglect seeking medical assistance to take care of their health problems for which they would have visited health professionals under normal circumstances. Furthermore, individuals that have had their incomes reduced may not be able to afford medical services (especially those not covered by insurances). Finally, the saturation of health systems because of COVID-19 made that numerous medical services (mostly non urgent) have been suspended is certain health care facilities. All these elements will reduce the quality of health care that populations receive and would worsen the pre-existing health conditions towards more serious diseases. These are additional factors strengthening the establishment and the persistence of obesity, diabetes and divers other metabolic disorders and chronic diseases in our societies.

Herein, we have illustrated how selected COVID-19 pandemic-related concepts do increase obesity risk and therefore multiple obesity-related morbidities including cardiovascular diseases, diabetes, etc. [23]. Obesity and those morbidities resulting from measures implemented to prevent COVID-19 spread and limit its mortality would, ironically, make the individuals more vulnerable to COVID-19 [24]. Importantly, it is of an extreme importance to make sure we do not replace COVID-19 pandemic with another pandemic(s) such as obesity which is already epidemic [25]. This can be achieved by implementing a variety of complimentary approaches to compensate the lost healthy practices and habits of the daily life. This could be reached, for instance, via the use of online tools to organise social activities, medical appointments, psychotherapies along with economic measures to reduce the heavy socioeconomic impacts on individuals as well.

Acknowledgement

Abdelaziz Ghanemi is a recipient of a Merit scholarship program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). The graphical abstract was created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License and Scidraw.io (Image's author: John Chilton).

Authors' contribution

Abdelaziz Ghanemi designed the manuscript structure and wrote it. Abdelaziz Ghanemi, Mayumi Yoshioka and Jonny St-Amand discussed the content and exchanged ideas and suggestions (concepts to add, the graphical abstract, references selection, etc) throughout the writing process and edited (and critically revised) the review. Jonny St-

Amand gave the final approval of the version to be published. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interests. This paper does not take any position neither for nor against any decision of a political or an economic nature.

5.5 References

[1] Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). Int J Surgery (London, England). 2020; 76:71–6.

[2] Hagerty SL, Williams LM. The impact of COVID-19 on mental health: the interactive roles of brain biotypes and human connection. Brain Behav Immun – Health 2020; 5:100078.

[3] Torales J, O'Higgins M, Castaldelli-Maia JM, Ventriglio A. The outbreak of COVID- 19 coronavirus and its impact on global mental health. Int J Soc Psychiatry 2020.

[4] Grover S, Dua D, Sahoo S, Mehra A, Nehra R, Chakrabarti S. Why all COVID-19 Hospitals should have Mental Health Professionals: the importance of mental health in a worldwide crisis!. Asian J Psychiatry 2020;102147.

[5] Altena E, Baglioni C, Espie CA, Ellis J, Gavriloff D, Holzinger B, et al. Dealing with sleep problems during home confinement due to the COVID-19 outbreak: practical recommendations from a task force of the European CBT-I Academy. J Sleep Res 2020: e13052.

[6] João K, Jesus SN, Carmo C, Pinto P. The impact of sleep quality on the mental health of a non-clinical population. Sleep Med 2018; 46:69–73.

[7] Stone KL, Xiao Q. Impact of poor sleep on physical and mental health in older women. Sleep Med Clinics 2018;13(3):457–65.

[8] Thorsteinsson EB, Brown RF, Owens MT. Modeling the effects of stress, anxiety, and depression on rumination, sleep, and fatigue in a nonclinical sample. J Nerv Ment Dis 2019; 207(5):355–9.

[9] Kalmbach DA, Anderson JR, Drake CL. The impact of stress on sleep: Pathogenic sleep reactivity as a vulnerability to insomnia and circadian disorders. J Sleep Res 2018; 27(6): e12710.

[10] St-Onge MP. Sleep-obesity relation: underlying mechanisms and consequences for treatment. Obesity Rev 2017; 18(Suppl 1):34–9.

[11] Bayon V, Leger D, Gomez-Merino D, Vecchierini MF, Chennaoui M. Sleep debt and obesity. Ann Med 2014;46(5): 264–72.

[12] Chan WS, Levsen MP, McCrae CS. A meta-analysis of associations between obesity and insomnia diagnosis and symptoms. Sleep Med Rev 2018; 40:170–82.

[13] Ghanemi A, Yoshioka M, St-Amand J. Broken energy homeostasis and obesity pathogenesis: the surrounding concepts. J Clin Med. 2018;7(11).

[14] Atlantis E, Goldney RD, Wittert GA. Obesity and depression or anxiety. BMJ (Clinical research ed). 2009;339: b3868.

[15] Gafoor R, Booth HP, Gulliford MC. Antidepressant utilisation and incidence of weight gain during 10 years' follow-up: population based cohort study. BMJ (Clinical research ed). 2018; 361:k1951.

[16] Traversy G, Chaput JP. Alcohol consumption and obesity: an update. Current Obesity Rep 2015; 4(1):122–30.

[17] Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: a systematic review. Nutr Rev 2011;69(8): 419–31.

[18] Sharif A, Aloui C, Yarovaya L. COVID-19 pandemic, oil prices, stock market, geopolitical risk and

policy uncertainty nexus in the US economy: fresh evidence from the wavelet-based approach. Int Rev Financ Anal 2020; 70:101496.
[19] Shammi M, Bodrud-Doza M, Towfiqul Islam ARM, Rahman MM. COVID-19 pandemic, socioeconomic crisis and human stress in resource-limited settings: a case from Bangladesh. Heliyon 2020;6(5): e04063.
[20] Payab M, Kelishadi R, Qorbani M, Motlagh ME, Ranjbar SH, Ardalan G, et al. Association of junk food consumption with high blood pressure and obesity in Iranian children and adolescents: the Caspian-IV Study. Jornal de Pediatria (Versão em Português) 2015;91(2): 196–205.
[21] Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ (Clinical research ed). 2020; 368:m1091.
[22] Hartman ME, Hernandez RA, Patel K, Wagner TE, Trinh T, Lipke AB, et al. COVID- 19 respiratory failure: targeting inflammation on VV-ECMO support. ASAIO J (American Society for Artificial Internal Organs: 1992) 2020.
[23] Ghanemi A, St-Amand J. Redefining obesity toward classifying as a disease. Eur J Intern Med. 2018;55: 20–2.
[24] Sanchis-Gomar F, Lavie CJ, Mehra MR, Henry BM, Lippi G. Obesity and outcomes in COVID-19: when an epidemic and pandemic collide. Mayo Clin Proc 2020.
[25] Bray GA, Macdiarmid J. The epidemic of obesity. Western J Med 2000;172(2):78–9.

Chapter 6. Review - Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars

Abdelaziz Ghanemi [1,2], Aicha Melouane [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 17 June 2020; Accepted: 28 July 2020; Published: 31 July 2020

6.1 Résumé (French abstract)

Les approches anti-obésité actuelles sont principalement le régime alimentaire contrôlé et l'exercice. Comme ces approches pourraient avoir des limites, des alternatives thérapeutiques sont nécessaires. Ici, nous mettons en lumière des cibles thérapeutiques potentielles pour l'obésité identifiées à la suite d'études basées sur l'expression différentielle de gènes dans diverses conditions en fonction de l'activité physique et de la diète riche en gras, deux facteurs clés influençant le développement de l'obésité. De telles approches de génomique fonctionnelle contribuent à élucider les mécanismes moléculaires qui contrôlent le développement de l'obésité. Nous pensons que cette exploration liée à la génomique fonctionnelle conduira non seulement à l'identification de nouveaux mécanismes, mais aussi à une nouvelle génération de thérapies pour l'obésité et les troubles métaboliques connexes, en particulier avec les progrès en pharmacologie et les techniques de génomique fonctionnelle.

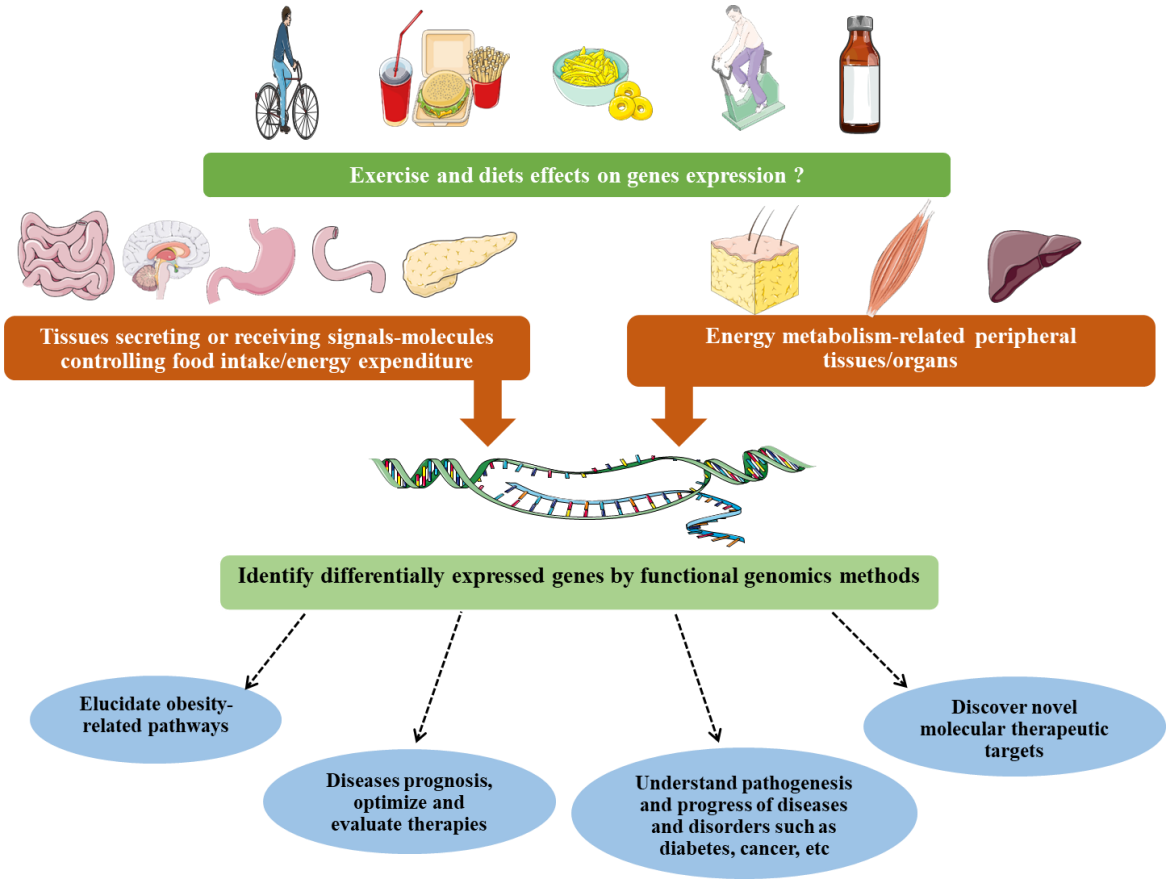
6.2 Abstract

The heavy impact of obesity on both the population general health and the economy makes clarifying the underlying mechanisms, identifying pharmacological targets, and developing efficient therapies for obesity of high importance. The main struggle facing obesity research is that the underlying mechanistic pathways are yet to be fully revealed. This limits both our understanding of pathogenesis and therapeutic progress toward treating the obesity epidemic. The current anti-obesity approaches are mainly a controlled diet and exercise which could have limitations. For instance, the "classical" anti-obesity approach of exercise might not be practical for patients suffering from disabilities that prevent them from routine exercise. Therefore, therapeutic alternatives are urgently required. Within this context, pharmacological agents could be relatively efficient in association to an adequate

diet that remains the most efficient approach in such situation. Herein, we put a spotlight on potential therapeutic targets for obesity identified following differential genes expression-based studies aiming to find genes that are differentially expressed under diverse conditions depending on physical activity and diet (mainly high-fat), two key factors influencing obesity development and prognosis. Such functional genomics approaches contribute to elucidate the molecular mechanisms that both control obesity development and switch the genetic biochemical, and metabolic pathways toward a specific energy balance phenotype. It is important to clarify that by "gene-related pathways", we refer to genes, the corresponding proteins and their potential receptors, the enzymes and molecules within both the cells in the intercellular space, that are related to the activation, the regulation, or the inactivation of the gene or its corresponding protein or pathways. We believe that this emerging area of functional genomics-related exploration will not only lead to novel mechanisms but also new applications and implications along with a new generation of treatments for obesity and the related metabolic disorders especially with the modern advances in pharmacological drug targeting and functional genomics techniques.

Keywords: obesity; differential genes expression; exercise; high-fat diet; pathways; potential therapeutic targets

6.3 Graphical abstract



6.4 Obesity as a Health Problem in Need of Novel Approaches

Obesity is defined as an abnormal or excessive fat accumulation [1] resulting from a broken energy homeostasis [2]. It has an epidemiological profile with a continuously increasing trend worldwide [3–5]. In the United States of America, at least 78.6 million people suffer from obesity [6]. Obesity is also linked to diabetes development (diabesity) [7]. In addition, not only many risk factors can increase obesity prevalence [8–10] but the obesity epidemic has also a major impact on health due to the complexity of its mechanisms, pathophysiology, and metabolic consequences [11]. Obesity has also been reported to increase risks and incidence of diseases and disorders such as advanced colorectal neoplasm [12], malnutrition [13], and mortality risk [14] in addition to decreasing life expectancy [15] among other diverse health impacts that could justify classifying obesity as a disease [16].

Diet control (caloric restriction), exercise, or the combination of both are the main anti-obesity approaches. For persons with morbid obesity, bariatric surgery can be an option [17] and medications are prescribed in some cases [18,19] as well. Although body weight management is a multibillion-dollar market, there are only few Food and Drug Administration-approved drugs available for long-term obesity treatment, but all have undesirable side effects [20,21].

In addition, some disabilities or heart diseases might limit the ability of individuals with obesity to exercise. In spite of the efforts of the diverse local, national, and international organizations in collaboration with health professionals and decision makers, obesity remains a major challenge with heavy consequences on life quality of the population and on healthcare budgets [22,23] especially that patients with obesity might require a specific or an adapted therapeutic care for some diseases compared to patients not suffering from obesity.

Therefore, there is an urgent need to further explore the obesity-related pathways in order to understand the underlying mechanisms and identify potential therapeutic targets. Herein, we focus on exercise and high-fat (HF) diet as they represent key factors for obesity prevention, development, and treatment area. We highlight how functional genomics allows exploring these factors via illustrative examples along with the research, pharmacological and clinical possible outcomes, and implications.

6.5 Exercise-Related Genes and Pathways: Towards an Exercise Pill

6.5.1 Exercise and Health

Along with resting energy expenditure, exercise-induced energy expenditure represents a key component of the total energy expenditure [24]. In addition to its place within the energy balance as the most variable part [24], exercise has benefits at different levels even for the older population [25]. Regular exercise contributes to reduced body weight, blood pressure, low-density lipoprotein, and total cholesterol and increases high

density lipoprotein cholesterol, muscular function, and strength as well as insulin sensitivity [26,27]. This makes exercise an important therapy both to prevent and manage obesity [28]. Although the purpose remains to create an accumulative negative caloric balance leading to weight loss [29], intensity, regularity, and duration of an exercise defines its type and the related outcomes and benefits.

The choice of exercise types depends on what we want to achieve in terms of muscle strength, fat mass loss, mitochondrial function enhancement, etc., as well as the ability of the individual depending on factors like age, cardiovascular health, and disability. For instance, an elderly person with cardiovascular disease would go for a walk to burn calories because of their limited exercise capacity [30]. The key metabolic tissue used during exercise is the skeletal muscle and its health represents a key factor for both an improved metabolic performance as well as a healthy ageing [31] which are two risk factors of obesity.

Exercise has a crucial role in maintaining skeletal muscle homeostasis [32] especially for the older population [33]. Biochemical profile of muscles is highly determined by protein synthesis (muscle contraction) and energy metabolism (energy expenditure) that govern the ability of energy usage via locomotion, which is a principal component of anti-obesity therapy involving exercise. Importantly, both body size and body composition, which are shaped by exercise, are determinants of resting energy expenditure. This shows that the benefits of exercise in terms of caloric use goes beyond the exercise-related energy expenditure. In addition, the benefits of exercise are not limited to energy metabolism, lipoprotein profile, or obesity treatment. Indeed, studies have shown how exercise could help to improve the prognosis, therapy, or prevent (reduce the risk) the onset of diverse diseases and conditions such as cancers [34,35], cancer-induced cardiac cachexia [36], multiple sclerosis [37], stroke [38], breast cancer-related lymphedema [39], as well as to counteract some treatments side effects [40] and can even be prescribed as a complimentary therapy (e.g., exercise oncology) [41].

6.5.2 Exercise Impacts Gene Expression

Identifying genes that are regulated by exercise (exercise-induced genes, especially in the skeletal muscle) has been among the focus of different research groups that have already identified a number of key exercise-related transcriptomes. For instance, numerous studies have obtained data that defined the effects of exercise on genes that are related to exercise benefits at the biochemical and metabolic levels. Indeed, they have shown that exercise induces the expression of genes that regulate or are related to mitochondrial biogenesis [42], oxidative phosphorylation (OXPHOS) [43], antioxidant defense mechanism [44], cell proliferation [45], and the amelioration of insulin resistance [46] which indicates links between exercise outcomes and transcriptome modifications.

Furthermore, other gene expression-based studies, mainly comparative [47] and under different conditions including exercise [48] and resting [49] have allowed the collation of data and increase our understanding of the skeletal muscle transcriptome and

functions in diverse contexts and depending on the population category. This contributes to a more precise mechanistic understanding of the genetic and biochemical changes at the molecular level. Thus, could guide to a muscle-targeting therapy development for obesity by defining the pathway associations with genes to optimize other therapies and even improve the pharmacovigilance based on genetic profiling. Beyond that, identifying exercise-induced genes would support further progress in understanding and treating different diseases other than those only depending on energy homeostasis which would expend the benefits of "exercise pills".

6.5.3 Gene Expression Patterns Underlie Muscular Adaptation to Exercise

Exploring such exercise-induced genes and pathways contributes to understand the molecular profiles that govern the adaptive responses of muscles to exercise. In addition, advances in epigenetics of muscle [50] in relation to exercise [51,52], diet [52], and aging [53] would further strengthen this field beyond genomics and put each of these pillars within a complementary network of data via which we can investigate potential therapies. For instance, exercise during pregnancy induces offspring changes [54,55], indicating that mother physical activity (intensity and frequency) impacts the health of the unborn child which opens an area in molecular pediatrics research.

Our team has also focused on gene expression in the skeletal muscle of endurance athletes compared to sedentary men and identified 33 genes that are differentially expressed [56]. This study, which supports the data reported above, highlight the global muscle gene expression including genes mostly related to muscle contraction and energy metabolism (two parameters improved by exercise). Moreover, these data further support our previous characterization of the global gene expression profile of sprinter's muscle, that shows transcripts mainly involved in contraction and energy metabolism as the most expressed in muscles of sprinters [57]. Such genetic expression pattern reflects a functional and metabolic adaptation of athletes toward an increased muscle contractile function along with an enhanced energy expenditure in the context of exercise training-induced muscle adaptations [58]. Furthermore, another study, involving healthy men, shows that moderate-intensity exercise at the lactate threshold induces the expression of transcriptomes involved in the tricarboxylic acid cycle, β-oxidation, antioxidant enzymes, contractile apparatus, and electron transport in the skeletal muscle [59].

Following the same line of thought, it was demonstrated that after 6 weeks of endurance training at lactate threshold intensity, the regulation of skeletal muscle transcriptome in elderly men includes increased expression of genes related to oxidative OXPHOS [60]. All these changes reflect an increase in the energy expenditure ability via an enhanced mitochondrial activity with an increased usage of biofuels which would be combined to reduced energy storage and lead to protection from obesity. This study [60] has also highlighted the importance of mitochondrial OXPHOS and extracellular matrix (ECM) remodeling in the skeletal muscle adaptation which correlates with a previously reported work in which genes of both ECM and calcium binding are upregulated and those

related to diabetes are modulated in human skeletal muscle following a 6 wks aerobic training [61]. We note that the exercise-induced genes are associated with a profile that counteracts the ageing process. Indeed, whereas ageing (risk factor for obesity) decreases metabolic performance (e.g., mitochondrial dysfunction [62]) and the strength of the muscle [63] and increases oxidative stress [64], exercise improves those biological patterns in the muscle.

One of the mild endurance training induced genes that draws particular attention is the secreted protein acidic and rich in cysteine (*SPARC*). This gene was characterized as an exercise-induced gene [60] as well as electrical pulse stimulation (considered as the in vitro form of exercise)-induced gene in C2C12 myoblasts [65]. In addition, studies have shown that SPARC increased in the skeletal muscle during training [66–68]. This same protein plays diverse roles in energy metabolism especially in the muscle [69,70], ECM remodeling and myoblast differentiation [71–74], inflammation [75], and cancer development [76], which would indicate that SPARC plays a role in exercise-induced benefit related processes involving inflammation, cancer, and tissue remodeling.

All these gene expression changes help to understand, at least in part, exercise induced pathways of mitochondrial biogenesis [77] and mitochondrial biochemistry [78] as well as muscle adaptation [79] and how exercise can reverse ageing impacts on skeletal muscle [80]. Such genomics studies are supported and complemented by proteomics studies that have explored the variations in protein expression in muscle depending on the physical activity [66,81–83] and reflects an adaptation of the proteinic profile, comparable to the transcriptomic changes, as well. This includes the increase in the expression of a peroxisome proliferator-activated receptor γ coactivator 1 α isoform PGC-1α4 that is involved in the regulation of skeletal muscle hypertrophy [84] which reflects an aspect from the correlation and complementarity between the functional genomics and functional proteomics.

Moreover, studies of exercise-related genes can be categorized depending on exercise type, e.g., endurance-based exercise and resistance-based exercise [85]. The transcriptomic signature of exercised muscle is also variable depending on muscle fibers and age [86]. This indicates a need of a classification strategy depending on the variables (age, muscle fibers, exercise type, etc.) that modify gene expression response to exercise. Such classification could also be extrapolated to the therapeutic target identification depending on the suitable pharmacological effects (enhance the metabolism, increase muscle strength, etc.).

6.5.4 Implications

Such exercise-related gene expression patterns explain some of the exercise benefits, including those seen even after detraining [87], including increased muscle contraction and energy metabolism improvement, thereby providing molecular and mechanistic links between the exercise benefits and the genes (over) expressed with or

following exercise which could potentially be used for drug development towards an "exercise pill" (Figure 6.1).

Importantly, the exercise benefits and their clinical outcomes are precisely what clinicians hope to observe in their patients (with obesity, diabetes, etc.) such as an improved blood lipoprotein profile [88,89], increased usage of lipids and glucose, ameliorated insulin resistance, as well as an enhanced energy expenditure. Obtaining these effects is exactly what functional genomics-based therapies aim to achieve via pharmacological agents. Indeed, identifying exercise-specific genes and exploring the pathways they control would allow the development of exercise pills. Such pills could therapeutically mimic the effects of exercise via targeting these "exercise-genes" pathways through pharmacological agents and thus, obtain the benefits of exercise without intensive training. This is of a particular importance for old (and suffering from heart diseases) or disabled individuals who have limited ability to exercise but who therapeutically require the benefits of exercise. Therefore, such "exercise pill" would allow to overcome this limitation of applying exercise as a therapy for obesity.

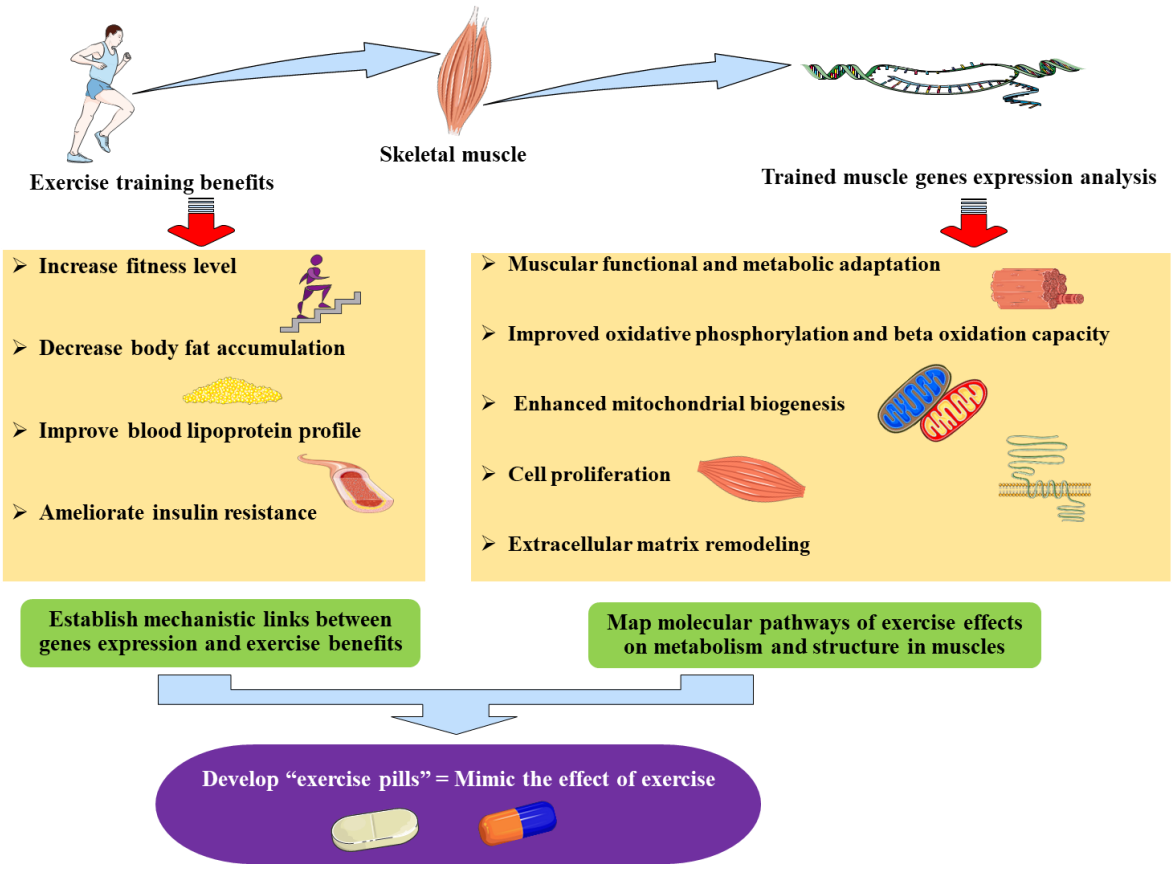


Figure 6.1. The implications of identifying genes differentially expressed during exercise training: exercise-induced genes.

6.6 Diet-Related Genes: A Focus on High-Fat Diet to Identify a Lipid-Specific Signal

6.6.1 High-Fat Diet Particularities in Obesity Context

As diet is the other pillar in obesity research and represents the energy intake and a key part of anti-obesity therapy, it is also an important factor for gene expression studies in the context of obesity. The diverse properties and impacts the diet has on metabolism pattern and biochemical adaptations made the identification and the exploration of associated specific gene expression patterns an important element in obesity molecular research. The effect of diet on obesity development is well known especially for HF diet [90–92]. The reason behind the focus on fat, beyond the concept of excess caloric intake, is that this nutrient, compared to both carbohydrates and proteins, has limited effect on satiety, is associated with high palatability, and has a high caloric density [93]. In addition, the lipid content in the modern Western diet increases fat consumption and is part of the unhealthy lifestyle. Indeed, following a HF meal ingestion, both caloric intake and energy expenditure favor weight gain because of the palatability, high caloric density, and low satiety effect of HF nutrients, as well as the weak potency for fat oxidation and energy expenditure associated with elevated fat intake [94–96]. The other pattern associated with HF diet is that the offspring have obesity risk and gene expression alterations [97] as a consequence of the maternal HF diet. This highlights the need to focus on HF diet especially as it impacts gene expression and epigenetics profile [98] as exemplified by studies showing that epigenetic changes can be consequences of the maternal HF diet [99–101].

The control of food intake represents a major determinant in the etiology of obesity especially with HF meals which acutely disrupt energy balance [102,103]. Feeding behavior is controlled by short-term circulating nutrients and hormones as well as signals derived from peripheral tissues in response to a meal and changes in energy stores. Within this context, the hypothalamus is a key brain center upon which all these peripheral signals converge to regulate feeding behavior and energy intake, thus it controls short-term as well as long-term energy balance and steady-state body weight [104,105]. Therefore, screening the changes in gene level following acute HF meal ingestions would reveal new elements within the gut brain axis leading to the development of novel approaches for the understanding and the control of energy homeostasis. In particular, the identification of transcriptomic changes induced by HF diet both in digestive and peripheral tissues as well as within the central energy metabolism control centers in the brain.

6.6.2 Digestive System (First Food “Receptors”)

Differentially expressed genes in the stomach and intestine are key elements since these two tissues represent the sites of most of the digestive processes and where the nutrients are first available in the simplest forms (that interact with endocrine system and

different receptors). Thus, stomach and intestine represent the starting point of signals controlling energy balance (including food intake). Importantly, variations (gene expression) within the digestive system may reflect changes at the digestive process that could impact the availability, the absorbance ratio, as well as the biochemical and endocrine effects of the diet nutrients. Since HF diet-induced transcriptomes would require more attention than the low-fat (LF)-induced genes, it is of a great importance to identify and more precisely distinguish between HF and LF specific genes. Therefore, the particularity of selected studies we report first herein is that fasting status was the reference (control) to study both HF and LF-specific genes. In fact, numerous previous studies that investigated HF-specific changes used LF conditions as a reference, therefore, were not able to characterize LF-specific genes nor to distinguish HF-specific from LF-specific transcriptomes. We first report a transcriptomic study that identified the peripheral signals of appetite and satiety from mice duodenum by investigating the transcriptomic changes in the duodenum mucosa 30 min, 1 h, and 3 h (to explore acute impact rather than chronic gene expression modifications) following HF and LF meal ingestion [106]. This study reveals that energy, protein, and fat intake transcriptome expression changes were higher in the HF groups compared to LF groups [106,107]. These data correlate with an intestinal mucosal mRNA analysis that demonstrates changes in the expression of genes related to anabolic and catabolic lipid metabolism pathways [108] and a recent paper shows that the expression of genes related to the uptake and transport of lipid and cholesterol as well as glucose storage are upregulated in the duodenum [109]. This changes specific patterns of HF-diet compared to LF-diet. Digestive mucosa is the first tissue that interacts with nutrients during the first digestive processes and has the ability to produce signal molecules that can act as hormones within the gut–brain axis [110]. Therefore, the key concept beyond identifying digestive mucosal diet-induced genes is to eventually identify new signals and responses to nutrient ingestion controlling food intake and energy expenditure. As an example of a potential signal molecule, the trefoil factor 2 (*Tff2*) has been identified as a newly found HF-specific gene [106] for which its deficiency in mice leads to a protection from HF diet-induced obesity [111,112]. Among the hundreds of genes that are modulated after HF or LF meal ingestion [106,113–116], we put a spotlight on the *Tff2* and its pathway as a potential targetable pathway for obesity molecular therapies. Indeed, this gene is upregulated by HF (and not LF) diet [106] which suggests it is a specific acute HF induced signals that may impact food intake regulation. At the peripheral level, HF-diet decreases the expression of genes involved in metabolizing glucose in porcine perirenal and subcutaneous adipose tissues [116] which would indicate the switch (as an adaptation) of the metabolism toward less glucose usage in the presence of lipid intake, probably to increase lipid metabolism following a LF-diet intake. In addition, it has been shown that in mesenteric adipose tissue, only LF meal upregulated transcripts implicated in lipid biosynthesis, whereas transcripts involved in lipid utilization and glucose production were downregulated in both HF and LF meals following 3 h of meal ingestion [114], also

pointing a metabolic adaptation of lipid metabolism depending on lipid ratio within the diet.

6.6.3 Adipose Tissue (Energy-Stocking Tissue) and Skeletal Muscle (Energy-Usage Tissue)

HF diet induces an increase in the expression of genes related to inflammation, whereas it downregulates genes related to lipid metabolism, adipocyte differentiation markers, and detoxification processes, and cytoskeletal structural components in mouse adipose tissue [117]. These observations highlight how the metabolic function reacts to HF diet in terms of adaptation and at the same time emphasizes health problems associated with obesity such as inflammation. These results, further indicate that the metabolism is shifted toward the usage of lipids rather than glucose, are in agreement with other studies showing that HF diet enhances the expression of genes related to lipid catabolism in the skeletal muscle [118]. Such data illustrate how the metabolic cellular system can adapt to the type and the quantity of nutrients received through different diets and the activated metabolic processes are chosen depending on such factors. Exploring such "diet-oriented" metabolic pathways might allow the development of pharmacological approaches that could mimic such pathways in order to increase lipid store usage by tissues as a part of anti-obesity therapies. Importantly, knowing the metabolism-related genes regulated by diet could optimize pharmacotherapies and diet-based therapies by selecting the type and the quantity of specific nutrients that could act towards a suitable metabolic phenotype for a specific patient. Herein, it is worth emphasizing that in order to correctly design a study, selecting the control group remains critical. Indeed, to study HF or LF diet, it is important to define the reference whether it is fasting status or fed control. In case of fed control, not only the caloric content but also the fat type and its chemical nature are also to be taken into account when reaching conclusions.

6.6.4 Brain (Energy Balance-Control Centers)

Besides identifying diet-related peripheral signals, changes induced by the diet at the central level have also been studied. For instance, the study of HF and LF meal ingestion induced changes in the hypothalamic transcriptome reveals that 3 h after the beginning of meal ingestion, 12 transcripts were regulated by food intake including two involved in mitochondrial functions [115]. This work also reveals the increased expression of the major urinary protein 1 (*Mup1*) gene in the hypothalamus of LF fed mice compared to fasting mice. MUP1 is a protein involved in metabolic profile improvement including energy balance toward skeletal muscle with increased mitochondrial function and energy expenditure in diabetic mice [119]. These MUP1 effects on metabolism regulation [120] including glucose and lipid metabolism [121], might explain the benefits of the LF diet. Such benefits are not only explained by the limited caloric intake in LF diet compared to HF diet but results from the switch of the metabolic profile toward more fuel usage and energy expenditure. In addition, we might also suggest that *Mup1*, with biochemical effects

protecting from obesity, is involved in the pathways that are blunted during obesity which would further increase energy storage and decrease energy expenditure. Indeed, in another study, a 8–12 d dietary restriction in LF-diet groups of mice led to a downregulation of *Mup1* in adipose tissue [122] which could be an adaptation to the dietary restriction in order to conserve energy stores and limit energy usage since the organism is under caloric privation. This further highlights the importance of *Mup1* in energy balance, both in energy expenditure and energy conservation, and presents its function as a potential molecular target for obesity as well.

Furthermore, regarding the hypothalamic (center of energy homeostasis control) transcriptome, high-fructose diet fed to Wistar rats throughout development lead to the remodeling of 966 genes and enhanced both depressive-like and anxiety-like behaviors [123] which could lead individuals to manifest either increase or loss of their appetite. In addition, the hypothalamic transcriptome pattern under HF diet condition (over 2 wks) exploring the neuropeptides involved in energy balance explains how ingesting a HF meal contributes to remodeling the expression of neuropeptide Y, agouti-related protein, and proopiomelanocortin over time [124]. This last element is extremely important to understand the establishment and the development of obesity by studying key molecular signals at different steps and reveal the underlying paths. Importantly, the data generated on preferentially expressed genes in the hypothalamus and pituitary gland [125,126] improve the understanding of the central control of energy metabolism and diet impact on gene expression.

6.6.5 Potential Applications

The characterization of novel fat-specific genes may contribute to the development of new therapeutic targets for appetite and satiety controls. Herein, it is worth mentioning that the existence of two levels of diet-dependent energy metabolism control (peripheral and central) provides wider therapeutic options and further choices depending on the patient's physiological or pathophysiological status. For instance, a patient with obesity suffering from a functional gastrointestinal disease might not respond well for an obesity therapy targeting the peripheral signals and would require targeting the central pathways. Mapping how the metabolic profiles (governed by selected genes) change according to the type of diet and the time between meal ingestion and gene expression analysis (and eventually at which time the meal is ingested) would allow the identification of selected signals that are specific and/or time dependent (Figure 6.2). Such data could allow to improve precise personal therapies for individuals.

Additional studies have examined the interaction between diet and gene expression regulation. HF and high-cholesterol (HFHC) diet, and HFHC plus high-sucrose diet [127] have been explored within the context of differentially expressed genes. Unlike the previous examples, blood RNA analysis was performed and revealed differential hyperlipidemia gene expression profiles even though levels of fasting plasma lipids and glucose corresponding to these two diets was similar [127]. This indicates that gene

expression might not reflect phenotypic changes and that corresponding in vivo metabolic and biochemical exploration is required to understand gene expression modifications. In addition to studying the effects of diet itself, it is highly relevant to explore the impacts of drugs that modify the effects and distribution of nutrients in vivo. For example, Salomäki et al. (2014), showed that administering metformin (prescribed to regulate glucose blood levels [128]) to pregnant female mice that were on a HF diet resulted in transcriptome related to mitochondrial ATP production and adipocytes differentiation of the offspring [129] resulting in an improved metabolic phenotype. From a therapeutic viewpoint (pharmacology and nutrition), understanding the pathways stimulated or deactivated depending on the type of diets would allow nutritionists and clinicians to adapt the diet for their patients based on the therapy they are following or based on their lifestyle to avoid possible adverse interactions between the diets, therapies, and activated pathways (genes, enzymes, etc.). This would help mitigate therapeutic failure, or pharmacotoxicity by reducing the drug clearance (metabolism) that could lead to a toxic accumulation. The goal herein remains to reach and adapt to the clinical and therapeutic needs.

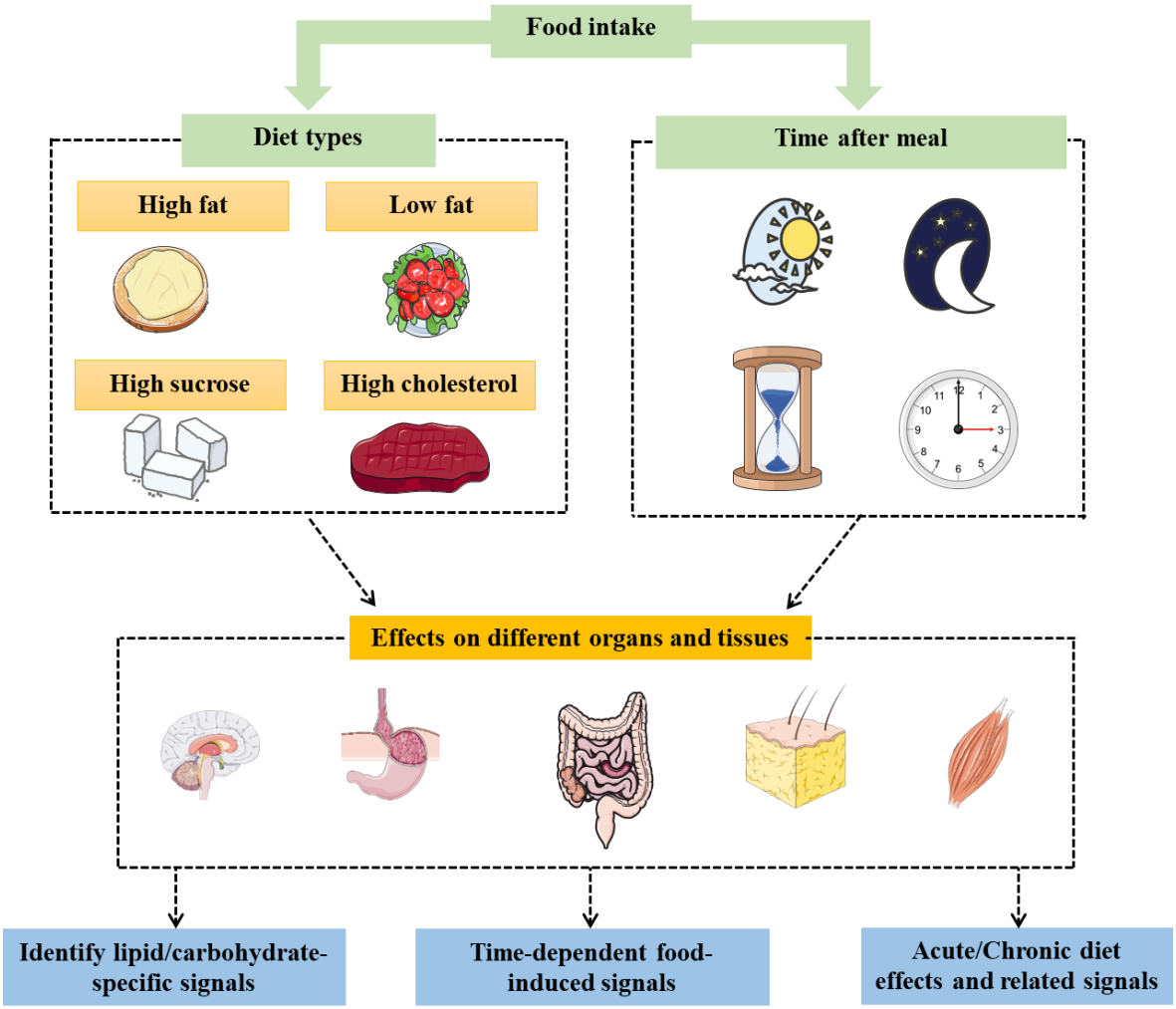


Figure 6.2. Studying the effects (expressed genes and the associated pathways) of different types of diets on the different organs/tissues involved in energy balance at different times allow to identify time-dependent specific signals (such as lipid-specific signals) regulating metabolism homeostasis.

Finally, the main potential application beyond focusing on HF-diet-induced genes remains the fact that lipid metabolism-related feedback hormones (mainly leptin) do not have an acute effect. In fact, their effects develop after a relatively long period of time compared to carbohydrate-induced hormones (for instance insulin) that are stimulated immediately following a carbohydrate intake. This highlights the importance of elucidating changes that are both acute and specific to HF diet intake in order to identify acute signals of lipid intake; based on which therapies (hormonal or pharmacological) can be developed. In addition, HF diet changed the expression of genes related to neurogenesis, calcium signaling, and synapse, in the brain cortex [130]. Such ability of the diet to impact neuronal-specific gene patterns could explain how diet and obesity establishment affect the ability of the brain to control energy balance and would require comparable studies in the hypothalamic region, the center of metabolic homeostasis control. Combining the study of changes in the intestinal mucosa (first tissue that comes in contact with the food) with those in the brain (centers that receive peripheral signals and control food intake) would provide the best combination to identify acute HF-specific signals of food intake regulation and, therefore, optimize the therapies based on these axes.

6.7 Conclusions, Discussion, and Perspectives

Overall, identifying such differentially expressed genes related to exercise and high-fat diet and their related pathways could suggest potential novel therapeutic targets for obesity treatments after elucidating the mechanisms linking those genes to the diverse energy metabolism phenotypes. Functional genomics would, therefore, lead to a new generation of therapeutic approaches that would, through targeting selected energy balance pathways, mimic the benefits and outcomes of physical activity, suitable diets, or even hormones.

For the diet, due to the properties of lipids (high caloric density, low satiety effect, etc.), we believe that one of the best strategies to develop pharmacotherapies for obesity would be to target HF intake at the appetizer time. Therefore, one of the primary strategies is to identify and study the HF diet-induced satiety hormone; usually transcriptionally regulated 30 min to 3 h after HF meal and to deliver it at the time of appetizer in order to control HF intake, obesity, and the related complex diseases and conditions. Herein, it is important to emphasize that adequate diet control is the key solution for obesity (especially if combined with exercise [131,132]) and that pharmacological options remain complementary in selected cases. Regarding identifying pathways of the exercise-induced genes is important for development of exercise pills (long-term objective) that could therapeutically mimic the effects of exercise via targeting these “exercise-genes” pathways through pharmacological agents and, thus, obtain the benefits of exercise without intensive

training. This is of a great importance for individuals who are not able to perform exercise because of physical handicap or diseases like heart failure.

Importantly, data generated by functional genomics, especially if combined with functional proteomics and the dynamic-dependent studies of the diverse related pathways will not only provide new insight into therapeutic options and research applications but also into clinical implications. Such implications will cover exercise, HF diet, but also other obesity-related factors such as hormones which are worth exploring within the functional genomics context.

Author Contributions: A.G. designed the review structure and wrote the review. A.G., A.M., M.Y., and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, figures, references selection, etc.) throughout the writing process and edited (and critically revised) the review. J.S.-A. gave the final approval of the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The graphical abstract and Figure 6.1; Figure 6.2 of this manuscript were created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ECM, extracellular matrix; HF, high-fat; HFHC, HF and high-cholesterol; LF, low-fat; MUP1/*Mup1*, major urinary protein 1; OXPHOS, oxidative phosphorylation; PGC1α (also known as PPARGC1A), peroxisome proliferator-activated receptor γ coactivator 1 α; *SPARC*/SPARC, secreted protein acidic and rich in cysteine; *Tff2*, trefoil factor 2.

6.8 References

1. Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. In *WHO Technical Report Series 894*; World Health Organization: Geneva, Switzerland, 2000; pp. i-xii, 1-253.
2. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
3. Inoue, Y.; Qin, B.; Poti, J.; Sokol, R.; Gordon-Larsen, P. Epidemiology of Obesity in Adults: Latest Trends. *Curr. Obes. Rep.* 2018, *7*, 276–288.
4. Ng, M.; Fleming, T.; Robinson, M.; Thomson, B.; Graetz, N.; Margono, C.; Mullany, E.C.; Biryukov, S.; Abbafati, C.; Abera, S.F.; et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014, *384*, 766–781.
5. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
6. Andolfi, C.; Fisichella, P.M. Epidemiology of Obesity and Associated Comorbidities. *J. Laparoendosc.*

Adv. Surg. Tech. A 2018, *28*, 919–924.
7. Bhupathiraju, S.N.; Hu, F.B. Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. *Circ. Res.* 2016, *118*, 1723–1735.
8. Wang, J.; Wu, Y.; Xiong, G.; Chao, T.; Jin, Q.; Liu, R.; Hao, L.; Wei, S.; Yang, N.; Yang, X. Introduction of complementary feeding before 4 months of age increases the risk of childhood overweight or obesity: A meta-analysis of prospective cohort studies. *Nutr. Res.* 2016, *36*, 759–770.
9. Segal, M.; Eliasziw, M.; Phillips, S.; Bandini, L.; Curtin, C.; Kral, T.V.E.; Sherwood, N.E.; Sikich, L.; Stanish, H.; Must, A. Intellectual disability is associated with increased risk for obesity in a nationally representative sample of U.S. children. *Disabil. Health J.* 2016, *9*, 392–398.
10. Michopoulos, V. Stress-Induced alterations in estradiol sensitivity increase risk for obesity in women. *Physiol. Behav.* 2016.
11. Heymsfield, S.B.; Wadden, T.A. Mechanisms, Pathophysiology, and Management of Obesity. *N. Engl. J. Med.* 2017, *376*, 254–266.
12. Kim, J.Y.; Park, D.I.; Yu, J.; Jung, Y.S.; Park, J.H.; Kim, H.J.; Cho, Y.K.; Sohn, C.I.; Jeon, W.K.; Kim, B.I.; et al. Increased Risk of Advanced Colorectal Neoplasia Among Korean Men With Metabolic Abnormality and Obesity. *Clin. Gastroenterol. Hepatol.* 2016, *14*.
13. Fu, M.C.; D0Ambrosia, C.; McLawhorn, A.S.; Schairer, W.W.; Padgett, D.E.; Cross, M.B. Malnutrition Increases With Obesity and Is a Stronger Independent Risk Factor for Postoperative Complications: A Propensity-Adjusted Analysis of Total Hip Arthroplasty Patients. *J. Arthroplast.* 2016.
14. Ricketts, T.A.; Sui, X.; Lavie, C.J.; Blair, S.N.; Ross, R. Addition of Cardiorespiratory Fitness Within an Obesity Risk Classification Model Identifies Men at Increased Risk of All-Cause Mortality. *Am. J. Med.* 2016, *129*, 536.e13–536.e20.
15. Fontaine, K.R.; Redden, D.T.; Wang, C.; Westfall, A.O.; Allison, D.B. Years of life lost due to obesity. *JAMA* 2003, *289*, 187–193.
16. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
17. le Roux, C.W.; Heneghan, H.M. Bariatric Surgery for Obesity. *Med. Clin. N. Am.* 2018, *102*, 165–182.
18. Nuffer, W. Chapter 5-Pharmacologic Agents Chapter for Abdominal Obesity. In *Nutrition in the Prevention and Treatment of Abdominal Obesity*, 2nd ed.; Watson, R.R., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 51–66.
19. Kim, G.W.; Lin, J.E.; Blomain, E.S.; Waldman, S.A. Antiobesity pharmacotherapy: New drugs and emerging targets. *Clin. Pharmacol. Ther.* 2014, *95*, 53–66.
20. Gadde, K.M.; Pritham Raj, Y. Pharmacotherapy of Obesity: Clinical Trials to Clinical Practice. *Curr. Diab. Rep.* 2017, *17*, 34.
21. Gadde, K.M.; Apolzan, J.W.; Berthoud, H.R. Pharmacotherapy for Patients with Obesity. *Clin. Chem.* 2017.
22. Canella, D.S.; Novaes, H.M.; Levy, R.B. Medicine expenses and obesity in Brazil: An analysis based on the household budget survey. *BMC Public Health* 2016, *16*, 54.
23. Verhaeghe, N.; De Greve, O.; Annemans, L. The potential health and economic effect of a Body Mass Index decrease in the overweight and obese population in Belgium. *Public Health* 2016.
24. Westerterp, K.R. Control of Energy Expenditure in Humans. In *Endotext*; Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., Dungan, K., Grossman, A., Hershman, J.M., Kaltsas, G., Koch, C., et al., Eds.; MDText.com, Inc.: South Dartmouth, MA, USA, 2000.
25. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. *Phys. Med. Rehabil. Clin. N Am.* 2017, *28*, 659–669.
26. Myers, J. Cardiology patient pages. Exercise and cardiovascular health. *Circulation* 2003, *107*, e2–e5.
27. Tran, Z.V.; Weltman, A. Differential effects of exercise on serum lipid and lipoprotein levels seen with changes in body weight. A meta-Analysis. *Jama* 1985, *254*, 919–924.
28. Blundell, J.E.; Gibbons, C.; Caudwell, P.; Finlayson, G.; Hopkins, M. Appetite control and energy

balance: Impact of exercise. *Obes. Rev.* 2015, *16* (Suppl. 1), 67–76.
29. Chin, S.H.; Kahathuduwa, C.N.; Binks, M. Physical activity and obesity: What we know and what we need to know. *Obes. Rev.* 2016, *17*, 1226–1244.
30. Marcin, T.; Eser, P.; Prescott, E.; Mikkelsen, N.; Prins, L.F.; Kolkman, E.K.; Lado-Baleato, Ó.; Cardaso-Suaréz, C.; Bruins, W.; van der Velde, A.E.; et al. Predictors of pre-Rehabilitation exercise capacity in elderly European cardiac patients-The EU-CaRE study. *Eur. J. Prev. Cardiol.* 2019.
31. McLeod, M.; Breen, L.; Hamilton, D.L.; Philp, A. Live strong and prosper: The importance of skeletal muscle strength for healthy ageing. *Biogerontology* 2016, *17*, 497–510.
32. Brook, M.S.; Wilkinson, D.J.; Phillips, B.E.; Perez-Schindler, J.; Philp, A.; Smith, K.; Atherton, P.J. Skeletal muscle homeostasis and plasticity in youth and ageing: Impact of nutrition and exercise. *Acta Physiol. (Oxf.)* 2016, *216*, 15–41.
33. Shad, B.J.; Wallis, G.; van Loon, L.J.; Thompson, J.L. Exercise prescription for the older population: The interactions between physical activity, sedentary time, and adequate nutrition in maintaining musculoskeletal health. *Maturitas* 2016, *93*, 78–82.
34. Figueira, A.C.C.; Figueira, M.C.; Silva, C.; Padrao, A.; Oliveira, P.A.; Ferreira, R.P.; Duarte, J.A. Exercise Training-Induced Modulation in Microenvironment of Rat Mammary Neoplasms. *Int. J. Sports Med.* 2018.
35. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. *Cell Metab.* 2016, *23*, 554–562.
36. Antunes, J.M.M.; Ferreira, R.M.P.; Moreira-Goncalves, D. Exercise Training as Therapy for Cancer-Induced Cardiac Cachexia. *Trends Mol. Med.* 2018, *24*, 709–727.
37. Wesnes, K.; Myhr, K.M.; Riise, T.; Cortese, M.; Pugliatti, M.; Bostrom, I.; Landtblom, A.M.; Wolfson, C.; Bjornevik, K. Physical activity is associated with a decreased multiple sclerosis risk: The EnvIMS study. *Mult. Scler.* 2018, *24*, 150–157.
38. Han, P.; Zhang, W.; Kang, L.; Ma, Y.; Fu, L.; Jia, L.; Yu, H.; Chen, X.; Hou, L.; Wang, L.; et al. Clinical Evidence of Exercise Benefits for Stroke. *Adv. Exp. Med. Biol.* 2017, *1000*, 131–151.
39. Panchik, D.; Masco, S.; Zinnikas, P.; Hillriegel, B.; Lauder, T.; Suttmann, E.; Chinchilli, V.; McBeth, M.; Hermann, W. The Effect of Exercise on Breast Cancer-Related Lymphedema: What the Lymphatic Surgeon Needs to Know. *J. Reconstr. Microsurg.* 2018.
40. Keilani, M.; Hasenoehrl, T.; Baumann, L.; Ristl, R.; Schwarz, M.; Marhold, M.; Sedghi Komandj, T.; Crevenna, R. Effects of resistance exercise in prostate cancer patients: A meta-analysis. *Support Care Cancer* 2017, *25*, 2953–2968.
41. Hart, N.H.; Galvao, D.A.; Newton, R.U. Exercise medicine for advanced prostate cancer. *Curr. Opin. Support Palliat. Care* 2017, *11*, 247–257.
42. Erlich, A.T.; Brownlee, D.M.; Beyfuss, K.; Hood, D.A. Exercise induces TFEB expression and activity in skeletal muscle in a PGC-1alpha-Dependent manner. *Am. J. Physiol. Cell Physiol.* 2017.
43. Vainshtein, A.; Tryon, L.D.; Pauly, M.; Hood, D.A. Role of PGC-1alpha during acute exercise-Induced autophagy and mitophagy in skeletal muscle. *Am. J. Physiol. Cell Physiol.* 2015, *308*, C710–C719.
44. Wang, P.; Li, C.G.; Qi, Z.; Cui, D.; Ding, S. Acute exercise stress promotes Ref1/Nrf2 signalling and increases mitochondrial antioxidant activity in skeletal muscle. *Exp. Physiol.* 2016, *101*, 410–420.
45. Choi, S.; Liu, X.; Li, P.; Akimoto, T.; Lee, S.Y.; Zhang, M.; Yan, Z. Transcriptional profiling in mouse skeletal muscle following a single bout of voluntary running: Evidence of increased cell proliferation. *J. Appl. Physiol. (Bethesda, Md. 1985)* 2005, *99*, 2406–2415.
46. Hu, Z.; Zhou, L.; He, T. Potential effect of exercise in ameliorating insulin resistance at transcriptome level. *J. Sports Med. Phys. Fit.* 2017.
47. Larose, M.; St-Amand, J.; Yoshioka, M.; Belleau, P.; Morissette, J.; Labrie, C.; Raymond, V.; Labrie, F. Transcriptome of mouse uterus by serial analysis of gene expression (SAGE): Comparison with skeletal muscle. *Mol. Reprod. Dev.* 2004, *68*, 142–148.

48. Vissing, K.; Schjerling, P. Simplified data access on human skeletal muscle transcriptome responses to differentiated exercise. *Sci. Data* 2014, *1*, 140041.
49. Lindholm, M.E.; Huss, M.; Solnestam, B.W.; Kjellqvist, S.; Lundeberg, J.; Sundberg, C.J. The human skeletal muscle transcriptome: Sex differences, alternative splicing, and tissue homogeneity assessed with RNA sequencing. *FASEB J.* 2014, *28*, 4571–4581.
50. Seaborne, R.A.; Strauss, J.; Cocks,M.; Shepherd, S.; O0Brien, T.D.; van Someren, K.A.; Bell, P.G.; Murgatroyd, C.; Morton, J.P.; Stewart, C.E.; et al. Human Skeletal Muscle Possesses an Epigenetic Memory of Hypertrophy. *Sci. Rep.* 2018, *8*, 1898.
51. Lindholm, M.E.; Marabita, F.; Gomez-Cabrero, D.; Rundqvist, H.; Ekstrom, T.J.; Tegner, J.; Sundberg, C.J. An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics* 2014, *9*, 1557–1569.
52. Sharples, A.P.; Stewart, C.E.; Seaborne, R.A. Does skeletal muscle have an 'epi'-memory? The role of epigenetics in nutritional programming, metabolic disease, aging and exercise. *Aging Cell* 2016, *15*, 603–616.
53. Gensous, N.; Bacalini, M.G.; Pirazzini, C.; Marasco, E.; Giuliani, C.; Ravaioli, F.; Mengozzi, G.; Bertarelli, C.; Palmas, M.G.; Franceschi, C.; et al. The epigenetic landscape of age-related diseases: The geroscience perspective. *Biogerontology* 2017, *18*, 549–559.
54. Li, S.; Chen, Y.; Zhang, Y.; Zhang, H.; Wu, Y.; He, H.; Gong, L.; Zeng, F.; Shi, L. Exercise during pregnancy enhances vascular function via epigenetic repression of Ca(V)1.2 channel in offspring of hypertensive rats. *Life Sci.* 2019, *231*, 116576.
55. Hopkins, S.A.; Baldi, J.C.; Cutfield, W.S.; McCowan, L.; Hofman, P.L. Exercise training in pregnancy reduces offspring size without changes in maternal insulin sensitivity. *J. Clin. Endocrinol. Metab.* 2010, *95*, 2080–2088.
56. Yoshioka, M.; Tanaka, H.; Shono, N.; Snyder, E.E.; Shindo, M.; St-Amand, J. Serial analysis of gene expression in the skeletal muscle of endurance athletes compared to sedentary men. *FASEB J.* 2003, *17*, 1812–1819.
57. Yoshioka, M.; Tanaka, H.; Shono, N.; Shindo, M.; St-Amand, J. Gene expression profile of sprinter's muscle. *Int. J. Sports Med.* 2007, *28*, 1053–1058.
58. Cochran, A.J.; Percival, M.E.; Tricarico, S.; Little, J.P.; Cermak, N.; Gillen, J.B.; Tarnopolsky, M.A.; Gibala, M.J. Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations. *Exp. Physiol.* 2014, *99*, 782–791.
59. Nishida, Y.; Tanaka, H.; Tobina, T.; Murakami, K.; Shono, N.; Shindo, M.; Ogawa, W.; Yoshioka, M.; St-Amand, J. Regulation of muscle genes by moderate exercise. *Int. J. Sports Med.* 2010, *31*, 656–670.
60. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
61. Timmons, J.A.; Larsson, O.; Jansson, E.; Fischer, H.; Gustafsson, T.; Greenhaff, P.L.; Ridden, J.; Rachman, J.; Peyrard-Janvid, M.; Wahlestedt, C.; et al. Human muscle gene expression responses to endurance training provide a novel perspective on Duchenne muscular dystrophy. *Faseb J.* 2005, *19*, 750–760.
62. Huang, J.H.; Hood, D.A. Age-Associated mitochondrial dysfunction in skeletal muscle: Contributing factors and suggestions for long-Term interventions. *IUBMB Life* 2009, *61*, 201–214.
63. Marzetti, E.; Lawler, J.M.; Hiona, A.; Manini, T.; Seo, A.Y.; Leeuwenburgh, C. Modulation of age induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle. *Free Radic Biol. Med.* 2008, *44*, 160–168.
64. Lourenço Dos Santos, S.; Baraibar, M.A.; Lundberg, S.; Eeg-Olofsson, O.; Larsson, L.; Friguet, B. Oxidative proteome alterations during skeletal muscle ageing. *Redox Biol.* 2015, *5*, 267–274.
65. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.

66. Norheim, F.; Raastad, T.; Thiede, B.; Rustan, A.C.; Drevon, C.A.; Haugen, F. Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. *Am. J. Physiol. Endocrinol. Metab.* 2011, *301*, E1013–E1021.
67. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
68. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
69. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
70. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
71. Sage, H.; Vernon, R.B.; Funk, S.E.; Everitt, E.A.; Angello, J. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. *J. Cell Biol.* 1989, *109*, 341–356.
72. Francki, A.; Motamed, K.; McClure, T.D.; Kaya, M.; Murri, C.; Blake, D.J.; Carbon, J.G.; Sage, E.H. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-Dependent signaling. *J. Cell Biochem.* 2003, *88*, 802–811.
73. Mason, I.J.; Taylor, A.; Williams, J.G.; Sage, H.; Hogan, B.L. Evidence from molecular cloning that SPARC, a major product of mouse embryo parietal endoderm, is related to an endothelial cell 'culture shock' glycoprotein of Mr 43,000. *Embo J.* 1986, *5*, 1465–1472.
74. Cho, W.J.; Kim, E.J.; Lee, S.J.; Kim, H.D.; Shin, H.J.; Lim, W.K. Involvement of SPARC in in vitro differentiation of skeletal myoblasts. *Biochem. Biophys. Res. Commun.* 2000, *271*, 630–634.
75. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
76. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
77. Hood, D.A. Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle. *Appl. Physiol. Nutr. Metab.* 2009, *34*, 465–472.
78. Hoffman, N.J.; Parker, B.L.; Chaudhuri, R.; Fisher-Wellman, K.H.; Kleinert, M.; Humphrey, S.J.; Yang, P.; Holliday, M.; Trefely, S.; Fazakerley, D.J.; et al. Global Phosphoproteomic Analysis of Human Skeletal Muscle Reveals a Network of Exercise-Regulated Kinases and AMPK Substrates. *Cell Metab.* 2015, *22*, 922–935.
79. Hamilton, M.T.; Booth, F.W. Skeletal muscle adaptation to exercise: A century of progress. *J. Appl. Physiol. (1985)* 2000, *88*, 327–331.
80. Melov, S.; Tarnopolsky, M.A.; Beckman, K.; Felkey, K.; Hubbard, A. Resistance exercise reverses aging in human skeletal muscle. *PLoS ONE* 2007, *2*, e465.
81. Petriz, B.A.; Gomes, C.P.; Almeida, J.A.; de Oliveira, G.P., Jr.; Ribeiro, F.M.; Pereira, R.W.; Franco, O.L. The Effects of Acute and Chronic Exercise on Skeletal Muscle Proteome. *J. Cell Physiol.* 2017, *232*, 257–269.
82. Holloway, K.V.; O0Gorman, M.; Woods, P.; Morton, J.P.; Evans, L.; Cable, N.T.; Goldspink, D.F.; Burniston, J.G. Proteomic investigation of changes in human vastus lateralis muscle in response to interval-Exercise training. *Proteomics* 2009, *9*, 5155–5174.
83. Burniston, J.G. Changes in the rat skeletal muscle proteome induced by moderate-intensity endurance exercise. *Biochim. Biophys. Acta* 2008, *1784*, 1077–1086.
84. Ruas, J.L.; White, J.P.; Rao, R.R.; Kleiner, S.; Brannan, K.T.; Harrison, B.C.; Greene, N.P.; Wu, J.; Estall, J.L.; Irving, B.A.; et al. A PGC-1alpha isoform induced by resistance training regulates skeletal muscle

hypertrophy. *Cell* 2012, *151*, 1319–1331.
85. Coffey, V.G.; Hawley, J.A. Concurrent exercise training: Do opposites distract? *J. Physiol.* 2017, *595*, 2883–2896.
86. Raue, U.; Trappe, T.A.; Estrem, S.T.; Qian, H.R.; Helvering, L.M.; Smith, R.C.; Trappe, S. Transcriptome signature of resistance exercise adaptations: Mixed muscle and fiber type specific profiles in young and old adults. *J. Appl. Physiol. (1985)* 2012, *112*, 1625–1636.
87. St-Amand, J.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Shono, N.; Tanaka, H. Effects of mild-exercise training cessation in human skeletal muscle. *Eur. J. Appl. Physiol.* 2012, *112*, 853–869.
88. Jacobs, K.A.; Krauss, R.M.; Fattor, J.A.; Horning, M.A.; Friedlander, A.L.; Bauer, T.A.; Hagobian, T.A.; Wolfel, E.E.; Brooks, G.A. Endurance training has little effect on active muscle free fatty acid, lipoprotein cholesterol, or triglyceride net balances. *Am. J. Physiol. Endocrinol. Metab.* 2006, *291*, E656–E665.
89. Shono, N.; Urata, H.; Saltin, B.; Mizuno, M.; Harada, T.; Shindo, M.; Tanaka, H. Effects of low intensity aerobic training on skeletal muscle capillary and blood lipoprotein profiles. *J. Atheroscler. Thromb.* 2002, *9*, 78–85.
90. Laguna-Camacho, A. Influence on Adiposity and Atherogenic Lipaemia of Fatty Meals and Snacks in Daily Life. *J. Lipids* 2017, *2017*, 1375342.
91. Ribaroff, G.A.; Wastnedge, E.; Drake, A.J.; Sharpe, R.M.; Chambers, T.J.G. Animal models of maternal high fat diet exposure and effects on metabolism in offspring: A meta-regression analysis. *Obes. Rev.* 2017, *18*, 673–686.
92. Flatt, J.P. The difference in the storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. *Ann. N. Y. Acad. Sci.* 1987, *499*, 104–123.
93. Maher, T.; Clegg, M.E. Dietary lipids with potential to affect satiety: Mechanisms and evidence. *Crit. Rev. Food Sci. Nutr.* 2018, 1–26.
94. Lissner, L.; Levitsky, D.A.; Strupp, B.J.; Kalkwarf, H.J.; Roe, D.A. Dietary fat and the regulation of energy intake in human subjects. *Am. J. Clin. Nutr.* 1987, *46*, 886–892.
95. Flatt, J.P.; Ravussin, E.; Acheson, K.J.; Jequier, E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J. Clin. Investig.* 1985, *76*, 1019–1024.
96. Schutz, Y.; Flatt, J.P.; Jequier, E. Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. *Am. J. Clin. Nutr.* 1989, *50*, 307–314.
97. Keleher, M.R.; Zaidi, R.; Shah, S.; Oakley, M.E.; Pavlatos, C.; El Idrissi, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. *PLoS ONE* 2018, *13*, e0192606.
98. Keleher, M.R.; Zaidi, R.; Hicks, L.; Shah, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. A high-Fat diet alters genome-Wide DNA methylation and gene expression in SM/J mice. *BMC Genom.* 2018, *19*, 888.
99. Suter, M.A.; Ma, J.; Vuguin, P.M.; Hartil, K.; Fiallo, A.; Harris, R.A.; Charron, M.J.; Aagaard, K.M. In utero exposure to a maternal high-Fat diet alters the epigenetic histone code in a murine model. *Am. J. Obstet. Gynecol.* 2014, *210*, 463.E1–463.E11.
100. Wankhade, U.D.; Zhong, Y.; Kang, P.; Alfaro, M.; Chintapalli, S.V.; Thakali, K.M.; Shankar, K. Enhanced offspring predisposition to steatohepatitis with maternal high-Fat diet is associated with epigenetic and microbiome alterations. *PLoS ONE* 2017, *12*, e0175675.
101. Glendining, K.A.; Fisher, L.C.; Jasoni, C.L. Maternal high fat diet alters offspring epigenetic regulators, amygdala glutamatergic profile and anxiety. *Psychoneuroendocrinology* 2018, *96*, 132–141.
102. Smilowitz, J.T.; German, J.B.; Zivkovic, A.M. Food Intake and Obesity: The Case of Fat. In *Fat Detection: Taste, Texture, and Post Ingestive Effects*; Montmayeur, J.P., le Coutre, J., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2010.
103. Astrup, A. The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of low-Fat diets. *Int. J. Obes. Relat. Metab. Disord. J. Int. Assoc. Study Obes.* 2001, *25* (Suppl. 1), S46–S50.
104. Liu, L.; Wang, Q.; Liu, A.; Lan, X.; Huang, Y.; Zhao, Z.; Jie, H.; Chen, J.; Zhao, Y. Physiological Implications of Orexins/Hypocretins on Energy Metabolism and Adipose Tissue Development. *ACS Omega*

2020, *5*, 547–555.
105. Milbank, E.; López, M. Orexins/Hypocretins: Key Regulators of Energy Homeostasis. *Front. Endocrinol.* 2019, *10*, 830.
106. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-Fat meal-Induced changes in the duodenum mucosa transcriptome. *Obes. (Silver Spring Md.)* 2008, *16*, 2302–2307.
107. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
108. Douglass, J.D.; Malik, N.; Chon, S.H.; Wells, K.; Zhou, Y.X.; Choi, A.S.; Joseph, L.B.; Storch, J. Intestinal mucosal triacylglycerol accumulation secondary to decreased lipid secretion in obese and high fat fed mice. *Front. Physiol.* 2012, *3*, 25.
109. Smolders, L.; Mensink, R.P.; Boekschoten, M.V.; de Ridder, R.J.J.; Plat, J. The acute effects on duodenal gene expression in healthy men following consumption of a low-Fat meal enriched with theobromine or fat. *Sci. Rep.* 2018, *8*, 1700.
110. Martin, C.R.; Osadchiy, V.; Kalani, A.; Mayer, E.A. The Brain-Gut-Microbiome Axis. *Cell Mol. Gastroenterol. Hepatol.* 2018, *6*, 133–148.
111. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.;St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-Fat diet-Induced obesity. *Obes. (Silver Spring)* 2013, *21*, 1389–1395.
112. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-Fat diet-Induced obesity. *Life Sci.* 2018, *215*, 190–197.
113. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding regulates the expression of pancreatic genes in gastric mucosa. *J. Obes.* 2010, *2010*, 371950.
114. Bolduc, C.; Yoshioka, M.; St-Amand, J. Acute molecular mechanisms responsive to feeding and meal constitution in mesenteric adipose tissue. *Obes. (Silver Spring)* 2010, *18*, 410–413.
115. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding induced changes in the hypothalamic transcriptome. *Clin. Chim. Acta* 2009, *406*, 103–107.
116. Gondret, F.; Vincent, A.; Houee-Bigot, M.; Siegel, A.; Lagarrigue, S.; Louveau, I.; Causeur, D. Molecular alterations induced by a high-Fat high-Fiber diet in porcine adipose tissues: Variations according to the anatomical fat location. *BMC Genom.* 2016, *17*, 120.
117. Moraes, R.C.; Blondet, A.; Birkenkamp-Demtroeder, K.; Tirard, J.; Orntoft, T.F.; Gertler, A.; Durand, P.; Naville, D.; Bégeot, M. Study of the Alteration of Gene Expression in Adipose Tissue of Diet-Induced Obese Mice by Microarray and Reverse Transcription-Polymerase Chain Reaction Analyses. *Endocrinology* 2003, *144*, 4773–4782.
118. Perez-Schindler, J.; Kanhere, A.; Edwards, L.; Allwood, J.W.; Dunn, W.B.; Schenk, S.; Philp, A. Exercise and high-fat feeding remodel transcript-metabolite interactive networks in mouse skeletal muscle. *Sci. Rep.* 2017, *7*, 13485.
119. Hui, X.; Zhu, W.; Wang, Y.; Lam, K.S.; Zhang, J.; Wu, D.; Kraegen, E.W.; Li, Y.; Xu, A. Major urinary protein-1 increases energy expenditure and improves glucose intolerance through enhancing mitochondrial function in skeletal muscle of diabetic mice. *J. Biol. Chem.* 2009, *284*, 14050–14057.
120. Zhou, Y.; Rui, L. Major urinary protein regulation of chemical communication and nutrient metabolism. *Vitam. Horm.* 2010, *83*, 151–163.
121. Zhou, Y.; Jiang, L.; Rui, L. Identification of MUP1 as a regulator for glucose and lipid metabolism in mice. *J. Biol. Chem.* 2009, *284*, 11152–11159.
122. van Schothorst, E.M.; Keijer, J.; Pennings, J.L.; Opperhuizen, A.; van den Brom, C.E.; Kohl, T.; Franssen-van Hal, N.L.; Hoebee, B. Adipose gene expression response of lean and obese mice to short-Term dietary restriction. *Obes. (Silver Spring)* 2006, *14*, 974–979.
123. Harrell, C.S.; Burgado, J.; Kelly, S.D.; Johnson, Z.P.; Neigh, G.N. High-Fructose diet during

periadolescent development increases depressive-Like behavior and remodels the hypothalamic transcriptome in male rats. *Psychoneuroendocrinology* 2015, *62*, 252–264.
124. Ziotopoulou, M.; Mantzoros, C.S.; Hileman, S.M.; Flier, J.S. Differential expression of hypothalamic neuropeptides in the early phase of diet-Induced obesity in mice. *Am. J. Physiol. Endocrinol. Metab.* 2000, *279*, E838–E845.
125. St-Amand, J.; Yoshioka, M.; Tanaka, K.; Nishida, Y. Transcriptome-Wide identification of preferentially expressed genes in the hypothalamus and pituitary gland. *Front. Endocrinol.* 2011, *2*, 111.
126. Bedard, K.; Bedard, J.; Rocheleau, G.; Ferland, G.; Gaudreau, P. Aging and diets regulate the rat anterior pituitary and hypothalamic transcriptome. *Neuroendocrinology* 2013, *97*, 146–159.
127. Takahashi, J.; Waki, S.; Matsumoto, R.; Odake, J.; Miyaji, T.; Tottori, J.; Iwanaga, T.; Iwahashi, H. Oligonucleotide microarray analysis of dietary-induced hyperlipidemia gene expression profiles in miniature pigs. *PLoS ONE* 2012, *7*, e37581.
128. Maruthur, N.M.; Tseng, E.; Hutfless, S.; Wilson, L.M.; Suarez-Cuervo, C.; Berger, Z.; Chu, Y.; Iyoha, E.; Segal, J.B.; Bolen, S. Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. *Ann. Intern. Med.* 2016, *164*, 740–751.
129. Salomaki, H.; Heinaniemi, M.; Vahatalo, L.H.; Ailanen, L.; Eerola, K.; Ruohonen, S.T.; Pesonen, U.; Koulu, M. Prenatal metformin exposure in a maternal high fat diet mouse model alters the transcriptome and modifies the metabolic responses of the offspring. *PLoS ONE* 2014, *9*, e115778.
130. Yoon, G.; Cho, K.A.; Song, J.; Kim, Y.-K. Transcriptomic Analysis of High Fat Diet Fed Mouse Brain Cortex. *Front. Genet.* 2019, *10*.
131. Hansen, D.; Dendale, P.; Berger, J.; van Loon, L.J.; Meeusen, R. The effects of exercise training on fat mass loss in obese patients during energy intake restriction. *Sports Med.* 2007, *37*, 31–46.
132. Miller, C.T.; Fraser, S.F.; Levinger, I.; Straznicky, N.E.; Dixon, J.B.; Reynolds, J.; Selig, S.E. The effects of exercise training in addition to energy restriction on functional capacities and body composition in obese adults during weight losss: A systematic review. *PLoS ONE* 2013, *8*, e81692.

Chapter 7. Opinion - Obese Animals as Models for Numerous Diseases: Advantages and Applications

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec Université Laval Research Center, 2705 Boul. Laurier, Québec, QC G1V 4G2, Canada

Received: 30 January 2021; Accepted: 20 April 2021; Published: 21 April 2021

7.1 Résumé (French abstract)

Une variété de modèles animaux ont été développés pour étudier l'obésité. De tels animaux nous permettent non seulement d'explorer l'obésité, mais représenteraient également des modèles pour étudier les maladies et les conditions qui se développent avec l'obésité ou où l'obésité représente un facteur de risque. En effet, les sujets obèses, comme les modèles animaux d'obésité, développent des pathologies telles que les maladies cardiovasculaires, le diabète, l'inflammation et les troubles métaboliques. Bien que ces maladies et conditions puissent être induites chez les animaux par des produits chimiques ou des médicaments sans développement de l'obésité, les avoir développées comme conséquences de l'obésité présente de nombreux avantages. Ces avantages comprennent l'imitation des processus naturels de pathogenèse, l'utilisation de diverse modèles animaux d'obésité pour étudier les variabilités connexes et l'exploration de l'intensité et de la réversibilité de la maladie en fonction du développement et des traitements de l'obésité.

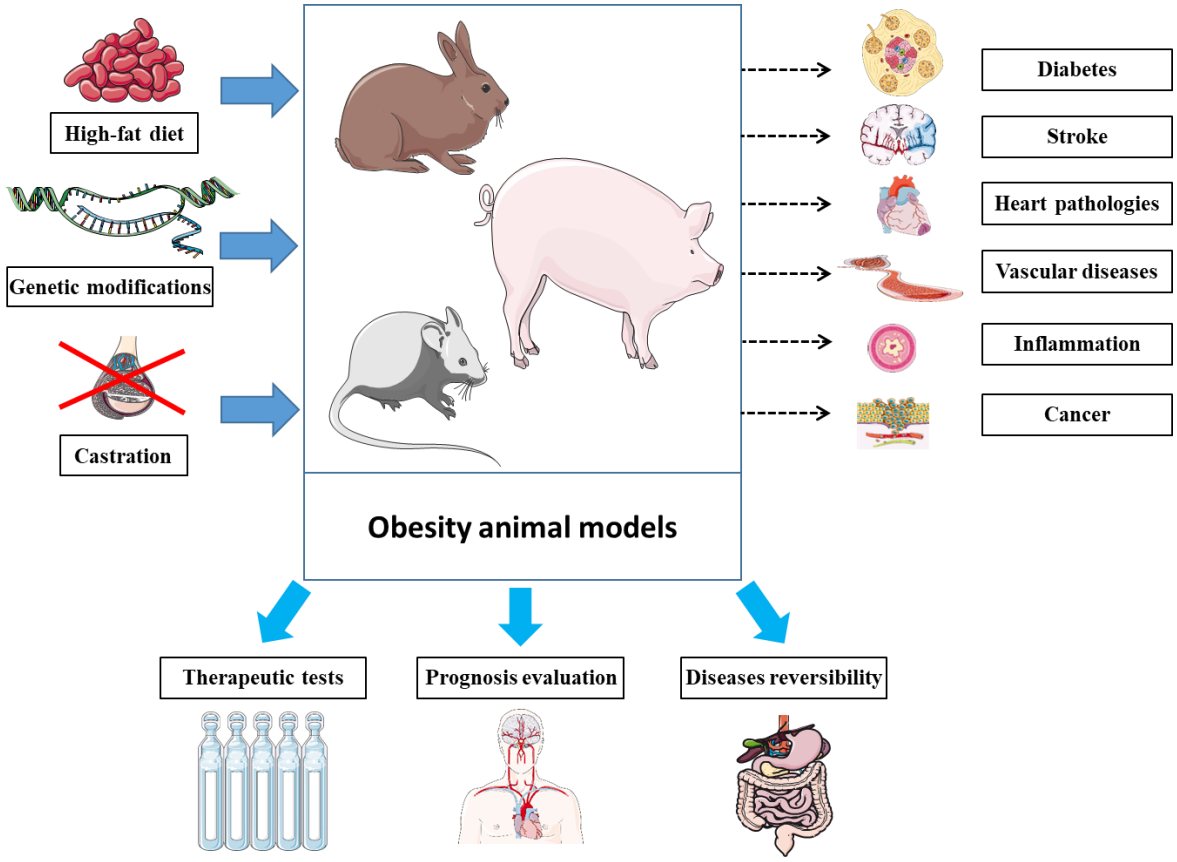
7.2 Abstract

With the advances in obesity research, a variety of animal models have been developed to investigate obesity pathogenesis, development, therapies and complications. Such obese animals would not only allow us to explore obesity but would also represent models to study diseases and conditions that develop with obesity or where obesity represents a risk factor. Indeed, obese subjects, as well as animal models of obesity, develop pathologies such as cardiovascular diseases, diabetes, inflammation and metabolic disorders. Therefore, obese animals would represent models for numerous diseases. Although those diseases can be induced in animals by chemicals or drugs without obesity development, having them developed as consequences of obesity has numerous advantages. These advantages include mimicking natural pathogenesis processes, using diversity in obesity models (diet, animal species) to study the related variabilities and exploring disease

intensity and reversibility depending on obesity development and treatments. Importantly, therapeutic implications and pharmacological tests represent key advantages too. On the other hand, obesity prevalence is continuously increasing, and, therefore, the likelihood of having a patient suffering simultaneously from obesity and a particular disease is increasing. Thus, studying diverse diseases in obese animals (either induced naturally or developed) would allow researchers to build a library of data related to the patterns or specificities of obese patients within the context of pathologies. This may lead to a new branch of medicine specifically dedicated to the diseases and care of obese patients, similar to geriatric medicine, which focuses on the elderly population.

Keywords: obese; animal; model; disease; obesity; pathogenesis

7.3 Graphical abstract



7.4 Introduction

Obesity remains one of the most challenging health problems worldwide, with increasing prevalence [1]. It impacts both public health and the economy [2], and it has even worsened within the ongoing context of COVID-19 [3]. In health science, obesity is defined based on body mass index (BMI), which represents a clinical measure of body composition using weight over height-squared [4,5]. Obesity involves abnormal fat

accumulation, initiated with adipocyte expansion via numerous underlying pathways as a result of an unhealthy lifestyle pattern (diet, physical inactivity, sleeping) combined with genetic factors, microbiota and psychology [6]. Obesity has even been hypothesized to have neuroendocrine reprogramming [7] that would make obesity hard to "reverse" once established. The medical danger of obesity is not limited to the increased body weight nor the social and psychological impacts on life quality [8]. In fact, the comorbidities and risks for health associated with obesity represent important and relevant medical problems [6]. Indeed, obesity is associated with a variety of diseases and health disorders. These include heart and cardiovascular diseases [9,10], inflammation [11,12], respiratory disease [13,14], stroke [15,16], diabetes [17,18], psychological disorders such as depression [19,20], iron deficiency [21], bone disorders [22] and even neurodegenerative diseases [23–25], cancer [26,27] and regeneration impairment [28]. In addition, the metabolically active tissues, including liver and adipose tissue and skeletal muscle, are also impacted during obesity. Indeed, obesity impacts on the liver can include portal inflammation, fibrosis and cirrhosis [29]. In a key tissue of energy expenditure, modified metabolic properties of muscle during obesity [30] can contribute to the development of sarcopenic obesity [31] and accumulate triacylglycerol in the lipid droplets that cause a lipotoxicity state [32]. Regarding adipose tissue, which is the main energy storage tissue, lipids are stored following the conversion of extra glucose into triglycerides [32]. Such impacts on these metabolically active tissues would worsen obesity-induced metabolic imbalances and lead to further metabolic disorders, including mitochondrial dysfunction [30] and insulin resistance [33], among other manifestations of metabolic syndrome during obesity [34]. Since obesity itself represents a status of imbalanced energy metabolism following broken homeostasis with a modified neuroendocrine profile [6,7], impacts on the main metabolic tissues worsen the metabolic phenotype. Other important changes are seen via the disturbed pattern of bioactive molecules produced by these metabolic tissues during obesity, including hormones, growth factors, cytokines and other adipokines [35] that impact a variety of tissues and biologically functional homeostasis. Within this context, whether to classify obesity as a disease represents a hot topic, with diverse medical and legal consequences [36–40].

Therefore, it is urgent to deepen our biological understanding of obesity. Within this perspective, different animal models have been developed through approaches aiming to induce obesity within a determined period of time. Once the obesity status is established, a variety of measures are conducted in order to explore diverse patterns, including obesity pathogenesis, obesity development and antiobesity therapy efficacy. These measures could be with or without exogenous interventions, including diet control, physical exercise and bariatric surgery.

Most importantly, the development and prognosis of obesity-related comorbidities and associated health problems such as strokes, metabolic disorders, lipid profile abnormalities, inflammations and cancer are key elements to explore as well. These animal

models aim to mimic the obesity status and allow the exploration of the related clinical and therapeutic outcomes along with their implications and applications.

On the other hand, an obese animal could develop (depending on numerous factors) one or more obesity-associated diseases and disorders. In this obese animal, those diseases and disorders are considered biological consequences or comorbidities associated with its obesity status. Therefore, obese animals would represent “natural models of choice” compared to the known obesity animal models to study the different aspects of those health problems using "naturally" conditioned animals over "artificially" induced ones. Below, we briefly expose the key reasons beyond such a statement.

7.5 In vivo mimicking of obesity-induced comorbidities and changes

Obesity is associated with various comorbidities and biological consequences. These same comorbidities can also be induced by exogenous treatments in addition to being the result of obesity. For instance, rather than inducing disease-like inflammation via lipopolysaccharides [41] or administering streptozotocin to induce diabetes [42], we would rather have these diseases “naturally” triggered by obesity and developed in obese animals. Compared to such chemically generated models of these conditions (inflammation and diabetes induced by lipopolysaccharides and streptozotocin, respectively, in these examples), obese animals are models that could develop these same conditions but in a naturally occurring manner. Importantly, the process occurs through pathogenic pathways similar to the in vivo mechanisms. Therefore, obese animals have the advantages of not only mimicking the status and the symptoms but also mimicking the biological processes leading to these outcomes and comorbidities (e.g., inflammation, diabetes) within obesity. This makes these models (obese animals) more “natural”. Indeed, the conditions would be the result of molecular and cellular processes caused by the obesity-generated environment, representing biochemically broken homeostasis [6,28] rather than an exogenous intervention (“artificial” model). Indeed, the “artificial” models lead only to the same final outcome but without all the underlying pathways. Therefore, we will miss the pathogenic patterns and links by only studying the comorbidities dissociated from obesity.

7.6 Diversity of animal models

Many animal models exist for obesity [43,44], and they vary in both species and the method of inducing obesity. The availability of different animals (e.g., mouse [45,46], rat [47], pig [48], rabbit [49]), allows diverse animal species choices for the development of an obese model. The choice is based on factors including genetics, size, life expectancy and generation time. This diversity facilitates the study of each obesity-related condition (e.g., brain modifications [50], epigenetics [51], impacts on pregnancy [52], metabolic phenotype [53]) in the most suitable animals. For instance, large mammals such as pigs would be closer to humans for studying the impacts of bariatric surgery due to size, whereas mice, also used to study bariatric surgeries [54], would be more suitable to study maternal diet

impact on offspring due to the short generation time as well as the important litter size. However, in many cases, financial reasons (animal cost) and the available animal care facilities would limit the choices and explain the wide usage of mice models rather than pigs, for example. The usage of more expensive models (for instance, pigs rather than mice, as well as monkeys) would be justified by practical design, including anatomical or physiological similarities (and fat cell size) to humans [55] or specific genetic patterns within some defined contexts, such as pathogenesis.

Some of the used approaches to generate obese animals in different studies are diet (mainly high-fat diet) [56–59], modified genes such as ob/ob [60,61] and db/db mice [62,63], and castration-induced obesity [64,65]. Even within the same method, variety can exist. For instance, diet-induced obesity is among the most widely used. However, the type of diet used in different studies [66–70] could be different from one model to another in terms of percentage of lipids, protein and carbohydrate as well as type of lipids, total calories and carbohydrate source. Therefore, these lead to diverse biological and pathological outcomes, especially where there is no standard protocol [71]. Having many choices is of particular importance when we want to (1) build an obesity model reflecting the diet of a specific region or population in order to mimic the obesity patterns that are developed by that population, and, therefore, (2) study geography-specific or population specific obesity patterns, such as the Western-style diet [72]. Within the context of diet, the role of the hypothalamus in the pathogenesis of obesity, pointed out even before leptin discovery [73], would contribute to the regulation of energy balance signals [74–76]. Indeed, the hypothalamus, particularly in rodent models of diet-induced obesity, is impacted by obesity and plays an important role in the pathogenesis of obesity [74,77–80]. This has critical importance for the development of neuropharmacological therapies for obesity [81,82] involving the hypothalamus [83–86].

This animal model diversity does significantly extend the possible choices in terms of animals, animal strains and types of diet. However, it remains important to mention the drawbacks to this diversity of animal models. For instance, there are the issues of whether we can make comparisons across studies and also the possible limited reproducibility (even when two studies use the same high-fat diet, would both use the same standard chow diet as a control?). This means that studies with similar designs can have different outcomes due to differences in animal strain, age, and diet content. Moreover, some studies use only male animals, neglecting to include both sexes, which represents another drawback that also requires optimizations towards standard parameters to further include sex- and gender-related variations. In addition, different designs might lead to different findings based on animal choice (e.g., genetics, age). Furthermore, it is also critical to consider the impacts of parameters external to the animal, such as temperature, housing conditions, and sacrifice time. Importantly, it is also worth stating that obesity impacts social and psychological well-being [87]. Such parameters might be hard to evaluate in animal models, which are among the limitations that animal models of obesity have. Differences between humans and mice represent the basis of most disadvantages and limitations of such

models, which includes the need for months of a high-fat diet to produce the full symptoms of type 2 diabetes mellitus [71]. This is why a struggle persists when extrapolating the results from animal studies to clinical applications.

7.7 Disease intensity and reversibility

Following the initial line of thought, presenting obesity as the inducer of specific diseases, it seems acceptable to say that disease developmental stage and severity scale are dependent on obesity intensity [88]. Therefore, we can compare the development of conditions such as inflammation, prediabetes and even psychological consequences [89] in obese animals based on different obesity intensity measured by selected parameters such as lipid percentage, body fat [90,91] and body weight [4]. Importantly, the ability to reverse obesity [92,93]-at least a short-term reversion by reducing body weight-would allow us to observe how the other diseases and disorders evolve. Most importantly, we would observe whether those diseases and disorders are also reversible once obesity is "treated". For instance, we can monitor diabetes or inflammation [94] marker changes following obesity treatment, which would be of great importance from clinical and therapeutic perspectives. Moreover, these changes may also be recorded depending on the approaches used to treat or reverse obesity (e.g., diet, physical activity [95], pharmacology [96], bariatric surgery [97]). This could be a methodology to evaluate and compare the different therapeutic approaches and add new evaluation and clinical tools for research. Importantly, this will allow us to establish pathological and mechanistic links between obesity and its associated comorbidities, which are among the most important missing patterns that limit our molecular and cellular understating of obesity.

7.8 Monitoring therapeutic implications

We can target-with antiobesity therapy-one disease among those existing in obese animals (such as diabetes) and see how the other related disease prognoses (such as stroke) evolve. This goes beyond therapeutic evaluation and could point to the chronological order of appearance of the different obesity-related disorders. Herein, it can also show whether a disorder (such as inflammation [98]) is a direct consequence of obesity or the indirect result of one of the diseases induced by obesity. This can be clarified by targeting the conditions of a pathway and then monitoring another condition to find out whether this second condition belongs to or is impacted by the same pathway as the first one. This will elucidate the mechanistic pathways and pathogenesis in chronological order. Recent molecular data characterizing gene activation in the context of obesity as well as diet and exercise [99–104] will also help the mapping of the chronology of such pathways, ultimately building bridges between gene activation and obesity-related comorbidities through identified molecular and cellular pathways in chronological order. Illustrative examples of obese animals that produce disease or health condition models have been reported in the literature (Table 7.1). This illustrates how complex health conditions and biological consequences of

obesity can be explored in obese animals. Therefore, these animals would be considered models for these specific health conditions or diseases as well.

Table 7.1. Examples of obese mice that are used or could be used as disease/health condition models.

Developed Disease (Models)	Obesity-Induction and Animal Models	References
Peripheral diabetic neuropathy	ob/ob mice	[105]
Hepatic steatosis	ob/ob mice	[106]
Type 2 diabetes and diabetic retinal neurodegeneration	db/db mice	[107,108]
Type 2 diabetes	ob/ob mice	[109]
Abnormal gut microbiota composition	db/db mice	[110]
Steatohepatitis	foz/foz mice fed a high-fat diet	[111]
Inflammation	Mice fed a high-fat diet	[112,113]
Insulin resistance	Mice fed a high-fat diet	[113]

Expanding the explored conditions to cover the remaining obesity-related comorbidities in different obese animals will complete the puzzle of both obesity-induced disease pathogenesis and the underlying mechanistic steps. Importantly, diseases such as cancer and COVID-19 include obesity among their risk factors (rather than causes) [26,114,115]. Therefore, an explorative comparison of obese animals that develop a disease such as cancer and the obese animals that do not could allow a better understanding of the mechanisms that convert a risk factor into a cause. This requires investigating key patterns of metabolism, physiology, gene expression and diet in both animal groups. This can, for instance, solve some mysteries beyond cancer, one of the leading causes of death [116,117] that is related to obesity. Among the emerging obesity-related research fields, microbiota has great potential for both understanding obesity pathogenesis and developing innovative therapeutic approaches [118]. Diet, an important factor in both obesity development and the building of obesity animal models, can modify the microbiota composition [119]. In addition, obese subjects have been reported to have a microbiota composition different from that seen in lean subjects [120]. Microbiota composition may modify the metabolism and energy balance of the host [67] via pathways suggested to either lead to obesity [67,120] or positively improve the metabolic profile [120]. Thus, scientists are exploring the use of fecal (microbiota) transplants as well as probiotics as therapies to improve obesity, among other diseases [121–127], through beneficially modifying microbiota composition. Importantly, we can also consider obese animals as models of dysbiosis since this microbiota composition change occurs naturally as a consequence of obesity.

7.9 Perspectives

These perspectives, presenting obese animals as models for a variety of diseases, could be extrapolated to other conditions that - like obesity - have multiple comorbidities such as diabetes [128–131]. Furthermore, obese animals can be used as starting models to build more complex models by inducing some disorders (such as infections) in obese animals and observing how a disease would evolve or how therapy efficacy would be affected in an obesity-induced environment compared to a nonobese environment. Such

studied control groups would be both obese animals not suffering from that disease (or not receiving the treatment) and nonobese animals suffering from that same disease (or receiving the treatment). Such experimental design will allow us to explore obesity as well as the induced disease or the applied treatment as variables and investigate their interactions. For clinical (and preclinical aspects), the properties linking obesity to its related diseases, explored in obese animals, can be extrapolated to humans, within the known limits [132], during clinical studies. Obesity prevalence [133] is continuously increasing. Thus, the likelihood of having a patient that is simultaneously suffering from obesity and another disease increases accordingly, especially during these times of the COVID-19 epidemic [3]. Therefore, studies within this context, supported by the results obtained from obese animals, as described above, would allow researchers to build a library of data related to the patterns or specificities of obese patients within the context of pathologies. Importantly, we look forward to a new branch of medicine that deals with the diseases and care of obese patients, similar to geriatric medicine, which focuses on the elderly population.

Author Contributions: A.G. drafted the manuscript; A.G., M.Y., and J.S.-A. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received of a merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d’excellence pour étudiants étrangers du Ministère de l’Éducation et de l’Enseignement supérieur du Québec, Le Fonds de recherche du Québec Nature et technologies (FRQNT) est responsable de la gestion du programme). The graphical abstract was created using images from http://smart.servier.com, accessed on 30 January 2021. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

7.10 References

1. Blüher, M. Obesity: Global epidemiology and pathogenesis. *Nat. Rev. Endocrinol.* 2019, *15*, 288–298.
2. Spieker, E.A.; Pyzocha, N. Economic impact of obesity. *Prim. Care* 2016, *43*, 83–95.
3. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
4. Weir, C.B.; Jan, A. *BMI Classification Percentile and Cut Off Points*; StatPearls Publishing: Treasure Island, FL, USA, 2020.
5. Gutin, I. In BMI we trust: Reframing the body mass index as a measure of health. *Soc. Theory Health* 2018, *16*, 256–271.
6. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken energy homeostasis and obesity pathogenesis: The surrounding concepts. *J. Clin. Med.* 2018, *7*, 453.
7. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a neuroendocrine reprogramming. *Medicina* 2021, *57*, 66.
8. Donini, L.M.; Rosano, A.; di Lazzaro, L.; Lubrano, C.; Carbonelli, M.; Pinto, A.; Giusti, A.M.; Lenzi, A.; Siervo, M. Impact of disability, psychological status, and comorbidity on health-related quality of life perceived by subjects with obesity. *Obes. Facts* 2020, *13*, 191–200.
9. Jokinen, E. Obesity and cardiovascular disease. *Minerva Pediatr.* 2015, *67*, 25–32.
10. Saliba, L.J.; Maffett, S. Hypertensive heart disease and obesity: A review. *Heart Fail. Clin.* 2019, *15*, 509–517.
11. Karczewski, J.; Sledzi´nska, E.; Baturo, A.; Jo´nczyk, I.; Maleszko, A.; Samborski, P.; Begier-Krasi´nska, B.; Dobrowolska, A. Obesity and inflammation. *Eur. Cytokine Netw.* 2018, *29*, 83–94.
12. Cox, A.J.; West, N.P.; Cripps, A.W. Obesity, inflammation, and the gut microbiota. *Lancet Diabetes Endocrinol.* 2015, *3*, 207–215.
13. Dixon, A.E.; Peters, U. The effect of obesity on lung function. *Expert Rev. Respir. Med.* 2018, *12*, 755–767.
14. Meurling, I.J.; Shea, D.O.; Garvey, J.F. Obesity and sleep: A growing concern. *Curr. Opin. Pulm. Med.* 2019, *25*, 602–608.
15. Tang, X.N.; Liebeskind, D.S.; Towfighi, A. The Role of Diabetes, Obesity, and Metabolic Syndrome in Stroke. *Semin. Neurol.* 2017, *37*, 267–273.
16. Yawoot, N.; Govitrapong, P.; Tocharus, C.; Tocharus, J. Ischemic stroke, obesity, and the anti-inflammatory role of melatonin. *BioFactors* 2021, *47*, 41–58.
17. Chobot, A.; Górowska-Kowolik, K.; Sokołowska, M.; Jarosz-Chobot, P. Obesity and diabetes—Not only a simple link between two epidemics. *Diabetes Metab. Res. Rev.* 2018, *34*, e3042.
18. Barnes, A.S. The epidemic of obesity and diabetes: Trends and treatments. *Texas Heart Inst. J.* 2011, *38*, 142–144.
19. Milaneschi, Y.; Simmons, W.K.; van Rossum, E.F.C.; Penninx, B.W. Depression and obesity: Evidence of shared biological mechanisms. *Mol. Psychiatry* 2019, *24*, 18–33.
20. Romain, A.J.; Marleau, J.; Baillot, A. Impact of obesity and mood disorders on physical comorbidities, psychological well-being, health behaviours and use of health services. *J. Affect. Disord.* 2018, *225*, 381–388.
21. Aigner, E.; Feldman, A.; Datz, C. Obesity as an emerging risk factor for iron deficiency. *Nutrients* 2014, *6*, 3587–3600.
22. Cohen, A.; Dempster, D.W.; Recker, R.R.; Lappe, J.M.; Zhou, H.; Zwahlen, A.; Müller, R.; Zhao, B.; Guo, X.E.; Lang, T.; et al. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: A transiliac bone biopsy study. *J. Clin. Endocrinol. Metab.* 2013, *98*, 2562–2572.
23. Lloret, A.; Monllor, P.; Esteve, D.; Cervera-Ferri, A.; Lloret, M.-A. Obesity as a risk factor for Alzheimer's disease: Implication of leptin and glutamate. *Front. Neurosci.* 2019, *13*, 508.
24. Shinohara, M.; Tashiro, Y.; Shinohara, M.; Hirokawa, J.; Suzuki, K.; Onishi-Takeya, M.; Mukouzono,

M.; Takeda, S.; Saito, T.; Fukumori, A.; et al. Increased levels of Aβ42 decrease the lifespan of ob/ob mice with dysregulation of microglia and astrocytes. *FASEB J.* 2020, *34*, 2425–2435.
25. Mazon, J.N.; de Mello, A.H.; Ferreira, G.K.; Rezin, G.T. The impact of obesity on neurodegenerative diseases. *Life Sci.* 2017, *182*,
22–28.
26. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metabolism* 2019, *92*, 121–135.
27. Pischon, T.; Nimptsch, K. Obesity and risk of cancer: An introductory overview. *Methods Mol. Biol.* 2016, *208*, 1–15.
28. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during obesity: An impaired homeostasis. *Animals* 2020, *10*, 2344.
29. Andersen, T.; Gluud, C. Liver morphology in morbid obesity: A literature study. *Int. J. Obes.* 1984, *8*, 97–106.
30. Di Meo, S.; Iossa, S.; Venditti, P. Improvement of obesity-linked skeletal muscle insulin resistance by strength and endurance training. *J. Endocrinol.* 2017, *234*, R159–R181.
31. Barazzoni, R.; Bischoff, S.; Boirie, Y.; Busetto, L.; Cederholm, T.; Dicker, D.; Toplak, H.; Van Gossum, A.; Yumuk, V.; Vettor, R. Sarcopenic Obesity: Time to Meet the Challenge. *Obes. Facts* 2018, *11*, 294–305.
32. Dashty, M. A quick look at biochemistry: Carbohydrate metabolism. *Clin. Biochem.* 2013, *46*, 1339–1352.
33. Mili´c, S.; Luli´c, D.; Štimac, D. Non-alcoholic fatty liver disease and obesity: Biochemical, metabolic and clinical presentations. *World J. Gastroenterol.* 2014, *20*, 9330–9337.
34. Engin, A. The Definition and Prevalence of Obesity and Metabolic Syndrome. *Adv. Exp. Med. Biol.* 2017, *960*, 1–17.
35. Unamuno, X.; Gómez-Ambrosi, J.; Rodríguez, A.; Becerril, S.; Frühbeck, G.; Catalán, V. Adipokine dysregulation and adipose tissue inflammation in human obesity. *Eur. J. Clin. Investig.* 2018, *48*, e12997.
36. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
37. Wilding, J.P.H.; Mooney, V.; Pile, R. Should obesity be recognised as a disease? *BMJ* 2019, *366*, l4258.
38. Arrieta, F.; Pedro-Botet, J. Recognizing obesity as a disease: A true challenge. *Rev. Clín. Esp.* 2020.
39. De Lorenzo, A.; Romano, L.; di Renzo, L.; di Lorenzo, N.; Cenname, G.; Gualtieri, P. Obesity: A preventable, treatable, but relapsing disease. *Nutrition* 2020, *71*, 110615.
40. Christensen, S. Recognizing obesity as a disease. *J. Am. Assoc. Nurse Pract.* 2020, *32*, 497–503.
41. Fuijkschot, W.W.; Morrison, M.C.; Zethof, I.P.; Krijnen, P.A.; Kleemann, R.; Niessen, H.W.; Smulders, Y.M. LPS-induced systemic inflammation does not alter atherosclerotic plaque area or inflammation in APOE3*LEIDEN mice in the early phase up to 15 days. *Shock* 2018, *50*, 360–365.
42. Furman, B.L. Streptozotocin-induced diabetic models in mice and rats. *Curr. Protoc. Pharmacol.* 2015, *70*, 5.47.1–5.47.20.
43. Lutz, T.A.; Woods, S.C. Overview of animal models of obesity. *Curr. Protoc. Pharmacol.* 2012, *58*, 5.61.1–5.61.18.
44. Kleinert, M.; Clemmensen, C.; Hofmann, S.M.; Moore, M.C.; Renner, S.; Woods, S.C.; Huypens, P.; Beckers, J.; de Angelis, M.H.; Schürmann, A.; et al. Animal models of obesity and diabetes mellitus. *Nat. Rev. Endocrinol.* 2018, *14*, 140–162.
45. Soto, M.; Orliaguet, L.; Reyzer, M.L.; Manier, M.L.; Caprioli, R.M.; Kahn, C.R. Pyruvate induces torpor in obese mice. *Proc. Natl. Acad. Sci. USA* 2018, *115*, 810–815.
46. Wang, C.Y.; Liao, J.K. A mouse model of diet-induced obesity and insulin resistance. *Methods Mol. Biol.* 2012, *821*, 421–433.
47. Shin, J.-H.; Nam, M.H.; Lee, H.; Lee, J.-S.; Kim, H.; Chung, M.-J.; Seo, J.-G. Amelioration of obesity-related characteristics by a probiotic formulation in a high-fat diet-induced obese rat model. *Eur. J. Nutr.* 2017, *57*, 2081–2090.

48. Val-Laillet, D. Review: Impact of food, gut–brain signals and metabolic status on brain activity in the pig model: 10 years of nutrition research using in vivo brain imaging. *Animal* 2019, *13*, 2699–2713.
49. Jerez, S.; Scacchi, F.; Sierra, L.; Karbiner, S.; de Bruno, M.P. Vascular hyporeactivity to angiotensin II and noradrenaline in a rabbit model of obesity. *J. Cardiovasc. Pharmacol.* 2012, *59*, 49–57.
50. Siino, V.; Amato, A.; di Salvo, F.; Caldara, G.F.; Filogamo, M.; James, P.; Vasto, S. Impact of diet-induced obesity on the mouse brain phosphoproteome. *J. Nutr. Biochem.* 2018, *58*, 102–109.
51. Lopomo, A.; Burgio, E.; Migliore, L. Epigenetics of obesity. *Prog. Mol. Biol. Transl. Sci.* 2016, *140*, 151–184.
52. Catalano, P.M.; Shankar, K. Obesity and pregnancy: Mechanisms of short term and long term adverse consequences for mother and child. *BMJ* 2017, *356*, j1.
53. Goossens, G.H. The metabolic phenotype in obesity: Fat mass, body fat distribution, and adipose tissue function. *Obes. Facts* 2017, *10*, 207–215.
54. Yin, D.P.; Gao, Q.; Ma, L.L.; Yan, W.; Williams, P.E.; McGuinness, O.P.; Wasserman, D.H.; Abumrad, N.N. Assessment of different bariatric surgeries in the treatment of obesity and insulin resistance in mice. *Ann. Surg.* 2011, *254*, 73–82.
55. Houpt, K.A.; Houpt, T.R.; Pond, W.G. The pig as a model for the study of obesity and of control of food intake: A review. *Yale J. Boil. Med.* 1979, *52*, 307–329.
56. Tang, L.-L.; Tang, X.-H.; Li, X.; Yu, H.-B.; Xie, Z.-G.; Liu, X.-Y.; Zhou, Z.-G. Effect of high-fat or high-glucose diet on obesity and visceral adipose tissue in mice. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 2014, *36*, 614–619.
57. Chen, H.; Guan, K.; Qi, X.; Wang, R.; Ma, Y. α-lactalbumin ameliorates hepatic lipid metabolism in high-fat-diet induced obese C57BL/6J mice. *J. Funct. Foods* 2020, *75*, 104253.
58. Chu, D.-T.; Malinowska, E.; Jura, M.; Kozak, L.P. C57BL/6J mice as a polygenic developmental model of diet-induced obesity. *Physiol. Rep.* 2017, *5*, e13093.
59. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
60. Zhou, A.; Ward, R. Milk polar lipids modulate lipid metabolism, gut permeability, and systemic inflammation in high-fat-fed C57BL/6J ob/ob mice, a model of severe obesity. *J. Dairy Sci.* 2019, *102*, 4816–4831.
61. Ahmad, A.; Ali, T.; Kim, M.W.; Khan, A.; Jo, M.H.; Rehman, S.U.; Khan, M.S.; Abid, N.B.; Khan, M.; Ullah, R.; et al. Adiponectin homolog novel osmotin protects obesity/diabetes-induced NAFLD by upregulating AdipoRs/PPARα signaling in ob/ob and db/db transgenic mouse models. *Metabolism* 2019, *90*, 31–43.
62. Yanagita, T.; Tsuge, K.; Koga, M.; Inoue, N.; Nagao, K. Eicosapentaenoic acid-containing polar lipids from seaweed Susabinori (*Pyropia yezoensis*) alleviate hepatic steatosis in obese db/db mice. *Arch. Biochem. Biophys.* 2020, *691*, 108486.
63. Noratto, G.D.; Murphy, K.; Chew, B.P. Quinoa intake reduces plasma and liver cholesterol, lessens obesity-associated inflammation, and helps to prevent hepatic steatosis in obese db/db mouse. *Food Chem.* 2019, *287*, 107–114.
64. Grigorova, N.; Ivanova, Z.; Bjorndal, B.; Vachkova, E.; Penchev, G.; Berge, R.; Ribarski, S.; Georgieva, T.M.; Yonkova, P.; Georgiev, I.P. Effect of fish oil supplementation and restricted feeding on body fat distribution and blood lipid profile in a rabbit model of castration-induced obesity. *Res. Vet. Sci.* 2019, *124*, 99–105.
65. Georgiev, I.P.; Georgieva, T.M.; Ivanov, V.; Dimitrova, S.; Kanelov, I.; Vlaykova, T.; Tanev, S.; Zaprianova, D.; Dichlianova, E.; Penchev, G.; et al. Effects of castration-induced visceral obesity and antioxidant treatment on lipid profile and insulin sensitivity in New Zealand white rabbits. *Res. Vet. Sci.* 2011, *90*, 196–204.
66. Keleher, M.R.; Zaidi, R.; Shah, S.; Oakley, M.E.; Pavlatos, C.; El Idrissi, S.; Xing, X.; Li, D.; Wang, T.;

Cheverud, J.M. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. *PLoS ONE* 2018, *13*, e0192606.
67. Liu, J.; He, Z.; Ma, N.; Chen, Z.Y. Beneficial Effects of Dietary Polyphenols on High-Fat Diet-Induced Obesity Linking with Modulation of Gut Microbiota. *J. Agric. Food Chem.* 2020, *68*, 33–47.
68. Herbert, D.; Franz, S.; Popkova, Y.; Anderegg, U.; Schiller, J.; Schwede, K.; Lorz, A.; Simon, J.C.; Saalbach, A. High-fat diet exacerbates early psoriatic skin inflammation independent of obesity: Saturated fatty acids as key players. *J. Investig. Dermatol.* 2018, *138*, 1999–2009.
69. Liu, Y.; Lu, X.; Li, X.; Du, P.; Qin, G. High-fat diet triggers obesity-related early infiltration of macrophages into adipose tissue and transient reduction of blood monocyte count. *Mol. Immunol.* 2020, *117*, 139–146.
70. Tung, Y.-T.; Chen, H.-L.; Wu, H.-S.; Ho, M.-H.; Chong, K.-Y.; Chen, C.-M. Kefir peptides prevent hyperlipidemia and obesity in high-fat-diet-induced obese rats via lipid metabolism modulation. *Mol. Nutr. Food Res.* 2018, *62*, 62.
71. Heydemann, A. An overview of murine high fat diet as a model for type 2 diabetes mellitus. *J. Diabetes Res.* 2016, *2016*, 2902351.
72. Varlamov, O. Western-style diet, sex steroids and metabolism. *Biochim. Biophys. Acta Mol. Basis Dis.* 2017, *1863*, 1147–1155.
73. Komorowski, J.M. Role of the hypothalamus in the pathogenesis of obesity in the light of modern studies. *Pol. Tyg. Lek.* 1978, *33*, 657–659.
74. Mercer, J.G.; Speakman, J.R. Hypothalamic neuropeptide mechanisms for regulating energy balance: From rodent models to human obesity. *Neurosci. Biobehav. Rev.* 2001, *25*, 101–116.
75. Nishida, Y.; Yoshioka, M.; St-Amand, J. Regulation of hypothalamic gene expression by glucocorticoid: Implications for energy homeostasis. *Physiol. Genomics* 2006, *25*, 96–104.
76. Nishida, Y.; Yoshioka, M.; St-Amand, J. The top 10 most abundant transcripts are sufficient to characterize the organs functional specificity: Evidences from the cortex, hypothalamus and pituitary gland. *Gene* 2005, *344*, 133–141.
77. Thaler, J.P.; Yi, C.X.; Schur, E.A.; Guyenet, S.J.; Hwang, B.H.; Dietrich, M.O.; Zhao, X.; Sarruf, D.A.; Izgur, V.; Maravilla, K.R.; et al. Obesity is associated with hypothalamic injury in rodents and humans. *J. Clin. Investig.* 2012, *122*, 153–162.
78. Velloso, L.A.; Schwartz, M.W. Altered hypothalamic function in diet-induced obesity. *Int. J. Obes. (Lond.)* 2011, *35*, 1455–1465.
79. Mercer, J.G.; Archer, Z.A. Putting the diet back into diet-induced obesity: Diet-induced hypothalamic gene expression. *Eur. J. Pharmacol.* 2008, *585*, 31–37.
80. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding induced changes in the hypothalamic transcriptome. *Clin. Chim. Acta* 2009, *406*, 103–107.
81. Stahl, S.M. Neuropharmacology of obesity: My receptors made me eat it. *J. Clin. Psychiatry* 1998, *59*, 447–448.
82. Moore, C.F.; Panciera, J.I.; Sabino, V.; Cottone, P. Neuropharmacology of compulsive eating. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2018, *373*, 20170024.
83. Leibowitz, S.F.; Alexander, J.T. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biol. Psychiatry* 1998, *44*, 851–864.
84. Weiss, G.F.; Rogacki, N.; Fueg, A.; Buchen, D.; Leibowitz, S.F. Impact of hypothalamic d norfenfluramine and peripheral d-fenfluramine injection on macronutrient intake in the rat. *Brain Res. Bull.* 1990, *25*, 849–859.
85. Weiss, G.F.; Rogacki, N.; Fueg, A.; Buchen, D.; Suh, J.S.; Wong, D.T.; Leibowitz, S.F. Effect of hypothalamic and peripheral fluoxetine injection on natural patterns of macronutrient intake in the rat. *Psychopharmacology* 1991, *105*, 467–476.
86. van Lingen, M.; Sidorova, M.; Alenina, N.; Klempin, F. Lack of Brain Serotonin Affects Feeding and Differentiation of Newborn Cells in the Adult Hypothalamus. *Front. Cell Dev. Biol.* 2019, *7*, 65.

87. Rao, D.P.; Patel, P.; Roberts, K.C.; Thompson, W. Obesity and healthy aging: Social, functional and mental well-being among older Canadians. *Health Promot. Chronic Dis. Prev. Can.* 2018, *38*, 437–444.
88. Anonymous. Body mass index (BMI) charts. *Pediatr. Dent.* 2017, *39*, 461–462.
89. Chu, D.-T.; Nguyet, N.T.M.; Nga, V.T.; Lien, N.V.T.; Vo, D.D.; Ngoc, V.T.N.; Son, L.H.; Le, D.-H.; Van Tu, P.; Van To, T.; et al. An update on obesity: Mental consequences and psychological interventions. *Diabetes Metab. Syndr. Clin. Res. Rev.* 2019, *13*, 155–160.
90. Oliosa, P.R.; Zaniqueli, D.; Alvim, R.O.; Barbosa, M.C.R.; Mill, J.G. Body fat percentage is better than indicators of weight status to identify children and adolescents with unfavorable lipid profile. *J. Pediatr.* 2019, *95*, 112–118.
91. Rogers, P.; Webb, G.P. Estimation of body fat in normal and obese mice. *Br. J. Nutr.* 1980, *43*, 83–86.
92. Air, E.L.; Strowski, M.Z.; Benoit, S.C.; Conarello, S.L.; Salituro, G.M.; Guan, X.-M.; Liu, K.; Woods, S.C.; Zhang, B.B. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. *Nat. Med.* 2002, *8*, 179–183.
93. Melega, W.P.; Lacan, G.; Gorgulho, A.A.; Behnke, E.J.; de Salles, A.A.F. Hypothalamic deep brain stimulation reduces weight gain in an obesity-animal model. *PLoS ONE* 2012, *7*, e30672.
94. Shoelson, S.E.; Herrero, L.; Naaz, A. Obesity, inflammation, and insulin resistance. *Gastroenterology* 2007, *132*, 2169–2180.
95. Fock, K.M.; Khoo, J. Diet and exercise in management of obesity and overweight. *J. Gastroenterol. Hepatol.* 2013, *28*, 59–63.
96. Rebello, C.J.; Greenway, F.L. Obesity medications in development. *Expert Opin. Investig. Drugs* 2020, *29*, 63–71.
97. Wolfe, B.M.; Kvach, E.; Eckel, R.H. Treatment of obesity: Weight loss and bariatric surgery. *Circ. Res.* 2016, *118*, 1844–1855.
98. Monteiro, R.; Azevedo, I. Chronic inflammation in obesity and the metabolic syndrome. *Mediat. Inflamm.* 2010, *2010*, 1–10.
99. Sonne, S.B.; Yadav, R.; Yin, G.; Dalgaard, M.D.; Myrmel, L.S.; Gupta, R.; Wang, J.; Madsen, L.; Kajimura, S.; Kristiansen, K. Obesity is associated with depot-specific alterations in adipocyte DNA methylation and gene expression. *Adipocyte* 2017, *6*, 124–133.
100. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and high-fat diet in obesity: Functional genomics perspectives of two energy homeostasis pillars. *Genes* 2020, *11*, 875.
101. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
102. Sardon Puig, L.; Pillon, N.J.; Näslund, E.; Krook, A.; Zierath, J.R. Influence of obesity, weight loss, and free fatty acids on skeletal muscle clock gene expression. *Am. J. Physiol. Endocrinol. Metab.* 2020, *318*, e1–e10.
103. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-fat diet-induced trefoil factor family member 2 (TFF2) to counteract the immunemediated damage in mice. *Animals* 2021, *11*, 258.
104. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine as a regeneration factor: Beyond the tissue repair. *Life* 2021, *11*, 38.
105. Drel, V.R.; Mashtalir, N.; Ilnytska, O.; Shin, J.; Li, F.; Lyzogubov, V.V.; Obrosova, I.G. The leptin-deficient (ob/ob) mouse: A new animal model of peripheral neuropathy of type 2 diabetes and obesity. *Diabetes* 2006, *55*, 3335–3343.
106. Malloy, V.L.; Perrone, C.E.; Mattocks, D.A.; Ables, G.P.; Caliendo, N.S.; Orentreich, D.S.; Orentreich, N. Methionine restriction prevents the progression of hepatic steatosis in leptin-deficient obese mice. *Metabolism* 2013, *62*, 1651–1661.
107. Neelankal John, A.; Ram, R.; Jiang, F.X. RNA-seq analysis of islets to characterise the dedifferentiation in type 2 diabetes model mice db/db. *Endocr. Pathol.* 2018, *29*, 207–221.
108. Bogdanov, P.; Corraliza, L.; Villena, J.A.; Carvalho, A.R.; García-Arumí, J.; Ramos, D.; Ruberte, J.;

Simó, R.; Hernández, C. The db/db mouse: A useful model for the study of diabetic retinal neurodegeneration. *PLoS ONE* 2014, *9*, e97302.
109. Vareniuk, I.; Pavlov, I.A.; Drel, V.R.; Lyzogubov, V.V.; Ilnytska, O.; Bell, S.R.; Tibrewala, J.; Groves, J.T.; Obrosova, I.G. Nitrosative stress and peripheral diabetic neuropathy in leptin-deficient (ob/ob) mice. *Exp. Neurol.* 2007, *205*, 425–436.
110. Yu, F.; Han, W.; Zhan, G.; Li, S.; Jiang, X.; Wang, L.; Xiang, S.; Zhu, B.; Yang, L.; Luo, A.; et al. Abnormal gut microbiota composition contributes to the development of type 2 diabetes mellitus in db/db mice. *Aging* 2019, *11*, 10454–10467.
111. Larter, C.Z.; Yeh, M.M.; van Rooyen, D.M.; Teoh, N.C.; Brooling, J.; Hou, J.Y.; Williams, J.; Clyne, M.; Nolan, C.J.; Farrell, G.C. Roles of adipose restriction and metabolic factors in progression of steatosis to steatohepatitis in obese, diabetic mice. *J. Gastroenterol. Hepatol.* 2009, *24*, 1658–1668.
112. Lauterbach, M.A.R.; Wunderlich, F.T. Macrophage function in obesity-induced inflammation and insulin resistance. *Pflügers Archiv* 2017, *469*, 385–396.
113. Van der Heijden, R.A.; Sheedfar, F.; Morrison, M.C.; Hommelberg, P.P.; Kor, D.; Kloosterhuis, N.J.; Gruben, N.; Youssef, S.A.; de Bruin, A.; Hofker, M.H.; et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. *Aging* 2015, *7*, 256–268.
114. Engin, A. Obesity-associated breast cancer: Analysis of risk factors. *Adv. Exp. Med. Biol.* 2017, *960*, 571–606.
115. Kompaniyets, L.; Goodman, A.B.; Belay, B.; Freedman, D.S.; Sucosky, M.S.; Lange, S.J.; Gundlapalli, A.V.; Boehmer, T.K.; Blanck, H.M. Body mass index and risk for COVID-19—Related hospitalization, intensive care unit admission, invasive mechanical ventilation, and death—United States, March–December 2020. *MMWR Morb. Mortal Wkly. Rep.* 2021, *70*, 355–361.
116. Torre, L.A.; Siegel, R.L.; Ward, E.M.; Jemal, A. Global cancer incidence and mortality rates and trends—An update. *Cancer Epidemiol. Biomark. Prev.* 2015, *25*, 16–27.
117. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* 2014, *136*, E359–E386.
118. Duranti, S.; Ferrario, C.; van Sinderen, D.; Ventura, M.; Turroni, F. Obesity and microbiota: An example of an intricate relationship. *Genes Nutr.* 2017, *12*, 18.
119. Madsen, L.; Myrmel, L.S.; Fjære, E.; Liaset, B.; Kristiansen, K. Links between Dietary Protein Sources, the Gut Microbiota, and Obesity. *Front Physiol.* 2017, *8*, 1047.
120. Cuevas-Sierra, A.; Ramos-Lopez, O.; Riezu-Boj, J.I.; Milagro, F.I.; Martinez, J.A. Diet, Gut Microbiota, and Obesity: Links with Host Genetics and Epigenetics and Potential Applications. *Adv. Nutr.* 2019, *10*, s17–s30.
121. Lee, P.; Yacyshyn, B.R.; Yacyshyn, M.B. Gut microbiota and obesity: An opportunity to alter obesity through faecal microbiota transplant (FMT). *Diabetes Obes. Metab.* 2019, *21*, 479–490.
122. Marotz, C.A.; Zarrinpar, A. Treating Obesity and Metabolic Syndrome with Fecal Microbiota Transplantation. *Yale J. Biol. Med.* 2016, *89*, 383–388.
123. Aron-Wisnewsky, J.; Clément, K.; Nieuwdorp, M. Fecal Microbiota Transplantation: A Future Therapeutic Option for Obesity/Diabetes? *Curr. Diab. Rep.* 2019, *19*, 51.
124. Kang, Y.; Cai, Y. Gut microbiota and obesity: Implications for fecal microbiota transplantation therapy. *Hormones (Athens)* 2017, *16*, 223–234.
125. Mazloom, K.; Siddiqi, I.; Covasa, M. Probiotics: How Effective Are They in the Fight against Obesity? *Nutrients* 2019, *11*, 258.
126. Fontané, L.; Benaiges, D.; Goday, A.; Llauradó, G.; Pedro-Botet, J. Influence of the microbiota and probiotics in obesity. *Clin. Investig. Arterioscler.* 2018, *30*, 271–279.
127. Nova, E.; Pérez de Heredia, F.; Gómez-Martínez, S.; Marcos, A. The Role of Probiotics on the Microbiota: Effect on Obesity. *Nutr Clin. Pract.* 2016, *31*, 387–400.
128. Katsiki, N.; Tousoulis, D. Diabetes mellitus and comorbidities: A bad romance. *Hellenic J. Cardiol.*

2020, *61*, 23–25.
129. Lehrke, M.; Marx, N. Diabetes mellitus and heart failure. *Am. J. Cardiol.* 2017, *120*, S37–S47.
130. Wojciechowska, J.; Krajewski, W.; Bolanowski, M.; Kr˛ecicki, T.; Zato´nski, T. Diabetes and cancer: A review of current knowledge. *Exp. Clin. Endocrinol. Diabetes* 2016, *124*, 263–275.
131. Cryer, M.J.; Horani, T.; DiPette, D.J. Diabetes and hypertension: A comparative review of current guidelines. *J. Clin. Hypertens.* 2016, *18*, 95–100.
132. Shifflett, K.; Marzi, A. Marburg virus pathogenesis—Differences and similarities in humans and animal models. *Virol. J.* 2019, *16*, 165.
133. Chooi, Y.C.; Ding, C.; Magkos, F. The epidemiology of obesity. *Metabolism* 2019, *92*, 6–10.

Chapter 8. Opinion - High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 22 December 2020; Accepted: 19 January 2021; Published: 21 January 2021

8.1 Résumé (French abstract)

Alors que la diète riche en gras (HF), par divers mécanismes, entraîne des dommages à médiation immunitaire, le trefoil factor family member 2 (*Tff2*) représente un gène induit par la diète HF. D'autre part, TFF2 favorise à la fois la réparation des tissus et réduit l'inflammation. Ces propriétés viseraient à contrer les dommages résultant de la diète HF. Ces observations suggèrent que l'induction du *Tff2* par la diète HF pourrait être une voie de régulation visant à contrer les dommages à médiation immunitaire résultant de la diète HF. En plus, puisque l'expression du *Tff2* augmente avec la diète HF et que *Tff2* est aussi exprimé dans le cerveau, nous émettons également l'hypothèse que TFF2 pourrait être un signal de contrôle de l'apport alimentaire induit par la diète HF pour réduire l'appétit et donc l'apport en diète HF.

8.2 Simple Summary

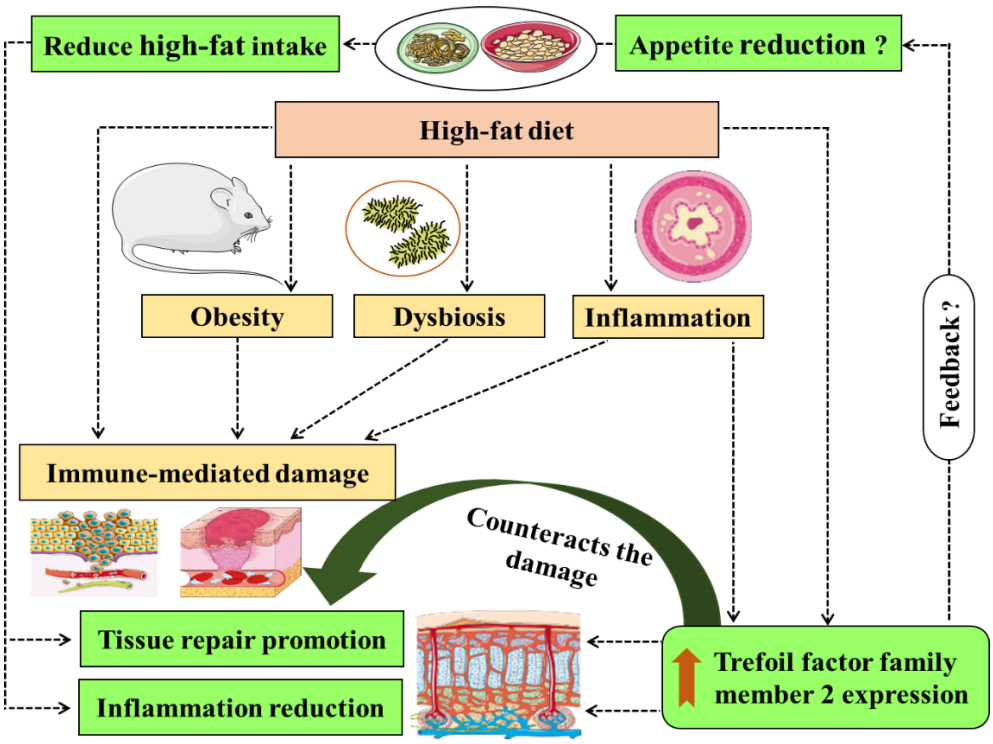
High-fat (HF) diet induces both immune-mediated damage and trefoil factor family member 2 (*Tff2*) expression. As TFF2 has tissue repair and protection properties, this suggests that HF diet-induced *Tff2* production and the resulting TFF2 mucosal protective effects would be a mechanism to counteract the HF diet-induced tissue damage. On the other hand, the induction of *Tff2* by HF diet could indicate that TFF2 is a food intake regulator (appetite control) since *Tff2* is also expressed in the brain. This highlights the importance of exploring TFF2-related pathways in the context of obesity management towards potential therapies.

8.3 Abstract

Physiological homeostasis requires a balance between the immunological functions and the resulting damage/side effects of the immunological reactions including those related to high-fat (HF) diet. Within this context, whereas HF diet, through diverse mechanisms (such as inflammation), leads to immune-mediated damage, trefoil factor family member 2 (*Tff2*) represents a HF diet-induced gene. On the other hand, TFF2 both promotes tissue repair and reduces inflammation. These properties are towards counteracting the immune mediated damage resulting from the HF diet. These observations suggest that the HF diet induction of *Tff2* could be a regulatory pathway aiming to counteract the immune-mediated damage resulting from the HF diet. Interestingly, since *Tff2* expression increases with HF diet and with *Tff2* also expressed in the brain, we also hypothesize that TFF2 could be a HF diet-induced food intake-control signal that reduces appetite. This hypothesis fits with counteracting the immune damage since reducing the food intake will reduce the HF intake and therefore, reduces the HF diet-induced tissue damage. Such food intake signaling would be an indirect mechanism by which TFF2 promotes tissue repair as well as a pathway worth exploring for potential obesity management pharmacotherapies.

Keywords: trefoil factor family member 2 (TFF2); high-fat diet; immunity; damage; mice

8.4 Graphical abstract



8.5 High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice

Animal physiological homeostasis requires a balance between the immunological functions and the damage/side effects of those immunological reactions. Knowing that immunological reactions can be triggered by diverse factors, the homeostasis supposes that parallel or secondary pathways are activated or stimulated with these immunological reactions to repair the damage. The immune system is a complex network of cells and circulating fluids that is modulated by the nervous system [1], endocrine system [2], infections [3], and even diet. Indeed, different types of diets, such as high-sucrose and high-fat (HF) diets, have been shown to impact immune functions [4,5], among other factors and genes [6,7]. HF diets characterize our modern life and are associated with diverse diseases and health problems, such as obesity, dyslipidemia, diabetes, fatty liver disease and cardiovascular diseases [7–10]. However, such HF diet-induced immune modulations, which could be implicated in the HF diet-induced risks and diseases, are yet to be fully understood. Within this context, the molecules and signals that are either upregulated or downregulated with HF diets could be the mechanistic answer, as per the examples we provide below from studies on mice.

For instance, trefoil factor family member 2 (TFF2), known as spasmolytic peptide [11], is well involved in mucosal repair, protection and proliferation, as it represents an important stabilizer of the gastric mucus, with roles in tissue remodeling [12]. Herein, we go beyond its mucosal protective role to explore the hypothesis linking this diet-induced molecule, TFF2, to the diet-induced immunomodulation. Indeed, whereas *Tff2* has been reported as a gene that is specifically induced by HF diets in mice [13,14], its knockout protected mice from HF diet-induced obesity [15] through a metabolic phenotype that contributes to more energy expenditure and reduced energy storage [16]. The importance of the studies that identified *Tff2* as a gene specifically induced by HF diets is that the control groups were, unlike in other studies, fasted mice [13,14]. Based on the HF induction of TFF2, we notice a correlation between the HF diet-induced immunological changes and the TFF2 related immunological effects and benefits (as illustrated below). This correlation suggests that TFF2 would be involved in mediating the protective effects against such HF diet damage.

On one side, a HF diet has important immunological impacts. For instance, a HF diet increases TNFα and IL1β in young mice's hippocampus [17], and leads to chronic systemic inflammation [18]. Moreover, a chronic HF diet is also associated with obesity [19,20], which also affects the immunity [21] and might explain some of the impacts obesity has on regeneration impairment through diverse processes, including inflammation [22], which is important in the context of TFF2's roles in tissues repair.

On the other hand, TFF2, beyond its well-known roles in injured mucosa healing [23–25], has a noticeable role in the immune response [25,26], as suggested by its expression in immune organs [27] and its expression during inflammations [12]. Indeed,

Helicobacter infection upregulated it in gastric tissues, macrophages and lymphocytes [11], whereas *Helicobacter pylori* eradication decreased TFF2 level in patients' sera [28]. Furthermore, TFF2 deficiency leads to a deregulation of macrophages' and lymphocytes' proliferative responses [11], and an accelerated gastritis progression [29] during *Helicobacter* infection. This correlates with both the ulceration role of *Helicobacter pylori* [30] and the tissue repair/protections roles of TFF2 in animal selected tissues [12].

TFF2 expression during such immunological changes seems to be an attempt to limit the negative impacts of these immune reactions, such as inflammation [12], due to the HF diet. For instance, TFF2 could both limit the recruitment of leukocytes and the monocyte production of nitric oxide [25], and decrease macrophage responsiveness [27], which would contribute to promoting the tissue repair environment. Therefore, this TFF2-induced downregulation of selected immunological responses would be a step required to accomplish the healing and protecting effects TFF2 governs.

These illustrative examples present TFF2 as a mediator of the HF diet-triggered mechanisms attempting to correct the HF diet's negative impacts, mediated through the immune system. Interestingly, unlike glucose, which causes insulin as a hormone to be secreted immediately following meal ingestion [31], there is no equivalent hormone for lipid ingestion. TFF2 could be that missing signal within animal endocrinology, since in the studies in which *Tff2* was shown to be unregulated at 3 h following a low-fat meal ingestion, it was upregulated with a HF meal [13,14]. The acute character of this expression indicates an immediate effect of the HF diet on Tff2 expression. Therefore, TFF2 could be a short-term lipid-specific signal that controls lipid intake by limiting lipid ingestion through a TFF2-dependant feedback acting on food intake centers. This is supported by the differential *Tff2* expression in the hypothalamus of fasted, and low-fat and HF diet-fed mice (lipid ratio-dependent expression) [15]. This hypothesis is further supported by the increase in the drive to consume a HF meal, as well as the appetite enhancement as a consequence of TFF2 deficiency [15]. This would suggest that TFF2 counteracts HF diet-induced damage indirectly through reducing the HF intake. The other remarkable link is that TFF2 is mostly expressed in the digestive system [32,33], which represents the site whereat the animal's neuroendocrine receptors first interact with the ingested food, including HF meals; this further suggests the acute responsiveness of the HF diet's induction of TFF2 in the mouse intestine. Always within the digestive system, the HF diet impacts the local microbiome [34,35], which could be another key link between the diet and the immunological changes, especially with the known interactions between the immune system and the microbiome [36–38], the microbiota richness reduction [39], and dysbiosis, in all of which the HF diet has been implicated [40]. In addition, since several effects of a HF diet are mediated by microbiota [18] with probiotics that upregulate TFF2 [41], these microbiota-mediated effects of the HF diet could be through TFF2 expression changes.

These elements highlight TFF2 expression (HF diet-induced) as a feedback aiming to counteract the immune-mediated HF diet-induced damage. However, the correcting

potential and efficacy of TFF2 would depend on the severity and the chronic or acute character of such a HF diet. This explains why during obesity (such as in HF diet-induced obesity in animal models), those TFF2-correcting mechanisms are less efficient due to the strong immune-mediated damage that overcomes the TFF2-counteracting ability. Further explorations of diets' impacts on TFF2 expression, such as high-salt diets [42], within an immunological context would expand this emerging field linking the type of diet to the immunological changes via identifying the linking factors. Importantly, combining these metabolic and immunological properties of TFF2 would allow us to further understand how mice immunologically react to a HF diet, and elucidate more diet-induced effects on immunology, infections and inflammation. Importantly, extrapolating these concepts from mice to humans and building clinical trials based on animal experiments could lead to developing novel TFF2-based therapies for diseases and conditions, such as inflammation, and, most importantly, a potential control for lipid intake (appetite control) towards a better obesity management strategy, which requires urgent solutions due obesity's epidemiological profile and its impacts on health and the economy [43–46].

Author Contributions: A.G. drafted the manuscript; A.G., M.Y. and J.S.-A. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). The graphical abstract was created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

8.6 References

1. Habek, M. Immune and autonomic nervous system interactions in multiple sclerosis: Clinical implications. *Clin. Auton. Res.* 2019, *29*, 267–275.
2. Renner, U.; Sapochnik, M.; Lucia, K.; Stalla, G.K.; Arzt, E. Intrahypophyseal Immune-Endocrine Interactions: Endocrine Integration of the Inflammatory Inputs. *Front. Horm. Res.* 2017, *48*, 37–47.
3. Brizi´c, I.; Hiršl, L.; Britt, W.J.; Krmpoti´c, A.; Jonji´c, S. Immune responses to congenital cytomegalovirus infection. *Microbes. Infect.* 2018, *20*, 543–551.
4. Brown, K.; DeCoffe, D.; Molcan, E.; Gibson, D.L. Diet-Induced Dysbiosis of the Intestinal Microbiota and the Effects on Immunity and Disease. *Nutrients* 2012, *4*, 1095–1119.
5. Sato Mito, N.; Suzui, M.; Yoshino, H.; Kaburagi, T.; Sato, K. Long term effects of high fat and sucrose diets on obesity and lymphocyte proliferation in mice. *J. Nutr. Health Aging* 2009, *13*, 602–606.
6. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
7. Keleher, M.R.; Zaidi, R.; Shah, S.; Oakley, M.E.; Pavlatos, C.; El Idrissi, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. *PLoS ONE* 2018, *13*, e0192606.
8. Heydemann, A. An Overview of Murine High Fat Diet as a Model for Type 2 Diabetes Mellitus. *J. Diabetes Res.* 2016, *2016*, 2902351.
9. Udomkasemsab, A.; Prangthip, P. High fat diet for induced dyslipidemia and cardiac pathological alterations in Wistar rats compared to Sprague Dawley rats. *Clin. Investig. Arterioscler.* 2019, *31*, 56–62.
10. Recena Aydos, L.; Aparecida do Amaral, L.; Serafim de Souza, R.; Jacobowski, A.C.; Freitas Dos Santos, E.; Rodrigues Macedo, M.L. Nonalcoholic Fatty Liver Disease Induced by High-Fat Diet in C57bl/6 Models. *Nutrients* 2019, *11*, 3067.
11. Kurt-Jones, E.A.; Cao, L.; Sandor, F.; Rogers, A.B.; Whary, M.T.; Nambiar, P.R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; et al. Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. *Infect. Immun.* 2007, *75*, 471–480.
12. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an Inflammatory-Induced and AntiInflammatory Tissue Repair Factor. *Animals* 2020, *10*, 1646.
13. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 1–10.
14. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. *Obesity (Silver Spring)* 2008, *16*, 2302–2307.
15. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity (Silver Spring)* 2013, *21*, 1389–1395.
16. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
17. Nakandakari, S.; Muñoz, V.R.; Kuga, G.K.; Gaspar, R.C.; Sant'Ana, M.R.; Pavan, I.C.B.; da Silva, L.G.S.; Morelli, A.P.; Simabuco, F.M.; da Silva, A.S.R.; et al. Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice. *Brain Behav. Immun.* 2019, *79*, 284–293.
18. Schachter, J.; Martel, J.; Lin, C.S.; Chang, C.J.; Wu, T.R.; Lu, C.C.; Ko, Y.F.; Lai, H.C.; Ojcius, D.M.; Young, J.D. Effects of obesity on depression: A role for inflammation and the gut microbiota. *Brain Behav. Immun.* 2018, *69*, 1–8.
19. Lissner, L.; Levitsky, D.A.; Strupp, B.J.; Kalkwarf, H.J.; Roe, D.A. Dietary fat and the regulation of

energy intake in human subjects. *Am. J. Clin. Nutr.* 1987, *46*, 886–892.
20. Schutz, Y.; Flatt, J.P.; Jéquier, E. Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. *Am. J. Clin. Nutr.* 1989, *50*, 307–314.
21. Andersen, C.J.; Murphy, K.E.; Fernandez, M.L. Impact of Obesity and Metabolic Syndrome on Immunity. *Adv. Nutr.* 2016, *7*, 66–75.
22. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
23. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. *Nat. Rev. Mol. Cell Biol.* 2003, *4*, 721–732.
24. Tran, C.P.; Cook, G.A.; Yeomans, N.D.; Thim, L.; Giraud, A.S. Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. *Gut* 1999, *44*, 636–642.
25. Baus-Loncar, M.; Kayademir, T.; Takaishi, S.; Wang, T. Trefoil factor family 2 deficiency and immune response. *Cell Mol. Life Sci.* 2005, *62*, 2947–2955.
26. Baus-Loncar, M.; Schmid, J.; Lalani el, N.; Rosewell, I.; Goodlad, R.A.; Stamp, G.W.; Blin, N.; Kayademir, T. Trefoil factor 2 (TFF2) deficiency in murine digestive tract influences the immune system. *Cell Physiol. Biochem.* 2005, *16*, 31–42.
27. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 2015, *308*, G12–G24.
28. Kaise, M.; Miwa, J.; Fujimoto, A.; Tashiro, J.; Tagami, D.; Sano, H.; Ohmoto, Y. Influence of Helicobacter pylori status and eradication on the serum levels of trefoil factors and pepsinogen test: Serum trefoil factor 3 is a stable biomarker. *Gastric Cancer* 2013, *16*, 329–337.
29. Fox, J.G.; Rogers, A.B.; Whary, M.T.; Ge, Z.; Ohtani, M.; Jones, E.K.; Wang, T.C. Accelerated progression of gastritis to dysplasia in the pyloric antrum of TFF2 -/- C57BL6 x Sv129 Helicobacter pylori-infected mice. *Am. J. Pathol.* 2007, *171*, 1520–1528.
30. Mobley, H.L. The role of Helicobacter pylori urease in the pathogenesis of gastritis and peptic ulceration. *Aliment. Pharmacol. Ther.* 1996, *10* (Suppl. 1), 57–64.
31. Kalwat, M.A.; Cobb, M.H. Mechanisms of the amplifying pathway of insulin secretion in the β cell. *Pharmacol. Ther.* 2017, *179*, 17–30.
32. Madsen, J.; Nielsen, O.; Tornøe, I.; Thim, L.; Holmskov, U. Tissue localization of human trefoil factors 1, 2, and 3. *J. Histochem. Cytochem.* 2007, *55*, 505–513.
33. Hoffmann, W.; Jagla, W.; Wiede, A. Molecular medicine of TFF-peptides: From gut to brain. *Histol. Histopathol.* 2001, *16*, 319–334.
34. Hasebe, K.; Rivera, L.R.; Smith, C.M.; Allnutt, T.; Crowley, T.; Nelson, T.M.; Dean, O.M.; McGee, S.L.; Walder, K.; Gray, L. Modulation of high fat diet-induced microbiome changes, but not behaviour, by minocycline. *Brain Behav. Immun.* 2019, *82*, 309–318.
35. Hassan, A.M.; Mancano, G.; Kashofer, K.; Fröhlich, E.E.; Matak, A.; Mayerhofer, R.; Reichmann, F.; Olivares, M.; Neyrinck, A.M.; Delzenne, N.M.; et al. High-fat diet induces depression-like behaviour in mice associated with changes in microbiome, neuropeptide Y, and brain metabolome. *Nutr. Neurosci.* 2019, *22*, 877–893.
36. Lambring, C.B.; Siraj, S.; Patel, K.; Sankpal, U.T.; Mathew, S.; Basha, R. Impact of the Microbiome on the Immune System. *Crit. Rev. Immunol.* 2019, *39*, 313–328.
37. Shi, N.; Li, N.; Duan, X.; Niu, H. Interaction between the gut microbiome and mucosal immune system. *Mil. Med. Res.* 2017, *4*, 14.
38. Ticinesi, A.; Lauretani, F.; Tana, C.; Nouvenne, A.; Ridolo, E.; Meschi, T. Exercise and immune system as modulators of intestinal microbiome: Implications for the gut-muscle axis hypothesis. *Exerc. Immunol. Rev.* 2019, *25*, 84–95.

39. Cândido, F.G.; Valente, F.X.; Grze´skowiak, Ł.M.; Moreira, A.P.B.; Rocha, D.; Alfenas, R.C.G. Impact of dietary fat on gut microbiota and low-grade systemic inflammation: Mechanisms and clinical implications on obesity. *Int. J. Food Sci. Nutr.* 2018, *69*, 125–143.
40. Netto Candido, T.L.; Bressan, J.; Alfenas, R.C.G. Dysbiosis and metabolic endotoxemia induced by high-fat diet. *Nutr. Hosp.* 2018, *35*, 1432–1440.
41. Khoder, G.; Al-Yassir, F.; Al Menhali, A.; Saseedharan, P.; Sugathan, S.; Tomasetto, C.; Karam, S.M. Probiotics Upregulate Trefoil Factors and Downregulate Pepsinogen in the Mouse Stomach. *Int. J. Mol. Sci.* 2019, *20*, 3901.
42. Chen, X.; Hu, Y.; Xie, Y.; Wang, Y. High salt diet can down-regulate TFF2 expression level in gastric mucosa of MGs after H. pylori infection. *Microb. Pathog.* 2018, *118*, 316–321.
43. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
44. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
45. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Méd. Hypotheses* 2020, *144*, 110042.
46. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.

Chapter 9. Letter - Trefoil Factor Family Member 2 (TFF2) as an Inflammatory-Induced and Anti-Inflammatory Tissue Repair Factor

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Endocrinology and Nephrology Axis, Functional Genomics Laboratory, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 24 August 2020; Accepted: 11 September 2020; Published: 14 September 2020

9.1 Résumé (French abstract)

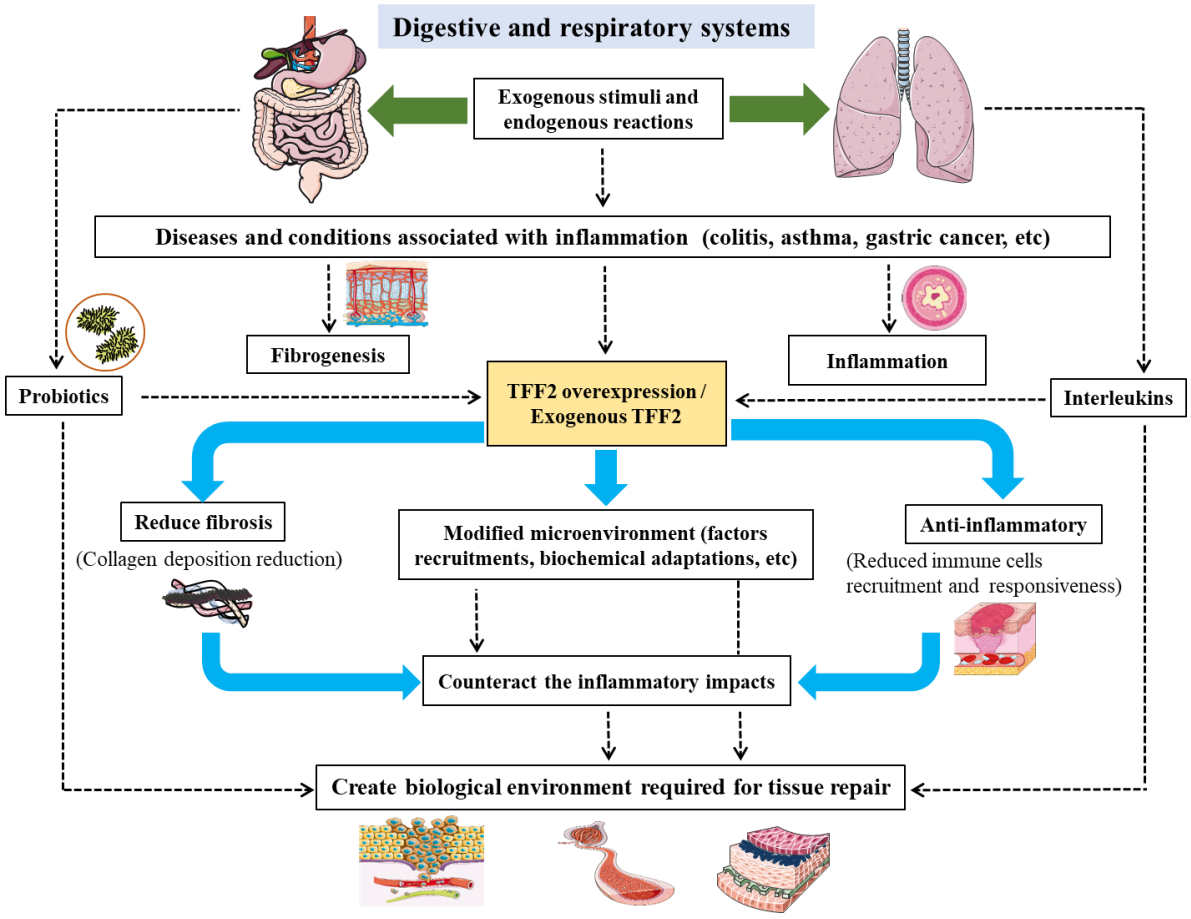
Le trefoil factor family member 2 (TFF2) est connu pour son implication dans la réparation des muqueuses. Alors qu'il est surexprimé pendant les processus inflammatoires, l'ajout de TFF2 conduit à un effet anti-inflammatoire qui contribuerait à créer un microenvironnement nécessaire à la réparation des tissus. Ces propriétés présentent TFF2 avec des propriétés homéostatiques au cours des processus inflammatoires, comme l'illustrent des exemples sélectionnés.

9.2 Abstract

Trefoil factor family member 2 (TFF2) is known for its involvement in mucosal repair. Whereas it is overexpressed during inflammatory processes, adding TFF2 leads to an anti-inflammatory effect that would contribute to create the microenvironment required for tissue repair. These properties present TFF2 with a homeostatic pattern during inflammatory processes as illustrated by selected examples.

Keywords: trefoil factor family member 2 (TFF2); inflammation; tissue repair

9.3 Graphical abstract



9.4 Trefoil Factor Family Member 2 (TFF2) as an Inflammatory-Induced and Anti-Inflammatory Tissue Repair Factor

Compared to the diverse physiological entities, digestive and respiratory systems represent the tissues that interact the most with exogenous organisms and molecules, as they represent the two “entrances” of the body. This anatomical property exposes these systems to diverse stimuli and injuries leading to inflammatory reactions, especially with their rich blood flow and close interactions with the immune system. In addition, their mucosa has a relatively high regenerative and repair activity. Within the context of mucosal repair, trefoil factor family member 2 (TFF2), also known as spasmolytic polypeptide and isolated in 1982 [1], is a biological factor known for its involvement in mucosal repair, protection and proliferation especially within both digestive and respiratory systems [2–8]. TFF2 represents an important component and a stabilizer of the gastric mucus with the property of binding to the mucin MUC6 [9] and is also involved in tissue remodeling [2,10]. It is expressed in different species such as mouse [11], cow [12], rat [13], pork [9]

and human [14]. In veterinary science, the animal models of TFF2-modified expression illustrate the importance of this protein in animal health as shown by studies investigating obesity, gastric secretion, asthma, etc [2,3,10,11].

These TFF2 properties are reflected by the increased susceptibility to injury seen in TFF2-deficient mice. Indeed, TFF2-deficient mice have an increased gastric ulceration degree compared to wild-type mice following indomethacin administration [3]. Since there are numerous inflammatory diseases [4,15–17] that develop in the digestive and respiratory systems, we would like to summarize hypothetic links between the TFF2 and selected inflammatory-related processes [2,18–20].

TFF2 has been shown to be overexpressed (or upregulated) following inflammations or inflammatory conditions [18] such as in asthma [2], gastrointestinal ulcerative disease [19] and allergic airway inflammation [20]. Furthermore, knowing that some interleukins (IL) have been linked to tissue repair [21–23], such regulation could also be under the control of selected cytokines since, for instance, IL-4 and IL-13 induce TFF2 in the lung [20]. Other treatments, also leading to cell damage, upregulate TFF2 or *TFF2* expression, such as hypoxia [24] and aspirin in which the damages are also associated with hypoxia [24,25]. This suggests that the upregulation would be a response of the inflammation-induced damage rather than the inflammation itself, which correlates with aspirin damage-induced activation of *Tff2* gene in rats [13]. This would mean that TFF2 would not be required to develop the inflammation but would rather increase with inflammation, either induced by inflammation or the factor triggering the inflammation. This TFF2 induction would initiate the healing and repairing process that counteracts the inflammation-induced damage, which could be a protective mechanism such as during chronic superficial gastritis [26].

Interestingly, other studies have pointed TFF2 with a potential anti-inflammatory effect. For instance, a recombinant human TFF2 was shown to reduce colitis inflammation in a rat model; it increases the colonic epithelial repair rate [27]. Within the same line, applying TFF2 does reduce inflammatory indexes in a hapten colitis rodent model and has even been suggested as a therapeutic scaffold for inflammatory bowel disease treatment [28]. Importantly, TFF2 treatment reduces fibrosis (subepithelial collagen deposition) in a murine model of chronic allergic airways disease [2], which could indicate a reduced fibrogenesis in tissues undergoing inflammation [29,30]. Thus, TFF2 effects are not limited to an anti-inflammatory effect but would also reduce the tissue fibrosis. Both effects are towards tissue repair and counteract the deteriorating inflammatory consequences (damage and fibrosis) as well. This could explain the TFF2 beneficial effect on intestinal inflammation in animal models, which would involve reducing both macrophage responsiveness [28] and leukocyte recruitment [31], regulating the NO-mediated inflammation (monocyte) [32] and blocking inflammatory cell recruitment [28] within its mechanism. On the same path, TFF2 is also expressed during gastric cancer [33,34]. This could indicate that the presence of TFF2 aims to limit the cancer-induced inflammatory damages. It cold also represent an attempt to limit cancer growth as suggested by an in vitro

study that shows the inhibition of the growth of gastric cancer cells by TFF2 expression [35].

It is worth precising that the anti-inflammatory effect or fibrosis reduction have been observed when exogenous TFF2 was added in different conditions [2,27,32] rather than when the inflammation-related endogenous TFF2 was overexpressed (since inflammation develops although the inflammation-induced upregulation of TFF2 expression [2,18–20]). This highlights TFF2 overexpression as an attempt to limit the inflammation and its consequences (such as fibrogenesis). Such an anti-inflammatory effect or fibrosis reduction would be among the main mechanisms underlying the pathways via which TFF2 mediates its mucosal protection. Although the inflammatory-induced TFF2 overexpression (not its exogenous addition) would not lead to a measurable effect on inflammation or fibrosis, inflammatory related TFF2 expression would probably contribute to create the biological environment required for tissue repair, but not only through recruiting selected factors and interacting with biomolecules such as mucins [18,36].

Interestingly, probiotics have been shown to increase the production of TFF2 in the mouse stomach [37]. Probiotics also have, in addition to roles in tissue repair [38], anti inflammatory effects especially in the intestine [39], which is one of the key tissues of TFF2 expression. Thus, TFF2 might be among the pathways linking probiotics to the anti inflammatory and tissue repair effects, probably involving immunological mechanisms impacted by probiotics [40]. In addition, the reported antibiotic activity of TFF2 [14] could be complimentary in both inflammation and immunological regulation towards reducing inflammation-related damages.

Tff2 has been recently characterized as a high-fat diet-induced gene in the intestinal mucosa [41] and the knockout of this gene lead to a protection from high-fat diet-induced obesity [11,42]. Both these facts could be further considered for the future exploration of the links between inflammation and metabolics. Indeed, obesity, for which a high-fat diet increases its development, also represents a risk factor for both inflammation and cancer development. Therefore, the metabolic implications of TFF2 could be behind a part of the inflammatory and cancer processes, especially based on known links between metabolic activities and the factors related to inflammation and cancer [43–45]. Within this context, IL could complete TFF2 roles during tissue repair. For instance, tissue injuries induce IL-6 production [46], which is required for gastric homeostasis [47] and has been shown to play metabolic roles [43]. This would indicate complementarity roles between TFF2 and IL during tissue repair by contributing to create the microenvironment as well as the metabolic conditions required for post-injury repair and counteracting the tissue damages. Within the context of diet, it is also worth mentioning that a diet rich in antioxidants would have a beneficial effect on inflammation development [48]. Moreover, the high-fat diet-induced *Tff2* gene expression could be related to counteracting inflammation damages, since high-fat diet induces oxidative stress [49] and is usually associated with obesity [50,51] which has both oxidative stress [52] and inflammation [53,54] in its context.

Moreover, the other TFFs (TFF1 and TFF3) would require additional exploration within the context of inflammation because of their implication in the inflammation process [4] as well as the possible expression interdependence linking TFF2, TFF1 and TFF3 [55,56]. Furthermore, the inflammatory properties of TFFs correlate with their immunological roles [28]. This could also justify the expression of TFFs (minute amounts) in the immune and central nervous systems [57] as well as in cancers [12,58] as regulatory factors. Deeper understudying of TFF2 implications in inflammation or inflammatory-related diseases and conditions would allow developing new methods to confirm diagnosis, make prognosis or follow a therapy efficiency based on TFF2 expression variation as a biological marker, such as in tumors [33]. Moreover, these implications of TFF2 in inflammation would suggest the potential usage of TFF2 or targeting TFF2-related pathways to develop novel therapies or optimize those in usage for diseases and conditions involving an inflammatory component. The "homeostatic property" of TFF2 exposed is similar to the one we reported for the secreted protein acidic and rich in cysteine (SPARC) during inflammation [59] and cancer [60]. Interestingly, SPARC is also involved in response to injury and tissue remodeling [61,62]. Such opposing effects may broaden the application horizons and these two examples of TFF2 and SPARC illustrate mechanistic links between the need to control inflammation as well as adapting cellular patterns (metabolism, structural shape, etc.) during tissue repair and regeneration processes. Elucidating these links will expand therapeutic perspectives based on molecular pathways of diseases in animals and humans.

Author Contributions: A.G. drafted the manuscript; A.G., M.Y. and J.S.-A. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Abdelaziz Ghanemi is a recipient of a Merit scholarship program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec Nature et technologies (FRQNT) est responsable de la gestion du programme). The graphical abstract was created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

9.5 References

1. Jørgensen, K.H.; Thim, L.; Jacobsen, H.E. Pancreatic spasmolytic polypeptide (PSP): I. Preparation and initial chemical characterization of a new polypeptide from porcine pancreas. *Regul. Pept.* 1982, *3*, 207–219.
2. Royce, S.G.; Lim, C.; Muljadi, R.C.; Samuel, C.S.; Ververis, K.; Karagiannis, T.C.; Giraud, A.S.; Tang, M.L. Trefoil factor-2 reverses airway remodeling changes in allergic airways disease. *Am. J. Respir. Cell Mol. Biol.* 2013, *48*, 135–144.
3. Farrell, J.J.; Taupin, D.; Koh, T.J.; Chen, D.; Zhao, C.-M.; Podolsky, D.K.; Wang, T.C. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J. Clin. Investig.* 2002, *109*, 193–204.
4. Aamann, L.; Vestergaard, E.M.; Grønbæk, H. Trefoil factors in inflammatory bowel disease. *World J. Gastroenterol.* 2014, *20*, 3223–3230.
5. Playford, R.J.; Marchbank, T.; Chinery, R.; Evison, R.; Pignatelli, M.; Boulton, R.A.; Thim, L.; Hanby, A.M. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. *Gastroenterology* 1995, *108*, 108–116.
6. McKenzie, C.; Marchbank, T.; Playford, R.J.; Otto, W.; Thim, L.; Parsons, M.E. Pancreatic spasmolytic polypeptide protects the gastric mucosa but does not inhibit acid secretion or motility. *Am. J. Physiol.* 1997, *273 Pt 1*, G112–G117.
7. Aihara, E.; Engevik, K.A.; Montrose, M.H. Trefoil Factor Peptides and Gastrointestinal Function. *Annu. Rev. Physiol.* 2017, *79*, 357–380.
8. Greeley, M.A.; Van Winkle, L.S.; Edwards, P.C.; Plopper, C.G. Airway trefoil factor expression during naphthalene injury and repair. *Toxicol. Sci.* 2010, *113*, 453–467.
9. Heuer, F.; Stürmer, R.; Heuer, J.; Kalinski, T.; Lemke, A.; Meyer, F.; Hoffmann, W. Different Forms of TFF2, A Lectin of the Human Gastric Mucus Barrier: In Vitro Binding Studies. *Int. J. Mol. Sci.* 2019, *20*, 5871.
10. Royce, S.G.; Lim, C.; Muljadi, R.C.; Tang, M.L. Trefoil factor 2 regulates airway remodeling in animal models of asthma. *J. Asthma* 2011, *48*, 653–659.
11. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
12. Katoh, M. Trefoil factors and human gastric cancer (review). *Int. J. Mol. Med.* 2003, *12*, 3–9.
13. Konturek, P.C.; Brzozowski, T.; Pierzchalski, P.; Kwiecien, S.; Pajdo, R.; Hahn, E.G.; Konturek, S.J. Activation of genes for spasmolytic peptide, transforming growth factor alpha and for cyclooxygenase (COX)-1 and COX-2 during gastric adaptation to aspirin damage in rats. *Aliment. Pharm.* 1998, *12*, 767–777.
14. Hanisch, F.G.; Bonar, D.; Schloerer, N.; Schroten, H. Human trefoil factor 2 is a lectin that binds α-GlcNAc-capped mucin glycans with antibiotic activity against Helicobacter pylori. *J. Biol. Chem.* 2014, *289*, 27363–27375.
15. Sébert, M.; Sola-Tapias, N.; Mas, E.; Barreau, F.; Ferrand, A. Protease-Activated Receptors in the Intestine: Focus on Inflammation and Cancer. *Front. Endocrinol. (Lausanne)* 2019, *10*, 717.
16. Ma, K.; Lu, N.; Zou, F.; Meng, F.Z. Sirtuins as novel targets in the pathogenesis of airway inflammation in bronchial asthma. *Eur. J. Pharm.* 2019, *865*, 172670.
17. Schuliga, M. NF-kappaB Signaling in Chronic Inflammatory Airway Disease. *Biomolecules* 2015, *5*, 1266–1283.
18. Hoffmann, W. TFF2, a MUC6-binding lectin stabilizing the gastric mucus barrier and more (Review). *Int. J. Oncol.* 2015, *47*, 806–816.
19. Ortiz-Masiá, D.; Hernández, C.; Quintana, E.; Velázquez, M.; Cebrián, S.; Riaño, A.; Calatayud, S.; Esplugues, J.V.; Barrachina, M.D. iNOS-derived nitric oxide mediates the increase in TFF2 expression associated with gastric damage: Role of HIF-1. *FASEB J.* 2010, *24*, 136–145.
20. Nikolaidis, N.M.; Zimmermann, N.; King, N.E.; Mishra, A.; Pope, S.M.; Finkelman, F.D.; Rothenberg,

M.E. Trefoil factor-2 is an allergen-induced gene regulated by Th2 cytokines and STAT6 in the lung. *Am. J. Respir. Cell Mol. Biol.* 2003, *29*, 458–464.
21. Knipper, J.A.; Willenborg, S.; Brinckmann, J.; Bloch, W.; Maaß, T.; Wagener, R.; Krieg, T.; Sutherland, T.; Munitz, A.; Rothenberg, M.E.; et al. Interleukin-4 Receptor α Signaling in Myeloid Cells Controls Collagen Fibril Assembly in Skin Repair. *Immunity* 2015, *43*, 803–816.
22. Balaji, S.; Wang, X.; King, A.; Le, L.D.; Bhattacharya, S.S.; Moles, C.M.; Butte, M.J.; de Jesus Perez, V.A.; Liechty, K.W.; Wight, T.N.; et al. Interleukin-10-mediated regenerative postnatal tissue repair is dependent on regulation of hyaluronan metabolism via fibroblast-specific STAT3 signaling. *FASEB J.* 2017, *31*, 868–881.
23. Hadian, Y.; Bagood, M.D.; Dahle, S.E.; Sood, A.; Isseroff, R.R. Interleukin-17: Potential Target for Chronic Wounds. *Mediat. Inflamm.* 2019, *2019*, 1297675.
24. Hernández, C.; Santamatilde, E.; McCreath, K.J.; Cervera, A.M.; Díez, I.; Ortiz-Masiá, D.; Martínez, N.; Calatayud, S.; Esplugues, J.V.; Barrachina, M.D. Induction of trefoil factor (TFF)1, TFF2 and TFF3 by hypoxia is mediated by hypoxia inducible factor-1: Implications for gastric mucosal healing. *Br. J. Pharmacol.* 2009, *156*, 262–272.
25. Azarschab, P.; Al-Azzeh, E.; Kornberger, W.; Götte, P. Aspirin promotes TFF2 gene activation in human gastric cancer cell lines. *FEBS Lett.* 2001, *488*, 206–210.
26. Hu, G.Y.; Yu, B.P.; Dong, W.G.; Li, M.Q.; Yu, J.P.; Luo, H.S.; Rang, Z.X. Expression of TFF2 and Helicobacter pylori infection in carcinogenesis of gastric mucosa. *World J. Gastroenterol.* 2003, *9*, 910–914.
27. Tran, C.P.; Cook, G.A.; Yeomans, N.D.; Thim, L.; Giraud, A.S. Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. *Gut* 1999, *44*, 636–642.
28. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2015, *308*, G12–G24.
29. Ellermann, M.; Gharaibeh, R.Z.; Fulbright, L.; Dogan, B.; Moore, L.N.; Broberg, C.A.; Lopez, L.R.; Rothemich, A.M.; Herzog, J.W.; Rogala, A.; et al. Yersiniabactin-Producing Adherent/Invasive Escherichia coli Promotes Inflammation-Associated Fibrosis in Gnotobiotic Il10(-/-) Mice. *Infect. Immun.* 2019, *87*, e00587-19.
30. Gordon, I.O.; Agrawal, N.; Willis, E.; Goldblum, J.R.; Lopez, R.; Allende, D.; Liu, X.; Patil, D.Y.; Yerian, L.; El-Khider, F.; et al. Fibrosis in ulcerative colitis is directly linked to severity and chronicity of mucosal inflammation. *Aliment. Pharmacol. Ther.* 2018, *47*, 922–939.
31. Soriano-Izquierdo, A.; Gironella, M.; Massaguer, A.; May, F.E.; Salas, A.; Sans, M.; Poulsom, R.; Thim, L.; Piqué, J.M.; Panés, J. Trefoil peptide TFF2 treatment reduces VCAM-1 expression and leukocyte recruitment in experimental intestinal inflammation. *J. Leukoc. Biol.* 2004, *75*, 214–223.
32. Giraud, A.S.; Pereira, P.M.; Thim, L.; Parker, L.M.; Judd, L.M. TFF-2 inhibits iNOS/NO in monocytes, and nitrated protein in healing colon after colitis. *Peptides* 2004, *25*, 803–809.
33. Dhar, D.K.; Wang, T.C.; Maruyama, R.; Udagawa, J.; Kubota, H.; Fuji, T.; Tachibana, M.; Ono, T.; Otani, H.; Nagasue, N. Expression of Cytoplasmic TFF2 Is a Marker of Tumor Metastasis and Negative Prognostic Factor in Gastric Cancer. *Lab. Investig.* 2003, *83*, 1343–1352.
34. Schmidt, P.H.; Lee, J.R.; Joshi, V.; Playford, R.J.; Poulsom, R.; Wright, N.A.; Goldenring, J.R. Identification of a metaplastic cell lineage associated with human gastric adenocarcinoma. *Lab. Investig.* 1999, *79*, 639–646.
35. Cai, Y.; Yi, M.; Chen, D.; Liu, J.; Guleng, B.; Ren, J.; Shi, H. Trefoil factor family 2 expression inhibits gastric cancer cell growth and invasion in vitro via interactions with the transcription factor Sp3. *Int. J. Mol. Med.* 2016, *38*, 1474–1480.
36. Longman, R.J.; Douthwaite, J.; Sylvester, P.A.; Poulsom, R.; Corfield, A.P.; Thomas, M.G.; Wright, N.A. Coordinated localisation of mucins and trefoil peptides in the ulcer associated cell lineage and the

gastrointestinal mucosa. *Gut* 2000, *47*, 792–800.
37. Khoder, G.; Al-Yassir, F.; Al Menhali, A.; Saseedharan, P.; Sugathan, S.; Tomasetto, C.; Karam, S.M. Probiotics Upregulate Trefoil Factors and Downregulate Pepsinogen in the Mouse Stomach. *Int. J. Mol. Sci.* 2019, *20*, 3901.
38. Lukic, J.; Chen, V.; Strahinic, I.; Begovic, J.; Lev-Tov, H.; Davis, S.C.; Tomic-Canic, M.; Pastar, I. Probiotics or pro-healers: The role of beneficial bacteria in tissue repair. *Wound Repair Regen.* 2017, *25*, 912–922.
39. Plaza-Díaz, J.; Ruiz-Ojeda, F.J.; Vilchez-Padial, L.M.; Gil, A. Evidence of the Anti-Inflammatory Effects of Probiotics and Synbiotics in Intestinal Chronic Diseases. *Nutrients* 2017, *9*, 555.
40. Liu, Y.; Alookaran, J.J.; Rhoads, J.M. Probiotics in Autoimmune and Inflammatory Disorders. *Nutrients* 2018, *10*, 1537.
41. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. *Obesity (Silver Spring)* 2008, *16*, 2302–2307.
42. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity (Silver Spring)* 2013, *21*, 1389–1395.
43. Ghanemi, A.; St-Amand, J. Interleukin-6 as a "metabolic hormone". *Cytokine* 2018, *112*, 132–136.
44. Hong, J.T.; Son, D.J.; Lee, C.K.; Yoon, D.Y.; Lee, D.H.; Park, M.H. Interleukin 32, inflammation and cancer. *Pharmacol. Ther.* 2017, *174*, 127–137.
45. Petruzzelli, M.; Wagner, E.F. Mechanisms of metabolic dysfunction in cancer-associated cachexia. *Genes Dev.* 2016, *30*, 489–501.
46. Tanaka, T.; Narazaki, M.; Kishimoto, T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb. Perspect. Biol.* 2014, *6*, a016295.
47. Judd, L.M.; Alderman, B.M.; Howlett, M.; Shulkes, A.; Dow, C.; Moverley, J.; Grail, D.; Jenkins, B.J.; Ernst, M.; Giraud, A.S. Gastric cancer development in mice lacking the SHP2 binding site on the IL-6 family co-receptor gp130. *Gastroenterology* 2004, *126*, 196–207.
48. Peritore, A.F.; Siracusa, R.; Crupi, R.; Cuzzocrea, S. Therapeutic Efficacy of Palmitoylethanolamide and Its New Formulations in Synergy with Different Antioxidant Molecules Present in Diets. *Nutrients* 2019, *11*, 2175.
49. Lasker, S.; Rahman, M.M.; Parvez, F.; Zamila, M.; Miah, P.; Nahar, K.; Kabir, F.; Sharmin, S.B.; Subhan, N.; Ahsan, G.U.; et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. *Sci. Rep.* 2019, *9*, 20026.
50. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
51. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes (Basel)* 2020, *11*, 875.
52. Sindhu, S.; Akhter, N.; Kochumon, S.; Thomas, R.; Wilson, A.; Shenouda, S.; Tuomilehto, J.; Ahmad, R. Increased Expression of the Innate Immune Receptor TLR10 in Obesity and Type-2 Diabetes: Association with ROS-Mediated Oxidative Stress. *Cell. Physiol. Biochem.* 2018, *45*, 572–590.
53. Karczewski, J.; Sledzi´nska, E.; Baturo, A.; Jo´nczyk, I.; Maleszko, A.; Samborski, P.; Begier-Krasi´nska, B.; Dobrowolska, A. Obesity and inflammation. *Eur. Cytokine Netw.* 2018, *29*, 83–94.
54. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
55. Taupin, D.; Wu, D.-C.; Jeon, W.-K.; Devaney, K.; Wang, T.C.; Podolsky, D.K. The trefoil gene family are coordinately expressed immediate-early genes: EGF receptor– and MAP kinase–dependent interregulation. *J. Clin. Investig.* 1999, *103*, R31–R38.
56. Lefebvre, O.; Chenard, M.-P.; Masson, R.; Linares, J.; Dierich, A.; LeMeur, M.; Wendling, C.; Tomasetto, C.; Chambon, P.; Rio, M.-C. Gastric Mucosa Abnormalities and Tumorigenesis in Mice Lacking the pS2 Trefoil Protein. *Science* 1996, *274*, 259–262.

57. Hoffmann, W. Trefoil Factor Family (TFF) Peptides and Their Diverse Molecular Functions in Mucus Barrier Protection and More: Changing the Paradigm. *Int. J. Mol. Sci.* 2020, *21*, 4535.
58. Kirikoshi, H.; Katoh, M. Expression of TFF1, TFF2 and TFF3 in gastric cancer. *Int. J. Oncol.* 2002, *21*, 655–659.
59. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
60. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
61. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
62. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.

Chapter 10. Research Article - Energy and metabolic pathways in trefoil factor family member 2 (*Tff2*) KO mice beyond the protection from high-fat diet-induced obesity

Abdelaziz Ghanemia [a,b], Aicha Melouane [a,b], Octave Mucunguzi [a,b], Mayumi Yoshioka [a], Jonny St-Amand [a,b]

[a] Functional Genomics Laboratory, CREMI, Québec Genome Center, CHUL-CHU de Québec Research Center, Québec, Québec G1V 4G2, Canada

[b] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

Received 16 August 2018; Received in revised form 27 October 2018; Accepted 4 November 2018; Available online 07 November 2018

10.1 Résumé (French abstract)

Une étude de notre laboratoire avait démontré que les souris ayant une déficience en trefoil factor family member 2 (*Tff2*) sont protégées contre l'obésité induite par une diète riche en gras. Donc, on souhaitait explorer les mécanismes expliquant cette protection aux niveaux génomique, protéique et biochimique.

On a utilisé du sang prélevé des souris pour mesurer les taux sériques d'acides gras libres, glucose, glycérol et triglycérides. Les niveaux d'expression de gènes et de protéines liés au métabolisme énergétique dans le muscle squelettique, le foie ainsi que le tissue adipeux ont aussi été mesurés.

Nos résultats révèlent un métabolisme orienté vers moins de stockage des lipides et plus de dépense énergétique, de phosphorylation oxydative et d'utilisation des lipides et du glucose. Ces observations présentent les voies liées au gène *Tff2* comme de potentielles cibles thérapeutiques moléculaires pour le traitement l'obésité et les syndromes et maladies qui y sont associés.

Highlights

- Trefoil factor family member 2 (TFF2) is involved in high-fat diet-induced obesity.
- *Tff2* KO mice are protected from the high-fat diet-induced obesity.
- We showed molecular mechanisms behind this protection.
- We mapped *Tff2* KO-related energetic and metabolic pathways.
- Our data point *Tff2*-Related pathways as potential therapeutic targets for obesity.

10.2 Abstract

Aims: Trefoil factor family member 2 (TFF2) is a small gut peptide. We have previously shown that *Tff2* knock out (KO) mice are protected from high-fat (HF) diet-induced obesity (De Giorgio et al., 2013a). Thus, exploring *Tff2* KO-related pathways of mice at the genomic, proteinic and biochemical levels would allow us to elucidate the processes behind this protection from obesity.

Main methods: To explore the metabolic and energetic effects related to *Tff2* deficiency, we used sampled blood from the previous study to measure levels of free fatty acids, glucose, glycerol and triglycerides in serum. Expression levels of selected genes and proteins related to energy metabolism in the skeletal muscle, liver and adipose tissue were also studied.

Key findings: Following the 12-wk challenging of *Tff2* KO and WT mice with both HF and low-fat diet, *Tff2* KO mice had lower levels of serum glucose, triglycerides and glycerol. Importantly, western blotting and Q_RT-PCR revealed that the expression levels of selected genes and proteins are toward less fat storage and increased energy expenditure by enhancing lipid and glucose utilization via oxidative phosphorylation.

Significance: We mapped a part of the metabolic and biochemical pathways of lipids and glucose involving the adipose tissue, liver, skeletal muscle and sympathetic nervous system that protect *Tff2* KO mice from the HF dietinduced obesity. Our data highlight *Tff2*-related pathways as potential targets for obesity therapies.

Keywords: Energy metabolism; High-fat diet; Obesity protection; Trefoil factor family member 2

Abbreviations*:* β-AR, β-adrenergic receptor; ADRB2, β2-adrenergic receptor; BAT, brown adipose tissue; CAPS, N-cyclohexyl-3-aminopropanesulfonic acid; CD36, fatty acids translocase; CNS, central nervous system; DF, density of each lane on the film; DM, density of each lane on the membrane; FFA, free fatty acids; GLUT2, glucose transporter 2 (solute carrier family 2); GLUT4, glucose transporter type 4; HF, high-fat; KO, knockout; LF, low-fat; NUR77, nerve growth factor IB or NGFIB; OXPHOS, oxidative phosphorylation; PC, positive control; PPARA, peroxisome proliferator-activated receptor alpha; Q_RT-PCR, quantitative real-time PCR; SNS, sympathetic nervous system; TFF2, trefoil factor 2; TG, triglyceride; *Ucp1*, mitochondrial uncoupling protein1; WAT, white adipose tissue; WT, wild type

10.3 Introduction

Obesity can result from the accumulated effect of minor imbalances between energy intake and expenditure [2]. In functional genomics, exploring genes-related energy metabolism variations represents a key step to understand the underlying mechanisms of obesity and identify potential therapeutic targets. Within this context, trefoil factor family

member 2 (TFF2), also known as spasmolytic polypeptide (SP) [3], is expressed in the gastrointestinal mucosa [4,5], where it plays a protective role [6], as well as in other tissues such as the central nervous system (CNS) [1,7]. TFF2 represents, beyond its roles in divers biological functions, a peptide which has been linked to food intake [8], specifically high-fat (HF) intake, in mice [9]. Thus, TFF2 may regulate feeding behavior and energy metabolism as a peripheral signal to the CNS.

Indeed, our previous study highlighted *Tff*2 knock out (KO) mice as protected from HF diet-induced obesity and that *Tff2* expression in hypothalamus is modulated by HF and low-fat (LF) meal [10]. Although *Tff2* KO mice had higher energy intake, they had less body weight and fat mass but a higher percentage of lean mass. This study demonstrated that TFF2 is involved in all components of energy balance: energy intake (+650 kJ in *Tff2* KO mice compared to wild type (WT) mice during the 12-wks feeding), expenditure (+608 kJ) and excretion (+129 kJ) [1]. Importantly, energy loss from energy excretion in *Tff2* KO mice accounted only for 17.5% whereas the majority (82.5%) of the energy loss resulted from energy expenditure [1].

The pathways beyond this specific energy metabolism pattern are yet to be identified. Herein, to characterize the metabolic mechanisms via which TFF2 is controlling energy balance and the implication of *Tff2* deficiency in the protection form the HF diet-induced obesity, the current work explores selected energy metabolic parameters via genes and proteins expression combined to biochemical measures.

10.4 Materials and methods

10.4.1 Experimental design and diet specifications

Our study has been carried out on male mice of our previous work involving both WT and *Tff2* KO mice fed HF (Research Diets #D08121503M) or LF diet (Research Diets #D12450CM) [1]. Briefly, at the age of 16 wks, the mice, divided into four groups: WT-LF (n = 7), WT-HF (n = 7), KO-LF (n = 7), KO-HF (n = 9), were fed with the corresponding diets and provided tap water ad libitum for 12 wks. Blood was collected from the mandibular vein at the 8th wk. of diet using a needle stick. Mice were sacrificed at the end of the 12-wk diet by cardiac puncture after being anesthetized with a commensurate dose of ketamine- xylazine. The blood and tissues were collected. The selected tissues were the adipose tissue, skeletal muscle and liver which are important targets for metabolic therapies [11].

10.4.2. Quantitative real-time PCR (Q_RT-PCR)

Total RNA was extracted from the skeletal muscles and the improved Q_RT-PCR method, with the technical specifications previously described [10,12], was used for the quantification of mRNAs corresponding to the following genes: nerve growth factor IB (*Nur77*), fatty acids translocase (*Cd36*), glucose transporter type 4 (*Glut4*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Ppargc1α*), uncoupling protein 3

(*Ucp3*) and mitochondrially encoded cytochrome C oxidase I (*Mtco1*). See the Appendix 10.1 for the related information on GenBank accession numbers, sizes and regions used for primers pairs and primers sequences.

10.4.3 Western blotting

Total proteins were extracted from the skeletal muscles, liver and adipose tissue using a radio-immunoprecipitation assay (RIPA) buffer and protease inhibitors cocktail (Sigma Aldrich Canada Co., Oakville, ON, Canada) and followed by a protein quantification of each protein extract. Five to thirty micrograms of proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the TGX Stain-Free FastCast acrylamide solutions (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada), and trihalo compound in the gels was activated under UV. Then, total proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad Laboratories Ltd.), and gels (before and after the transfer) and membranes were visualized under UV by using the AlphaImagerTM 1220 (Alpha Innotech Co., San Leandro, CA, USA). Appendix 10.2 represents examples of images of gel (before and after the transfer) and membrane visualized under UV.

Membranes were blocked using the Pierce™ Protein-Free (TBS) blocking buffer (Life Technologies Inc., ON, Canada), incubated with 1/ 100–1/400 dilution of primary antibodies (Santa Cruz Biotechnology Inc., Dallas, Texas, USA) and secondary antibodies (sc-2004 or sc-2005, 1/10000 dilution: Santa Cruz Biotechnology Inc.), and finally visualized with the Clarity™ Western ECL Blotting Substrate on a film (BioRad Laboratories Ltd.). The visualized total proteins on the membranes and target proteins on the films were quantified using the ImageJ software [13]. In order to optimize the western blot experimental conditions for each protein and before measuring its expression levels, pooled sample (constituted of a mixture of equal proteins amount of all the samples) were used to determine both the quantity of proteins to load (tested quantities: 0–40 μg) as well as the primary antibody dilution (tested dilutions: 1/100–1/800) for the incubation. The same pooled sample was loaded in each gel and used as a positive control (PC) to normalize the differences between membranes/films. The density of each lane on the membrane (DM) (loading control) and film (DF) was expressed as a ratio to each PC on the same membrane/film. Finally, the quantity of protein loaded was normalized via dividing DF by DM, as previously suggested [14,15]. Results are expressed as mean ± standard error of the mean (SEM) of the ratio DF/DM. Whereas the images of the membrane and the film were used to calculate the DM and the DF, respectively, the images of the gel (before and after the transfer) reflects the quality of both the electrophoresis and the transfer.

For the oxidative phosphorylation (OXPHOS)-related proteins, some different western bloating conditions were applied. Indeed, we used a pre-casted ready Tris-HCl gel (161-1124, Bio-Rad Laboratories Ltd.) for the proteins separation. After the electrophoresis, gel was soaked in the N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) transfer buffer (C2632, Sigma-Aldrich Canada Co.) for 30 min before assembling the

transfer sandwich. Following the transfer, the membrane was soaked in CAPS transfer buffer then in TrueBlot enhancer solution before being placed in 5% TrueBlot Blocker (in TrueBlot Assay Buffer) overnight at 4 °C. For the primary antibodies, we used the OXPHOS rodent western blot antibody cocktail (Abcam Inc., Toronto, ON, Canada) at a dilution of 1/250.

The changes in the expression levels of one or more of the following proteins: β2-adrenergic receptor (ADRB2), NUR77, CD36, GLUT4, glucose transporter 2 (GLUT2) and peroxisome proliferator-activated receptor alpha (PPARA) and selected mitochondrial OXPHOS-related enzymes and proteins were studied for each of the three key metabolic tissues.

10.4.4 Blood and serum analysis

Serum for free fatty acids (FFA) measurement was collected at the 8th wk. of diet (following 12-h fasting), whereas the collection of the serum for insulin, triglyceride (TG) and glycerol measurement were performed at wk 12 of the diet, following a 4-h fasting (postprandial period) at sacrifice. Blood glucose measurement was performed both at the 8^{th} wk. (following 12-h fasting) and at wk 12 (following a 4-h fasting at sacrifice) of the diet.

Serum insulin levels were measured by immunoassay ELISA kits (ALPCO, Salem, NH, USA). Glucose was measured with a glucometer (Accu-Chek® Aviva, Roche, Basel, Switzerland). Serum TG and glycerol concentrations were measured by a colorimetric enzymatic method, using the Triglyceride Quantification Kit (BioVision, Inc., CA, USA). Measurement of FFA was performed by a colorimetric kit (Abcam Inc.).

10.4.5 Statistical analyses

The data were analyzed by the two-way (genotype and diet) ANOVA ($p < 0.05$). When the ANOVA revealed a significant interaction between two variables, the Tukey Kramer post-hoc test was performed to identify the significant difference between the two groups ($p < 0.05$). A trend corresponds to $0.05 \leq p < 0.1$.

10.5 Results

10.5.1 Increased expression of genes and proteins related to glucose and lipids uptake and utilization in the skeletal muscle of *Tff2* KO mice

Following the 12 wks of diet, both gene and protein expressions levels have been studied in the skeletal muscle. The obtained results (Figure 10.1), put together, indicate an increase in both glucose and lipid metabolism. Indeed, compared to WT mice, KO mice had an increased expression of *Cd36*, *Nur77* (trend), NUR77, CD36, NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8 (NDUFB8) as well as GLUT4 (trend). The HF diet (compared to the LF diet) resulted in an increased expression of *Cd36*. In addition, the Tukey Kramer post-hoc test revealed that in HF-fed mice, *Nur77*, *Cd36* and *Glut4* are more expressed in KO mice compared to WT.

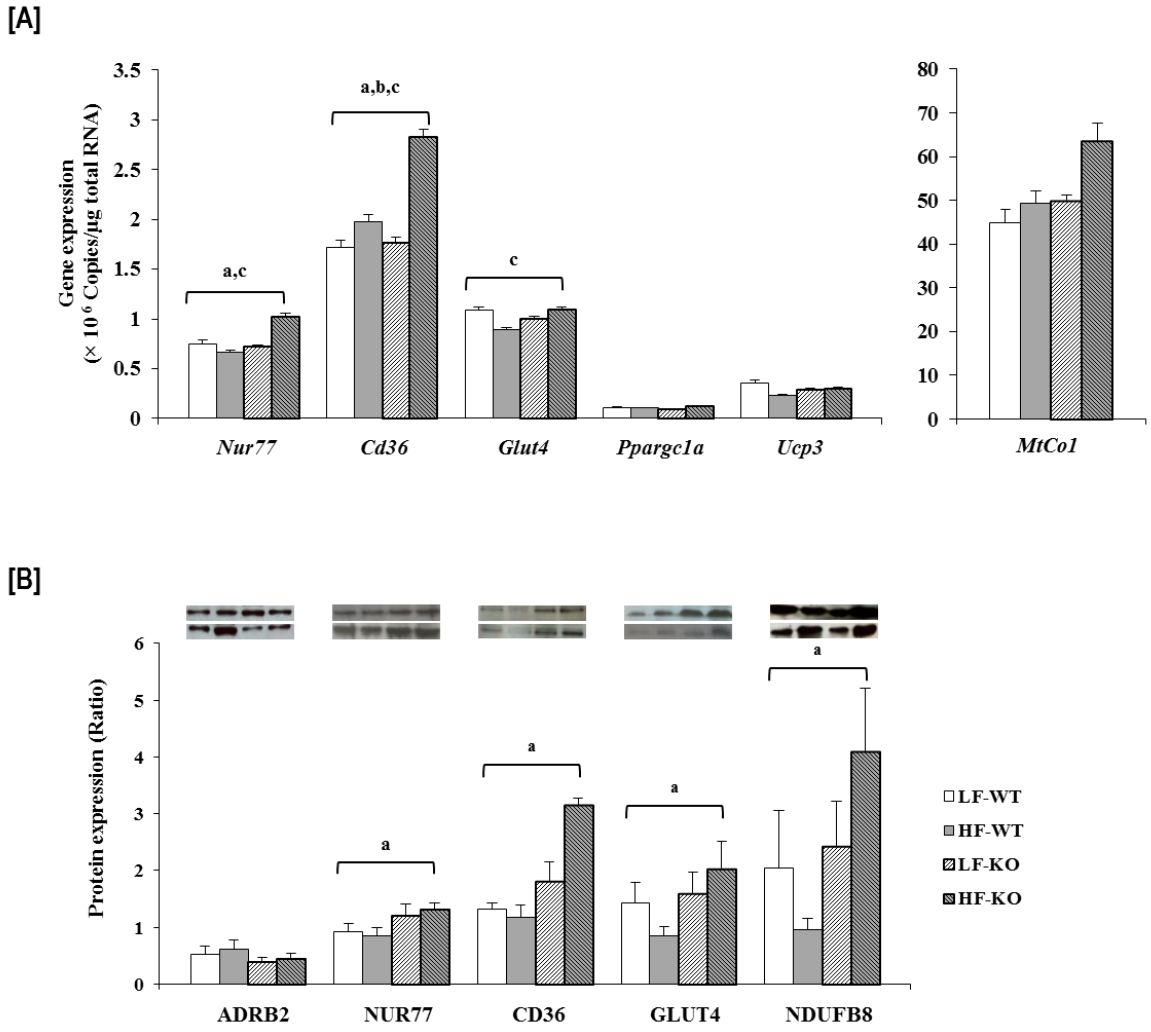


Figure 10.1. Effect of *Tff2* deficiency on genes (A) and protein (B) expressions in the skeletal muscle after 12 wks of HF or LF diet.

[A] For the muscular genes expression, the two-way ANOVA revealed a significant effect of genotype[a] (KO > WT) for *Cd36* and a trend effect of genotype[a] (KO > WT) for *Nur77*. Significant effects of diet[b] (HF > LF) for *Cd36* and diet × genotype[c] for *Nur77*, *Cd36* and *Glut4* (KO > WT in HF-fed mice by the Tukey-Kramer post-hoc test) were also revealed.

[B] For the protein expression in the skeletal muscle, the same statistical approach showed a significant effect of genotype[a] (KO > WT) for NUR77, CD36 and NDUFB8, in addition to a trend effect of genotype[a] (KO > WT) for GLUT4.

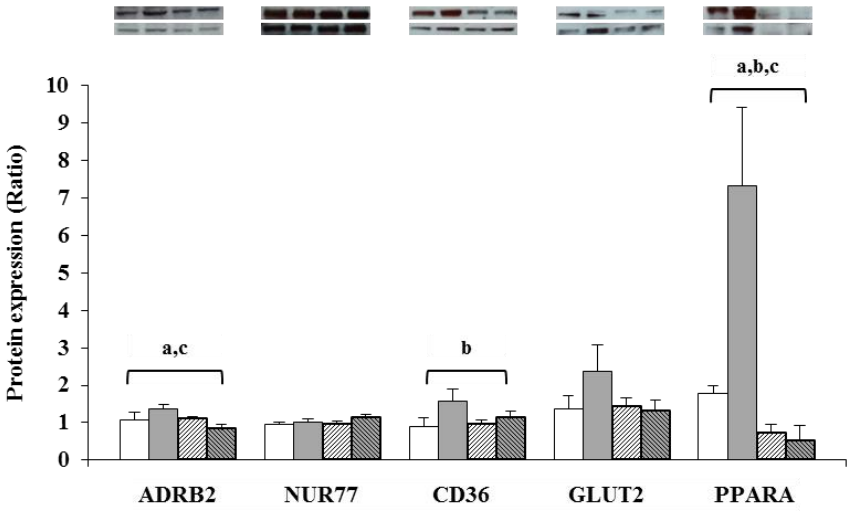
All data are mean ± SEM. Number of mice: WT-LF (7), WT-HF (6), KO-LF (7) and KO-HF (9).

Abbreviations: ADRB2, β-2 adrenergic receptor; *Cd36*/CD36, fatty acids translocase; *Glut4*/GLUT4, glucose transporter type 4; HF, high-fat, KO, knockout; LF, low-fat; *MtCo1*, mitochondrially encoded cytochrome *c* oxidase I; NDUFB8, NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8; *Nur77*/NUR77, nerve growth factor IB; *Ppargc1a*, peroxisome proliferator-activated receptor γ coactivator 1α; *Tff2*, trefoil factor 2; *Ucp3*, mitochondrial uncoupling protein 3; WT, wild type.

10.5.2 Decreased expression of energy metabolism related proteins in the liver and adipose tissue of *Tff2* KO mice

The two-way ANOVA analysis of the western blotting data of liver (Figure 10.2A) and adipose tissue (Figure 10.2B) revealed the impact of *Tff2* deficiency and diet type on these two tissues. Data indicate a decrease in the proteins involved in the energy metabolism of these two tissues in *Tff2* KO mice compared to WT mice (Figure 10.2). Indeed, the KO decreased the expression of both PPARA and ADRB2 (trend) in the liver and the NUR77 (trend) in the adipose tissue. The HF diet, compared to LF diet, increased the expression of PPARA and CD36 (trend) in the liver. In HF fed mice, the KO decreased the expression of PPARA and ADRB2 in the liver and of NUR 77 in the adipose tissue. Additionally, in WT mice, the HF diet compared to LF diet increased the expression of NUR77 in the adipose tissue and of PPARA in the liver.

[A]



[B]

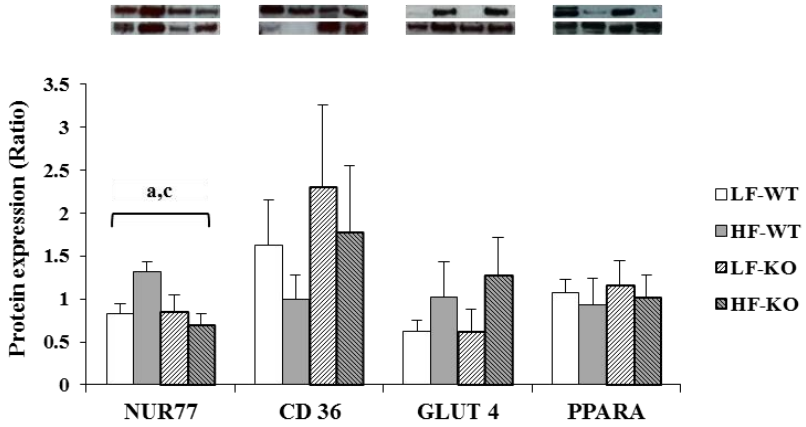


Figure 10.2. Effect of *Tff2* deficiency on proteins expression in the liver (A) and adipose tissue (B) after 12 wks of HF or LF diet.

[A] In the liver, we report significant effects of genotype[a] (KO < WT) and dietb (HF > LF) for the protein PPARA along with significant effects of diet × genotype[c] (KO < WT in HF fed mice by the Tukey-Kramer post-hoc test) for the PPARA and ADRB2. In addition, trend in effects of genotype[a] (KO < WT) for the ADRB2 and of diet[b] (HF > LF) for CD36 were also observed.

[B] For the adipose tissue, our data point a trend in effect of genotype[a] (KO < WT) and significant effect of genotype × diet[c] (KO < WT in HF-fed mice and HF > LF in WT mice by the Tukey-Kramer post-hoc test) for the NUR77. All data are mean ± SEM. Number of mice: WT-LF (7), WTHF (7), KO-LF (7) and KO HF (9).

Abbreviations: ADRB2, β-2 adrenergic receptor; CD36, fatty acids translocase; GLUT2, glucose transporter 2 (solute carrier family 2); GLUT4, glucose transporter type 4; HF, high-fat; KO, knockout, LF, low-fat; NUR77, nerve growth factor IB; PPARA, peroxisome proliferator-activated receptor alpha; *Tff2*, trefoil factor 2, WT, wild type.

10.5.3 Tff2 KO mice have decreased gastrocnemius muscle, mesenteric adipose tissue, brown adipose tissue and liver mass whereas increased percentage of gastrocnemius muscle mass

Mice were sacrificed at the end of the 12-wk diet and after 4-h fasting and tissues weights were analyzed via the two-way ANOVA (Table 10.1). The analysis revealed significant effects of genotype for both gastrocnemius muscle mass (KO < WT) and mass percentage (KO > WT) and an effect of diet (HF < LF) for the gastrocnemius muscle mass percentage. For the mesenteric adipose tissue (both mass and masse percentage), we noticed significant effects of both genotype (KO < WT) and diet (HF > LF). The statistics showed also significant effects of genotype (KO < WT) for the liver weight, diet (HF < LF) for the liver mass percentage. Significant effects of genotype × diet interaction were also revealed for both mesenteric adipose tissue and liver masse (HF: KO < WT). Other significant effects of genotype × diet interaction (trends) were revealed for the mass percentages of the mesenteric adipose tissue (HF: KO < WT), the liver (LF: KO > WT) and the gastrocnemius muscle (HF: KO > WT). Finally, a genotype effect (KO < WT) for both brown adipose tissue (BAT) mass and mass percentage was noticed (Table 10.1).

Table 10.1. Tissues weights of *Tff2* KO and WT mice fed LF and HF diet.

	WT-LF	WT-HF	KO-LF	KO-HF	2-way ANOVA		
					Genotype	Diet	Genotype×Diet
Body weight (g)	32.4 ± 1.0	41.9 ± 2.1	28.0 ± 0.5	31.6 ± 1.2	KO<WT	HF>LF	HF: KO<WT
Mesenteric adipose tissue (g)	0.40 ± 0.05	1.15 ± 0.20	0.27 ± 0.03	0.50 ± 0.06	KO<WT	HF>LF	HF: KO<WT
Mesenteric adipose tissue (%)	1.20 ± 0.14	2.68 ± 0.45	0.96 ± 0.10	1.42 ± 0.17	KO<WT	HF>LF	HF: KO<WT*
Brown adipose tissue (g)	0.21 ± 0.03	0.25 ± 0.03	0.13 ± 0.02	0.16 ± 0.02	KO<WT	ns	ns
Brown adipose tissue (%)	0.62 ± 0.06	0.59 ± 0.05	0.46 ± 0.06	0.50 ± 0.05	KO<WT	ns	ns
Liver (g)	1.27 ± 0.03	1.55 ± 0.19	1.21 ± 0.05	1.06 ± 0.05	KO<WT	ns	HF: KO<WT
Liver (%)	3.90 ± 0.08	3.60 ± 0.26	4.30 ± 0.20	3.40 ± 0.18	ns	HF<LF	LF: KO>WT*
Gastrocnemius muscle (g)	0.141 ± 0.004	0.144 ± 0.007	0.126 ± 0.004	0.133 ± 0.002	KO<WT	ns	ns
Gastrocnemius muscle (%)	0.44 ± 0.02	0.35 ± 0.02	0.45 ± 0.01	0.43 ± 0.02	KO>WT	HF<LF	HF: KO>WT*

Data are mean ± SEM. Number of mice: WT-LF (7), WT-HF (7), KO-LF (7) and KO-HF (9).

ns: No significant effect. ∗ Trend ($p < 0.09$).

Abbreviations: HF, high-fat, KO, knockout; LF, low-fat; *Tff2*, trefoil factor 2; WT, wild type.

10.5.4 Tff2 KO mice have lower blood glucose as well as serum FFA, TG, glycerol and insulin levels

The analysis of the blood and serum (sampled at the 8th wk. of diet and following 12-h fasting) by the two-way ANOVA was shown in Table 10.2. The data showed a trend in effect of genotype (KO > WT) for the FFA but no effect on glucose. At 12-wks diet (4-h fasting), it revealed significant effects of genotype (KO < WT) for glucose, TG and glycerol levels. The glucose levels were higher in HF groups. A genotype × diet interaction (LF: KO < WT) was observed for TG levels. A trend in the effect of genotype (KO < WT) was also showed for insulin levels at the wk 12 of the diet (Table 10.2).

Table 10.2. Serum analysis of selected energy metabolism-related biochemicals of *Tff2* KO and WT mice depending on the type of diet.

		WT-LF	WT-HF	KO-LF	KO-HF	2-way ANOVA		
						Genotype	Diet	Genotype×Diet
8 wks of feeding: 12-h fasting								
Glucose	(mmol/L)	6.10 ± 0.44	6.40 ± 0.56	5.33 ± 0.36	6.43 ± 0.49	ns	ns	ns
Free fatty acids	(mmol/L)	0.02 ± 0.01	0.08 ± 0.05	0.15 ± 0.05	0.10 ± 0.05	KO>WT*	ns	ns
12 wks of feeding: 4-h fasting								
Glucose	(mmol/L)	8.03 ± 0.59	9.36 ± 0.57	6.64 ± 0.18	7.84 ± 0.40	KO<WT	HF>LF	ns
Insulin	(pmol/L)	214 ± 46	667 ± 326	81 ± 10	172 ± 26	KO<WT*	ns	ns
Triglyceride	(mmol/L)	1.18 ± 0.13	0.60 ± 0.08	0.94 ± 0.13	0.79 ± 0.13	KO<WT	ns	LF: KO<WT
Glycerol	(mmol/L)	0.64 ± 0.11	0.54 ± 0.12	0.33 ± 0.07	0.44 ± 0.05	KO<WT	ns	ns

Data are mean ± SEM. Number of mice: WT-LF (7), WT-HF (7), KO-LF (7) and KO-HF (9).
ns: No significant effect. ∗ Trend ($p < 0.1$).
Abbreviations: HF, high-fat, KO, knockout; LF, low-fat; *Tff2*, trefoil factor 2; WT, wild type.

10.6 Discussion

From a biochemical viewpoint, obesity is mainly related to both lipidic and glucidic metabolism. Therefore, the current study selected genes, proteins, tissues and biochemicals that are specifically involved within the lipidic and/or glucidic metabolic pathways. In addition, selected tissues have been weighed to further map the *Tff2* deficiency-related pathways in mice. Therefore, our discussion focuses on the variations seen in *Tff2* KO mice compared to WT mice as well as their significance in term of energy metabolism.

10.6.1 Elevated energy expenditure

Leptin enhances energy expenditure and decreases food intake [16]. This explains why *Tff2* KO mice, with decreased leptin levels, had higher food intake [1]. However, despite the decreased leptin concentration [1], *Tff2* KO mice had a higher energy expenditure which is due to leptin-independent energy expenditure pathways, including mitochondrial uncoupling protein1 (*Ucp1*)-dependent energy expenditure. Indeed, the higher expression of *Ucp1* gene in the BAT [1] indicates that the mitochondrion oxidation is uncoupled from ATP production and suggests that the energy is dissipated as thermogenesis resulting from the sympathetic nerves system (SNS) activity after β-

adrenergic receptor (β-AR) stimulation [17]. This explains the higher energy expenditure observed in *Tff2* KO mice [1] following the excessive caloric intake probably detected by the brain. Indeed, the higher expression of agouti-related protein in the hypothalamus of *Tff2* KO mice [1] indicates an increased feeding stimulation rather than an obesity pattern [18,19] because *Tff2* KO mice were lean although they had a higher energy intake [1]. These changes highlight the involvement of the central regulation in the energy metabolism of *Tff2* KO mice. Herein, we mention that since leptin is produced by adipose tissue [20], the lower fat mass of *Tff2* KO mice also correlates with their decreased leptin level.

The increased *Ucp1* expression leading to diet-induced thermogenesis involves pathways similar to cold-response in BAT [21,22]. Importantly, this activity in BAT includes an increased uptake of glucose [23] as well as an accelerated plasma clearance of TG [24]. The higher BAT *Ucp1* expression seen in *Tff2* KO mice correlates with the lower glycemia and TG level in the postprandial serum of *Tff2* KO mice and the higher glucose uptake (higher GLUT4 expression) in skeletal muscle despite the lower insulin concertation indicating an improvement in insulin sensitivity. Within this context, it seems logic to suppose that energy consuming components of adipose tissue are similar or higher in *Tff2* KO mice, although the decreased body fat, white adipose tissue (WAT) and BAT masses were seen. Indeed, *Tff2* KO mice had higher total RNA contents in BAT (both μg/tissue and μg/mg tissue) and protein contents percentage in WAT (mesenteric), whereas no difference of total protein contents was found in WAT (data not shown). Moreover, *Tff2* KO mice had less abdominal WAT [1], probably due to the increased metabolic activity within the BAT that uses lipids as a fuel and thus, limit the depot of energy storage in WAT.

Stimuli such as diet, including long-chain fatty acids, induce UCP1 expression in BAT [25]. Therefore, upregulation of *Ucp1* expression observed during increased caloric intake can stimulate BAT thermogenesis, since BAT represents a key tissue of energy conversion into heat [26]. On the other hand, during fasting, FFA increases in serum following increased SNS activity (β-AR stimulation) [27,28], which correlates with the FFA elevation and the non-modification of glucose level following the 12-h fasting (wk 8). These indicate enhanced metabolic switch toward the use of lipids rather than glucose as an energy source during fasting in *Tff2* KO mice.

10.6.2 Increased glucose and lipid uptake and utilization in the skeletal muscle

The increased expression of *Cd36/*CD36 and *Glut4*/GLUT4 show the important uptake of the fatty acids and glucose, respectively. The increased expression of the mitochondrial protein NDUFB8 (Complex I of the mitochondrial respiratory chain) reflects a higher OXPHOS and therefore, a higher utilization of the imported glucose, lipids (FFA and TG) and glycerol within the mitochondrion. This is confirmed by the decreased concentrations of glucose, TG (in LF condition) and glycerol in postprandial serum as well as fasting serum FFA indicating that these “fuels” are imported into the skeletal muscle in addition to the BAT. This elevated energy production is required to cover, among other

needs, the energy required for the enhanced spontaneous locomotor activity observed for the *Tff2* KO mice [1]. Especially that the skeletal muscle accounts for an important ratio (50%) of resting energy expenditure of the body and around 40% of the body mass and is responsible for around 70% to 80% of insulin-stimulated postprandial glucose uptake [29,30]. Although *Tff2* KO mice had decreased body weight and gastrocnemius muscle mass, muscle protein content was similar (data not shown). When we calculate protein expression in whole gastrocnemius muscle, KO mice had an increased expression of CD36, GLUT4 (trend) and NDUFB8 as well as succinate dehydrogenase complex, subunit B (trend) was seen (data not shown). These indicate that *Tff2* KO mice had a higher mitochondrial energy production in the skeletal muscle as well as an important energy utilization, which further contribute to increase energy expenditure. These metabolically active and energy consuming alterations in muscle might compensate the excess energy ingestion (HF condition), and led to the higher percentage of muscle mass in HF-fed *Tff2* KO mice.

The muscular metabolic activity is confirmed by the higher expression of both the gene *Nur77* and its protein NUR77 in the skeletal muscle of *Tff2* KO mice. Indeed, this represents a higher lipolysis [31] as well as an enhanced oxidative metabolism in the skeletal muscle [32] with a regulation of expression of genes linked to glucose metabolism [33]. This is further supported by the increased expression of both CD36 and *Glut4* (in HF-fed mice)/GLUT4 in the skeletal muscle due to its role in both fatty acid oxidation and glucose utilization. Importantly, in the skeletal muscle, *Nur77*, whose protein plays roles in glucose [33] and lipid metabolism [31], can be induced by the exercise [34]. Thus, the increased locomotor activity of *Tff2* KO mice might result in increased *Nur77*/NUR77 expression.

Although *Tff2* KO mice had a lower insulin concentration, they had a higher uptake and utilization of the glucose in muscles which indicates a higher insulin sensitivity. Such fact might be of a significant importance for a possible *Tff2*-targeting therapy for diseases involving insulin resistance. The decreased insulin concentration in *Tff2* KO mice could be the consequence of the implication of TFF2 in pancreatic β- cells proliferation [35].

10.6.3 Decreased lipids uptake and increased lipolysis in adipose tissue

Whereas adipocyte differentiation is inhibited by NUR77 [36], the inhibition of *Nur77* expression has no effect on the adipogenesis [37]. This correlates with the decreased NUR77 expression in the mesenteric adipose tissue as well as no difference in retroperitoneal adipocytes number (data not shown) and protein contents in the mesenteric adipose tissue of *Tff2* KO mice (data not shown). In addition, the lower serum insulin concentration of *Tff2* KO mice is also in accordance with this observation due to the facts that insulin was shown to induce *Nur77* in adipocytes [38] in addition to the role of insulin in the stimulation of adipogenesis [39] and the uptake and storage of lipids [40]. Put together, these observations correlate with the reduced WAT (reduced lipid storage). In addition, increases in NUR77 expression of WAT and serum insulin levels in WT mice fed

HF diet are supported by the data showing an upregulation of nuclear receptors (including NUR77) in human obesity [11].

In order to map the pathways, it is important to describe the role that SNS plays in controlling TG metabolism [41] especially in the adipose tissue [42]. Indeed, the effects of the SNS on the adipose tissue, such as the lipolysis of stored TG [42] and the release of FFA [43], explains the increased fasting FFA in *Tff2* KO mice serum. The innervation of BAT by the SNS is incontrovertible and represents the main stimulator of BAT thermogenesis via UCP1 activation [44]. Increased expressions of *Ucp1* in BAT and NUR77 in skeletal muscle of *Tff2* KO mice indicate increased activity of the SNS in these mice.

On the other hand, during a postprandial period, there is no change in the FFA uptake (CD36) in the adipose tissue whereas an increase was observed in the skeletal muscle of *Tff2* KO mice, indicating that lipid uptake and utilization is shifted toward the muscles and BAT rather than the adipose tissue. This would cover the metabolic needs of the muscles especially with the increased locomotor activity of *Tff2* KO mice [1]. These results, put together, suggest no effect on postprandial adipogenesis as well as increased fasting lipolysis in the adipose tissue of *Tff2* KO mice leading to reduced fat storage as well as the adjustment of the excessive uptake of the FFA by the skeletal muscle and BAT. This would explain the reduced body fat mass and the smaller retroperitoneal fat cell diameter in the *Tff2* KO mice [1]. Additionally, the elevated lipolysis followed by an increased liberation of FFA from the adipose tissue into the blood rather than a beta-oxidation of fatty acids (no difference in PPARA) explains the high serum FFA concentration.

Importantly, GLUT4 is involved in the glucose uptake in the adipose tissue [45]. In addition, the glucose is an important source of fat storage via insulin-induced lipogenesis. However, in this study, GLUT4 expression in the adipose tissue was not modulated in *Tff2* KO mice, which indicates that the contribution of postprandial adipogenesis in the adipose tissue is minimal.

10.6.4 Decreased lipolysis and glycolysis in the liver

The decrease of the PPARA expression, a transcription factor involved in the lipid metabolism [46], indicate a reduced lipid metabolism (β-oxidation) in the liver during a postprandial period. In addition, unaltered expression of CD36 involved in importing FFA into hepatocyte in *Tff2* KO mice indicate that there is no difference of the FFA uptake. Although *Tff2* KO mice hepatocytes had reduced β-oxidation rate for fatty acids, both the non-increase of FFA uptake in the liver and the reduced TG and glycerol serum levels would limit the liver lipid accumulation. This makes *Tff2* deficiency worth exploring to understand metabolic syndromes such as nonalcoholic fatty liver disease for which obesity is one of the main risk factors [47].

It has been shown that hepatic β-AR play a key role in the stimulation of both gluconeogenesis and glycogenolysis [48], which result in an elevated glycemia. In our study, *Tff2* KO mice showed a decreased expression of ADRB2 in the liver especially in

HF-fed condition, whereas no change has been seen in the skeletal muscle. This correlates with a work which has shown that a mechanism of downregulation of the β-AR is observed following agonist stimulation [49]. Furthermore, injection of β-AR agonists stimulates the expression of *Nur77* in the liver [50]. Importantly, *Nur77* induction in the liver increases the gluconeogenesis [51]. Such facts explain our observed data in term of decreased expression of ADRB2 and no change in NUR77 expression in the liver of the *Tff2* KO mice. Indeed, these suggest reduced gluconeogenesis and glycogenolysis in the hepatocytes that also contributes to maintain the low glycemia in *Tff2* KO mice during a postprandial period. In addition, there is no difference in glucose uptake by GLUT2, which further contributes to the glycemia maintenance.

While the reduced postprandial gluconeogenesis correlates with the limited uptake of FFA, that are mainly imported and used by the skeletal muscle and BAT, the reduced glycogenolysis corresponds well with the lower glycemia. The lower weight of liver and no difference in liver protein contents (data not shown) in *Tff2* KO mice could indicate a lower hepatic storage of glycogen [52] as well as water in hydrated glycogen [53] in addition to limited lipid accumulation, which further support that *Tff2* KO mice had less tendency to store energy.

10.6.5 Mapping *Tff2* deficiency-related pathways and conclusive perspectives

Our results, combined with our previous study [1], allow us to suggest pathways (Figure 10.3) behind the metabolic variations seen in *Tff2* KO mice compared to WT mice. This highlights the impact of *Tff2* deficiency on energy balance leading to anti-obesity effect, which could be either controlled by *Tff2* genes or influenced by pathways affected by *Tff2* related pathways. Indeed, although *Tff2* KO mice had a higher energy intake [1], they also had an increased energy utilization and expenditure and a lower energy storage. *Tff2* KO mice had an increased uptake and utilization of cell “fuels”, mostly lipids and glucose, mainly by the skeletal muscle and BAT which explains the decreased level of glucose, TG and glycerol levels in the serum. Moreover, the high TG fecal concentration [1] explains, in part, the de lower serum TG concentration in *Tff2* KO mice. In addition, since TFF2 is expressed in the gastrointestinal mucosa [4], the high TG fecal concentration [1] in *Tff2* KO mice may suggest that TFF2 plays a role in the digestion and absorbance of lipids at the gastrointestinal level especially that *Tff2* is a gene for which the expression is specifically increased following the ingestion of a HF meal [9].

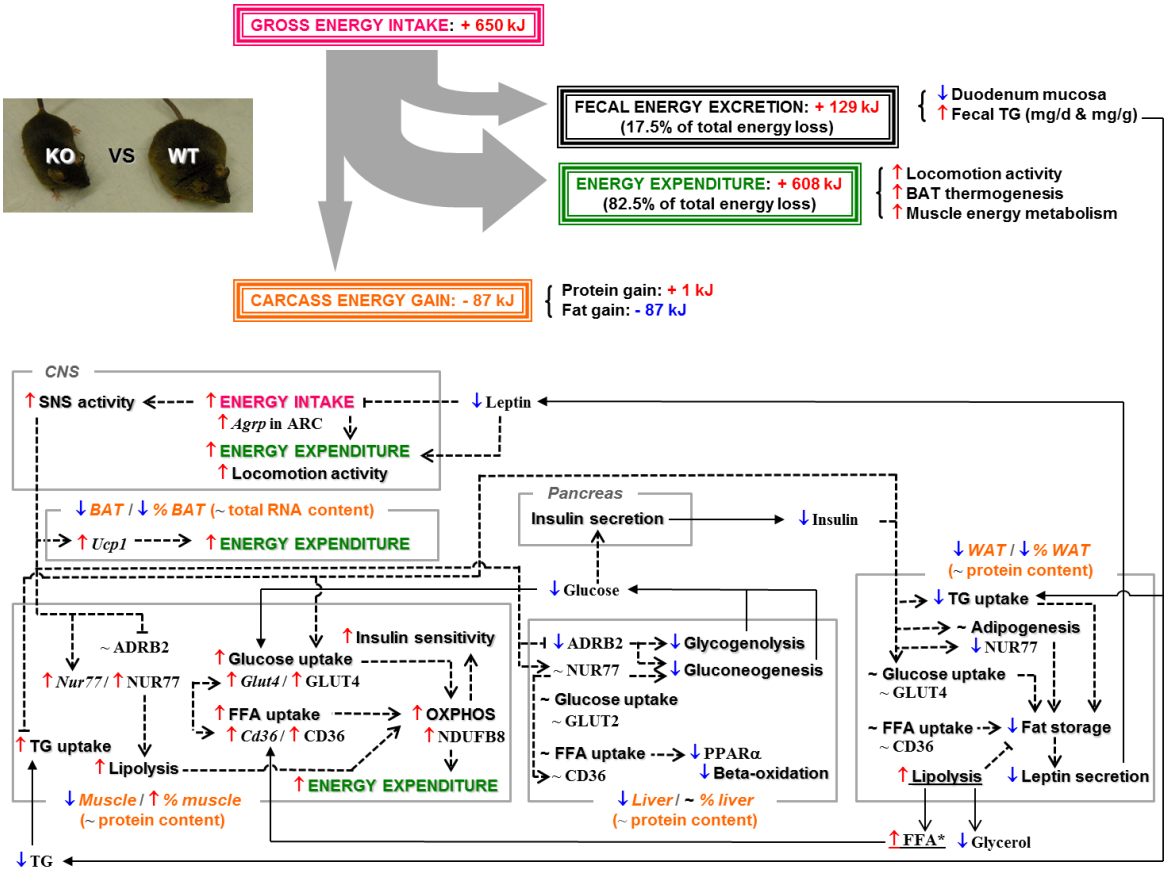


Figure 10.3. Suggested metabolic pathways variations seen in *Tff2* KO mice, compared to WT, and explaining the protection from high-fat diet-induced obesity.

Tff2 KO mice had a higher energy intake (+650 kJ) compared to WT mice, which was remarkably accompanied by a concomitant increase in energy expenditure (94% of energy intake) and to a smaller extent energy excretion (20% of energy intake), which resulted in a smaller body fat gain [1]. Lower serum leptin levels [1] and higher *Agrp* expression in the ARC [1] contributed to the increased energy intake which will leads to higher diet induced thermogenesis. On the other hand, increased *Ucp1* expression contributed to BAT thermogenesis, which indicates a higher SNS activity in *Tff2* KO mice. The higher SNS activity will further contribute to produce energy needed for increased locomotion activity by inducing muscle *Nur77*/NUR77. This led to increase in glucose (*Glut4*/GLUT4) and FFA (*Cd36*/CD36) uptakes, lipolysis and oxidative energy production (NDUFB8), which will improve insulin sensitivity. Thus, the decreased serum insulin levels limited TG uptake and adipogenesis in the white adipose tissue, which resulted in less fat storage and thus less leptin secretion. In addition, reduced intestinal mucosal length in *Tff2* KO mice will limit TG absorption, thus, increased TG (energy) excretion, which led to reduced serum TG levels and further contributed to the reduced fat storage.

*12-h fasting.

Abbreviations: ADRB2, β-2 adrenergic receptor; *Agrp*, agouti-related protein; ARC, arcuate nucleus of the hypothalamus; BAT, brown adipose tissue; CD36, fatty acids translocase; CNS, central nervous system; FFA, free fatty acids; GLUT2, glucose transporter 2 (solute carrier family 2); GLUT4, glucose transporter type 4; HF, high-fat; KO, knockout; NDUFB8, NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8; NUR77, nerve growth factor IB or NGFIB; OXPHOS, oxidative phosphorylation; PPARα, peroxisome proliferator-activated receptor alpha; SNS, sympathetic nervous system; *Tff2*, trefoil factor 2; TG, triglyceride; *Ucp1*, mitochondrial uncoupling protein1; WAT, white adipose tissue; WT, wild type. (For

interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The effects of *Tff2* deficiency could result from the fact that *Tff2* acts as a signaling molecule (probably HF-specific) acting on the central system to regulate the energy homeostasis at different levels. Moreover, the fact that no glucose concentration difference was seen after 12 h fasting but a significant difference was seen after 4 h fasting (KO < WT) (Table 10.2) strengthens the hypothesis of TFF2 acting as a signaling peptide regulating energy balance following meal ingestion (short-term regulating signal). Such hypothesis is further supported by the expression of this peptide in both gastrointestinal mucosa [4,5] and the CNS [7,54] and its specific modulation by HF intake [55,56] (lipid-induced signal). Importantly, knowing the effects TFF3 has on metabolism [57,58], the *Tff2*-deficiency effects could also result from modifications in the expression of TFF1 or TFF3 due what seems to be an interdependence of expression between TFF1, TFF2 and TFF3 [59,60]. Proceeding to similar investigations of the other trefoil factors could also lead to novel metabolic findings especially that they are also related to food intake and share similar biological functions with TFF2 [57,58].

Interestingly, the fact that *Tff2* KO mice had less fat mass and higher muscle mass percentage (similar muscle protein contents) whereas WT mice had less lean mass percentage [1] might highlight a role of *Tff2* is muscle development and could indicate a possible involvement of *Tff2* is sarcopenia which makes this gene worth exploring within the context of sarcopenia especially that sarcopenia is related to age [61] and in elderly, an age-related weight loss due to anorexia of aging was reported [62]. Additionally, *Tff2* KO mice showed increased appetite and food intake [1] which is also worth looking into for the anorexia.

In addition to contributing to our knowledge about TFF2 via studying *Tff2*-defecient models, this work provides new elements to better understand and develop novel therapies for obesity and the related diseases, including diabetes, metabolic syndrome and hyperlipidemia, and hopefully overcome the undesirable side effects known for the long-term obesity treatments. Our data would be strengthened by a further exploration of *Tff2* KO metabolic profile through analyses such as oral glucose tolerance test, insulin tolerance test and VO_2-VCO_2 exchange measurements that would provide substantial in-depth information about the respective role of metabolic organs to the phenotype of these *Tff2* KO mice.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2018.11.006.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Dr. Nikolaus Blin for providing our research group with three couples of each *Tff2* KO and WT mice with the same genetic background. We also thank Nesrine Nachi for performing a part of the western blot.

This work was supported by funding from the Heart and Stroke Foundation of Canada (HSFC), GIA number G-11-ST-4777.

Compliance with ethical requirements

All animal experimentation was conducted in accord with the guidelines of the Canadian Council on Animal Care and approved by the Animal Protection Committee of Laval University. This article does not contain any studies with human subjects.

10.7 References

[1] M.R. De Giorgio, et al., Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity, Obesity 21 (7) (2013) 1389–1395.
[2] E. Jequier, Leptin signaling, adiposity, and energy balance, Ann. N. Y. Acad. Sci. 967 (2002) 379–388.
[3] L. Thim, Trefoil peptides: from structure to function, Cell. Mol. Life Sci. 53 (11-12) (1997) 888–903.
[4] J. Madsen, et al., Tissue localization of human trefoil factors 1, 2, and 3, J. Histochem. Cytochem. 55 (5) (2007) 505–513.
[5] W. Hoffmann, W. Jagla, A. Wiede, Molecular medicine of TFF-peptides: from gut to brain, Histol. Histopathol. 16 (1) (2001) 319–334.
[6] D. Taupin, D.K. Podolsky, Trefoil factors: initiators of mucosal healing, Nat Rev Mol Cell Biol 4 (9) (2003) 721–732.
[7] M. Hinz, et al., Trefoil factor family (TFF) expression in the mouse brain and pituitary: changes in the developing cerebellum, Peptides 25 (5) (2004) 827–832.
[8] C. Bolduc, M. Yoshioka, J. St-Amand, Acute molecular mechanisms responsive to feeding and meal constitution in mesenteric adipose tissue, Obesity 18 (2) (2010) 410–413.
[9] M. Yoshioka, et al., High-fat meal-induced changes in the duodenum mucosa transcriptome, Obesity 16 (10) (2008) 2302–2307.
[10] M.R. De Giorgio, M. Yoshioka, J. St-Amand, Feeding induced changes in the hypothalamic transcriptome, Clin. Chim. Acta 406 (1–2) (2009) 103–107.
[11] V.L. Veum, et al., The nuclear receptors NUR77, NURR1 and NOR1 in obesity and during fat loss, Int. J. Obes. 36 (9) (2012) 1195–1202.
[12] V. Luu-The, et al., Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction, BioTechniques 38 (2) (2005) 287–293.
[13] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH image to ImageJ: 25 years of image analysis, Nat. Methods 9 (7) (2012) 671–675.
[14] S.C. Taylor, et al., A defined methodology for reliable quantification of Western blot data, Mol. Biotechnol. 55 (3) (2013) 217–226.
[15] S.C. Taylor, A. Posch, The Design of a Quantitative Western Blot Experiment, Biomed. Res. Int. 2014 (2014) 8.
[16] J.L. Halaas, et al., Weight-reducing effects of the plasma protein encoded by the obese gene, Science 269 (5223) (1995) 543–546.
[17] A. Matthias, et al., Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scidinduced thermogenesis, J. Biol. Chem. 275 (33) (2000) 25073–25081.
[18] J.A. Harrold, G. Williams, P.S. Widdowson, Changes in hypothalamic agouti-related protein (AGRP),

but not alpha-MSH or pro-opiomelanocortin concentrations in dietary-obese and food-restricted rats, Biochem. Biophys. Res. Commun. 258 (3) (1999) 574–577.
[19] M.M. Ollmann, et al., Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein, Science 278 (5335) (1997) 135–138.
[20] Y. Zhang, et al., Positional cloning of the mouse obese gene and its human homologue, Nature 372 (6505) (1994) 425–432.
[21] F. Depocas, The calorigenic response of cold-acclimated white rats to infused noradrenaline, Can. J. Biochem. Physiol. 38 (2) (1960) 107–114.
[22] G.M. Heaton, et al., Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation, Eur. J. Biochem. 82 (2) (1978) 515–521.
[23] J. Orava, et al., Different metabolic responses of human brown adipose tissue to activation by cold and insulin, Cell Metab. 14 (2) (2011) 272–279.
[24] A. Bartelt, et al., Brown adipose tissue activity controls triglyceride clearance, Nat. Med. 17 (2) (2011) 200–205.
[25] A. Fedorenko, P.V. Lishko, Y. Kirichok, Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria, Cell 151 (2) (2012) 400–413.
[26] N.J. Rothwell, M.J. Stock, Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis, Pflugers Arch. 389 (3) (1981) 237–242.
[27] M.A. Paschoalini, R.H. Migliorini, Participation of the CNS in the control of FFA mobilization during fasting in rabbits, Physiol. Behav. 47 (3) (1990) 461–465.
[28] S. Klein, et al., Effect of short- and long-term beta-adrenergic blockade on lipolysis during fasting in humans, Am. J. Phys. 257 (1 Pt 1) (1989) E65–E73.
[29] R.A. Defronzo, et al., The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization, Diabetes 30 (12) (1981) 1000–1007.
[30] P. Nuutila, et al., Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans, Am. J. Phys. 267 (6 Pt 1) (1994) E941–E946.
[31] M.A. Maxwell, et al., Nur77 regulates lipolysis in skeletal muscle cells. Evidence for cross-talk between the beta-adrenergic and an orphan nuclear hormone receptor pathway, J. Biol. Chem. 280 (13) (2005) 12573–12584.
[32] L.C. Chao, et al., Skeletal muscle Nur77 expression enhances oxidative metabolism and substrate utilization, J. Lipid Res. 53 (12) (2012) 2610–2619.
[33] L.C. Chao, et al., Nur77 coordinately regulates expression of genes linked to glucose metabolism in skeletal muscle, Mol. Endocrinol. 21 (9) (2007) 2152–2163.
[34] D.J. Mahoney, et al., Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise, FASEB J. 19 (11) (2005) 1498–1500.
[35] K. Orime, et al., Trefoil factor 2 promotes cell proliferation in pancreatic beta-cells through CXCR-4-mediated ERK1/2 phosphorylation, Endocrinology 154 (1) (2013) 54–64.
[36] L.C. Chao, et al., Inhibition of adipocyte differentiation by Nur77, Nurr1, and Nor1, Mol. Endocrinol. 22 (12) (2008) 2596–2608.
[37] W.S. Au, et al., The NR4A family of orphan nuclear receptors are not required for adipogenesis, Int. J. Obes. 32 (2) (2008) 388–392.
[38] Y. Fu, et al., NR4A orphan nuclear receptors modulate insulin action and the glucose transport system: potential role in insulin resistance, J. Biol. Chem. 282 (43) (2007) 31525–31533.
[39] D.J. Klemm, et al., Insulin-induced adipocyte differentiation. Activation of CREB rescues adipogenesis from the arrest caused by inhibition of prenylation, J. Biol. Chem. 276 (30) (2001) 28430–28435.
[40] K.N. Frayn, et al., Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to postprandial transition, Am. J. Phys. 266 (3 Pt 1) (1994) E308–E317.
[41] J.J. Geerling, et al., Sympathetic nervous system control of triglyceride metabolism: novel concepts derived from recent studies, J. Lipid Res. 55 (2) (2014) 180–189.

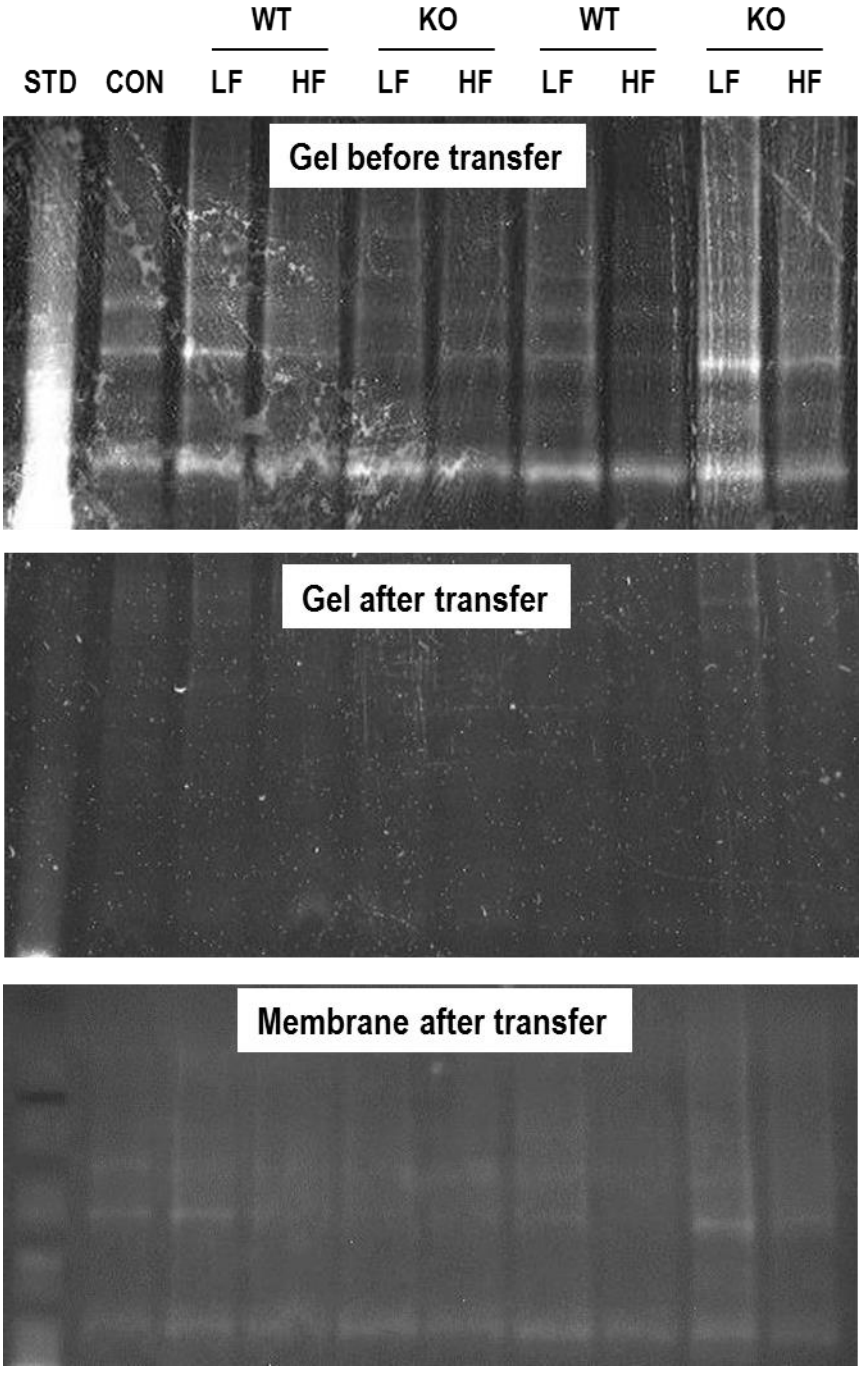
[42] T.J. Bartness, C.K. Song, Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue, J. Lipid Res. 48 (8) (2007) 1655–1672.
[43] S. Rosell, Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation, Acta Physiol. Scand. 67 (3) (1966) 343–351.
[44] T.J. Bartness, C.H. Vaughan, C.K. Song, Sympathetic and sensory innervation of brown adipose tissue, Int. J. Obes. 34 (2010) S36.
[45] P.R. Shepherd, B.B. Kahn, Glucose transporters and insulin action–implications for insulin resistance and diabetes mellitus, N. Engl. J. Med. 341 (4) (1999) 248–257.
[46] W. Wahli, O. Braissant, B. Desvergne, Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more, Chem. Biol. 2 (5) (1995) 261–266.
[47] P. Angulo, Nonalcoholic fatty liver disease, N. Engl. J. Med. 346 (16) (2002) 1221–1231.
[48] P.H. Schmelck, J. Hanoune, The hepatic adrenergic receptors, Mol. Cell. Biochem. 33 (1–2) (1980) 35–48.
[49] J.L. Benovic, et al., Regulation of adenylyl cyclase-coupled beta-adrenergic receptors, Annu. Rev. Cell Biol. 4 (1988) 405–428.
[50] S.A. Myers, et al., Beta-adrenergic signaling regulates NR4A nuclear receptor and metabolic gene expression in multiple tissues, Mol. Cell. Endocrinol. 309 (1–2) (2009) 101–108.
[51] L. Pei, et al., NR4A orphan nuclear receptors are transcriptional regulators of hepatic glucose metabolism, Nat. Med. 12 (9) (2006) 1048–1055.
[52] G.A. Leveille, K. Chakrabarty, Diurnal variations in tissue glycogen and liver weight of meal-fed rats, J. Nutr. 93 (4) (1967) 546–554.
[53] S.N. Kreitzman, A.Y. Coxon, K.F. Szaz, Glycogen storage: illusions of easy weight loss, excessive weight regain, and distortions in estimates of body composition, Am. J. Clin. Nutr. 56 (1 Suppl) (1992) 292S–293S.
[54] M.R. De Giorgio, et al., Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity, Obesity (Silver Spring) 21 (7) (2013) 1389–1395.
[55] M. Yoshioka, et al., High-fat meal-induced changes in the duodenum mucosa transcriptome, Obesity (Silver Spring) 16 (10) (2008) 2302–2307.
[56] O. Mucunguzi, et al., Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine, Nutr. Metab. (Lond.) 14 (2017) 66.
[57] H. Ge, et al., Trefoil factor 3 (TFF3) is regulated by food intake, improves glucose tolerance and induces mucinous metaplasia, PLoS One 10 (6) (2015) e0126924.
[58] Y. Xue, et al., Tff3, as a novel peptide, regulates hepatic glucose metabolism, PLoS One 8 (9) (2013) e75240.
[59] D. Taupin, et al., The trefoil gene family are coordinately expressed immediateearly genes: EGF receptor- and MAP kinase-dependent interregulation, J. Clin. Invest. 103 (9) (1999) R31–R38.
[60] O. Lefebvre, et al., Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein, Science 274 (5285) (1996) 259–262.
[61] I.H. Rosenberg, Summary comments, Am. J. Clin. Nutr. 50 (5) (1989) 1231–1233.
[62] J.E. Morley, Protein-energy malnutrition in older subjects, Proc. Nutr. Soc. 57 (4) (1998) 587–592.

10.8 Appendix 10.1. Q_RT-PCR primers information

Gene name	GenBank Accession	Size	Region used for primer pairs	Primer sequence
Cd36	NM_007643	199	1216-138	AGCCTCCTTTCCACCTTTTGTTA/ ATTCTGGAGGGGTGATGCAAAG
Glut4	NM_009204	207	1571-174	TATGGGTCCTTACGTCTTCCTTCTAT/ TTTGCCCCTCAGTCATTCTCATC
Nur77	NM_010444	179	1203-138	CCTCCAGATGCCTCCCCTACC/ TCCAGGAACCAGAGAGCAAGTC
Ppargc1a	NM_008904	177	922-1038	GACCCCAGAGTCACCAAATGA/ GGAGGAGTTGTGGGAGGAGTTA
Ucp3	NM_009464	198	835-1032	TCCCCTGTCACTTTGTCTCTGC/ GACGCAGAAAGGAGGGCACAA
Mtco1	NC_005089	159	6516-667	TTCCCATTATTTTCAGGCTTCACCC/ TGTGGTGTAAGCATCGGGTAGTCT

Abbreviations: ***Cd36,*** fatty acids translocase; ***Glut4,*** glucose transporter type 4; ***MtCo1,*** mitochondrially encoded cytochrome c oxidase I; ***Nur77,*** nerve growth factor IB; ***Ppargc1a,*** peroxisome proliferator-activated receptor γ coactivator 1α; ***Ucp3,*** mitochondrial uncoupling protein 3.

10.9 Appendix 10.2. Example of gel and membrane images



Abbreviations: **STD**, molecular weight standard; **CON**, pooled sample (positive control); **WT**, wild type; **KO**, knockout; **LF**, low-fat; **HF**, high-fat.

Chapter 11. Review - Trefoil Factor Family Member 2: From a High-Fat-Induced Gene to a Potential Obesity Therapy Target

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, CREMI, Québec Genome Center, CHUL-CHU de Québec Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

Received: 30 July 2021; Accepted: 10 August 2021; Published: 12 August 2021

11.1 Résumé (French abstract)

Le trefoil factor family member 2 (*Tff2*) a été caractérisé comme spécifiquement induit par la diète riche en gras (HFD). TFF2 a également été lié à divers mécanismes neurologiques et propriétés métaboliques suggérant son rôle dans l'équilibre énergétique. L'hypothèse est que TFF2 serait un signal induit par la HFD qui régule le métabolisme, principalement lipidique. Dans le cadre de cette revue, nous mettons l'accent sur les principales conclusions mettant en évidence cette hypothèse. Il est important de noter que les mécanismes hypothétiques mis en évidence sont que TFF2 est un contributeur important du développement de l'obésité via l'augmentation de l'absorption intestinale des lipides et de l'anabolisme. Par conséquent, une perspective pour les activités expérimentales futures et l'évaluation du potentiel thérapeutique de l'inhibition de TFF2 est donnée. En effet, son renversement ou sa régulation négative contribuerait à un phénotype d'anti-obésité.

11.2 Abstract

Obesity has its epidemiological patterns continuously increasing. With controlling both diet and exercise being the main approaches to manage the energy metabolism balance, a high-fat (HF) diet is of particular importance. Indeed, lipids have a low satiety potential but a high caloric density. Thus, focusing on pharmacologically targetable pathways remains an approach with promising therapeutic potential. Within this context, trefoil factor family member 2 (*Tff2*) has been characterized as specifically induced by HF diet rather than low-fat diet. TFF2 has also been linked to diverse neurological mechanisms and metabolic patterns suggesting its role in energy balance. The hypothesis is that TFF2 would be a HF diet-induced signal that regulates metabolism with a focus on lipids. Within this review, we put the spotlight on key findings highlighting this line of thought. Importantly, the hypothetical mechanisms pointed highlight TFF2 as an important

contributor to obesity development via increasing lipids intestinal absorption and anabolism. Therefore, an outlook for future experimental activities and evaluation of the therapeutic potential of TFF2 inhibition is given. Indeed, its knockdown or downregulation would contribute to an antiobesity phenotype. We believe this work represents an addition to our understanding of the lipidic molecular implications in obesity, which will contribute to develop therapies aiming to manage the lipidic metabolic pathways including the absorption, storage and metabolism via targeting TFF2-related pathways. We briefly discuss important relevant concepts for both basic and clinical researchers.

Keywords: trefoil factor family member 2; high-fat; metabolism; obesity

11.3 Focusing on Lipids in Obesity and Metabolic Disorders

Since lipid metabolism is the main component of energy balance, we can expect that humans will have hormone(s) which are specific sensors for high-fat (HF) intake in order to command the brain to stop eating. Still, no such hormone has been identified. The best strategy to develop a treatment for fat intake is to target the time before meal ingestion [1]. Based on this principle, our idea is to identify the HF-diet induced satiety hormone/peptide differentially regulated between 30 min to 3 h after HF compared to low-fat/high-carbohydrate (LF) meal and fasting in order to develop a potential drug given before the meal as a part of antiobesity therapy. Obesity, metabolic syndrome, cardiovascular disease (CVD) and type 2 diabetes (T2D) are complex multifactorial clinical conditions with altered energy balance and potentially similar candidate genes and therapeutic factors, involving complex gene–gene and gene–environment interactions [2–18]. Dietary lipids are among the most important environmental factors in all these diseases [19–21]. Obesity, considered as a disease [22] and epidemic in industrialized countries brings obesity as a very critical risk factor for various diseases, [23] including the ongoing COVID-19 pandemic [24,25]. For instance, in 2015, there were over 600 million adults and over 100 million children that were obese worldwide and the numbers are continuously increasing [26]. This epidemic has a tremendous impact on public health, since obesity, especially intra-abdominal fat mass, is associated with a dysregulation of lipoprotein-lipid metabolism and several pathologies and health problems including CVD, T2D, liver disease, impaired regeneration, and cancers [8,27–33]. Studies have shown that obesity also independently impacts mortality [28,29,34,35]. Weight loss based on either caloric restrictions or pharmacological agents simultaneously improves most CVD risk factors [36–38]. However, limited FDA-approved drugs are available, and all of them have important drawbacks. Moreover, body weight management represents a growing and multibillion dollar market [39].

Obesity, also suggested to be a neuroendocrine reprogramming combined to a broken energy homeostasis [40,41], can result from the cumulative effect of a repeated

energy imbalance, with minor excesses in energy intake (EI) over energy expenditure (EE) [42]. HF food promotes weight gain through both EI and EE, according to the high caloric density, low satiety effect and high palatability of HF nutrients, as well as the weak potency for fat oxidation and EE that are associated to fat ingestion [19–21]. Thus, the acute control of fat intake is a major determinant in the etiology of obesity. Feeding behaviour is controlled by short-term circulating nutrients and hormones, as well as signals derived from the peripheral tissues in response to a meal and changes in energy stores. The hypothalamus is a key brain center upon which all these peripheral signals converge to regulate feeding behaviour and EI [43]. Therefore, as a preliminary step to discover the peripheral factor controlling fat intake and other determinants of energy balance, we previously used functional genomic strategy to investigate gastric, intestinal, fat, and hypothalamic genes differentially regulated by the ingestion of HF and LF meals [44–47].

Importantly, whereas we have insulin as a signal triggered by glucose that contributes to balancing metabolism and glycemia, there is a need to further explore lipids specific signals. This would represent an additional step toward controlling/influencing the energy balance with a focus on the HF diet and its related signals.

11.4 Trefoil Factor 2 (*Tff2*) as a High-Fat-Induced Gene

Functional genomics have been proved as a strong tool to characterize many genes specifically induced by different conditions including those related to obesity and within the context of diet and exercise [48–50]. For instance, we have identified hundreds of genes modulated after HF or LF meals using the serial analysis of gene expression (SAGE) method [44–47].

In our previous study [51], the differentially expressed transcripts after LF or HF meals compared to fasting condition were classified into one of the three following patterns: Meal responsive (commonly modulated by both meals), LF-specific (modulated only in LF condition) and HF-specific (modulated only in HF condition). Then, using the following

criteria, we have selected new candidate genes for controlling appetite and satiety: (1) HF or LF-specific genes, since lowering fat appetite or increasing satiety could lead to efficient and safe therapies for obesity compared to the interventions decreasing the overall food intake. (2) Genes coding for secreted proteins. Feeding behaviour is controlled by short-term circulating nutrients and hormones as well as signals derived from the peripheral tissues in response to a meal and changes in energy stores. In addition, when the facility of drug delivery method is taken into account, it is reasonable to target secreted proteins. (3) Genes not coding for nutrient's digestion and absorption. The characterization of proteins involved in lipid digestion and absorption has already been studied for decades. Since the lipase inhibitor Orlistat has undesirable side effects [39], new drugs targeting different pathways or functions are needed. (4) Gene not coding for known appetite/satiety signals, since new candidates are of interest. (5) Genes with potential interest based on

published literature. These include genes whose relationship with known appetite/satiety genes have been reported, as well as genes whose involvement in energy balance has been reported but not in feeding behaviour. (6) Patterns and magnitude of expression. These include genes strongly modulated by LF or HF meal, genes showing modulation at interesting time points, and groups of genes showing similar pattern of expressions. (7) Genes with their expression levels confirmed by other methods, such as quantitative real-time PCR (Q_RT-PCR) and Western blot. As a result, trefoil factor 2 (*Tff2*) was the top ranked new candidate gene. Since we found no work focusing on TFF2 implications within either obesity or HF diet, it was important to focus our next exploration on the mechanisms of TFF2 involvement in energy balance from in vitro to in vivo.

11.5 TFF2 Metabolic Properties and Implications

TFFs constitute a family of polypeptides with a distinctive structural module characterized by six cysteine residues [52]. This structural feature has a stabilizing effect via intramolecular disulfide bridges which are responsible for the remarkable protease resistance of TFFs [53], resulting in a distinct and unusual supersecondary structure clearly identifying the trefoil polypeptide domain as a unique growth factor-associated module, structurally unrelated to other highly disulfide-linked modules such as those in epidermal growth factor and insulin-like growth factor-I [54–57]. TFFs are small secreted proteins involved in protection of the mucosal lining of the gastrointestinal tract [58,59]. They protect against gastrointestinal damage by stimulating the migration of adjacent epithelial cells, a process termed “restitution”, and by virtue of their interaction with mucins and other proteins [60–62].

Tissue localization analyses show the highest expression of TFF2 (also known as SP; SML1) in stomach/duodenum [63]. TFF2 has structural homology with growth hormone and has been detected in blood streams [54,64,65]. Moreover, *Tff2* is specifically upregulated by HF meals in gastrointestinal mucosa, whereas downregulated in hypothalamus [44,66]. This is in agreement with the expression pattern of a peripheral signal secreted to inform the brain to stop eating fat. We have shown that *Tff2* knockout (KO) mice have increased EI, EE, and excretion, as well as lower abdominal adipose tissue [66].

Studies with *Tff2*-deficient mice have not only shown the evidence of decreased gastric cell proliferation, increased acid secretion, and increased susceptibility to gastric mucosal injury [67], but have also revealed new aspects of *Tff2*’s role in the immune response such as changes in the expression of diverse crucial genes involved in innate and adaptive immunity [68,69], including defensins which regulate the composition of the intestinal bacterial microbiome [70]. The innate and adaptive immunity as well as microbiota are dysregulated in obesity and metabolic disorders such as insulin resistance [71,72]. Moreover, TFF2 secretion can be regulated by both pro-inflammatory and anti-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin 4 (IL-

4), and IL-13, and in turn influence cytokine release and activation (i.e., IL-1, IL-6) [69], as well as immune cell recruitment [73]. This correlates with its suggested roles both as anti-inflammatory [74] and in reducing immune-mediated damage [75].

Furthermore, expression of apolipoprotein A-IV *(Apoa4*) which has an anti-inflammatory effect and inhibits gastric motility, emptying and acid secretion [76,77], has been shown to be upregulated in the stomach and duodenum of *Tff2*-deficient mice [68,69]. Importantly, Apolipoprotein A-IV (apoA-IV) is a satiety signal induced by lipid ingestion [78]. Moreover, the circadian rhythm of pancreatic polypeptide expression, which is known to regulate food intake, is negatively correlated with gastric *Tff2* circadian rhythm [79]. *Tff2* expression has been shown to be regulated by peroxisome proliferator-activated receptor (PPAR) γ [80], which is established as an important target for the treatment of T2D and other disorders associated with HF intake [81]. Finally, the repression of *Apoa4* was followed by *Tff2* whose modulation was concomitant with fat intake [44]. Therefore, this evidence suggest that *Tff2* might be a novel candidate gene for fat satiety control. However, the roles of this protein in the regulation of feeding behaviour, energy balance and development of obesity are yet to be fully understood. Therefore, the characterization of the mechanisms will be the first step to understand its roles, as well as to develop new therapeutic targets to control food and fat intakes.

It is widely accepted that TFFs exert their biological action through a cell surface receptor [82]. Concordantly, several membrane proteins are found to interact with TFF2 [83–86]. Recently, Dubeykovskaya et al. demonstrated an ability of TFF2 peptide to activate Ca^{2+}-Akt-ERK1/2 (for extracellular-signal-regulated kinases 1/2) signaling pathway via chemokine (C-X-C motif) receptor 4 (CXCR4). Interestingly, CXCR4 was first reported as bovine neuropeptide Y (NPY) Y3 receptor [87]. Moreover, following 12 weeks of feeding, *Tff2* KO mice exhibited lower abdominal adipose tissue and diameter of retroperitoneal adipocytes compared to the wild-type mice [66]. Furthermore, the *Tff2* KO mice showed increased EE and excretion, whereas higher EI were observed. Digestive energy efficiency, duodenum mucosal length, glycemia, as well as insulin and leptin levels, were lower in KO mice [66]. Appetite signal, agouti-related protein (*Agrp*), expression was higher in the hypothalamus [66]. Thus, TFF2 seems to be a mastermind regulator of overall energy balance. Consequently, it is important to investigate the various mechanisms of action of TFF2 on energy balance corresponding not only to new, but also multiple opportunities to eventually prevent and treat obesity, which remains to this day a major challenge to public health providers.

In order to identify the mechanisms whereby TFF2 regulates feeding, we need to explore the pathways of TFF2 action on feeding. *Tff2* KO mice showed HF-specific increase in meal intake [66]. Any satiety signal induced by food intake (FI) may reach the central nervous system (CNS) either through blood circulation or vagal nerve afferences relaying gastrointestinal stimuli. Since *Tff2* is expressed in the gastric mucosa, and that oral/systemic administration of radioactive TFF2 was found in blood, digestive tracts, and

brain, TFF2 might act as a peripheral signal to the CNS [64]. It is worth noting that no adverse metabolic effect has been reported with oral/systemic administration of TFF2 [64].

On the other hand, regarding the biological links, there are connections between CXCR4 and appetite/satiety signals (possibly involving TFF2, Figure 11.1). Indeed, TFF2 receptor, CXCR4, is localized within areas rich in dopaminergic (DA) neurons [88]. Mesolimbic/mesocortical DA neurons are essential for reward and motivational behaviours [89]. Thus, there would be an important addition to elucidate whether CXCR4 is expressed in the same neurons expressing appetite (*Npy*/*Agrp*)/satiety (proopiomelanocortin, *Pomc*/cocaineamphetamine regulated transcript, *Cart* and corticotrophin releasing hormone, *Crh*) signals. We have shown higher *Agrp* expression in the hypothalamus of *Tff2* KO mice [66]. To elucidate how TFF2 modulates energy expenditure, there is a need to focus on energy expenditure since there are evidence that a regulatory control on EE is exerted through the sympathetic nervous system, especially on BAT thermogenesis by uncoupling protein 1 (UCP1) [90]. The TFF2 receptor, CXCR4 is involved in mesolimbic/mesocortical DA system to modulate locomotor activity and our data showed higher locomotion activity in *Tff2* KO mice [91]. These suggest the involvement of mesolimbic/mesocortical DA system in the modulation of locomotion activity in *Tff2* KO mice via CXCR4. Therefore, behavioural activity after TFF2 injection with or without pre infusion of CXCR4 is to be investigated as well [66,91]. Importantly, expression of CXCR4 in other tissues including digestive tract and metabolic tissues [92–100], could suggest a metabolic action of TFF2 via CXCR4 within these tissues.

Regarding the EE during HF or LF feeding, we have demonstrated that *Tff2* KO mice exhibited higher locomotor activity and total EE than WT animals [66]. The metabolic exploration of *Tff2* KO mice revealed interesting patterns for the lipid intake and excretion as well as the energy balance within the key metabolic tissues, as well the body and tissue weights [101]. Briefly, the *Tff2* KO in mice resulted in lower glucose, triglycerides (TG), and glycerol serum levels with a metabolism towards less fat storage and increased EE by enhancing lipid and glucose utilization via oxidative phosphorylation in the key metabolic tissues (muscle, liver and adipose tissues) [101]. The *Tff2* KO also led to reduced body and adipose tissues weights [101].

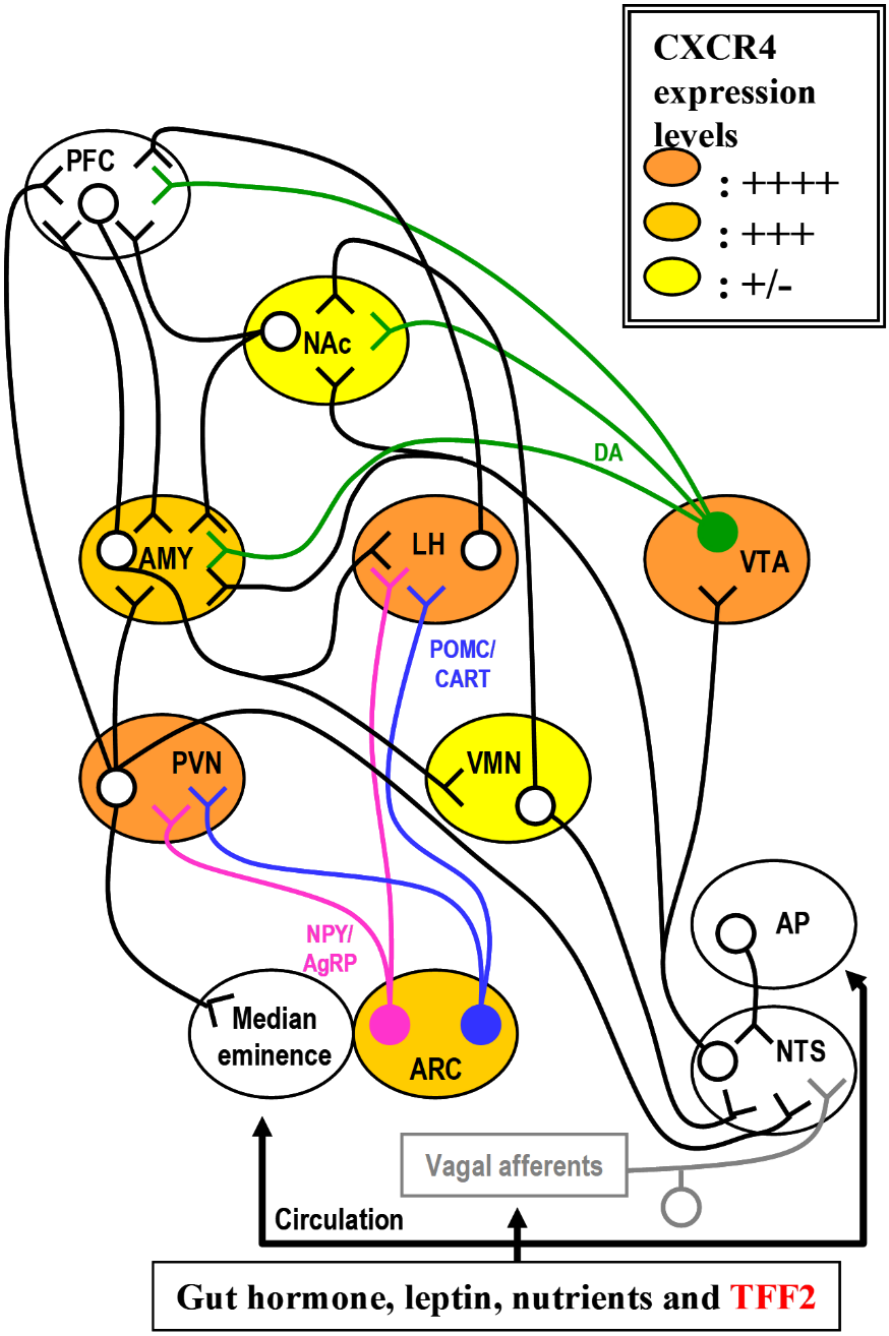


Figure 11.1. Possible connection between CXCR4 and appetite/satiety signals.
Meal-modulated hormonal and neuronal signals from the gut are received via the blood in the area postrema (AP) and through vagal afferent fibers in the nucleus of the solitary tract (NTS), respectively. These sensory inputs are transmitted via the ventral tegmental area (VTA) to other centers, including the amygdala (AMY) and nucleus accumbens (Nac), where dopaminergic (DA) and other signals act in reward processes. Inputs from these pathways are integrated with circulating signals of nutritional state which are detected in the arcuate nucleus (ARC) via the median eminence. Within the ARC, the activity of neurons expressing proopiomelanocortin (POMC) is stimulated, while that of neurons expressing neuropeptide Y (NPY) is inhibited by leptin. Axons from both types of neurons project in parallel to the paraventricular nuclei (PVN) and lateral hypothalamus (LH). Release of α melanocytestimulating hormone by POMC-expressing neurons leads to activation of the melanocortin receptor 4 (MC4R), which lowers food intake and increases energy

expenditure (EE). By contrast, release of NPY activates Y1 and Y5 receptors, which increases food intake and reduces EE. NPY-expressing neurons also release agouti-related protein (AgRP), an antagonist of MC4R. This dual innervation within the PVN modulates EE via the thyroid and adrenal axis and the sympathetic nervous system. *Abbreviations*: CART, cocaine–amphetamine regulated transcript; CXCR4, chemokine (C-X-C motif) receptor 4; PFC, prefrontal cortex; TFF2, trefoil factor family member 2; VMN, ventromedial nuclei.

11.6 Experimental Perspectives

In order to complete the puzzle surrounding the molecular mechanisms and pathways of the multifunctional TFF2 in the context of lipid-related signals along with a possible connection between fat sensing in gut and the satiety signals in hypothalamus, further studies can be suggested.

Following fat digestion by lipases, the lipolytic products are absorbed by the enterocytes where chylomicrons are formed and secreted. The assembly of these TG-rich lipoproteins within the enterocyte is a multistep pathway including: (1) the uptake (CD36 and FA transport protein 4, FATP4) and translocation of lipolytic products from the brush border membrane to the endoplasmic reticulum by FA binding protein 1/2 (FABP1/2); (2) TG synthesis via monoacylglycerol pathway (monoacylglycerol acyltransferase 2, MGAT2 and diglyceride acyltransferase 2, DGAT2) and phosphatidic acid pathway; (3) the packaging of lipid and apolipoprotein components into lipoprotein particles (apoB-48 and apoA-IV); (4) the transport into the Golgi for secretion (microsomal TG transport protein, MTP) [102]. We have already reported that many genes listed above have been modulated by HF and LF meal ingestion [44]. Thus, the expression levels of such key genes and proteins involved in lipid absorption are important to understand.

There are several practical steps that would also allow further exploration of TFF2 metabolic implications. These include injection of this secreted (recombinant protein) protein to mice orally, intravenously and intracerebroventricularly [103–113]. Brain sections can be processed for immunocytochemical detection of CXCR4 [88], and in situ hybridization of *Npy*, *Agrp*, *Pomc*, *Cart* and *Crh* in mice either fasted or refeeding with HF or LF meal to make a link between the expression of the molecules and the corresponding *Tff2* expression depending on the status (fasting, LF feeding and HF feeding). Along with the expression of other peripheral appetite/satiety signals (ghrelin, cholecystokinin, PYY, and apoA-IV) [114] combined with c-Fos expression that is particularly reliable to assess brain activations in response to feeding [103], an in situ hybridization of *Npy*, *Agrp*, *Pomc*, *Cart* and *Crh* mRNA can be performed to determine whether NPY/AgRP, POMC/CART and CRH cells will be activated [103,105,106]. It has been shown that c-Fos expression is induced by refeeding [103], and the subcutaneous injection of peptide YY (PYY) in the nucleus of the solitary tract (NTS) [115] which represents an additional molecular link. In addition, an in vitro model such as Caco-2 cells, used as a valid model of study for lipid/lipoprotein homeostasis [114,116], would allow us to study the lipid synthesis, secretion, and metabolism variations with both *Tff2* knockdown and overexpression.

Similar studies can be conducted in the diverse existent animal models of obesity as well [117].

11.7 Conclusion

The functional genomics studies that revealed *Tff2* as a HF-induced gene combined with the observations of the metabolic implications of TFF2, as well as the metabolic phenotype of *Tff2* KO mice allow us to build a hypothetic path regarding a TFF2 mechanism as a response to HF diet (Figure 11.2). The HF diet induces an overexpression of TFF2 which acts not only towards energy balance centers, (Figure 11.1) but would also facilitate the lipids intestinal absorption. This explains that the *Tff2* KO mice had an increased energy excretion in form of TG (lipids not absorbed). In addition, the *Tff2* KO resulting in an increased metabolic activity of both lipids and glucose in the mice (that ultimately led to the protection from the HF diet-induced obesity) points TFF2 as a lipid anabolic (and possibly a carbohydrates conversion into lipids) factor and explains the reduced adiposity in *Tff2* KO mice. On the other hand, the other properties associated to TFF2 such as anti-inflammatory [74] and reducing immune-mediated damage [75] would indicate roles that would balance some of the HF diet consequences, such as inflammation and immune induction. This correlates with the protective roles TFF2 plays in the digestive mucosa as well. Since during fat absorption apoA-IV is secreted into intestinal lymph [118], the upregulation of Apoa4 in *Tff2*-deficient mice [68,69] could be a regulatory mechanism aiming to compensate the reduced lipid absorption resulting from the absence of TFF2. Taken together, these elements highlight TFF2 as a molecule induced by HF diet in order to facilitate the absorption of the lipids, their storage, as well as their anabolism, along with the induction of mechanism that would correct or reduce the negative impacts of HF diet in terms of inflammation and immune impacts. Ultimately, we would conclude that TFF2, as a lipid anabolic factor and a lipid absorption facilitator, would contribute to obesity establishment. Such important molecular implication in obesity pathogenesis fits well and explains the reported protection from HF diet-induced obesity seen in *Tff2* KO mice.

Within this context, there is a potential to develop new treatments for obesity and related diseases by characterizing the molecular mechanisms by which TFF2 controls energy balance and target the related pathways. The next objectives are to identify the mechanisms by which TFF2 regulates feeding, EE, and energy excretion, as well as explore the roles of TFF2 in obesity and related diseases. Future studies will allow the characterization of potential therapeutic targets which can be used for the treatment of obesity and related diseases by the administration of the pharmaceutical inhibitors of TFF2 pathway among other options. The ultimate goal is to develop a novel generation of treatments and therapeutic targets for obesity and metabolic disorders that would pharmacologically target TFF2 pathways to mimic the antiobesity effects of *Tff2* KO (Figure 11.2). The putative advantages of interference with intestinal lipid absorption in

course of TFF2 blockade compared to the well-established therapeutic option of lipase inhibition by orlistat could be summarized within several points. Unlike orlistat [119,120], the *Tff2* KO mice did not have diarrhea, although they had an increased TG excretion [66]. TFF2 blockade would also lead to an increased EE. In addition, targeting TFF2 pathway would lead to an antiobesity effect not only via the interference with intestinal lipid absorption but also through the energy metabolism control center as well as the key metabolic tissues. However, the involvement of TFF2 in different functions [86,121–123], such as mucosa protection, imposes a specific pharmacovigilance for such potential pharmacological approaches such as prioritizing targeting the peripheral (intestinal) TFF2 pathways rather than the central (brain) pathways, such as using a CXCR4 antagonist.

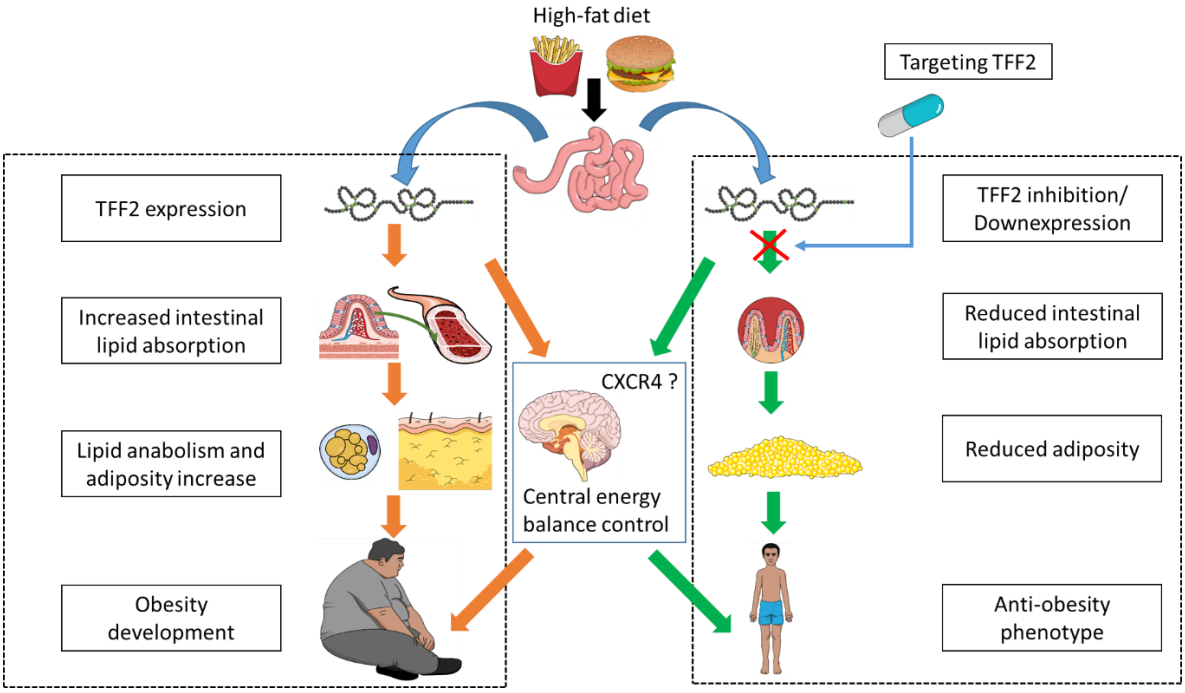


Figure 11.2. TFF2 hypothetic implications in obesity development and its potential therapeutic targeting. The high-fat diet increases TFF2 expression. This would lead to the facilitation of the intestinal lipid absorption, as well as the increase in lipids storage in part, at least, via increasing anabolism. All these contribute to obesity development. Thus, inhibiting or reducing the expression or the action of TFF2 would reduce the lipid intestinal absorption as well as the adiposity. That points targeting TFF2 and its related pathways as a potential antiobesity therapeutic approach. Both TFF2 action and inhibiting TFF2 mechanisms would involve CXCR4 pathways. *Abbreviations*: CXCR4, chemokine (C-X-C motif) receptor 4; TFF2, trefoil factor family member 2.

Importantly, since intestinal villi increase the absorptive area and the surface area of the intestinal wall and the implication of TFF2 in the mucosa, possible morphological changes of such pharmacological targeting could affect nutrients absorption as well. In addition, an assessment of metabolic markers in the key metabolic tissues including those revealed by the metabolic exploration of *Tff2* KO mice [101] would be important for such

pharmacological agents. That includes the fatty acids translocase in the skeletal muscle, as well as the nerve growth factor IB (NGFIB, also known as *Nur77*) that both coordinately regulates the expression of genes linked to glucose metabolism, including insulin sensitive glucose transporter type 4 (*Glut4*) [124] and regulates lipolysis and gene expression of *Ucp2/3*, *Pgc1a, Cd36* [125,126]. In the liver, Nur77 stimulates glucose production and induces expression of genes involved in gluconeogenesis [126]. Adiponectin, which is exclusively secreted from adipose tissue, also affects these insulin-sensitizing processes by stimulating fatty acids oxidation in the skeletal muscle and repressing hepatic-glucose production [127]. PPARγ is a master regulator of adipocyte differentiation and function [128]. Thus, along with leptin and adiponectin, these elements are at the heart of the molecular explorations required towards developing "lipid metabolism-controlling pills".

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content, exchanged ideas and suggestions (concepts to add, the figures, references selection, etc.) throughout the writing process, edited, and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi is the recipient of the scholarship « Bourse Tremplin Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec–Sante (FRQS), Quebec, Canada. Figure 11.2 was created using images from https://mindthegraph.com/ and http://smart.servier.com, accessed on 25 July 2021. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License. (Accessed on 25 July 2021).

Conflicts of Interest: The authors declare that there is no conflict of interest.

11.8 References

1. Yoshioka, M.; St-Pierre, S.; Drapeau, V.; Dionne, I.; Doucet, E.; Suzuki, M.; Tremblay, A. Effect of red pepper on feeding behavior and energy intake. *Br. J. Nutr.* 1999, *82*, 115–123.
2. Lusis, A.J. Genetic factors affecting blood lipoproteins: The candidate gene approach. *J. Lipid Res.* 1988, *29*, 397–429.
3. Da Silva, M.S.; Julien, P.; Perusse, L.; Vohl, M.-C.; Rudkowska, I. Natural Rumen-Derived trans Fatty Acids Are Associated with Metabolic Markers of Cardiac Health. *Lipids* 2015, *50*, 873–882.
4. Perusse, L.; Tremblay, A.; Leblanc, C.; Cloninger, C.R.; Reich, T.; Rice, J.; Bouchard, C. Familial resemblance in energy intake: Contribution of genetic and environmental factors. *Am. J. Clin. Nutr.* 1988, *47*, 629–635.
5. Locke, A.; Kahali, B.; Berndt, S.I.; Justice, A.E.; Pers, T.; Day, F.; Powell, C.; Vedantam, S.; Buchkovich, M.L.; Yang, J.; et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015, *518*, 197–206.
6. Rudkowska, I.; Pérusse, L.; Bellis, C.; Blangero, J.; Després, J.-P.; Bouchard, C.; Vohl, M.-C. Interaction between Common Genetic Variants and Total Fat Intake on Low-Density Lipoprotein Peak Particle Diameter: A Genome-Wide Association Study. *J. Nutr. Nutr.* 2015, *8*, 44–53.
7. Perusse, L.; Rankinen, T.; Zuberi, A.; Chagnon, Y.C.; Weisnagel, S.J.; Argyropoulos, G.; Walts, B.; Snyder, E.E.; Bouchard, C. The Human Obesity Gene Map: The 2004 Update. *Obes. Res.* 2005, *13*, 381–490.
8. Bierman, E.L.; Bagdade, J.D.; Porte, D. Obesity and Diabetes: The Odd Couple. *Am. J. Clin. Nutr.* 1968, *21*, 1434–1437.
9. Government of Canada. Statistics Canada. Overweight and Obese Adults (Self-Reported). 2016. Available online: http://www.statcan.gc.ca/eng/help/bb/info/obesity (accessed on 11 October 2016).
10. Shungin, D.; Winkler, T.W.; Croteau-Chonka, D.C.; Ferreira, T.; Locke, A.; Mägi, R.; Strawbridge, R.; Pers, T.; Fischer, K.; Justice, A.E.; et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015, *518*, 187–196.
11. Paffenbarger, R.S.J.; Lee, I.-M. Age-specific physical activities and life style patterns as related to all-causes mortality and to longevity. In *Exercise for Preventing Common Diseases*; Tanaka, H., Shindo, M., Eds.; Springer: Tokyo, Japan, 1999; pp. 121–130.
12. Paffenbarger, R.S., Jr. Factors predisposing to fatal stroke in longshoremen. *Prev. Med.* 1972, *1*, 522–528.
13. Després, J.P.; Pouliot, M.C.; Moorjani, S.; Nadeau, A.; Tremblay, A.; Lupien, P.J.; Thériault, G.; Bouchard, C. Loss of abdominal fat and metabolic response to exercise training in obese women. *Am. J. Physiol. Metab.* 1991, *261 (Pt 1)*, E159–E167.
14. Pérusse, L.; Bouchard, C. Genotype-environment interaction in human obesity. *Nutr. Rev.* 1999, *57*, S31–S37.
15. Pérusse, L.; Bouchard, C. Gene-diet interactions in obesity. *Am. J. Clin. Nutr.* 2000, *72*, 1285s–1290s.
16. St-Amand, J.; Prud'Homme, D.; Moorjani, S.; Nadeau, A.; Tremblay, A.; Bouchard, C.; Lupien, P.J.; Després, J.P. Apolipoprotein E polymorphism and the relationships of physical fitness to plasma lipoprotein-lipid levels in men and women. *Med. Sci. Sports Exerc.* 1999, *31*, 692–697.
17. St-Amand, J.; Moorjani, S.; Lupien, P.J.; Prud'Homme, D.; Després, J.P. The relation of plasma triglyceride, apolipoprotein B and high-density lipoprotein-cholesterol to postheparin lipoprotein lipase activity is dependent upon apolipoprotein E polymorphism. *Metabolism* 1996, *45*, 261–268.
18. St-Amand, J.; Okamura, K.; Matsumoto, K.; Shimizu, S.; Sogawa, Y. Characterization of control and immobilized skeletal muscle: An overview from genetic engineering. *FASEB J.* 2001, *15*, 684–692.
19. Lissner, L.; Levitsky, D.A.; Strupp, B.J.; Kalkwarf, H.J.; Roe, D.A. Dietary fat and the regulation of energy intake in human subjects. *Am. J. Clin. Nutr.* 1987, *46*, 886–892.
20. Flatt, J.P.; Ravussin, E.; Acheson, K.J.; Jéquier, E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *J. Clin. Investig.* 1985, *76*, 1019–1024.
21. Schutz, Y.; Flatt, J.P.; Jéquier, E. Failure of dietary fat intake to promote fat oxidation: A factor favoring

the development of obesity. *Am. J. Clin. Nutr.* 1989, *50*.
22. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
23. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic*; Report of a WHO Consultation; World Health Organization Technical Report Series; WHO: Geneva, Switzerland, 2000; Volume 894, pp. 1–253.
24. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
25. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
26. Afshin, A. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N. Engl. J. Med.* 2017, *377*, 13–27.
27. Després, J.P. Abdominal obesity and the risk of coronary artery disease. *Can. J. Cardiol.* 1992, *8*, 561–562.
28. Hubert, H.B.; Feinleib, M.; McNamara, P.M.; Castelli, W.P. Obesity as an independent risk factor for cardiovascular disease: A 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 1983, *67*, 968–977.
29. Rabkin, S.W.; Mathewson, F.A.; Hsu, P.-H. Relation of body weight to development of ischemic heart disease in a cohort of young north American men after a 26 year observation period: The manitoba study. *Am. J. Cardiol.* 1977, *39*, 452–458.
30. Mobley, D.; Baum, N. The Obesity Epidemic and Its Impact on Urologic Care. *Rev. Urol.* 2015, *17*, 165–170.
31. Corey, K.E.; Kaplan, L.M. Obesity and liver disease: The epidemic of the twenty-first century. *Clin. Liver Dis.* 2014, *18*, 1–18.
32. McElroy, S.L. The epidemic of depression with obesity. *J. Clin. Psychiatry* 2015, *76*.
33. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
34. D'Apuzzo, M.R.; Novicoff, W.M.; Browne, J.A. The John Insall Award: Morbid Obesity Independently Impacts Complications, Mortality, and Resource Use After TKA. *Clin. Orthop. Relat. Res.* 2015, *473*, 57–63.
35. Nasraway, S.A.; Albert, M.; Donnelly, A.M.; Ruthazer, R.; Shikora, S.A.; Saltzman, E. Morbid obesity is an independent determinant of death among surgical critically ill patients. *Crit. Care Med.* 2006, *34*, 964–970, quiz 971.
36. Wood, P.D.; Stefanick, M.L.; Dreon, D.M.; Frey-Hewitt, B.; Garay, S.C.; Williams, P.T.; Superko, H.R.; Fortmann, S.P.; Albers, J.J.; Vranizan, K.M.; et al. Changes in Plasma Lipids and Lipoproteins in Overweight Men during Weight Loss through Dieting as Compared with Exercise. *N. Engl. J. Med.* 1988, *319*, 1173–1179.
37. Anderson, J.W.; Brinkman-Kaplan, V.L.; Lee, H.; Wood, C.L. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. *J. Am. Coll. Nutr.* 1994, *13*, 256–261.
38. Dumont, M.; Mauriège, P.; Bergeron, J.; Despres, J.P.; Prud'homme, D. Effect of a six month gemfibrozil treatment and dietary recommendations on the metabolic risk profile of visceral obese men. *Int. J. Obes. Relat. Metab. Disord.* 2001, *25*, 1136–1143.
39. Bray, G.A.; Greenway, F.L. Current and potential drugs for treatment of obesity. *Endocr. Rev.* 1999, *20*, 805–875.
40. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.
41. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
42. Jequier, E. Pathways to obesity. *Int. J. Obes. Relat. Metab. Disord.* 2002, *26* (Suppl. S2), S12–S17.
43. Kalra, S.P.; Dube, M.G.; Pu, S.; Xu, B.; Horvath, T.L.; Kalra, P.S. Interacting Appetite-Regulating

Pathways in the Hypothalamic Regulation of Body Weight. *Endocr. Rev.* 1999, *20*, 68–100.
44. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat Meal-induced Changes in the Duodenum Mucosa Transcriptome. *Obesity* 2008, *16*, 2302–2307.
45. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding Regulates the Expression of Pancreatic Genes in Gastric Mucosa. *J. Obes.* 2010, *2010*, 1–10.
46. Bolduc, C.; Yoshioka, M.; St-Amand, J. Acute Molecular Mechanisms Responsive to Feeding and Meal Constitution in Mesenteric Adipose Tissue. *Obesity* 2010, *18*, 410–413.
47. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding induced changes in the hypothalamic transcriptome. *Clin. Chim. Acta* 2009, *406*, 103–107.
48. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
49. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
50. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res.* 2019, *781*, 175–185.
51. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.-L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
52. Thim, L.; May, F.E. Structure of mammalian trefoil factors and functional insights. *Cell Mol. Life Sci.* 2005, *62*, 2956–2973.
53. Hoffmann, W.; Jagla, W.; Wiede, A. Molecular medicine of TFF-peptides: From gut to brain. *Histol. Histopathol.* 2001, *16*, 319–334.
54. Gajhede, M.; Petersen, T.; Henriksen, A.; Petersen, J.; Dauter, Z.; Wilson, K.; Thim, L. Pancreatic spasmolytic polypeptide: First three-dimensional structure of a member of the mammalian trefoil family of peptides. *Structure* 1993, *1*, 253–262.
55. Dignass, A.U.; Sturm, A. Peptide growth factors in the intestine. *Eur. J. Gastroenterol. Hepatol.* 2001, *13*, 763–770.
56. Beld, J.; Woycechowsky, K.J.; Hilvert, D. Diselenides as universal oxidative folding catalysts of diverse proteins. *J. Biotechnol.* 2010, *150*, 481–489.
57. Hua, Q.X.; Mayer, P.J.; Jia, W.; Zhang, J.; Weiss, A.M. The folding nucleus of the insulin superfamily: A flexible peptide model foreshadows the native state. *J. Biol. Chem.* 2006, *281*, 28131–28142.
58. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. *Nat. Rev. Mol. Cell Biol.* 2003, *4*, 721–732.
59. Thim, L. Trefoil peptides: From structure to function. *Cell. Mol. Life Sci.* 1997, *53*, 888–903.
60. Playford, R.J.; Marchbank, T.; Chinery, R.; Evison, R.; Pignatelli, M.; Boulton, R.A.; Thim, L.; Hanby, A.M. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. *Gastroenterology* 1995, *108*, 108–116.
61. Tomasetto, C.; Masson, R.; Linares, J.; Wendling, C.; Lefebvre, O.; Chenard, M.; Rio, M. pS2/TFF1 interacts directly with the VWFC cysteine-rich domains of mucins. *Gastroenterology* 2000, *118*, 70–80.
62. Westley, B.R.; Griffin, S.M.; May, F.E.B. Interaction between TFF1, a Gastric Tumor Suppressor Trefoil Protein, and TFIZ1, a Brichos Domain-Containing Protein with Homology to SP-C. *Biochemistry* 2005, *44*, 7967–7975.
63. Madsen, J.; Nielsen, O.; Tornøe, I.; Thim, L.; Holmskov, U. Tissue Localization of Human Trefoil Factors 1, 2, and 3. *J. Histochem. Cytochem.* 2007, *55*, 505–513.
64. Poulsen, S.S.; Thulesen, J.; Christensen, L.; Nexo, E.; Thim, L. Metabolism of oral trefoil factor 2 (TFF2) and the effect of oral and parenteral TFF2 on gastric and duodenal ulcer healing in the rat. *Gut* 1999, *45*, 516–522.
65. Huang, Z.; Zhang, X.; Lu, H.; Wu, L.; Wang, D.; Zhang, Q.; Ding, H. Serum trefoil factor 3 is a

promising non-invasive biomarker for gastric cancer screening: A monocentric cohort study in China. *BMC Gastroenterol.* 2014, *14*, 74.
66. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.-G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity* 2013, *21*, 1389–1395.
67. Farrell, J.J.; Taupin, D.; Koh, T.J.; Chen, D.; Zhao, C.M.; Podolsky, D.K.; Wang, T.C. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J. Clin. Investig.* 2002, *109*, 193–204.
68. Baus-Loncar, M.; Schmid, J.; Lalani, E.-N.; Rosewell, I.; Goodlad, R.; Stamp, G.W.H.; Blin, N.; Kayademir, T. Trefoil Factor 2 (Tff2) Deficiency in Murine Digestive Tract Influences the Immune System. *Cell. Physiol. Biochem.* 2005, *16*, 31–42.
69. Baus-Loncar, M.; Schmid, J.; Lalani, E.N.; Rosewell, I.; Goodlad, R.; Stamp, G.; Blin, N.; Kayademir, T. Trefoil factor family 2 deficiency and immune response. *Cell Mol. Life Sci.* 2005, *62*, 2947–2955.
70. Salzman, N.H.; Hung, K.; Haribhai, D.; Chu, H.; Karlsson-Sjöberg, J.; Amir, E.; Teggatz, P.; Barman, M.; Hayward, M.; Eastwood, D.; et al. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* 2010, *11*, 76–83.
71. Sell, H.; Habich, C.; Eckel, J. Adaptive immunity in obesity and insulin resistance. *Nat. Rev. Endocrinol.* 2012, *8*, 709–716.
72. Zhao, L. The gut microbiota and obesity: From correlation to causality. *Nat. Rev. Microbiol.* 2013, *11*, 639–647.
73. Kurt-Jones, E.A.; Cao, L.; Sandor, F.; Rogers, A.B.; Whary, M.T.; Nambiar, P.R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; et al. Trefoil Family Factor 2 Is Expressed in Murine Gastric and Immune Cells and Controls both Gastrointestinal Inflammation and Systemic Immune Responses. *Infect. Immun.* 2007, *75*, 471–480.
74. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an Inflammatory-Induced and AntiInflammatory Tissue Repair Factor. *Animals* 2020, *10*, 1646.
75. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice. *Animals* 2021, *11*, 258.
76. Vowinkel, T.; Mori, M.; Krieglstein, C.F.; Russell, J.; Saijo, F.; Bharwani, S.; Turnage, R.H.; Davidson, W.; Tso, P.; Granger, D.N.; et al. Apolipoprotein A-IV inhibits experimental colitis. *J. Clin. Investig.* 2004, *114*, 260–269.
77. Okumura, T.; Fukagawa, K.; Tso, P.; Taylor, I.L.; Pappas, T. Intracisternal injection of apolipoprotein A-IV inhibits gastric secretion in pylorus-ligated conscious rats. *Gastroenterology* 1994, *107*, 1861–1864.
78. Tso, P.; Sun, W.; Liu, M. Gastrointestinal satiety signals IV. Apolipoprotein A-IV. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2004, *286*, G885–G890.
79. Johns, C.E.; Newton, J.L.; Westley, B.R.; May, F.E. Human pancreatic polypeptide has a marked diurnal rhythm that is affected by ageing and is associated with the gastric TFF2 circadian rhythm. *Peptides* 2006, *27*, 1341–1348.
80. Shimada, T.; Fujii, Y.; Koike, T.; Tabei, K.; Namatame, T.; Yamagata, M.; Tajima, A.; Yoneda, M.; Trano, A.; Hiraishi, H. Peroxisome proliferator-activated receptor gamma (PPARgamma) regulates trefoil factor family 2 (TFF2) expression in gastric epithelial cells. *Int. J. Biochem. Cell Biol.* 2007, *39*, 626–637.
81. Vázquez, M.; Silvestre, J.S.; Prous, J.R. Experimental approaches to study PPAR gamma agonists as antidiabetic drugs. *Methods Find. Exp. Clin. Pharmacol.* 2002, *24*, 515–523.
82. Dubeykovskaya, Z.; Dubeykovskiy, A.; Solal-Cohen, J.; Wang, T.C. Secreted Trefoil Factor 2 Activates the CXCR4 Receptor in Epithelial and Lymphocytic Cancer Cell Lines. *J. Biol. Chem.* 2009, *284*, 3650–3662.
83. Chinery, R.; Cox, H.M. Immunoprecipitation and characterization of a binding protein specific for the peptide, intestinal trefoil factor. *Peptides* 1995, *16*, 749–755.
84. Tan, X.-D.; Hsueh, W.; Chang, H.; Wei, K.R.; Crussi, F.G. Characterization of a Putative Receptor for Intestinal Trefoil Factor in Rat Small Intestine: Identification byin SituBinding and Ligand Blotting. *Biochem.*

Biophys. Res. Commun. 1997, *237*, 673–677.
85. Thim, L.; Mørtz, E. Isolation and characterization of putative trefoil peptide receptors. *Regul. Pept.* 2000, *90*, 61–68.
86. Otto, W.R.; Patel, K.; McKinnell, I.; Evans, M.D.; Lee, C.-Y.; Frith, D.; Hanrahan, S.; Blight, K.; Blin, N.; Kayademir, T.; et al. Identification of blottin: A novel gastric trefoil factor family-2 binding protein. *Proteomics* 2006, *6*, 4235–4245.
87. Rimland, J.; Xin, W.; Sweetnam, P.; Saijoh, K.; Nestler, E.J.; Duman, R.S. Sequence and expression of a neuropeptide Y receptor cDNA. *Mol. Pharmacol.* 1991, *40*, 869–875.
88. Banisadr, G.; Fontanges, P.; Haour, F.; Kitabgi, P.; Rostene, W.; Parsadaniantz, S.M. Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. *Eur. J. Neurosci.* 2002, *16*, 1661–1671.
89. Vucetic, Z.; Reyes, T.M. Central dopaminergic circuitry controlling food intake and reward: Implications for the regulation of obesity. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 2010, *2*, 577–593.
90. Richard, D. Energy expenditure: A critical determinant of energy balance with key hypothalamic controls. *Minerva Endocrinol.* 2007, *32*, 173–183.
91. Trecki, J.; Unterwald, E. Modulation of cocaine-induced activity by intracerebral administration of CXCL12. *Neuroscience* 2009, *161*, 13–22.
92. Pervin, B.; Aydın, G.; Visser, T.; Uçkan-Çetinkaya, D.; Aerts-Kaya, F.S.F. CXCR4 expression by mesenchymal stromal cells is lost after use of enzymatic dissociation agents, but preserved by use of non enzymatic methods. *Int. J. Hematol.* 2021, *113*, 5–9.
93. Mousessian, A.S.; da Silva, C.P.N.; Oba-Shinjo, S.M.; Kolias, A.G.; Paiva, W.S.; Marie, S.K.N. CXCR7, CXCR4, and Their Ligand Expression Profile in Traumatic Brain Injury. *World Neurosurg.* 2021, *147*, e16–e24.
94. Singh, A.K.; Arya, R.K.; Trivedi, A.; Sanyal, S.; Baral, R.; Dormond, O.; Briscoe, D.; Datta, D. Chemokine receptor trio: CXCR3, CXCR4 and CXCR7 crosstalk via CXCL11 and CXCL12. *Cytokine Growth Factor Rev.* 2013, *24*, 41–49.
95. Hsiao, J.J.; Ng, B.H.; Smits, M.M.; Wang, J.; Jasavala, R.J.; Martinez, H.D.; Lee, J.; Alston, J.J.; Misonou, H.; Trimmer, J.S.; et al. Androgen receptor and chemokine receptors 4 and 7 form a signaling axis to regulate CXCL12-dependent cellular motility. *BMC Cancer* 2015, *15*, 204.
96. Jiang, Q.; Sun, Y.; Liu, X. CXCR4 as a prognostic biomarker in gastrointestinal cancer: A meta-analysis. *Biomarkers* 2019, *24*, 510–516.
97. Li, L.-N.; Jiang, K.-T.; Tan, P.; Wang, A.-H.; Kong, Q.-Y.; Wang, C.-Y.; Lu, H.-R.; Wang, J. Prognosis and Clinicopathology of CXCR4 in Colorectal Cancer Patients: A Meta-analysis. *Asian Pac. J. Cancer Prev.* 2015, *16*, 4077–4080.
98. Mouhieddine, T.H.; Ahmad, Y.; Barlogie, B.; Jagannath, S.; Teruya-Feldstein, J.; Richter, J. Increased Muscle CXCR4 Expression in the Setting of Rare Muscle-invasive Multiple Myeloma. *Clin. Lymphoma Myeloma Leuk.* 2020, *20*, e341–e344.
99. Guo, Z.; Chen, W.; Dai, G.; Huang, Y. Cordycepin suppresses the migration and invasion of human liver cancer cells by downregulating the expression of CXCR4. *Int. J. Mol. Med.* 2020, *45*, 141–150.
100. Yang, J.; Zhang, L.; Jiang, Z.; Ge, C.; Zhao, F.; Jiang, J.; Tian, H.; Chen, T.; Xie, H.; Cui, Y.; et al. TCF12 promotes the tumorigenesis and metastasis of hepatocellular carcinoma via upregulation of CXCR4 expression. *Theranostics* 2019, *9*, 5810–5827.
101. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
102. Iqbal, J.; Hussain, M.M. Intestinal lipid absorption. *Am. J. Physiol. Endocrinol. Metab.* 2009, *296*, E1183–E1194.
103. Timofeeva, E.; Baraboi, E.D.; Richard, D. Contribution of the vagus nerve and lamina terminalis to brain activation induced by refeeding. *Eur. J. Neurosci.* 2005, *22*, 1489–1501.

104. Guesdon, B.; Samson, P.; Richard, D. Effects of intracerebroventricular and intra-accumbens melanin-concentrating hormone agonism on food intake and energy expenditure. *Am. J. Physiol. Integr. Comp. Physiol.* 2009, *296*, R469–R475.
105. Arvaniti, K.; Huang, Q.; Richard, D. Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. *Neuroendocrinology* 2001, *73*, 227–236.
106. Huang, Q.; Rivest, R.; Richard, D. Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. *Endocrinology* 1998, *139*, 1524–1532.
107. Timofeeva, E.; Picard, F.; Duclos, M.; Deshaies, Y.; Richard, D. Neuronal activation and corticotropin releasing hormone expression in the brain of obese (fa/fa) and lean (fa/?) Zucker rats in response to refeeding. *Eur. J. Neurosci.* 2002, *15*, 1013–1029.
108. Coppola, A.; Liu, Z.-W.; Andrews, Z.B.; Paradis, E.; Roy, M.-C.; Friedman, J.M.; Ricquier, D.; Richard, D.; Horvath, T.L.; Gao, X.-B.; et al. A Central Thermogenic-like Mechanism in Feeding Regulation: An Interplay between Arcuate Nucleus T3 and UCP2. *Cell Metab.* 2007, *5*, 21–33.
109. Nadreau, E.; Baraboi, E.-D.; Samson, P.; Blouin, A.; Hould, F.-S.; Marceau, P.; Biron, S.; Richard, D. Effects of the biliopancreatic diversion on energy balance in the rat. *Int. J. Obes.* 2006, *30*, 419–429.
110. Cabanac, M.; Richard, D. Acute intraventricular CRF lowers the hoarding threshold in male rats. *Physiol. Behav.* 1995, *57*, 705–710.
111. Dagnault, A.; Ouerghi, D.; Richard, D. Treatment with alpha-helical-CRF(9-41) prevents the anorectic effect of 17-beta-estradiol. *Brain Res. Bull.* 1993, *32*, 689–692.
112. Richard, D.; Rivest, R.; Naimi, N.; Timofeeva, E.; Rivest, S. Expression of corticotropin-releasing factor and its receptors in the brain of lean and obese Zucker rats. *Endocrinology* 1996, *137*, 4786–4795.
113. Rivest, S.; Richard, D. Hypothalamic paraventricular nucleus lesions do not prevent anorectic effect of exercise in male rats. *Am. J. Physiol. Integr. Comp. Physiol.* 1990, *259 (Pt 2)*, R579–R584.
114. Mailhot, G.; Ravid, Z.; Barchi, S.; Moreau, A.; Rabasa-Lhoret, R.; Levy, E. CFTR knockdown stimulates lipid synthesis and transport in intestinal Caco-2/15 cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2009, *297*, G1239–G1249.
115. Baraboi, E.-D.; Michel, C.; Smith, P.; Thibaudeau, K.; Ferguson, A.V.; Richard, D. Effects of albumin-conjugated PYY on food intake: The respective roles of the circumventricular organs and vagus nerve. *Eur. J. Neurosci.* 2010, *32*, 826–839.
116. Levy, E.; Harmel, E.; Laville, M.; Sanchez, R.; Emonnot, L.; Sinnett, D.; Ziv, E.; Delvin, E.; Couture, P.; Marcil, V.; et al. Expression of Sar1b Enhances Chylomicron Assembly and Key Components of the Coat Protein Complex II System Driving Vesicle Budding. *Arterioscler. Thromb. Vasc. Biol.* 2011, *31*, 2692–2699.
117. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.
118. Wang, F.; Kohan, A.B.; Lo, C.-M.; Liu, M.; Howles, P.; Tso, P. Apolipoprotein A-IV: A protein intimately involved in metabolism. *J. Lipid Res.* 2015, *56*, 1403–1418.
119. Filippatos, T.D.; Derdemezis, C.S.; Gazi, I.F.; Nakou, E.S.; Mikhailidis, D.P.; Elisaf, M.S. Orlistat associated adverse effects and drug interactions: A critical review. *Drug Saf.* 2008, *31*, 53–65.
120. Khalil, H.; Ellwood, L.; Lord, H.; Fernandez, R. Pharmacological Treatment for Obesity in Adults: An Umbrella Review. *Ann. Pharmacother.* 2020, *54*, 691–705.
121. Al-Azzeh, E.; Dittrich-Breiholz, O.; Vervoorts, J.; Blin, N.; Gött, P.; Lüscher, B. Gastroprotective peptide trefoil factor family 2 gene is activated by upstream stimulating factor but not by c-Myc in gastrointestinal cancer cells. *Gut* 2002, *51*, 685–690.
122. Shah, A.A.; Leidinger, P.; Keller, A.; Wendschlag, A.; Meese, E.; Blin, N. Altered miRNA expression patterns in Tff2 knock-out mice correlate with cellular pathways of neoplastic development and caloric metabolism. *Int. J. Mol. Med.* 2012, *29*, 637–643.
123. Shah, A.A.; Mihalj, M.; Ratkaj, I.; Lubka-Pathak, M.; Balogh, P.; Klingel, K.; Bohn, E.; Blin, N.; Baus-

Loncar, M. Increased Susceptibility to *Yersinia enterocolitica* Infection of *Tff2* Deficient Mice. *Cell. Physiol. Biochem.* 2012, *30*, 853–862.
124. Chao, L.C.; Zhang, Z.; Pei, L.; Saito, T.; Tontonoz, P.; Pilch, P. Nur77 Coordinately Regulates Expression of Genes Linked to Glucose Metabolism in Skeletal Muscle. *Mol. Endocrinol.* 2007, *21*, 2152–2163.
125. Maxwell, M.A.; Cleasby, M.E.; Harding, A.; Stark, A.; Cooney, G.J.; Muscat, G.E. Nur77 regulates lipolysis in skeletal muscle cells. Evidence for cross-talk between the beta-adrenergic and an orphan nuclear hormone receptor pathway. *J. Biol. Chem.* 2005, *280*, 12573–12584.
126. Pearen, M.A.; Muscat, G.E.O. Minireview: Nuclear Hormone Receptor 4A Signaling: Implications for Metabolic Disease. *Mol. Endocrinol.* 2010, *24*, 1891–1903.
127. Guerremillo, M. Adiponectin: An update. *Diabetes Metab.* 2008, *34*, 12–18.
128. Siersbaek, R.; Nielsen, R.; Mandrup, S. PPARgamma in adipocyte differentiation and metabolism Novel insights from genomewide studies. *FEBS Lett.* 2010, *584*, 3242–3249.

Chapter 12. Opinion - Trefoil Factor Family Member 2 Expression as an Indicator of the Severity of the High-Fat Diet-Induced Obesity

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec Université Laval Research Center, 2705 Boul. Laurier, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

Received: 15 September 2021; Accepted: 24 September 2021; Published: 26 September 2021

12.1 Résumé (French abstract)

Le développement de mesures exploratrices de l'obésité est un élément clé dans la recherche sur l'obésité. Le trefoil factor family member 2 (*Tff2*), connu pour son rôle dans la protection des muqueuses, a été caractérisé comme un gène induit par une diète riche en gras (HFD). Par conséquent, TFF2 représenterait un lien mécanistique moléculaire entre la HFD et le développement de l'obésité. Nous décrivons une application potentielle du modèle d'expression TFF2/*Tff2* lié à la HFD. Nous suggérons que la mesure de l'expression du TFF2/*TFF2*/*Tff2* dans des échantillons biologiques après l'ingestion d'une HFD peut refléter la « réactivité » biologique à l'ingestion de lipides et refléterait la gravité de l'obésité qui en résulte. Une telle propriété pourrait être exploitée, par exemple, pour dépister des modèles d'animaux d'obésité, évaluer la prédisposition à l'obésité induite par une HFD, ainsi que dans des domaines biomédicaux et cliniques de la recherche sur l'obésité.

12.2 Abstract

Trefoil Factor Family Member 2 (TFF2) belongs to TFF family peptides that includes TFF1, TFF2, TFF3. TFF2 is mainly known for its roles in the mucosal protection. In the context of obesity and high-fat diet (HFD), *Tff2* has been characterized as a HFD-induced gene. The knock-out of *Tff2* in mice lead to the protection from HFD-induced obesity with a metabolic profile towards a negative energy balance. Such HFD-specific expression gives *Tff2* a pattern worth exploring in biomedical research. Indeed, measuring TFF2/*TFF2*/*Tff2* expression in biological samples following the ingestion of high-fat diet reflects the biological "responsiveness" to the lipids ingestion and would reflect the severity of obesity establishment afterwards. Such property could be explored for instance to screen animal models, evaluate the predisposition to HFD-induced obesity as well as in biomedical and clinical applications. Results might advance obesity research especially in terms of understanding lipid-induced signals, appetite control and adiposity storage.

Keywords: Trefoil Factor Family Member 2; expression; indicator; high-fat; diet; obesity

12.3 Trefoil Factor Family Member 2 Expression as an Indicator of the Severity of the High-Fat Diet-Induced Obesity

Obesity represents a growing challenge for health professionals and officials which represents a risk factor for a variety of diseases (including during the ongoing COVID-19 crisis [1–4]) and various diseases [5–8]; and it is also considered a disease itself [9]. It also represents a huge economic burden [10,11]. The main challenging pattern facing the development of obesity research and therapies is the limited understanding of its molecular and cellular pathways [12,13]. Therefore, providing new molecular tools to explore obesity, its development and its pathogenesis remains of a high importance. Within this piece of writing, we describe a potential application of trefoil factor family member 2 (TFF2/*Tff2*) expression pattern related to high-fat (HF) diet (HFD).

TFF2 belongs to TFF family peptides that includes TFF1, TFF2 and TFF3 [14,15]. TFF2 is mainly known for its roles in the mucosal protection including in the gastrointestinal tracts [15–17], but it is also implicated in a variety of functions including anti-inflammatory process [18], tissue repair [19] and cancer [20]. Interestingly, recent studies have highlighted metabolic implications of TFF2 especially in the context of obesity and HFD. Indeed, using functional genomics approaches [21], *Tff2* has been characterized as a HF-induced gene in mice intestinal mucosa. The HF specificity has been revealed through an experimental design that used fasted status (instead of low-fat) as a control to which both HF and low-fat fed mice have been compared [22,23]. Indeed, the gene expression, studied based on serial analysis of gene expression and confirmed with microarray analysis, revealed that in the intestinal mucosa the *Tff2* is overexpressed following the ingestion of a HF meal and not a low-fat meal [22,23]. Therefore, highlights *Tff2* as a HF specifically-induced gene.

In order to elucidate the implications of TFF2 in the context of obesity, and more specifically in the HF-diet obesity, *Tff2* knock-out (KO) mice were challenged with HFD [24]. The study has shown that the *Tff2* KO mice, compared to the wild-type (WT) mice, are in fact protected from HFD-induced obesity with an increased lipids excretion as well [24] which correlates with the exacerbation of weight loss by TFF2 deficiency shown by Judd et al. [25]. Moreover, the metabolic exploration of key metabolic tissues of these mice revealed mechanisms that explain such protection. Indeed, *Tff2* KO mice have a metabolic phenotype towards an increased energy expenditure with reduced energy storage [26]. In our recent review [27], we have detailed a hypothesis that aims to explain how HFD induces *Tff2* overexpression and at the same time the KO of this same gene, *Tff2*, lead to the protection from the HF-diet-induced obesity via metabolic changes. Briefly, the TFF2 expression would be a signal leading to metabolic adaptation, which facilitates the lipid digestion, anabolism and storage. Therefore, HFD would induces its overexpression to facilitate the digestion and the anabolism of lipids coming from such HFD, whereas *Tff2* KO would deprive the metabolic machinery from molecular tools required to use the ingested lipids through an increased lipid absorption and storage, which leads to a

protection from the HFD-induced obesity. It is worth pointing that gut microbiota, which contributes significantly to metabolic disorders [28] including obesity [29], impaired glucose [30] and lipid metabolism [31], can also be altered by diets [32,33] including HFD [34,35]. In the obesity context, the interactions between TFF2 and gut microbiota [36] could be involved in the mechanisms of HFD-induced obesity. Therefore, TFF2 would represent a molecular mechanistic link between HFD and obesity development [27].

Based on such properties of *Tff2* induction by HDF and its implications in HFD induced obesity, potential applications can derive from and range from biomedical research to clinical practice (Figure 12.1). The concept would be to challenge biological systems (animals, cell cultures, isolated tissues, etc.) with HFD followed by the measure of *Tff2* or TFF2 expression in the intestinal mucosa [22,23], blood [37] or other tissues [38–40]. This could allow for instance to evaluate the "predisposition" to develop HFD-induced obesity based on the expression intensity of TFF2/*Tff2* following HFD. Obesity animal models are diverse [41], among them different species have been used to generate animal models of HFD-induced obesity. Within this context, measuring *Tff2* expression following the ingestion of a HFD could represent a standard approach to compare the different animal models and therefore optimize the selections of the one(s) suitable to build the obesity model for the experiments depending on the experimental contexts and goals. The same principle can be applied to select, following genetic modifications (KO, overexpression, etc.), the animals to be used for breeding and used for the obesity-related studies.

For clinical perspectives, we can estimate the risk of HFD-induced obesity in individuals by the same approaches. For the pharmacological studies and research applications, obesity drugs can be tested as a purpose to reduce *Tff2* expression and therefore mimic *Tff2* KO and lead to a metabolic profile similar to the one seen in *Tff2* KO mice described above (protection for HFD-induced obesity) [24,26]. Therefore, adapt the individuals' diet, not only in terms of lipids content but even depending on lipids types. Indeed, for the pharmacological studies and research applications such measures can be used also to adapt a diet, test a drug or evaluate a treatment in the context of HFD-induced obesity. This might be achieved by measuring TFF2/*Tff2* expression depending on the type of lipids that can also be tested in cells to study the molecular changes. Therefore, the studies would go deeper by comparing, among the HFD, the different types of lipids and test a variety of combination to gain new knowledge on links between diets composition and its ability to induce obesity (through *Tff2* expression) especially while comparing diets that have similar number of calories. Within this context, the variety of functional genomics methods [42,43] allowing to measure the *Tff2* expression are molecular tools that provide a flexibility to such applications as well.

We believe that the measure of TFF2/*TFF2*/*Tff2* expression level in response to HFD could expand our cellular and molecular understanding of obesity and strengthen therapeutic research especially that TFF2 could be a lipid-specifically induced signal we are yet to confirm to complete the puzzle of fat-induced signals, appetite control and adiposity storage; all key elements in energy homeostasis and obesity development.

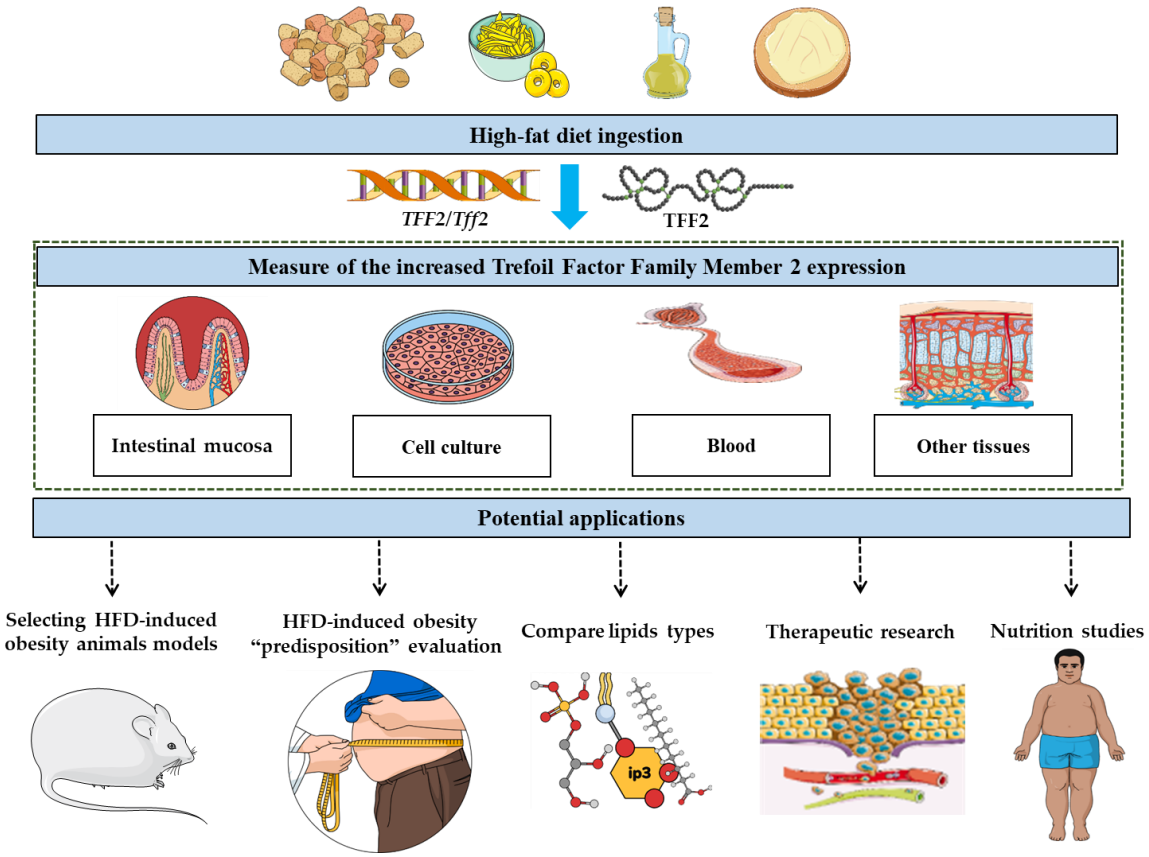


Figure 12.1. Measuring Trefoil Factor Family Member 2 (TFF2/*TFF2*/*Tff2*) expression in biological samples following the ingestion of high-fat diet reflects the biological "responsiveness" to the lipids ingestion and would reflect the severity of obesity establishment. Such property could be explored for instance to screen animal models, evaluate the predisposition to high-fat diet-induced obesity as well as in biomedical and clinical applications.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, the figure, references selection, etc.) throughout the writing process, edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have
read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship « Bourse Tremplin-Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec–Sante (FRQS), Quebec, Canada. Figure 12.1 was created using images from https://mindthegraph.com/ (accessed on 31 August 2021) and http://smart.servier.com (accessed on 31 August 2021). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported0License.

Conflicts of Interest: The authors declare that there is no conflict of interest.

12.4 References

1. Albashir, A.A.D. The potential impacts of obesity on COVID-19. *Clin. Med.* 2020, *20*, e109–e113.
2. Petrakis, D.; Margină, D.; Tsarouhas, K.; Tekos, F.; Stan, M.; Nikitovic, D.; Kouretas, D.; Spandidos, D.A.; Tsatsakis, A. Obesity—A risk factor for increased COVID-19 prevalence, severity and lethality (Review). *Mol. Med. Rep.* 2020, *22*, 9–19.
3. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
4. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
5. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
6. Zachariae, C.; Skov, L. Obesity as a risk factor for psoriasis. *J. Eur. Acad. Dermatol. Venereol.* 2020, *34*, 915–916.
7. Mandviwala, T.; Khalid, U.; Deswal, A. Obesity and Cardiovascular Disease: A Risk Factor or a Risk Marker? *Curr. Atheroscler. Rep.* 2016, *18*, 21.
8. De Pergola, G.; Silvestris, F. Obesity as a major risk factor for cancer. *J. Obes.* 2013, *2013*, 291546.
9. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
10. Withrow, D.; Alter, D.A. The economic burden of obesity worldwide: A systematic review of the direct costs of obesity. *Obes Rev.* 2011, *12*, 131–141.
11. Tremmel, M.; Gerdtham, U.G.; Nilsson, P.M.; Saha, S. Economic Burden of Obesity: A Systematic Literature Review. *Int. J. Environ. Res. Public Health* 2017, *14*, 435.
12. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
13. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.
14. Hoffmann, W. Trefoil Factor Family (TFF) Peptides and Their Diverse Molecular Functions in Mucus Barrier Protection and More: Changing the Paradigm. *Int. J. Mol. Sci.* 2020, *21*, 4535.

15. Braga Emidio, N.; Hoffmann, W.; Brierley, S.M.; Muttenthaler, M. Trefoil Factor Family: Unresolved Questions and Clinical Perspectives. *Trends Biochem. Sci.* 2019, *44*, 387–390.
16. Hoffmann, W.; Jagla, W.; Wiede, A. Molecular medicine of TFF-peptides: From gut to brain. *Histol. Histopathol.* 2001, *16*, 319–334.
17. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. *Nat. Rev. Mol. Cell Biol.* 2003, *4*, 721–732.
18. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an Inflammatory-Induced and AntiInflammatory Tissue Repair Factor. *Animals* 2020, *10*, 1646.
19. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice. *Animals* 2021, *11*, 258.
20. Ge, Y.; Ma, G.; Liu, H.; Lin, Y.; Zhang, G.; Du, M.; Wang, M.; Chu, H.; Zhang, H.; Zhang, Z. MUC1 is associated with TFF2 methylation in gastric cancer. *Clin. Epigenet.* 2020, *12*, 37.
21. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
22. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. *Obesity* 2008, *16*, 2302–2307.
23. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
24. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity* 2013, *21*, 1389–1395.
25. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. *Am. J. Physiol Gastrointest. Liver Physiol.* 2015, *308*, G12–G24.
26. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
27. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2: From a High-Fat-Induced Gene to a Potential Obesity Therapy Target. *Metabolites* 2021, *11*, 536.
28. Fan, Y.; Pedersen, O. Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* 2021, *19*, 55–71.
29. Karlsson, F.H.; Tremaroli, V.; Nookaew, I.; Bergström, G.; Behre, C.J.; Fagerberg, B.; Nielsen, J.; Bäckhed, F. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013, *498*, 99–103.
30. Wu, H.; Tremaroli, V.; Schmidt, C.; Lundqvist, A.; Olsson, L.M.; Krämer, M.; Gummesson, A.; Perkins, R.; Bergström, G.; Bäckhed, F. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based Cross-Sectional Study. *Cell Metab.* 2020, *32*, 379–390.e373.
31. Yu, Y.; Raka, F.; Adeli, K. The Role of the Gut Microbiota in Lipid and Lipoprotein Metabolism. *J. Clin. Med.* 2019, *8*, 2227.
32. Festi, D.; Schiumerini, R.; Eusebi, L.H.; Marasco, G.; Taddia, M.; Colecchia, A. Gut microbiota and metabolic syndrome. *World J. Gastroenterol.* 2014, *20*, 16079–16094.
33. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. *Eur. Rev. Med. Pharmacol. Sci.* 2016, *20*, 4742–4749.
34. Yin, J.; Li, Y.; Han, H.; Chen, S.; Gao, J.; Liu, G.; Wu, X.; Deng, J.; Yu, Q.; Huang, X.; et al. Melatonin reprogramming of gut microbiota improves lipid dysmetabolism in high-fat diet-fed mice. *J. Pineal Res.* 2018, *65*, e12524.
35. Bisanz, J.E.; Upadhyay, V.; Turnbaugh, J.A.; Ly, K.; Turnbaugh, P.J. Meta-Analysis Reveals

Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. *Cell Host Microbe* 2019, *26*, 265–272.e264.
36. Khoder, G.; Al-Yassir, F.; Al Menhali, A.; Saseedharan, P.; Sugathan, S.; Tomasetto, C.; Karam, S.M. Probiotics Upregulate Trefoil Factors and Downregulate Pepsinogen in the Mouse Stomach. *Int. J. Mol. Sci.* 2019, *20*, 3901.
37. Ishibashi, Y.; Ohtsu, H.; Ikemura, M.; Kikuchi, Y.; Niwa, T.; Nishioka, K.; Uchida, Y.; Miura, H.; Aikou, S.; Gunji, T.; et al. Serum TFF1 and TFF3 but not TFF2 are higher in women with breast cancer than in women without breast cancer. *Sci. Rep.* 2017, *7*, 4846.
38. Hoffmann, W. TFF2, a MUC6-binding lectin stabilizing the gastric mucus barrier and more (Review). *Int. J. Oncol.* 2015, *47*, 806–816.
39. May, F.E.; Semple, J.I.; Newton, J.L.; Westley, B.R. The human two domain trefoil protein, TFF2, is glycosylated in vivo in the stomach. *Gut* 2000, *46*, 454–459.
40. Cook, G.A.; Familari, M.; Thim, L.; Giraud, A.S. The trefoil peptides TFF2 and TFF3 are expressed in rat lymphoid tissues and participate in the immune response. *FEBS Lett.* 1999, *456*, 155–159.
41. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.
42. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat Res. Rev. Mutat Res.* 2019, *781*, 175–185.
43. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.

Chapter 13. Review - Diet Impact on Obesity beyond Calories and Trefoil Factor Family 2 (TFF2) as an Illustration: Metabolic Implications and Potential Applications

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 4G2, Canada

Received: 28 October 2021; Accepted: 3 December 2021; Published: 4 December 2021

13.1 Résumé (French abstract)

La gestion de l'obésité est principalement basée sur l'exercice et l'alimentation. Au-delà de la contribution à l'apport calorique, les éléments de l'alimentation ont des propriétés indépendantes du bilan énergétique et des effets métaboliques indirects. Alors que les propriétés indépendantes du bilan énergétique sont proches des effets « pharmacologiques » et comprennent des effets tels que celles des antioxydants et des anti-inflammatoires, les effets métaboliques indirects comprennent le contrôle de l'apport alimentaire et les changements métaboliques. À titre illustratif, nous avons également décrit l'implication métabolique et les voies hypothétiques du gène induit par la diète riche en gras, le trefoil factor family 2. Les propriétés du régime alimentaire peuvent avoir une variété d'implications principalement en pharmacologie et en nutrition et encouragent à explorer davantage les aliments « pharmacologiquement » actifs vers de potentielles applications thérapeutiques.

13.2 Abstract

Obesity is a health problem with increasing impacts on public health, economy and even social life. In order to reestablish the energy balance, obesity management focuses mainly on two pillars: exercise and diet. Beyond the contribution to the caloric intake, the diet nutrients and composition govern a variety of properties. This includes the energy balance-independent properties and the indirect metabolic effects. Whereas the energy balance-independent properties are close to "pharmacological" effects and include effects

such as antioxidant and anti-inflammatory, the indirect metabolic effects represent the contribution a diet can have on energy metabolism beyond the caloric contribution itself, which include the food intake control and metabolic changes. As an illustration, we also described the metabolic implication and hypothetical pathways of the high-fat diet-induced gene Trefoil Factor Family 2. The properties the diet has can have a variety of applications mainly in pharmacology and nutrition and further explore the "pharmacologically" active food towards potential therapeutic applications.

Keywords: obesity; diet; calories; energy metabolism; trefoil factor family member 2; high-fat diet

13.3 Obesity and the Ongoing Coronavirus Disease 2019 Crisis

Obesity, as an epidemic health problem [1], represents a topic of a continuously increasing number of studies surrounding different fields, ranging from obesity development and pathogenesis to the related diseases and health problems, at different life stages and physiological or pathological statuses. The basic definition of obesity is a lost balance between energy intake and energy expenditure that leads to an accumulation of excessive calories, mainly as lipid storage within the adipocytes in a distribution pattern that depends on genetic and environmental factors [2,3]. Such energy imbalance results from the superiority of the energy intake represented by the food intake compared to the energy expenditure represented mainly by the basal metabolic rate [4], physical activity/exercise [5,6] and thermogenesis [7–9]. Obesity can also develop as results of gene disruption or deficiency, such as the Brd2 gene [10,11] and the leptin gene [12,13], both leading to metabolic pathways towards an obesity-related phenotype. In addition, obesity has also epigenetics factors [14–17]. The related adiposity distribution depends on many factors and also impacts the pathological outcomes of obesity [18]. The pathogenesis of obesity has been discussed from a variety of perspectives, including broken energy homeostasis [18], microbiota contribution [19], neuroendocrine reprogramming [20] and even toward considering obesity as a disease [2,21]. The complexity of the molecular and cellular mechanisms governing obesity establishment, development and pathogenesis are poorly understood, which limits its research advances and therefore the development of efficient molecular therapies. The danger of obesity and increased adiposity results from their various impacts on health in terms of diseases risks and disturbed biological functions [22]. That includes metabolic disorders [23], cardiovascular diseases [24,25], dyslipidemia [26], impaired regeneration [27], inflammation [28], kidney disease [29], cancer [30], diabetes [24], impacts on immunity and antibodies [31–34], age-related cognitive decline [35], respiratory complications [36,37] and infertility [38,39].

It is worth noting that the ongoing coronavirus disease 2019 (COVID-19) crisis has increased the importance of obesity research because of both the impact of the COVID-19 pandemic (and the related measures [40,41]) on obesity development [42] as well as the obesity-induced increased vulnerability to COVID-19 [43–45]. The measures imposed

by the governments and health authorities, including confinement and its consequences (sedentary lifestyle, reduced physical activity, increased food intake, disturbed sleeping, etc. [41,42,46]) are towards worsening obesity rates worldwide. This includes the greater stress that was related to a higher body mass index, an increased working time, a higher anxiety level and less time to spend on weight management efforts [47] during this COVID-19 crisis. On the other hand, obesity consequences on the prognosis of patients with COVID-19 include increased hospitalization, intensive care unit admission and mortality [48]. The mechanisms beyond such increased vulnerability are diverse and include the chronic inflammatory character of obesity [49], immune dysregulation [50], excessive oxidative stress [51] and probably metabolic dysfunction and endothelium imbalance as well [52]. Furthermore, in addition to the direct impact of obesity on COVID-19 severity, obesity would also have indirect impacts on COVID-19. Obesity-related diseases and health conditions represent risk factors toward increasing the vulnerability to COVID-19. Diabetes, one of the most known diseases associated with obesity [53,54], represents a risk factor for severe COVID-19 [55–57]. Cardiovascular diseases, which have their risks increased in obese patients [25,58,59], also represent risk factors for COVID-19 [60]. Other obesity-related health problems, such as hypertension and kidney and liver diseases [57], do represent risk factor for the severe forms of COVID-19. In addition, studies have also explored the fact that COVID-19 could also worsen or lead to other health problems, including diabetes [56,61], and possibly lead to a post-COVID-19 multi-level health crisis [62]. That would lead to a vicious cycle involving obesity, diabetes and COVID-19, among other pathologies that also increase COVID-19 severity. Moreover, the impacts of obesity on immunity [31–34] threaten to reduce the efficacy of anti-COVID-19 vaccines [63] that represent the best hope for humans to see an end of this pandemic.

The heavy consequences of obesity on health as well as on economy [64] and society [65,66] have led to the development of various pharmacotherapies [67] for obesity and the necessity to have other medications in development [68]. However, the main approaches to manage obesity remain exercise [69,70] and diet [71]. Along with the energy expenditure (exercise [6], thermogenesis [72], etc.), the diet represents the energy intake part of the caloric balance. Therefore, it is important to describe the nutritional aspects of the diet. Thus, the main focus of the nutritional research in obesity emphasizes the caloric density and the need to limit the caloric intake [73] to create a caloric deficiency leading to adiposity reduction and weight loss. Within this context, high-fat diet (HFD) and high-sugar diet have a high importance. HFD diet is characterized by both its high caloric density and a limited satiety. In addition, sugar consumption has even been described in the context of addiction [74] that contributes to obesity development [75]. Furthermore, the combined effect of high fat and high sugar has the most deleterious effects, as suggested by the work of Ghosh et.al. [76]. This gives both HFD and high-sugar diet a special status within nutrition research in the context of obesity and weight management.

13.4 Diet and Calories-Independent Patterns

The important diet caloric contribution to obesity is well documented, and various diets have been studied and compared in the context of prevention and treatment of obesity [77–83]. The research "obesity-diet" mainly focuses on controlling/limiting the caloric intake to manage obesity or reduce the adiposity. We describe fat and sugar diets as key contributors to the caloric intake. However, sugar and lipids cannot be seen only as caloric resources or "fuel"; they also play important biological roles within the cellular and subcellular structures, biochemical pathways, heat insulation, etc. Similarly, and in addition to the important direct impacts diet has on obesity, the in vivo diet consequences include two main patterns, energy balance-independent and those indirectly impacting the energy metabolism. In these two, the diet not only directly impacts the energy balance by increasing the caloric intake. The energy balance-independent diet benefits are close to "pharmacological" effects in which the diet elements can be described as functional foods or nutraceuticals [84–89]. Indeed, diet contents have been reported with a variety of properties that can be pharmacologically explored, including antioxidant pattern [90,91], anti-inflammatory [92,93], anti-microbial, anti-fungal, anti-diabetic and anti-atherosclerotic activity [94], anticancer properties [95,96], antiviral effects [97], body weight lowering fibers [98], protection against endogenous exposure to persistent organic pollutants [99] and gut microbiota changes [99]. In addition, consumption of elements, such as calcium (calories-free), has also been shown to impact obesity development [100,101]. Furthermore, some compounds from vegetables and fruits can be converted to hormone-like active substances with biological actions [102]. All these elements illustrate how the diet impacts on obesity go beyond the caloric density (quantity) but also include the diet composition (quality) as exemplified in Figure 13.1. Thus, a balanced diet within an anti-obesity approach should not be limited to a caloric intake control but needs also to make use of such "pharmacological" properties to both optimize the anti-obesity effects and also obtain effects such as anti-inflammation that will counteract outcomes seen during obesity (inflammation, increased risk cancer, etc.). Within this context, some concepts reported in the literature, such as "eat to treat" [103] would find their explanatory mechanisms and underlying pathways in those calorie-independent impacts of diet. Some of these properties (potential antimicrobial, antioxidant and anti-inflammatory) have also led researchers to suggest the Mediterranean diet as a nutritional approach for COVID-19 [104].

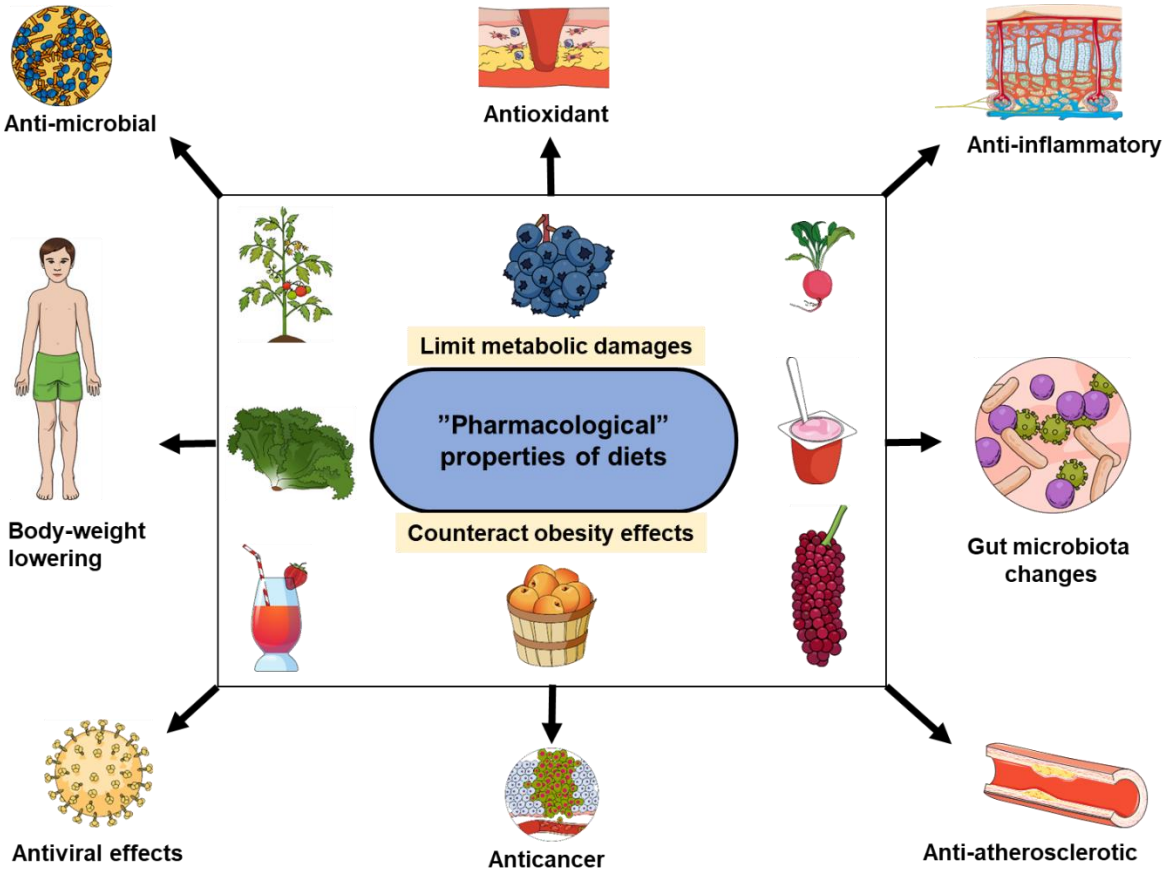


Figure 13.1. The energy balance-independent diet benefits are close to pharmacological effects. The diverse benefits related to food include properties that counteract consequences of obesity, such as inflammation, cancer risk and metabolic damages.

On the other hand, the second perspective is that dietary elements can indirectly impact the energy metabolism. Among the good examples are: (i) the red pepper decreases appetite [105], reduces fat intake [106] and increases satiety [107], (ii) oligofructose reduces hunger [108], (iii) dietary fiber potentiates weight regulation [109] and (iv) fenugreek fiber reduces energy intake and increases satiety [110]. In addition, the increased insulin resistance following the ingestion of a protein source mirroring western diet (compared to provision of casein) [111], prevention of obesity-related glucose intolerance by the salmon peptide fraction [112], the benefits of fish oil on diet-induced insulin resistance [113], the fish oil diet induced both reduction in body weight gain in ob/ob mice [114] and improvement of glucose intolerance in HFD-fed mice [115], the role of n-3 polyunsaturated fatty acid in insulin resistance prevention [116] that involve controlling adipose tissue inflammation in its mechanism [117], HFD effects on gut hormones production [118], the HFD-induced reduction of the sensitivity to satiety signals [119], blood lipid profile and body fat distribution improvement with fish oil [120], and appetite control [121] and food intake modification [122] of coffee/caffeine are also important illustrative properties mediated by diet and represent an indirect energy balance

management. All these paths represent illustrations of how selecting diet can impact the energy balance and the metabolism beyond the simple caloric intake related to the diet (Figure 13.2). These properties can be explored to optimize nutritional approaches and therapeutic development. This field is well supported by functional genomics studies exploring the transcriptomic changes depending on the diet [123] which highlight the links between the regulated genes and the related underlying metabolic pathways.

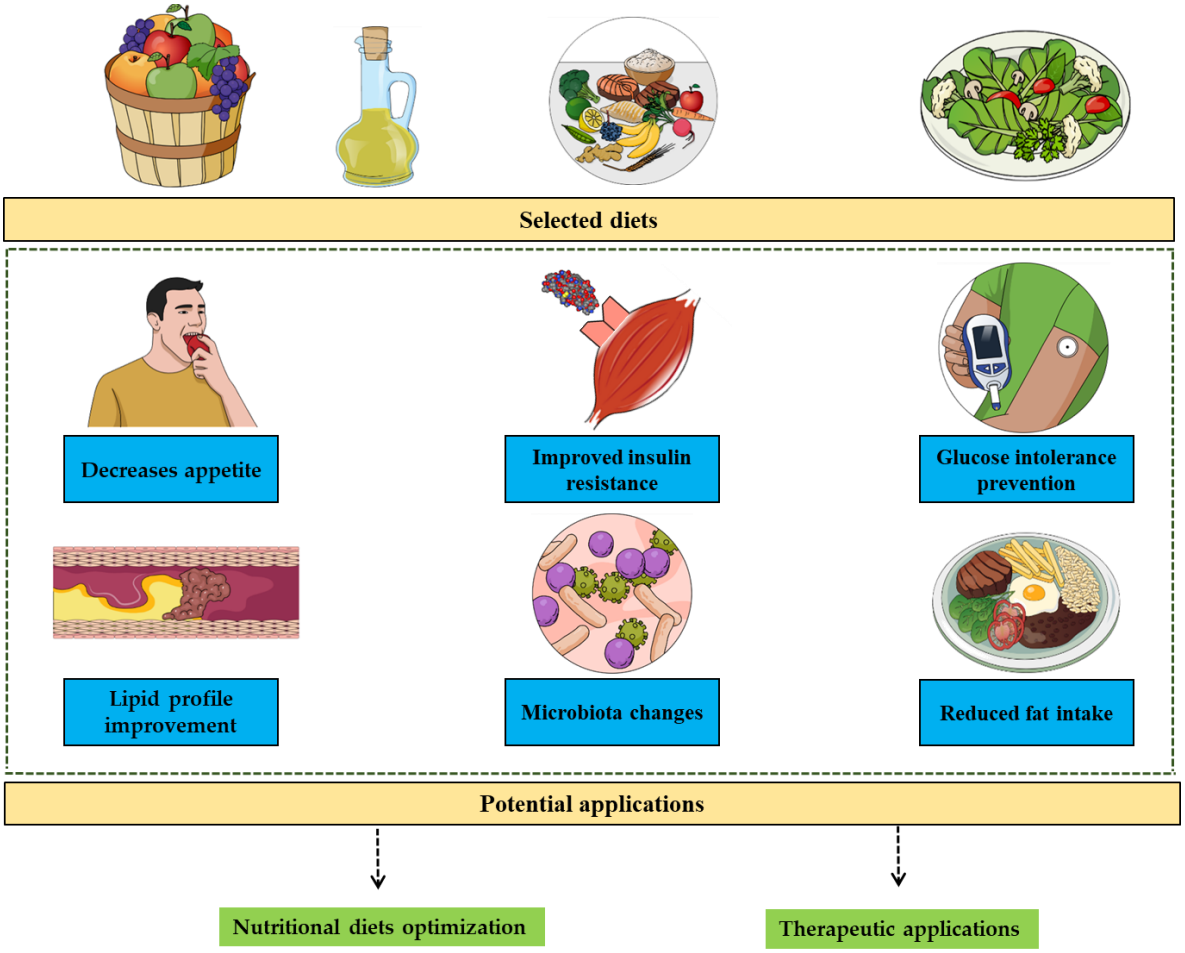


Figure 13.2. Indirect impacts diet has on energy metabolism. Some diets have the ability to impact the energy metabolism through insulin sensitivity improvement, appetite control, microbiota changes-related metabolic changes and improving the lipids profile. These properties are independent from the caloric intake that comes with such diets due to caloric-independent patterns.

Moreover, the key consequences of the diet on microbiota composition, which has a significant metabolic importance, reflects another indirect impact diet has on energy balance. Indeed, some diets rich in or with a supplementation of probiotics, prebiotics or synbiotics can modulate the microbiota [124]. For instance, a pro-inflammatory gut microbiota development is promoted by HFD [125], and no microbiota composition pattern was associated with vegan or vegetarian diets [126] which reflects some microbiota effects. The microbiota changes result in adaptations, including metabolic improvement [127,128] and even improving/treating obesity [19,124,129]. Importantly, the fact that different diets

lead to different changes in gut microbiota composition [130] and that different diets also lead to different transcriptome expression [123] both suggest a diet-specific metabolic phenotype. Beyond the aspects of potential therapeutic effect and the impacts a diet has on biological processes, including genes and metabolic pathways represents a growing area that could lead to therapeutic applications.

In addition to obesity, ageing also represents an important risk factor for a variety of diseases and health problems including cardiovascular disease [131], loss of neuroplasticity [132], cerebral ischemia [133], osteoporosis [134], pulmonary disease, cancer [135] and neurodegenerative disease [136]. Therefore, healthy ageing will contribute to reducing the risk and the severity of various diseases. For that, a healthy lifestyle that includes, in addition to exercise [137–139], sleep quality [140] and metal health [141], optimized diets choice is a key to achieve healthy ageing. Within this context, and based on the ageing-related pathways, mainly the oxidative stress [142] and redox regulation [143] involving reactive oxygen and nitrogen species [135] and free radicals [144,145], a diet of selected properties would contribute to healthy ageing. Indeed, food and beverages with properties ranging from antioxidant [146] and anti-inflammatory to anticancer and metabolic benefits will reduce the risks associated with ageing. For instance, diet choice toward white meat and fish can reduce the risk of age-related cognitive decline [147], and dietary antioxidants contribute to healthy ageing [148]. Such impacts of diet management in ageing [146] will lead to further development of geriatrics and elderly care beyond pharmacology.

13.5 Diet and Trefoil Factor Family: From Gastrointestinal Protection to Metabolic Implications

Following the same line of thought highlighting the indirect impacts of the diet, we provide an illustrative example of the indirect impacts of diet on energy balance mediated by trefoil factor family (TFF). Indeed, the indirect effect on metabolism can also be illustrated by the diet consequences on selected metabolic patterns related to the energy balance through the TFF. The TFF is a family of secreted protease-resistant peptides [149] with intramolecular disulfide bonds [150], trefoil domain(s) [151] and C-terminal dimerization domain, and which have important roles in mucosal protection and post-injury mucosal repair [152], epithelial migration promotion [153] and mucosal healing [154]. Whereas *Tff1*, *Tff2* or *Tff3* knock-out (KO) in mice led to gastrointestinal impairment [155], TFF have a therapeutic potential to treat gastrointestinal disorders [156]. While TFF1, or pS2, is expressed mainly in the stomach [152] without a known receptor (although a basolateral binding site in the gastrointestinal epithelial was suggested for the three TFFs [150]) or detailed metabolic implications, TFF3, or ITF, expressed in both the small (duodenum, jejunum and ileum) and large intestine [152] and in other tissues, such as the liver [157] and the nervous system [158], is still without a validated receptor [159,160], but its expression is regulated by food intake [161]. The metabolic implications of TFF3, although not well known, are also worth exploring as it has been shown to regulate the

glucose metabolism in the liver [157], and its deficiency leads to an affected hepatic lipid metabolism but with an improved glucose utilization [162]. On the other hand, TFF3 improves HFD-induced hepatic steatosis [163] and, in a diet-induced obesity mouse model, TFF3 improved glucose tolerance [161].

On the other hand, TFF2, known as spasmolytic peptide (SP), is a very stable small protein [164] expressed in the duodenum [152], the stomach, the colon, immune organs, leucocytes [164], macrophages, spleen cells [165], lymphocytes [165] hypothalamus [166] and the kidneys [167]. Unlike TFF1 and TFF3, it has been further studied in diverse context, including the energy metabolism. Indeed, TFF2 has been shown/suggested to play roles in anti-inflammatory pathways [165,168], counteracting the immune-mediated damage resulting from the HFD [169] and immune response [165,170]. Among the TFFs, TFF2 has been shown to play a role in obesity and metabolic disorders, mainly after its characterization as an HFD-induced gene and the work that explored its metabolic implications. Briefly, functional genomics studies characterized *Tff2* as an HFD-induced gene [171,172]. Thus, TFF2/*Tff2* expression has been suggested as an indicator of the severity of the HFD-induced obesity with a variety of potential applications [173]. Importantly, *Tff2* KO protected the mice from the HFD-induced obesity [166], which was explained by a metabolic phenotype toward an increased energy expenditure [174]. As an attempt to explain all these findings, we have hypothesized that the HFD-induced overexpression of TFF2 would represent a mechanism of adaptation to the HFD ingestion leading to an increased lipid absorption and storage via TFF2-stimulated receptors, as we previously reviewed [175]. This correlates with the exacerbated weight loss resulting from TFF2 deficiency [164].

Regarding the molecular and cellular pathways beyond such TFF2 metabolic roles, we highlight the receptor chemokine (C-X-C motif) receptor 4 (CXCR4), that belongs to the important family of G protein-coupled receptor [176,177], which is a known TFF2 receptor, including in cancer cell lines [178]. For instance, during development, the embryonic insulin-producing cells are maintained by the TFF2/CXCR4 axis [179]. This could indicate an insulin-mediated metabolic implication of TFF2, especially that cell proliferation in pancreatic β-cells is promoted by TFF2 through CXCR-4-mediated phosphorylation [180], and the *Tff2* KO reduced insulin serum levels in mice [166]. Furthermore, the expression of CXCR4 in key metabolic tissues, such as the liver [181], adipose tissue [182] and muscles [183] as well as the central nervous system [184] and the digestive tract [185], correlates with the metabolic changes observed in *Tff2* KO mice. Moreover, the increased expression of CXCR4 in situations in which TFF2 is also overexpressed, such as cancer [181,186], further supports such molecular links. Importantly, the CXCR4-defeciency in adipocytes leads to exacerbated obesity and impairs the brown adipose tissue thermoregulatory process [182]. This correlated with our previous hypothesis in which TFF2 would be a signal aiming to limit the food intake and obesity development since CXCR4 deficiency would prevent TFF2 from playing the related metabolic roles in limiting obesity and thus, explains the exacerbated obesity.

In addition, the fact that *Tff2* KO mice have an increased expression of agouti-related protein (AgRP) in the arcuate nucleus of the hypothalamus [166] may suggest that the HFD-induction of *Tff2* [171,172] could limit the expression (or at least prevent the increased expression seen in *Tff2* KO mice) of AgRP and thus limit its orexigenic and energy expenditure reduction effects [187], thus supporting the hypothesis that TFF2 could be a lipid-induced signal aiming to shift the energy balance towards counteracting the HFD-related excessive caloric intake. On the other hand, the melanocortin system, involved in the control of energy balance [188–190], receptors have AgRP as their antagonists [191]. Thus, suggesting a possible molecular link between TFF2 and the melanocortin system via the AgRP. The underlying molecular pathways are yet to be elucidated, and other works pointed out the need to clarify the roles of miRNAs in the TFF2-related processes [149,155].

The interesting part of TFF is that they are mainly expressed in the gastrointestinal system [152,192–194] which is the location in which the nutrients are fully digested into their basic forms to be absorbed [195,196]. Thus, there would be potential pathways according to which there is a detection of those nutrients [197] in the gastrointestinal system resulting in regulatory signals sent to the energy metabolism tissues (both regulatory as well as the key metabolic tissues) to trigger biological processes toward a metabolic adaptation [198] to such energy intake depending on the detected nutrients. This explains that stomach and intestine represent the starting point of energy balance regulatory signals as candidates for pharmacological targeting of TFF. Regarding TFF2 perspectives, the ultimate goal would be to identify a targetable lipid-specific signaling pathway(s) in order to control the food intake as well as other metabolic patterns. Unlike glucoses, for which we have the insulin as an induced acute regulatory metabolic signal, we are yet to identify a lipid-specifically induced acute signals equivalent to insulin. Identifying such signal(s) would allow researchers to target the food intake and control the appetite. The rationale beyond the potential targeting of TFF2 is that *Tff2* expression is induced by HFD within 30 min from the meal intake, a perfect therapeutic timing. Although we have the leptin concertation that changes with adiposity/obesity development [199–201] as well as fasting and re-feeding [202–204], identifying acute appetite/metabolic signals specific for lipids would still represent a key breakthrough with various therapeutic and mechanistic potential applications and implications.

13.6 Perspectives

The traditional approach of measuring the impact of diet on energy metabolism and obesity was limited to the mathematical evaluation of the caloric intake as compared to the energy expenditure. Now, the new advances on food chemistry and the related biological impacts allow researchers to expand the approach of diet impacts on obesity development beyond the direct caloric input. The diet can directly influence the energy balance toward certain metabolic phenotypes or can induce signals, such as TFF2, that would modify

metabolic pathways (indirect impact of the diet). These concepts related to the non-caloric patterns of the diet would allow a deeper exploration of how diet choices affect the energy balance. Therefore, allowing an optimization of the diet in order to target specific pathways depending on the metabolic needs. Within this context, we can think about a dietary "metabolic enhancer" as a potential approach within an anti-obesity therapy.

There is also a need to consider the potential pharmacotherapies that might be prescribed at the same time with a specific diet. For instance, a specific diet could activate a pathway or a biochemical reaction that deactivates a pharmacological agent and thus results in therapeutic inefficacy. Therefore, related pharmacovigilance [205] to map the line between the pharmacology and the toxicology [206,207] both in vivo and in vitro [208] remains required. However, the interaction between diet and pharmacology could be positive as shown by the omega-3 polyunsaturated fatty acid that improve the responsiveness to ursodeoxycholic acid during autoimmune liver diseases and cholestatic [209]. Thus, a specific diet choice could turn into a therapeutic adjuvant.

The education and the culture of the lifestyle habits related to diet should not be limited to the caloric balance and nutritional needs, such as vitamins and minerals, but should go beyond that toward the concept of "nutraceutical" [87,210] as a pharmacologically active food with potential therapeutic applications. Such approaches will provide additional tools to manage diseases, such as obesity, ageing and metabolic disorders and thus, optimize medical care.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, the figures, references selection, etc.) throughout the writing process, edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du

programme). Abdelaziz Ghanemi received the scholarship « Bourse Tremplin-Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec-Sante (FRQS), Quebec, QC, Canada. Figures were created using images from https: //mindthegraph.com/ and http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (accessed on 18 October 2021).

Conflicts of Interest: The authors declare no conflict of interest.

13.7 References

1. Caballero, B. Humans against Obesity: Who Will Win? *Adv. Nutr.* 2019, *10*, S4–S9.
2. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
3. Apovian, C.M. Obesity: Definition, comorbidities, causes, and burden. *Am. J. Manag. Care* 2016, *22*, s176–s185.
4. Hulbert, A.J.; Else, P.L. Basal metabolic rate: History, composition, regulation, and usefulness. *Physiol. Biochem. Zool.* 2004, *77*, 869–876.
5. van Baak, M.A. Physical activity and energy balance. *Public Health Nutr.* 1999, *2*, 335–339.
6. Westerterp, K.R. Exercise, energy balance and body composition. *Eur. J. Clin. Nutr.* 2018, *72*, 1246–1250.
7. Saito, M.; Matsushita, M.; Yoneshiro, T.; Okamatsu-Ogura, Y. Brown Adipose Tissue, Diet-Induced Thermogenesis, and Thermogenic Food Ingredients: From Mice to Men. *Front. Endocrinol.* 2020, *11*, 222.
8. Roesler, A.; Kazak, L. UCP1-independent thermogenesis. *Biochem. J.* 2020, *477*, 709–725.
9. Fenzl, A.; Kiefer, F.W. Brown adipose tissue and thermogenesis. *Horm. Mol. Biol. Clin. Investig.* 2014, *19*, 25–37.
10. Wang, F.; Deeney, J.T.; Denis, G.V. Brd2 gene disruption causes “metabolically healthy” obesity: Epigenetic and chromatin-based mechanisms that uncouple obesity from type 2 diabetes. *Vitam. Horm.* 2013, *91*, 49–75.
11. Wang, F.; Liu, H.; Blanton, W.P.; Belkina, A.; Lebrasseur, N.K.; Denis, G.V. Brd2 disruption in mice causes severe obesity without Type 2 diabetes. *Biochem. J.* 2009, *425*, 71–83.
12. Rossi, H.L.; Raj, N.R.; Marquez de Prado, B.; Kuburas, A.; Luu, A.K.S.; Barr, G.A.; Recober, A. Trigeminal Pain Responses in Obese ob/ob Mice Are Modality-Specific. *Neuroscience* 2019, *415*, 121–134.
13. Lindström, P. beta-cell function in obese-hyperglycemic mice [ob/ob Mice]. *Adv. Exp. Med. Biol.* 2010, *654*, 463–477.
14. Lopomo, A.; Burgio, E.; Migliore, L. Epigenetics of Obesity. *Prog. Mol. Biol. Transl. Sci.* 2016, *140*, 151–184.
15. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. *Cell Metab.* 2019, *29*, 1028–1044.
16. Rohde, K.; Keller, M.; la Cour Poulsen, L.; Blüher, M.; Kovacs, P.; Böttcher, Y. Genetics and epigenetics in obesity. *Metab. Clin. Exp.* 2019, *92*, 37–50.
17. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. *Diseases* 2021, *9*, 87.
18. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
19. Lim, Y.Y.; Lee, Y.S.; Ooi, D.S.Q. Engineering the Gut Microbiome for Treatment of Obesity: A Review of Current Understanding and Progress. *Biotechnol. J.* 2020, *15*, e2000013.
20. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021,

57, 66.
21. Conway, B.; Rene, A. Obesity as a disease: No lightweight matter. *Obes. Rev.* 2004, *5*, 145–151.
22. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.
23. Després, J.P.; Lemieux, I. Abdominal obesity and metabolic syndrome. *Nature* 2006, *444*, 881–887.
24. Piché, M.E.; Tchernof, A.; Després, J.P. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. *Circ. Res.* 2020, *126*, 1477–1500.
25. Piché, M.E.; Poirier, P.; Lemieux, I.; Després, J.P. Overview of Epidemiology and Contribution of Obesity and Body Fat Distribution to Cardiovascular Disease: An Update. *Prog. Cardiovasc. Dis.* 2018, *61*, 103–113.
26. Vekic, J.; Zeljkovic, A.; Stefanovic, A.; Jelic-Ivanovic, Z.; Spasojevic-Kalimanovska, V. Obesity and dyslipidemia. *Metabolism* 2019, *92*, 71–81.
27. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
28. Stolarczyk, E. Adipose tissue inflammation in obesity: A metabolic or immune response? *Curr. Opin. Pharm.* 2017, *37*, 35–40.
29. Silva Junior, G.B.; Bentes, A.C.; Daher, E.F.; Matos, S.M. Obesity and kidney disease. *J. Bras. Nephrol.* 2017, *39*, 65–69.
30. Kolb, R.; Sutterwala, F.S.; Zhang, W. Obesity and cancer: Inflammation bridges the two. *Curr. Opin. Pharm.* 2016, *29*, 77–89.
31. Fang, X.; Henao-Mejia, J.; Henrickson, S.E. Obesity and immune status in children. *Curr. Opin. Pediatr.* 2020, *32*, 805–815.
32. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. *Medicines* 2021, *8*, 57.
33. Andersen, C.J.; Murphy, K.E.; Fernandez, M.L. Impact of Obesity and Metabolic Syndrome on Immunity. *Adv. Nutr.* 2016, *7*, 66–75.
34. Rojas-Osornio, S.A.; Cruz-Hernández, T.R.; Drago-Serrano, M.E.; Campos-Rodríguez, R. Immunity to influenza: Impact of obesity. *Obes. Res. Clin. Pract.* 2019, *13*, 419–429.
35. Bischof, G.N.; Park, D.C. Obesity and Aging: Consequences for Cognition, Brain Structure, and Brain Function. *Psychosom. Med.* 2015, *77*, 697–709.
36. Xanthopoulos, M.; Tapia, I.E. Obesity and common respiratory diseases in children. *Paediatr. Respir. Rev.* 2017, *23*, 68–71.
37. Jubber, A.S. Respiratory complications of obesity. *Int. J. Clin. Pract.* 2004, *58*, 573–580.
38. Best, D.; Bhattacharya, S. Obesity and fertility. *Horm. Mol. Biol. Clin. Investig.* 2015, *24*, 5–10.
39. Silvestris, E.; de Pergola, G.; Rosania, R.; Loverro, G. Obesity as disruptor of the female fertility. *Reprod. Biol. Endocrinol.* 2018, *16*, 22.
40. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis Measures: Health Protective Properties? *Medicines* 2021, *8*, 49.
41. Ammar, A.; Brach, M.; Trabelsi, K.; Chtourou, H.; Boukhris, O.; Masmoudi, L.; Bouaziz, B.; Bentlage, E.; How, D.; Ahmed, M.; et al. Effects of COVID-19 Home Confinement on Eating Behaviour and Physical Activity: Results of the ECLB-COVID19 International Online Survey. *Nutrients* 2020, *12*, 1583.
42. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
43. Kwok, S.; Adam, S.; Ho, J.H.; Iqbal, Z.; Turkington, P.; Razvi, S.; Le Roux, C.W.; Soran, H.; Syed, A.A. Obesity: A critical risk factor in the COVID-19 pandemic. *Clin. Obes.* 2020, *10*, e12403.
44. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
45. Albashir, A.A.D. The potential impacts of obesity on COVID-19. *Clin. Med.* 2020, *20*, e109–e113.
46. Castañeda-Babarro, A.; Arbillaga-Etxarri, A.; Gutiérrez-Santamaría, B.; Coca, A. Physical Activity

Change during COVID-19 Confinement. *Int. J. Environ. Res. Public Health* 2020, *17*, 6878.
47. Pellegrini, C.A.; Webster, J.; Hahn, K.R.; Leblond, T.L.; Unick, J.L. Relationship between stress and weight management behaviors during the COVID-19 pandemic among those enrolled in an internet program. *Obes. Sci. Pract.* 2021, *7*, 129–134.
48. Popkin, B.M.; Du, S.; Green, W.D.; Beck, M.A.; Algaith, T.; Herbst, C.H.; Alsukait, R.F.; Alluhidan, M.; Alazemi, N.; Shekar, M. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. *Obes. Rev.* 2020, *21*, e13128.
49. Petrakis, D.; Marginǎ, D.; Tsarouhas, K.; Tekos, F.; Stan, M.; Nikitovic, D.; Kouretas, D.; Spandidos, D.A.; Tsatsakis, A. Obesity—A risk factor for increased COVID-19 prevalence, severity and lethality (Review). *Mol. Med. Rep.* 2020, *22*, 9–19.
50. Korakas, E.; Ikonomidis, I.; Kousathana, F.; Balampanis, K.; Kountouri, A.; Raptis, A.; Palaiodimou, L.; Kokkinos, A.; Lambadiari, V. Obesity and COVID-19: Immune and metabolic derangement as a possible link to adverse clinical outcomes. *Am. J. Physiol. Endocrinol. Metab.* 2020, *319*, E105–E109.
51. Caci, G.; Albini, A.; Malerba, M.; Noonan, D.M.; Pochetti, P.; Polosa, R. COVID-19 and Obesity: Dangerous Liaisons. *J. Clin. Med.* 2020, *9*, 2511.
52. Ritter, A.; Kreis, N.N.; Louwen, F.; Yuan, J. Obesity and COVID-19: Molecular Mechanisms Linking Both Pandemics. *Int. J. Mol. Sci.* 2020, *21*, 5793.
53. Verma, S.; Hussain, M.E. Obesity and diabetes: An update. *Diabetes Metab. Syndr.* 2017, *11*, 73–79.
54. Malone, J.I.; Hansen, B.C. Does obesity cause type 2 diabetes mellitus (T2DM)? Or is it the opposite? *Pediatr. Diabetes* 2019, *20*, 5–9.
55. Zhou, Y.; Chi, J.; Lv, W.; Wang, Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (COVID-19). *Diabetes Metab. Res. Rev.* 2021, *37*, e3377.
56. Landstra, C.P.; de Koning, E.J.P. COVID-19 and Diabetes: Understanding the Interrelationship and Risks for a Severe Course. *Front. Endocrinol.* 2021, *12*, 649525.
57. Rajpal, A.; Rahimi, L.; Ismail-Beigi, F. Factors leading to high morbidity and mortality of COVID-19 in patients with type 2 diabetes. *J. Diabetes* 2020, *12*, 895–908.
58. Neeland, I.J.; Poirier, P.; Després, J.P. Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. *Circulation* 2018, *137*, 1391–1406.
59. Ferrara, D.; Montecucco, F.; Dallegri, F.; Carbone, F. Impact of different ectopic fat depots on cardiovascular and metabolic diseases. *J. Cell Physiol.* 2019, *234*, 21630–21641.
60. Del Turco, S.; Vianello, A.; Ragusa, R.; Caselli, C.; Basta, G. COVID-19 and cardiovascular consequences: Is the endothelial dysfunction the hardest challenge? *Thromb. Res.* 2020, *196*, 143–151.
61. Lima-Martínez, M.M.; Carrera Boada, C.; Madera-Silva, M.D.; Marín, W.; Contreras, M. COVID-19 and diabetes: A bidirectional relationship. *Clin. Investig. Arterioscler.* 2021, *33*, 151–157.
62. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Post-Coronavirus Disease-2019 (COVID-19): Toward a Severe Multi-Level Health Crisis? *Med. Sci.* 2021, *9*, 68.
63. Soleimanpour, S.; Yaghoubi, A. COVID-19 vaccine: Where are we now and where should we go? *Expert Rev. Vaccines* 2021, *20*, 23–44.
64. Tremmel, M.; Gerdtham, U.G.; Nilsson, P.M.; Saha, S. Economic Burden of Obesity: A Systematic Literature Review. *Int. J. Environ. Res. Public Health* 2017, *14*, 435.
65. Cole, A.G.; Laxer, R.E.; Leatherdale, S. Social Consequences of Overweight and Obesity among Ontario High School Students. *Can. J. Diabetes* 2015, *39*, S67.
66. Bacchini, D.; Licenziati, M.R.; Garrasi, A.; Corciulo, N.; Driul, D.; Tanas, R.; Fiumani, P.M.; Di Pietro, E.; Pesce, S.; Crinò, A.; et al. Bullying and Victimization in Overweight and Obese Outpatient Children and Adolescents: An Italian Multicentric Study. *PLoS ONE* 2015, *10*, e0142715.
67. Jackson, V.M.; Breen, D.M.; Fortin, J.P.; Liou, A.; Kuzmiski, J.B.; Loomis, A.K.; Rives, M.L.; Shah, B.; Carpino, P.A. Latest approaches for the treatment of obesity. *Expert Opin. Drug Discov.* 2015, *10*, 825–839.
68. Rebello, C.J.; Greenway, F.L. Obesity medications in development. *Expert Opin. Investig. Drugs* 2020, *29*, 63–71.

69. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. *Metabolism* 2019, *92*, 163–169.
70. Fonseca-Junior, S.J.; Sá, C.G.; Rodrigues, P.A.; Oliveira, A.J.; Fernandes-Filho, J. Physical exercise and morbid obesity: A systematic review. *Arq. Bras. Cir. Dig.* 2013, *26* (Suppl. 1), 67–73.
71. Fock, K.M.; Khoo, J. Diet and exercise in management of obesity and overweight. *J. Gastroenterol. Hepatol.* 2013, *28* (Suppl. 4), 59–63.
72. Ikeda, K.; Yamada, T. UCP1 Dependent and Independent Thermogenesis in Brown and Beige Adipocytes. *Front. Endocrinol.* 2020, *11*, 498.
73. Harvey-Berino, J. Calorie restriction is more effective for obesity treatment than dietary fat restriction. *Ann. Behav. Med.* 1999, *21*, 35–39.
74. Avena, N.M.; Rada, P.; Hoebel, B.G. Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci. Biobehav. Rev.* 2008, *32*, 20–39.
75. Lennerz, B.; Lennerz, J.K. Food Addiction, High-Glycemic-Index Carbohydrates, and Obesity. *Clin. Chem.* 2018, *64*, 64–71.
76. Ghosh, S.; O'Connell, J.F.; Carlson, O.D.; González-Mariscal, I.; Kim, Y.; Moaddel, R.; Ghosh, P.; Egan, J.M. Linoleic acid in diets of mice increases total endocannabinoid levels in bowel and liver: Modification by dietary glucose. *Obes. Sci. Pract.* 2019, *5*, 383–394.
77. Lecheminant, J.D.; Gibson, C.A.; Sullivan, D.K.; Hall, S.; Washburn, R.; Vernon, M.C.; Curry, C.; Stewart, E.; Westman, E.C.; Donnelly, J.E. Comparison of a low carbohydrate and low fat diet for weight maintenance in overweight or obese adults enrolled in a clinical weight management program. *Nutr. J.* 2007, *6*, 36.
78. Brinkworth, G.D.; Wycherley, T.P.; Noakes, M.; Buckley, J.D.; Clifton, P.M. Long-term effects of a very-low-carbohydrate weight-loss diet and an isocaloric low-fat diet on bone health in obese adults. *Nutrition* 2016, *32*, 1033–1036.
79. LeCheminant, J.D.; Smith, B.K.; Westman, E.C.; Vernon, M.C.; Donnelly, J.E. Comparison of a reduced carbohydrate and reduced fat diet for LDL, HDL, and VLDL subclasses during 9-months of weight maintenance subsequent to weight loss. *Lipids Health Dis.* 2010, *9*, 54.
80. Meckling, K.A.; O'Sullivan, C.; Saari, D. Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. *J. Clin. Endocrinol. Metab.* 2004, *89*, 2717–2723.
81. Schlundt, D.G.; Hill, J.O.; Pope-Cordle, J.; Arnold, D.; Virts, K.L.; Katahn, M. Randomized evaluation of a low fat ad libitum carbohydrate diet for weight reduction. *Int. J. Obes. Relat. Metab. Disord.* 1993, *17*, 623–629.
82. Astrup, A.; Astrup, A.; Buemann, B.; Flint, A.; Raben, A. Low-fat diets and energy balance: How does the evidence stand in 2002? *Proc. Nutr. Soc.* 2002, *61*, 299–309.
83. Astrup, A. The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of low-fat diets. *Int. J. Obes. Relat. Metab. Disord.* 2001, *25* (Suppl. 1), S46–S50.
84. Hunter, P.M.; Hegele, R.A. Functional foods and dietary supplements for the management of dyslipidaemia. *Nat. Rev. Endocrinol.* 2017, *13*, 278–288.
85. Asgary, S.; Rastqar, A.; Keshvari, M. Functional Food and Cardiovascular Disease Prevention and Treatment: A Review. *J. Am. Coll. Nutr.* 2018, *37*, 429–455.
86. Alkhatib, A.; Tsang, C.; Tiss, A.; Bahorun, T.; Arefanian, H.; Barake, R.; Khadir, A.; Tuomilehto, J. Functional Foods and Lifestyle Approaches for Diabetes Prevention and Management. *Nutrients* 2017, *9*, 1310.
87. Aronson, J.K. Defining Nutraceuticals: Neither nutritious nor pharmaceutical. *Br. J. Clin. Pharm.* 2017, *83*, 8–19.
88. Sachdeva, V.; Roy, A.; Bharadvaja, N. Current Prospects of Nutraceuticals: A Review. *Curr. Pharm. Biotechnol.* 2020, *21*, 884–896.
89. Tramontin, N.D.S.; Luciano, T.F.; Marques, S.O.; de Souza, C.T.; Muller, A.P. Ginger and avocado as

nutraceuticals for obesity and its comorbidities. *Phytother. Res.* 2020, *34*, 1282–1290.
90. Manganaris, G.A.; Goulas, V.; Vicente, A.R.; Terry, L.A. Berry antioxidants: Small fruits providing large benefits. *J. Sci. Food Agric.* 2014, *94*, 825–833.
91. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive Compounds and Antioxidant Activity in Different Types of Berries. *Int. J. Mol. Sci.* 2015, *16*, 24673–24706.
92. Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Crit. Rev. Food Sci. Nutr.* 2018, *58*, 1260–1270.
93. Maleki, S.J.; Crespo, J.F.; Cabanillas, B. Anti-inflammatory effects of flavonoids. *Food Chem* 2019, *299*, 125124.
94. ¸Sanlier, N.; Gökcen, B.B.; Sezgin, A.C. Health benefits of fermented foods. *Crit. Rev. Food Sci. Nutr.* 2019, *59*, 506–527.
95. Baby, B.; Antony, P.; Vijayan, R. Antioxidant and anticancer properties of berries. *Crit. Rev. Food Sci. Nutr.* 2018, *58*, 2491–2507.
96. Boivin, D.; Blanchette, M.; Barrette, S.; Moghrabi, A.; Béliveau, R. Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFkappaB by edible berry juice. *Anticancer Res.* 2007, *27*, 937–948.
97. Sekizawa, H.; Ikuta, K.; Mizuta, K.; Takechi, S.; Suzutani, T. Relationship between polyphenol content and anti-influenza viral effects of berries. *J. Sci. Food Agric.* 2013, *93*, 2239–2241.
98. Slavin, J. Fiber and prebiotics: Mechanisms and health benefits. *Nutrients* 2013, *5*, 1417–1435.
99. Choi, B.S.; Varin, T.V.; St-Pierre, P.; Pilon, G.; Tremblay, A.; Marette, A. A polyphenol-rich cranberry extract protects against endogenous exposure to persistent organic pollutants during weight loss in mice. *Food Chem. Toxicol.* 2020, *146*, 111832.
100. Pannu, P.K.; Calton, E.K.; Soares, M.J. Calcium and Vitamin D in Obesity and Related Chronic Disease. *Adv. Food Nutr. Res.* 2016, *77*, 57–100.
101. Kuroda, M.; Sakaue, H. Role of vitamin D and calcium in obesity and type 2 diabetes. *Clin. Calcium.* 2016, *26*, 349–354.
102. Sathyamoorthy, N.; Wang, T.T.; Phang, J.M. Stimulation of pS2 expression by diet-derived compounds. *Cancer Res.* 1994, *54*, 957–961.
103. Van Horn, L.; Huffman, M.D. Eat to Treat Heart Failure. *Circ. Heart Fail.* 2018, *11*, e005367.
104. Angelidi, A.M.; Kokkinos, A.; Katechaki, E.; Ros, E.; Mantzoros, C.S. Mediterranean diet as a nutritional approach for COVID-19. *Metabolism* 2021, *114*, 154407.
105. Yoshioka, M.; St-Pierre, S.; Drapeau, V.; Dionne, I.; Doucet, E.; Suzuki, M.; Tremblay, A. Effects of red pepper on appetite and energy intake. *Br. J. Nutr.* 1999, *82*, 115–123.
106. Yoshioka, M.; Imanaga, M.; Ueyama, H.; Yamane, M.; Kubo, Y.; Boivin, A.; St-Amand, J.; Tanaka, H.; Kiyonaga, A. Maximum tolerable dose of red pepper decreases fat intake independently of spicy sensation in the mouth. *Br. J. Nutr.* 2004, *91*, 991–995.
107. Westerterp-Plantenga, M.S.; Smeets, A.; Lejeune, M.P. Sensory and gastrointestinal satiety effects of capsaicin on food intake. *Int. J. Obes.* 2005, *29*, 682–688.
108. Cani, P.D.; Joly, E.; Horsmans, Y.; Delzenne, N.M. Oligofructose promotes satiety in healthy human: A pilot study. *Eur. J. Clin. Nutr.* 2006, *60*, 567–572.
109. Howarth, N.C.; Saltzman, E.; Roberts, S.B. Dietary fiber and weight regulation. *Nutr. Rev.* 2001, *59*, 129–139.
110. Mathern, J.R.; Raatz, S.K.; Thomas, W.; Slavin, J.L. Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. *Phytother. Res.* 2009, *23*, 1543–1548.
111. Choi, B.S.; Daniel, N.; Houde, V.P.; Ouellette, A.; Marcotte, B.; Varin, T.V.; Vors, C.; Feutry, P.; Ilkayeva, O.; Ståhlman, M.; et al. Feeding diversified protein sources exacerbates hepatic insulin resistance via increased gut microbial branched-chain fatty acids and mTORC1 signaling in obese mice. *Nat. Commun.* 2021, *12*, 3377.
112. Chevrier, G.; Mitchell, P.L.; Rioux, L.E.; Hasan, F.; Jin, T.; Roblet, C.R.; Doyen, A.; Pilon, G.; St-

Pierre, P.; Lavigne, C.; et al. LowMolecular-Weight Peptides from Salmon Protein Prevent Obesity-Linked Glucose Intolerance, Inflammation, and Dyslipidemia in LDLR-/-/ApoB100/100 Mice. *J. Nutr.* 2015, *145*, 1415–1422.
113. Samane, S.; Christon, R.; Dombrowski, L.; Turcotte, S.; Charrouf, Z.; Lavigne, C.; Levy, E.; Bachelard, H.; Amarouch, H.; Marette, A.; et al. Fish oil and argan oil intake differently modulate insulin resistance and glucose intolerance in a rat model of dietary-induced obesity. *Metabolism* 2009, *58*, 909–919.
114. Steerenberg, P.A.; Beekhof, P.K.; Feskens, E.J.; Lips, C.J.; Höppener, J.W.; Beems, R.B. Long-term effect of fish oil diet on basal and stimulated plasma glucose and insulin levels in ob/ob mice. *Diabetes Nutr. Metab.* 2002, *15*, 205–214.
115. Gao, X.; Xu, J.; Jiang, C.; Zhang, Y.; Xue, Y.; Li, Z.; Wang, J.; Xue, C.; Wang, Y. Fish oil ameliorates trimethylamine N-oxideexacerbated glucose intolerance in high-fat diet-fed mice. *Food Funct.* 2015, *6*, 1117–1125.
116. Fedor, D.; Kelley, D.S. Prevention of insulin resistance by n-3 polyunsaturated fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care* 2009, *12*, 138–146.
117. Baynes, H.W.; Mideksa, S.; Ambachew, S. The role of polyunsaturated fatty acids (n-3 PUFAs) on the pancreatic β-cells and insulin action. *Adipocyte* 2018, *7*, 81–87.
118. Richards, P.; Pais, R.; Habib, A.M.; Brighton, C.A.; Yeo, G.S.; Reimann, F.; Gribble, F.M. High fat diet impairs the function of glucagon-like peptide-1 producing L-cells. *Peptides* 2016, *77*, 21–27.
119. Grabauskas, G.; Wu, X.; Zhou, S.; Li, J.; Gao, J.; Owyang, C. High-fat diet-induced vagal afferent dysfunction via upregulation of 2-pore domain potassium TRESK channel. *JCI Insight* 2019, *4*, e130402.
120. Grigorova, N.; Ivanova, Z.; Bjorndal, B.; Vachkova, E.; Penchev, G.; Berge, R.; Ribarski, S.; Georgieva, T.M.; Yonkova, P.; Georgiev, I.P. Effect of fish oil supplementation and restricted feeding on body fat distribution and blood lipid profile in a rabbit model of castration-induced obesity. *Res. Vet. Sci.* 2019, *124*, 99–105.
121. Schubert, M.M.; Irwin, C.; Seay, R.F.; Clarke, H.E.; Allegro, D.; Desbrow, B. Caffeine, coffee, and appetite control: A review. *Int. J. Food Sci. Nutr.* 2017, *68*, 901–912.
122. Gavrieli, A.; Karfopoulou, E.; Kardatou, E.; Spyreli, E.; Fragopoulou, E.; Mantzoros, C.S.; Yannakoulia, M. Effect of different amounts of coffee on dietary intake and appetite of normal-weight and overweight/obese individuals. *Obesity* 2013, *21*, 1127–1132.
123. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
124. Choi, B.S.; Daoust, L.; Pilon, G.; Marette, A.; Tremblay, A. Potential therapeutic applications of the gut microbiome in obesity: From brain function to body detoxification. *Int. J. Obes.* 2020, *44*, 1818–1831.
125. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. *Eur. Rev. Med. Pharmacol. Sci.* 2016, *20*, 4742–4749.
126. Trefflich, I.; Jabakhanji, A.; Menzel, J.; Blaut, M.; Michalsen, A.; Lampen, A.; Abraham, K.; Weikert, C. Is a vegan or a vegetarian diet associated with the microbiota composition in the gut? Results of a new cross-sectional study and systematic review. *Crit. Rev. Food Sci. Nutr.* 2020, *60*, 2990–3004.
127. Barengolts, E. Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: Review of randomized controlled trials. *Endocr. Pract.* 2016, *22*, 1224–1234.
128. da Silva, T.F.; Casarotti, S.N.; de Oliveira, G.L.V.; Penna, A.L.B. The impact of probiotics, prebiotics, and synbiotics on the biochemical, clinical, and immunological markers, as well as on the gut microbiota of obese hosts. *Crit. Rev. Food Sci. Nutr.* 2021, *61*, 337–355.
129. Aoun, A.; Darwish, F.; Hamod, N. The Influence of the Gut Microbiome on Obesity in Adults and the Role of Probiotics, Prebiotics, and Synbiotics for Weight Loss. *Prev. Nutr. Food Sci.* 2020, *25*, 113–123.
130. Gual-Grau, A.; Guirro, M.; Mayneris-Perxachs, J.; Arola, L.; Boqué, N. Impact of different hypercaloric diets on obesity features in rats: A metagenomics and metabolomics integrative approach. *J. Nutr. Biochem.* 2019, *71*, 122–131.
131. Jaiswal, S.; Libby, P. Clonal haematopoiesis: Connecting ageing and inflammation in cardiovascular

disease. *Nat. Rev. Cardiol.* 2020, *17*, 137–144.
132. Bektas, A.; Schurman, S.H.; Sen, R.; Ferrucci, L. Aging, inflammation and the environment. *Exp. Gerontol.* 2018, *105*, 10–18.
133. Popa-Wagner, A.; Petcu, E.B.; Capitanescu, B.; Hermann, D.M.; Radu, E.; Gresita, A. Ageing as a risk factor for cerebral ischemia: Underlying mechanisms and therapy in animal models and in the clinic. *Mech. Ageing Dev.* 2020, *190*, 111312.
134. Aspray, T.J.; Hill, T.R. Osteoporosis and the Ageing Skeleton. *Subcell. Biochem.* 2019, *91*, 453–476.
135. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; et al. Oxidative stress, aging, and diseases. *Clin. Interv. Aging* 2018, *13*, 757–772.
136. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* 2019, *15*, 565–581.
137. Kozakova, M.; Palombo, C. Vascular Ageing and Aerobic Exercise. *Int. J. Environ. Res. Public Health* 2021, *18*, 666.
138. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. *Am. Fam. Physician* 2017, *95*, 425–432.
139. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. *Phys. Med. Rehabil. Clin. N. Am.* 2017, *28*, 659–669.
140. Ohayon, M.; Wickwire, E.M.; Hirshkowitz, M.; Albert, S.M.; Avidan, A.; Daly, F.J.; Dauvilliers, Y.; Ferri, R.; Fung, C.; Gozal, D.; et al. National Sleep Foundation's sleep quality recommendations: First report. *Sleep Health* 2017, *3*, 6–19.
141. Flicker, L.; Lautenschlager, N.T.; Almeida, O.P. Healthy mental ageing. *J. Br. Menopause Soc.* 2006, *12*, 92–96.
142. Luo, J.; Mills, K.; le Cessie, S.; Noordam, R.; van Heemst, D. Ageing, age-related diseases and oxidative stress: What to do next? *Ageing. Res. Rev.* 2020, *57*, 100982.
143. Davies, K.J.A. Free radicals and redox regulation in ageing. *Free Radic. Biol. Med.* 2019, *134*, 688–689.
144. Viña, J.; Borrás, C.; Miquel, J. Theories of ageing. *IUBMB Life* 2007, *59*, 249–254.
145. Pajares, M.; Cuadrado, A.; Engedal, N.; Jirsova, Z.; Cahova, M. The Role of Free Radicals in Autophagy Regulation: Implications for Ageing. *Oxidative Med. Cell. Longev.* 2018, *2018*, 2450748.
146. Dhanjal, D.S.; Bhardwaj, S.; Sharma, R.; Bhardwaj, K.; Kumar, D.; Chopra, C.; Nepovimova, E.; Singh, R.; Kuca, K. Plant Fortification of the Diet for Anti-Ageing Effects: A Review. *Nutrients* 2020, *12*, 3008.
147. Bramorska, A.; Zarzycka, W.; Podolecka, W.; Kuc, K.; Brzezicka, A. Age-Related Cognitive Decline May Be Moderated by Frequency of Specific Food Products Consumption. *Nutrients* 2021, *13*, 2504.
148. De la Fuente, M. Effects of antioxidants on immune system ageing. *Eur. J. Clin. Nutr.* 2002, *56* (Suppl. 3), S5–S8.
149. Yin, Y.; Shan, H.Q.; Huang, W.; Wu, Y.M.; Lu, H.; Jin, Y. Bioinformatic analysis of miRNA expression patterns in TFF2 knock-out mice. *Genet. Mol. Res.* 2014, *13*, 8502–8510.
150. Aihara, E.; Engevik, K.A.; Montrose, M.H. Trefoil Factor Peptides and Gastrointestinal Function. *Annu. Rev. Physiol.* 2017, *79*, 357–380.
151. Thim, L.; May, F.E. Structure of mammalian trefoil factors and functional insights. *Cell Mol. Life Sci.* 2005, *62*, 2956–2973.
152. Katoh, M. Trefoil factors and human gastric cancer (review). *Int. J. Mol. Med.* 2003, *12*, 3–9.
153. Dignass, A.; Lynch-Devaney, K.; Kindon, H.; Thim, L.; Podolsky, D.K. Trefoil peptides promote epithelial migration through a transforming growth factor beta-independent pathway. *J. Clin. Investig.* 1994, *94*, 376–383.
154. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. *Nat. Rev. Mol. Cell Biol.* 2003, *4*, 721–732.
155. Shah, A.A.; Leidinger, P.; Keller, A.; Wendschlag, A.; Meese, E.; Blin, N. Altered miRNA expression patterns in Tff2 knock-out mice correlate with cellular pathways of neoplastic development and caloric

metabolism. *Int. J. Mol. Med.* 2012, *29*, 637–643.
156. Braga Emidio, N.; Brierley, S.M.; Schroeder, C.I.; Muttenthaler, M. Structure, Function, and Therapeutic Potential of the Trefoil Factor Family in the Gastrointestinal Tract. *ACS Pharm. Transl. Sci.* 2020, *3*, 583–597.
157. Xue, Y.; Shen, L.; Cui, Y.; Zhang, H.; Chen, Q.; Cui, A.; Fang, F.; Chang, Y. Tff3, as a novel peptide, regulates hepatic glucose metabolism. *PLoS ONE* 2013, *8*, e75240.
158. Arnold, P.; Rickert, U.; Helmers, A.K.; Spreu, J.; Schneppenheim, J.; Lucius, R. Trefoil factor 3 shows anti-inflammatory effects on activated microglia. *Cell Tissue Res.* 2016, *365*, 3–11.
159. Braga Emidio, N.; Meli, R.; Tran, H.N.T.; Baik, H.; Morisset-Lopez, S.; Elliott, A.G.; Blaskovich, M.A.T.; Spiller, S.; Beck-Sickinger, A.G.; Schroeder, C.I.; et al. Chemical Synthesis of TFF3 Reveals Novel Mechanistic Insights and a Gut-Stable Metabolite. *J. Med. Chem.* 2021, *64*, 9484–9495.
160. Belle, N.M.; Ji, Y.; Herbine, K.; Wei, Y.; Park, J.; Zullo, K.; Hung, L.Y.; Srivatsa, S.; Young, T.; Oniskey, T.; et al. TFF3 interacts with LINGO2 to regulate EGFR activation for protection against colitis and gastrointestinal helminths. *Nat. Commun.* 2019, *10*, 4408.
161. Ge, H.; Gardner, J.; Wu, X.; Rulifson, I.; Wang, J.; Xiong, Y.; Ye, J.; Belouski, E.; Cao, P.; Tang, J.; et al. Trefoil Factor 3 (TFF3) Is Regulated by Food Intake, Improves Glucose Tolerance and Induces Mucinous Metaplasia. *PLoS ONE* 2015, *10*, e0126924.
162. Bujak, M.; Bujak, I.T.; Sobo˘canec, S.; Mihalj, M.; Novak, S.; Cosi´c, A.; Levak, M.T.; Kopa˘cin, V.; Mihaljevi´c, B.; Balog, T.; et al. ´ Trefoil Factor 3 Deficiency Affects Liver Lipid Metabolism. *Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* 2018, *47*, 827–841.
163. Wu, X.; Zheng, H.; Yang, R.; Luan, X.; Zhang, L.; Jin, Q.; Jin, Y.; Xue, J. Mouse trefoil factor 3 ameliorated high-fat-diet-induced hepatic steatosis via increasing peroxisome proliferator-activated receptor-α-mediated fatty acid oxidation. *Am. J. Physiol. Endocrinol. Metab.* 2019, *317*, E436–E445.
164. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2015, *308*, G12–G24.
165. Kurt-Jones, E.A.; Cao, L.; Sandor, F.; Rogers, A.B.; Whary, M.T.; Nambiar, P.R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; et al. Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. *Infect. Immun.* 2007, *75*, 471–480.
166. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity* 2013, *21*, 1389–1395.
167. Lebherz-Eichinger, D.; Tudor, B.; Ankersmit, H.J.; Reiter, T.; Haas, M.; Einwallner, E.; Roth-Walter, F.; Krenn, C.G.; Roth, G.A. Increased trefoil factor 2 levels in patients with chronic kidney disease. *PLoS ONE* 2017, *12*, e0174551.
168. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an Inflammatory-Induced and AntiInflammatory Tissue Repair Factor. *Animals* 2020, *10*, 1646.
169. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice. *Animals* 2021, *11*, 258.
170. Baus-Loncar, M.; Kayademir, T.; Takaishi, S.; Wang, T. Trefoil factor family 2 deficiency and immune response. *Cell. Mol. Life Sci.* 2005, *62*, 2947–2955.
171. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. *Obesity* 2008, *16*, 2302–2307.
172. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
173. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 Expression as an Indicator of

the Severity of the High-Fat Diet-Induced Obesity. *Genes* 2021, *12*, 1505.
174. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
175. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2: From a High-Fat-Induced Gene to a Potential Obesity Therapy Target. *Metabolites* 2021, *11*, 536.
176. Wu, B.; Chien, E.Y.; Mol, C.D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F.C.; et al. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. *Science* 2010, *330*, 1066–1071.
177. Ghanemi, A. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. *Saudi Pharm. J.* 2015, *23*, 115–129.
178. Dubeykovskaya, Z.; Dubeykovskiy, A.; Solal-Cohen, J.; Wang, T.C. Secreted trefoil factor 2 activates the CXCR4 receptor in epithelial and lymphocytic cancer cell lines. *J. Biol. Chem.* 2009, *284*, 3650–3662.
179. Hirata, K.; Kodama, S.; Nakano, Y.; Minaki-Nakagawa, Y.; Aoyama, Y.; Sakikubo, M.; Goto, T.; Yoshida, M.; Masui, T.; Yamamoto, T.; et al. Exocrine tissue-driven TFF2 prevents apoptotic cell death of endocrine lineage during pancreas organogenesis. *Sci Rep.* 2019, *9*, 1636.
180. Orime, K.; Shirakawa, J.; Togashi, Y.; Tajima, K.; Inoue, H.; Ito, Y.; Sato, K.; Nakamura, A.; Aoki, K.; Goshima, Y.; et al. Trefoil factor 2 promotes cell proliferation in pancreatic β-cells through CXCR-4 mediated ERK1/2 phosphorylation. *Endocrinology* 2013, *154*, 54–64.
181. Guo, Z.; Chen, W.; Dai, G.; Huang, Y. Cordycepin suppresses the migration and invasion of human liver cancer cells by downregulating the expression of CXCR4. *Int. J. Mol. Med.* 2020, *45*, 141–150.
182. Yao, L.; Heuser-Baker, J.; Herlea-Pana, O.; Zhang, N.; Szweda, L.I.; Griffin, T.M.; Barlic-Dicen, J. Deficiency in adipocyte chemokine receptor CXCR4 exacerbates obesity and compromises thermoregulatory responses of brown adipose tissue in a mouse model of diet-induced obesity. *FASEB J.* 2014, *28*, 4534–4550.
183. Mouhieddine, T.H.; Ahmad, Y.; Barlogie, B.; Jagannath, S.; Teruya-Feldstein, J.; Richter, J. Increased Muscle CXCR4 Expression in the Setting of Rare Muscle-invasive Multiple Myeloma. *Clin. Lymphoma Myeloma Leuk.* 2020, *20*, e341–e344.
184. Banisadr, G.; Fontanges, P.; Haour, F.; Kitabgi, P.; Rostène, W.; Mélik Parsadaniantz, S. Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. *Eur. J. Neurosci.* 2002, *16*, 1661–1671.
185. Jiang, Q.; Sun, Y.; Liu, X. CXCR4 as a prognostic biomarker in gastrointestinal cancer: A meta-analysis. *Biomarkers* 2019, *24*, 510–516.
186. Urosevic, J.; Blasco, M.T.; Llorente, A.; Bellmunt, A.; Berenguer-Llergo, A.; Guiu, M.; Cañellas, A.; Fernandez, E.; Burkov, I.; Clapés, M.; et al. ERK1/2 Signaling Induces Upregulation of ANGPT2 and CXCR4 to Mediate Liver Metastasis in Colon Cancer. *Cancer Res.* 2020, *80*, 4668–4680.
187. Shibata, M.; Banno, R.; Sugiyama, M.; Tominaga, T.; Onoue, T.; Tsunekawa, T.; Azuma, Y.; Hagiwara, D.; Lu, W.; Ito, Y.; et al. AgRP Neuron-Specific Deletion of Glucocorticoid Receptor Leads to Increased Energy Expenditure and Decreased Body Weight in Female Mice on a High-Fat Diet. *Endocrinology* 2016, *157*, 1457–1466.
188. Lu, X.Y. Role of central melanocortin signaling in eating disorders. *Psychopharmacol. Bull.* 2001, *35*, 45–65.
189. Benoit, S.; Schwartz, M.; Baskin, D.; Woods, S.C.; Seeley, R.J. CNS melanocortin system involvement in the regulation of food intake. *Horm. Behav.* 2000, *37*, 299–305.
190. Biebermann, H.; Kühnen, P.; Kleinau, G.; Krude, H. The neuroendocrine circuitry controlled by POMC, MSH, and AGRP. *Handb. Exp. Pharm.* 2012, *209*, 47–75.
191. Seeley, R.J.; Drazen, D.L.; Clegg, D.J. The critical role of the melanocortin system in the control of energy balance. *Annu. Rev. Nutr.* 2004, *24*, 133–149.
192. Farrell, J.J.; Taupin, D.; Koh, T.J.; Chen, D.; Zhao, C.M.; Podolsky, D.K.; Wang, T.C. TFF2/SP-

deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. *J. Clin. Investig.* 2002, *109*, 193–204.
193. Scholven, J.; Taras, D.; Sharbati, S.; Schön, J.; Gabler, C.; Huber, O.; Meyer zum Büschenfelde, D.; Blin, N.; Einspanier, R. Intestinal expression of TFF and related genes during postnatal development in a piglet probiotic trial. *Cell. Physiol. Biochem. Int. J. Exp.*
Cell. Physiol. Biochem. Pharmacol. 2009, *23*, 143–156.
194. Dossinger, V.; Kayademir, T.; Blin, N.; Gött, P. Down-regulation of TFF expression in gastrointestinal cell lines by cytokines and nuclear factors. *Cell. Physiol. Biochem.* 2002, *12*, 197–206.
195. Capuano, E.; Janssen, A.E.M. Food Matrix and Macronutrient Digestion. *Annu. Rev. Food Sci. Technol.* 2021, *12*, 193–212.
196. Nieva-Echevarría, B.; Goicoechea, E.; Guillén, M.D. Food lipid oxidation under gastrointestinal digestion conditions: A review. *Crit. Rev. Food Sci. Nutr.* 2020, *60*, 461–478.
197. Raka, F.; Farr, S.; Kelly, J.; Stoianov, A.; Adeli, K. Metabolic control via nutrient-sensing mechanisms: Role of taste receptors and the gut-brain neuroendocrine axis. *Am. J. Physiol. Endocrinol. Metab.* 2019, *317*, E559–E572.
198. Vettor, R.; Di Vincenzo, A.; Maffei, P.; Rossato, M. Regulation of energy intake and mechanisms of metabolic adaptation or maladaptation after caloric restriction. *Rev. Endocr. Metab. Disord.* 2020, *21*, 399–409.
199. Jequier, E. Leptin signaling, adiposity, and energy balance. *Ann. N. Y. Acad. Sci.* 2002, *967*, 379–388.
200. Frühbeck, G.; Catalán, V.; Rodríguez, A.; Gómez-Ambrosi, J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Adipocyte* 2018, *7*, 57–62.
201. Rebello, C.J.; Kirwan, J.P.; Greenway, F.L. Obesity, the most common comorbidity in SARS-CoV-2: Is leptin the link? *Int. J. Obes.* 2020, *44*, 1810–1817.
202. Ishioka, K.; Hatai, H.; Komabayashi, K.; Soliman, M.M.; Shibata, H.; Honjoh, T.; Kimura, K.; Saito, M. Diurnal variations of serum leptin in dogs: Effects of fasting and re-feeding. *Vet. J.* 2005, *169*, 85–90.
203. Kiani, A. Temporal Changes in Plasma Concentration of Leptin, IGF-1, Insulin and Metabolites Under Extended Fasting and Re-Feeding Conditions in Growing Lambs. *Int. J. Endocrinol. Metab.* 2013, *11*, 34–40.
204. Lee, Y.C.; Ko, Y.H.; Hsu, Y.P.; Ho, L.T. Plasma leptin response to oral glucose tolerance and fasting/re-feeding tests in rats with fructose-induced metabolic derangements. *Life Sci.* 2006, *78*, 1155–1162.
205. Ronis, M.J.J.; Pedersen, K.B.; Watt, J. Adverse Effects of Nutraceuticals and Dietary Supplements. *Annu. Rev. Pharm. Toxicol.* 2018, *58*, 583–601.
206. Ghanemi, A. Is mapping borders between pharmacology and toxicology a necessity? *Saudi Pharm. J.* 2014, *22*, 489–490.
207. Ghanemi, A. How to define a pharmacological or a toxic food? *Alex. J. Med.* 2015, *51*, 359–360.
208. Ghanemi, A. Cell cultures in drug development: Applications, challenges and limitations. *Saudi Pharm. J.* 2015, *23*, 453–454.
209. Thérien, A.; Cie´slak, A.; Verreault, M.; Perreault, M.; Trottier, J.; Gobeil, S.; Vohl, M.-C.; Barbier, O. Omega-3 Polyunsaturated Fatty Acid: A Pharmaco-Nutraceutical Approach to Improve the Responsiveness to Ursodeoxycholic Acid. *Nutrients* 2021, *13*, 2617.
210. Kalra, E.K. Nutraceutical–definition and introduction. *AAPS Pharm. Sci.* 2003, *5*, E25.

Chapter 14. Review - Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism

Abdelaziz Ghanemi [a,b], Aicha Melouane [a,b], Mayumi Yoshioka [a], Jonny St-Amand [a,b]

[a] Functional Genomics Laboratory, CREMI, Québec Genome Center, CHUL-CHU de Québec Research Center, Québec, Québec G1V 4G2, Canada

[b] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

Received 9 May 2019; Received in revised form 20 September 2019; Accepted 1 October 2019; Available online 04 October 2019

14.1 Résumé (French abstract)

La matrice extracellulaire joue un rôle important dans le remodelage des adipocytes et dans le métabolisme du muscle squelettique (SM). La secreted protein acidic and rich in cysteine (SPARC) est exprimée dans divers tissus, y compris le tissu adipeux et le muscle squelettique où elle a un impact sur une variété de fonctions. De nombreuses recherches ont tenté d'élucider les implications de SPARC dans ces deux tissus métaboliques clés. Alors que la déficience en SPARC a tendance à remodeler les adipocytes, cette déficience diminue les propriétés métaboliques du SM. D'autre part, SPARC semble être un amplificateur du métabolisme et un médiateur de l'adaptation induite par l'exercice dans le SM ainsi qu'un inhibiteur de l'adipogenèse. Ces propriétés représenteraient un point de départ pour un ciblage thérapeutique des voies liées au SPARC dans l'obésité, la sarcopénie et le diabète.

Highlights

- SPARC is a glycoprotein that provides ECM with metabolic and functional properties.
- The ECM remodeling plays important roles in adipocytes shape/expansion remodeling.
- SPARC is involved in adipose tissue and skeletal muscle metabolism and remodeling.
- Specific therapeutic targeting of SPARC for diseases such as obesity.

14.2 Abstract

The extracellular matrix (ECM) remodeling plays important roles in both adipocytes shape/expansion remodeling and the skeletal muscle (SM) metabolism. Secreted protein acidic and rich in cysteine (SPARC) is expressed in divers tissues including adipose tissue

(AT) and SM where it impacts a variety of remodeling as well as metabolic functions. SPARC, also known as osteonectin or BM-40, is a glycoprotein associated with the ECM.

Numerous researches attempted to elucidate the implications of SPARC in these two key metabolic tissues under different conditions. Whereas SPARC deficiency tends to shape the remodeling of the adipocytes and the fat distribution, this deficiency decreases SM metabolic properties. On the other hand, SPARC seems to be an enhancer of the metabolism and a mediator of the exercise-induced adaptation in the SM and as well as an adipogenesis inhibitor.

Some findings about the SPARC effects on AT and SM seem "contradictory" in terms of tissue development and energy profile therefore highlighting the mechanistic role of SPARC in both is a priority. Yet, within this review, we expose selected research and compare the results. We conclude with explanations to "reconcile" the different observations, hypothesize the feedback and regulatory character of SPARC and put its roles within the energetic and structural maps of both adipocytes and myocytes in homeostasis and in situations such as obesity or exercise.

These properties explain the modifications and the remodeling seen in AT and SM undergoing adaptive changes (obesity, exercise, etc.) and represent a starting point for precise therapeutic targeting of SPARC-related pathways is conditions such as obesity, sarcopenia and diabetes.

Keywords: Secreted protein acidic and rich in cysteine; Extracellular matrix; Adipose tissue; Skeletal muscle; Energy metabolism homeostasis

14.3 Secreted protein acidic and rich in cysteine (SPARC): from biological remodeling to energy balance

Disorders in energy homeostasis and metabolic biofunctions represent the origins of divers health problems and diseases such as obesity and diabetes. Obesity can result from the accumulated effects of minor imbalances between energy intake and expenditure (Jequier, 2002). Overweight and obesity are defined by the World Health Organization as abnormal or excessive fat accumulation that presents a risk factor for diabetes, cardiovascular disease, dyslipidemia, cancer and other chronic diseases (Health topics, 2019; Ghanemi and St-Amand, 2018; Kilov and G, 2018). These energy imbalances are the consequence of the modern unhealthy diet characterized by an increased intake of food, mainly with high caloric density, combined to a sedentary lifestyle, in addition to factors such as psychological impacts and sleep shortage (Ghanemi et al., 2018a). Creating a deficiency in the energy balance is the basis of anti-obesity approaches. It is achieved either via a caloric restriction (limit the food intake), exercise (increase energy expenditure) or the combination of both.

Studying the dynamic expression of genes related to factors involved in obesity development and therapies such as diet, mainly highfat (HF) diet (Yoshioka et al., 2008;

Mucunguzi et al., 2017; Ghanemi et al., 2018b) and exercise (Nishida et al., 2010; St-Amand et al., 2012) would allow a better understanding of the underlying mechanisms and lead to the identification of new molecular therapeutic targets in key tissues such as adipose tissue (AT), skeletal muscle (SM) and digestive mucosa. Functional genomics represents one of the strong tools for such genes characterization and allows exploring genes expression changes/ adaptations under dynamic conditions including exercise. For instance, gene-encoding SPARC was characterized as an exercise-induced gene (Riedl et al., 2010). This represents one of the key steps toward understanding underlying molecular mechanisms implicated in the SM adaptation and adiposity modification during exercise and identify potential therapeutic targets not only for obesity but also for diabetes, sarcopenia and related metabolic disorders.

SPARC (known as osteonectin or BM-40) is a 32-kDa glycoprotein expressed mainly when tissues undergo changes such as tissue renewal, remodeling and repair (Termine et al., 1981). It is made of three domains (Lane and Sage, 1994; Bradshaw and Sage, 2001; Brekken and Sage, 2000) and is encoded by the gene SPARC in the chromosomal site at 5q31-q33 in human (Swaroop et al., 1988) and localized to the central region of chromosome 11 in mouse (Mason et al., 1986a). SPARC is a calcium binding matricellular glycoprotein secreted by several types of cells in many organisms, and it is associated with extracellular matrix (ECM) organization and remodeling, growth, cellular differentiation, wound repair and tissue response to injury (Bradshaw and Sage, 2001; Tai and Tang, 2008; Basu et al., 2001; Rosset and Bradshaw, 2016). SPARC binds to matrix proteins and controls cellular interaction with the ECM (Brekken and Sage, 2000). ECM represents a network of macromolecules including fibronectin, collagens and glycosaminoglycans that control a variety of biological functions such as signals transduction and allow cellular adhesion to form tissues and organs (Theocharis et al., 2016). This implication of SPARC in ECM functions suggest that alterations, including the deficiency and over expression, of SPARC would have an impact on the ECM remodeling, thus, influences how the cells receive surrounding signals and respond to them and also impact the divers cellular functions and properties such as cell shape and polarity, growth, tissue expansion pattern and energy metabolism such as those reported in studies linking SPARC and cancer (Naczki et al., 2018; Said et al., 2007; Neuzillet et al., 2013).

It is worth mentioning that in spite of these important functions modulated by SPARC, SPARC-deficient mice have similar growth, fertility, and viability compared to wild-type (WT) mice (Norose et al., 1998). This allows reproducing, observing and, most importantly, studying this genetically modified model and learn more about this gene and its encoded protein. In addition, results obtained from studying SPARC in mice could be reasonably extrapolated to humans due the high homology between mouse and human SPARC (Norose et al., 1998). Herein, we focus on how changes in SPARC expression impact two keys metabolic tissues, AT and SM, mainly through the ECM remodeling and the metabolic changes involving the mitochondria. These two tissues represent the main

site of energy storages and energy expenditure, respectively and have the highest importance within obesity and metabolic homeostasis research.

14.4 SPARC and adipocytes: ECM impacts adiposity distribution

Development of obesity is known to be under the influence of diver factors such as genetics, environment (including diet), exercise, sleep shortage and psychology (Ghanemi et al., 2018a). Obesity is considered as a status in which cells undergo selected modification in order to adapt to the new energetic status. Within this context, describing the ECM remodeling implication in both adipocytes development and fat distribution represents the key to understand the role of SPARC in obesity development. AT is the main tissue of energy storage and the key tissue in obesity pathogenesis (Ghanemi et al., 2018a).

Different studies and observations linking gene-encoding SPARC to adiposity and adipogenesis have been reported in the literature. However, not all results have similar conclusions. These differences seen among the effects of SPARC deficiency could be due to different factors such as the mice age, genetic background, investigated tissues, their localization and diet (type and composition). For instance, the work of (Bradshaw et al. (2003a)) have reported SPARC-deficient mice with increased fat deposition in epididymal and dermal (with increased-diameter adipocytes) but not in major organs including liver, lung, and kidney with a HF diet but not with a low-fat (LF) diet although the food intake was similar (Nie et al., 2011). This suggests that a reduced energy expenditure is, at least in part, beyond this enhanced HF diet-induced weight gain. Especially that when handled, SPARC deficient mice have been reported as passive and with reduced physical activity (lower mobility-related energy expenditure) compared to WT mice (Norose et al., 1998). Importantly, the differences in the ECM between subcutaneous AT of WT and SPARC deficient mice could be explained by the adaptive properties of the adipocytes ECM (Mori et al., 2014; Lin and Kang, 2016) and indicate why SPARC-deficient mice fed with HF-diet had more fat deposit within the dermal AT compared to WT. Indeed, since SPARC is an important component of the ECM, its deficiency reduces the ECM (mesangial cells) (Taneda et al., 2003) and would reduce the “rigidity” of the ECM (SPARC-deficiency-induced ECM remodeling) and thus, increases the expansion ability of the adipocytes (Figure 14.1) especially in the subcutaneous AT in which SPARC expression is predominant compared to visceral AT (Kos et al., 2009). Therefore, the subcutaneous AT would be more affected by SPARC deficiency and develop a greater ability to expand and store fat compared to the visceral AT (whereas the opposite is seen WT mice).

Obesity development is not only about the fat tissue percentage, but also about the ability of adipocyte to expand both in number (hyperplasia) and size (hypertrophy) (Ghanemi et al., 2018a). Thus, histological studies of adipocytes remain an essential element. Bradshaw et al. showed that although SPARC-deficient mice had increased fat accumulation and total number of adipocytes in epididymal pads, those adipocytes were bigger and thus the number of fat cells per gram of epididymal pads tissue was lower

(Bradshaw et al., 2003b). These suggest that this tissue expansion resulted from the increase of adipocyte size, likely due to the decrease of collagen I (Francki et al., 1999) in the same reported tissue (Bradshaw et al., 2003a). This is similar to what was seen in the dermis of SPARC-deficient mice of another study as well (Bradshaw et al., 2002) and which is also similar to what has been reported by Mansergh et al. (increased in bone marrow adipocyte size and not number in SPARC deficient mice at the age of 4 months) (Mansergh et al., 2007). Therefore, this indicates that in this situation, SPARC-deficient mice have an increased size of adipocytes rather than increased differentiation of preadipocytes into adipocytes in the epididymal AT (hypertrophic fat accumulation). Importantly, the fact that in SPARC-deficient mice the increased adiposity was observed in epididymal and subdermal AT but not in liver, lung or kidney (Bradshaw et al., 2003a), further supports that this enhanced adiposity affects more subcutaneous AT rather than the ectopic localizations. Interestingly, the increased adipocytes size rather than preadipocytes differentiation into adipocytes reported in SPARC-deficient mice further emphasize the importance of SPARC. This highlights SPARC in cell differentiation as a macromolecule expressed specifically when tissues undergo changes which explains why the SPARC-deficient adipocytes have limited differentiation. Therefore, points once again obesity-developing status as a biological change requiring cell differentiation and ECM remodeling.

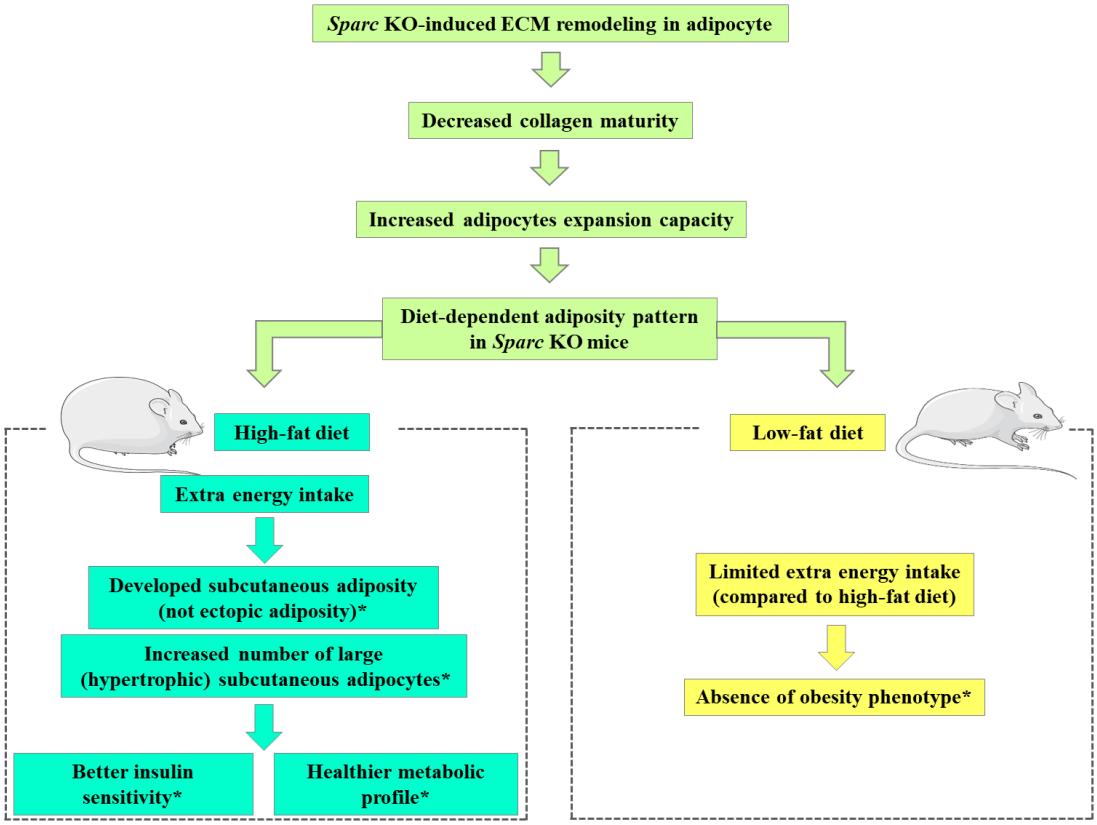


Figure 14.1. The difference in ECM between subcutaneous and other adipocytes localizations makes that only an extra energy intake "reveals" a significant difference in adiposity development (development ability) pattern between different localizations.

*: Compared to WT mice.
Abbreviations: ECM: Extracellular matrix; SPARC: Secreted protein acidic and rich in cysteine; WT: Wild type.

The ECM remodeling phenomenon, involving different enzymes and factors, is not limited to AT. It has been reported in different tissues such as cardiac tissue (McCurdy et al., 2010), lung (Tomos et al., 2017), brain (Miyata and Kitagawa, 2017), peripheral neurons (Moustafa et al., 2018), and in conditions including tumors (Erdogan and Webb, 2017; Despotovic et al., 2017) some of which undergo a SPARC-dependent ECM remodeling (Tanaka et al., 2019) which further point the implication of SPARC in ECM remodeling. Importantly, therapeutic targeting of ECM remodeling has also been described (Agarwal and Agrawal, 2017; van der Steen et al., 2017; Ito and Ohno, 2018; Islam et al., 2018). The ECM remodeling properties and its implications both in maintaining homeostasis and in pathological conditions (Ford and Rajagopalan, 2018) provide explanations on how adipocytes are modified in terms of expansion and tissue differentiation during a developing obesity which accrues when the energy balance is switched from a balanced status into a broken homeostasis (Ghanemi et al., 2018a). Thus, cellular and functional properties of remodeled adipocytes would require ECM remodeling that also depends on the adipocytes localization as well as on the ECM properties of each type of adipocytes (subcutaneous or visceral). Following the same line of thoughts, the SPARC deficiency would have an impact on the adipocyte ECM remodeling occurring during obesity and therefore, govern fat distribution. Such conclusion would be explained by the property of SPARC to bind and interact with different component of the ECM such as collagen type IV, vitronectin and fibrillar collagens (types I, II, III, and V) (Brekken and Sage, 2000) and thus affect adipocytes “rigidity” and expansion ability when compared to the adipocytes under balanced homeostasis.

Therefore, when fed with a HF diet, SPARC-deficient mice would store the extra energy in the subcutaneous AT rather than in the visceral AT. Such adiposity distribution pattern is towards a better metabolic profile because cardiometabolic benefits have been associated to subcutaneous AT (Tran et al., 2008a; Chen et al., 2018; Hocking et al., 2008; Tran et al., 2008b), whereas the visceral AT is associated with a variety of health problems including metabolic syndrome (Despres and Lemieux, 2006), cardiometabolic risk (Smith et al., 2012), coronary artery diseases and dysregulation of lipoprotein-lipid metabolism (Despres, 1992). However, this difference in fat storage between the subcutaneous AT and visceral AT is not seen in LF-fed mice. Indeed, although there is an increased adipocyte expansion in subcutaneous adipocytes compared to visceral adipocytes, the LF diet does not provide enough extra energy intake to create a positive energy balance (Figure 14.1) (as in the HF diet) to reveal such differential ability to store fat between these two locations of AT. Therefore, no such difference is seen in LF-fed mice. This explains the contrast of the results reported above and point that links between SPARC and the fat distribution (visceral vs subcutaneous) which is in fact more important than the body weight in terms of health prognosis (Ghanemi et al., 2018a; Park and Lee, 2005; Neeland et al., 2019). The previous

critical analysis of the works linking SPARC to adiposity allows us to hypothesize that SPARC-deficient mice will not become obese nor develop increased adiposity compared to WT mice when fed with a LF diet (Figure 14.1). This is supported by a recent study showing that whereas both SPARC-deficient and WT mice fed with regular laboratory chow have similar body weights, SPARC-deficient mice fed with high-calorie diet (HF chow and sucrose added to drinking water) had an increased body weight compared to WT mice fed with the same high-calorie diet (Atorrasagasti et al., 2019).

Moreover, stage of adipogenesis is also affected by the type of expressed collagen which would also be affected by SPARC. Indeed, preadipocytes synthesize primarily collagen I (fibrillar) but differentiated adipocytes express primarily collagen IV (the prevalent collagen in basement membranes) as part of the ECM network (Smas and Sul, 1995) with the increased secretion of type IV collagen in association with adipocytes differentiation (Aratani and Kitagawa, 1988). This is of interest knowing that the association between collagen IV and adipocytes differentiation might also be behind the difference in adipogenesis between visceral and subcutaneous adiposity based on the dynamics of ECM (Mariman and Wang, 2010) leading to a fat distribution toward a developed subcutaneous adiposity (rather than a visceral AT) and eventually results in an improved insulin sensitivity and a lower metabolic risk (compared to a developed visceral adiposity rather than subcutaneous adiposity) (Despres et al., 1990; Després, 2012). In addition, SPARC-deficient mice had no significant difference in the circulating amount of insulin compared to WT (Bradshaw et al., 2003a). This would further indicate a higher insulin sensitivity because these SPARC-deficient mice had increased adiposity (Bradshaw et al., 2003a) for which insulin action is required due to the implication of insulin in AT development (Dimitriadis et al., 2011; Emanuel Anna et al., 2017). However, the fact that SPARC-deficient mice have a developed subcutaneous AT, does not necessary mean less visceral AT but could indicate an increased ability to store the extra energy in the dermal AT. These SPARC-deficiency induced modifications in the fat distribution could contribute to reduce central obesity and thus, resulting in a better metabolic profile with reduced risk for the metabolic syndrome and insulin resistance (Figure 14.1).

Yet, the expressions of SPARC and the associated proteins (herein, collagens) pattern would be different depending on tissues, mouse strain and also age (Mansergh et al., 2007). For instance, whereas in the study of Norose et al. only 1.5 months were enough to observe the first posterior cortical opacities (cataract) in C57BL/6 J × 129 Sv F2 (exon 4 was targeted) mice (Norose et al., 1998), in the study that used MF1 × 129 Sv F2 background mouse strain (exon 6 was targeted for disruption) cataract was observed at around 6 months of age (Gilmour et al., 1998). These illustrates the effect of mouse strain on the effects of SPARC deficiency seen at different ages. This indicates that the degree of implication of SPARC in ECM remodeling (effect of SPARC deficiency) is different depending on the age (development stage) and thus can explain those “contradictory” results seen among the different SPARC deficiency studies (Norose et al., 1998; Gilmour et al., 1998). Studies in other species and different tissues have also been carried out and

showed the different effects of either SPARC deficiency or SPARC overexpression that can be seen. As an illustration, the overexpression of SPARC decreased type IV collagen levels in the basement membrane ECM of anchor cells in Caenorhabditis elegans (Morrissey et al., 2016) and SPARC-deficient mesangial cells have a reduced expression of collagen type I (Francki et al., 1999). Such data further support the involvement of SPARC in cellular remodeling.

SPARC is known to inhibit adipogenesis, a pathway shown to involve beta-catenin signaling enhancement (Nie and Sage, 2009a,b). Following a HF diet, SPARC has been shown to be upregulated (epididymal adipose) in three different models of obesity (Tartare Deckert et al., 2001). This SPARC upregulation could represent a homeostatic response to the HF diet in order to limit adipogenesis as regulatory feedback (Figure 14.3) and maintain the energy balance. The possible induction of AT fibrosis resulting from the hyperleptinemia induced upregulation of SPARC (Petersson et al., 2013) would also participate in limiting adiposity development. However, the existence of factors that are also involved in adipogenesis such as collagens and that are also affected by SPARC expression (Francki et al., 1999; Bradshaw et al., 2002) could make that although SPARC inhibits adipogenesis, SPARC deficiency might not stimulate adipogenesis and could just remove the SPARC induced adipogenesis inhibition (Nakamura et al., 2012). Within this context, Delany et al. (Delany et al., 2003) indicated that the absence of SPARC might influence adipocytes differentiations. However, the adipocytes described in this paper are bone marrow AT (BMAT) that have different properties from the adipocytes we focus on in obesity research which are white AT (both subcutaneous and visceral) in addition to the brown adipose tissue (BAT) as described by Hardouin et al. (Hardouin et al., 2016). In addition, the loss of bone due to the SPARC deficiency would affect the BMAT properties, which means that the modifications seen in the adipocytes of SPARC-deficient bone marrow would be the consequence of the reduced ability of osteoblasts for formation, maturation and survival that resulted from the SPARC deficiency. Adipocytes could even be a “replacement tissue” due to the fibrosis tissue limited ability to develop because it requires SPARC as a fibrosis promoter (Kos et al., 2009) which is absent in those SPARC-deficient mice. Importantly, (Hardouin et al., 2016) conclude that their finding does fit with the idea that SPARC governs and antiadipogenic signal.

SPARC is involved in cell differentiation and development (Bradshaw and Sage, 2001). Since obesity is considered as a status of a “development” and turnover that seems to require SPARC, difference in SPARC expression between obese and non-obese models requires attention. In addition, SPARC expression should be interpreted differentially depending on whether it is expressed during an ongoing tissue development (such as adiposity growth during obesity (Tartare-Deckert et al., 2001)) conditions or rather is a non developing tissue. Such way of thinking could emphasize that the overexpression of SPARC in obesity could be an adaptive feedback attempting to limit the adiposity expansion. This both correlates with results associating SPARC expression and AT hyperplasia in obese transgenic mice (Chavey et al., 2006) and the inhibitory properties of

SPARC toward adipogenesis (Nie and Sage, 2009a,b). In addition, since ageing is a factor of body fat gain (St-Onge and Gallagher, 2010), SPARC deficiency effect on adiposity would be more noticeable in old subjects. In addition, since SPARC is important in muscle functions (as detailed in the next section), especially during exercise, the SPARC-deficiency could reduce muscular activity-related energy expenditure and therefore, with HF diet, would further enhance the positive energy balance and therefore, increase the energy storage which explains the increase of the HF diet-induced weight gain and AT depots in SPARC-deficient mice. Because of the impact of SPARC on the adipocytes ECM remodeling, the SPARC deficiency would increase the expansion ability of adipocytes. This ability is different between subcutaneous and visceral adiposity, and the distribution pattern would be towards an increased accumulation of the extra energy in the subcutaneous AT, thus leading to an improved metabolic profile and a healthier outcome compared to an adiposity accumulation within the visceral or ectopic locations.

14.5 The gene encoding SPARC as an "exercise-induced gene": a metabolic enhancer of the skeletal muscle

SM represents a key tissue of energy consumption since it accounts for an important ratio (50%) of energy expenditure of the body and around 40% of the body mass (Frontera and Ochala, 2015). Moreover, SM is responsible for around 70%–80% of insulin-stimulated postprandial glucose uptake (DeFronzo et al., 1981; Nuutila et al., 1994). Changes observed in the SM following exercise in term of cell differentiation, proliferation, mitochondrial functions and ECM remodeling are important to study at the molecular level in different ages. It allows to both elucidate the underlying mechanisms of important physiological and pathological pathways and the identification of potential therapeutic targets especially if we consider changes of muscle mass and metabolic functions associated with aging and the link with obesity (Biolo et al., 2014). Indeed, aging leads to muscle loss and dysfunction which affects the energy metabolism, including resting metabolic rate (St-Onge and Gallagher, 2010). Thus, aging represents an influencing factor in obesity development via increasing fat tissue especially for sedentary individuals (Biolo et al., 2014). Therefore, genetic factors affecting muscular metabolic performance would be easier to observe and evaluate in young subject since this metabolic performance (energy usage, contractility, etc.) decreases physiologically with age. Following this line of thought, effects of SPARC deficiency would be more significant in young subjects especially that SPARC expression in SM decreases with age as well (Scime et al., 2010; Nakamura et al., 2013).

Numerous research teams have shown that exercise enhances the expression of mitochondrial genes such as oxidative phosphorylation (OXPHOS) and improves physical fitness, lipid profile and insulin sensitivity (Riedl et al., 2010; JM, 1994). A cellular model of exercise (electrical pulse stimulation applied in myotubes culture) also showed that gene encoding SPARC is an electrical pulse stimulation-induced gene (Melouane et al., 2019).

Its activity in linking mitochondrial properties and ECM would involve interaction with integrin-linked kinase/adenosine monophosphate-activated protein kinase (AMPK) pathway (Melouane et al., 2019). A study reported genes modulated in SM by mild exercise which can easily and safely be performed by elderly individuals (Riedl et al., 2010). This study has highlighted the importance of mitochondrial OXPHOS and ECM remodeling in the SM adaptation. Importantly, these results showed that the training at lactate threshold (LT) induced 3 transcripts related to ECM, namely collagen type III alpha 1 (COL3A1), collagen type IV alpha 1 (COL4A1) and gene encoding SPARC, which accounted for 25% (3/12) of modulated transcripts in elderly (Riedl et al., 2010). However, in young adults, only 2 transcripts in the same function including collagen type I alpha 2 (COL1A2) were modulated, which corresponds to approximately 0.5% (Nishida et al., 2010). Although the only other study examining the effects of exercise on SM transcriptome in elderly subjects has used high-intensity (80% maximal heart rate) exercise, the exercise has also induced ECM-related genes such as genes encoding SPARC and COL3A1 (Radom-Aizik et al., 2005). The ECM associated protein, SPARC, specifically binds several ECM molecules including collagen types I, III and IV (Sage et al., 1989), thus influence both fibrous and basal lamina organization through growth factors binding, such as insulin-like growth factor (IGF), to mediate cell-matrix interactions (Francki et al., 2003; Mason et al., 1986b).

A Recent study has confirmed the induction of SPARC mRNA and protein expressions after exercise in SM of human and mice (Aoi et al., 2013). More importantly, increases in C2C12 myoblasts differentiation and expression levels of myogenin, COL1A1 and OXPHOS proteins after adding a recombinant SPARC protein have been described (Melouane et al., 2018). In addition, an improved collagen maturation was also reported following the overexpression of SPARC in WT mice (Schellings et al., 2009). These elements strongly suggest that SPARC represents an important factor in exercise-induced metabolic benefits and improvement of muscle structure and energy homeostasis profile; especially that exercise has been linked to ECM remodeling (Duarte et al., 2017) and an upregulation of SPARC has been reported during SM regeneration as well (Petersson et al., 2013). In contrast, lack of SPARC has been linked to modified expression of actin isoforms, led to post-fatigue recover inability (Jorgensen et al., 2017) and myofiber atrophy (Nakamura et al., 2013). This further support the concepts defining SPARC as specifically expressed according to a ‘temporal” pattern (Petersson et al., 2013) during tissue modifications including remodeling and repair (Jorgensen et al., 2009) rather than regeneration (SPARC deficiency does not affect SM regeneration (Jorgensen et al., 2017)) although there is an upregulation of SPARC during SM regeneration (Petersson et al., 2013). This could indicate that SPARC would be involved in complementary remodeling and repair functions during regeneration. In addition, the inhibitory properties of SPARC towards myoblast differentiation (Petersson et al., 2013) could be seen as a regulatory effect to control the SM hyperplasia and rather enhance SM metabolic performance instead of increasing SM cells number.

All together, these data allow us to map the links between SPARC expression and SM modification including during exercise and development (metabolic and functional) mediated by ECM and mitochondria as well as cytoskeletal (Figure 14.2) because SPARC interacts, at least in part, with SM actin (Jorgensen et al., 2017). Since exercise induces the release of SPARC from the SM, considered therefore as a myokine (So et al., 2014), it would act, among other tissues, on the SM itself and therefore would be an "autocrine" factor.

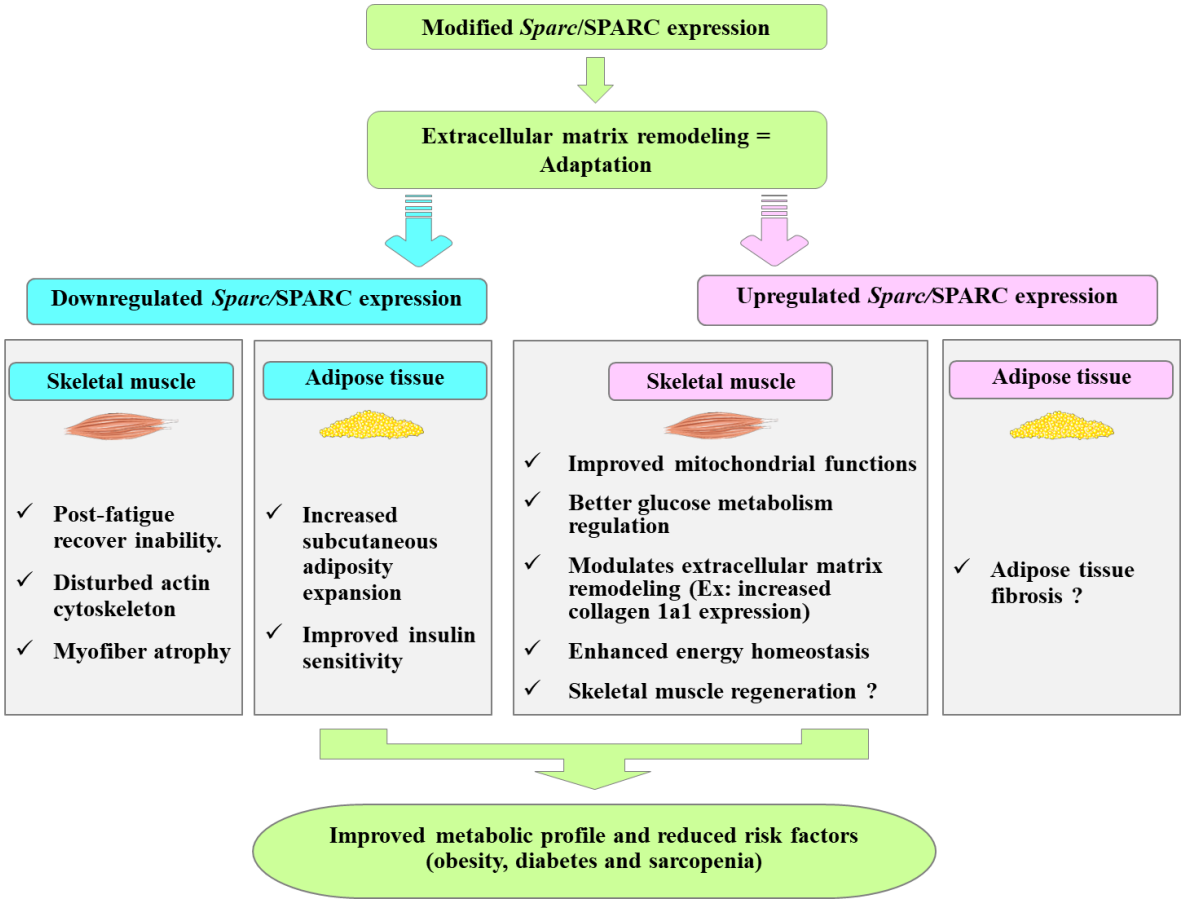


Figure 14.2. SPARC seems to play different roles in these two tissues. Whereas it ameliorates metabolic and structural properties of the skeletal muscles, SPARC-deficiency in adipocytes seems metabolically beneficial.
Abbreviation: SPARC: Secreted protein acidic and rich in cysteine

Moreover, there is a decrease in both collagen type I (increase in AT) and physical activity in SPARC-deficient mice (Norose et al., 1998; Delany et al., 2000). In addition, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR)-stimulated AMPK phosphorylation is reduced by small interfering RNA (siRNA) of gene encoding SPARC (Song et al., 2010). This is of interest because AMPK phosphorylation induces mitochondrial biogenesis via the activation/induction of the key regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma coactivator 1 alpha

(PPARGC1A, also known as PGC1α) (Lira et al., 2010; Jager et al., 2007; Wu et al., 1999) and thus, siRNA of gene encoding SPARC would reduce the mitochondrial biogenesis. This indicates that the deficiency or the down expression of gene encoding SPARC negatively affect the mitochondrial biogenesis and thus the OXPHOS capacity of the SM (cellular bioenergetics). In addition, the increase of adiposity without an increase in the body weight in SPARC deficient mice (compared to WT) (Bradshaw et al., 2003a) could indicate a loss of lean mass (SM) as well. This would suggest an implication of SPARC not only in the ECM or mitochondrial functions but also in the SM building (myocytoskeletal). Whereas exercise is known to increase protein synthesis in aged SM (Reynolds et al., 2004), myoblasts from endurance-trained men exhibit higher glucose uptake (Berggren et al., 2005), probably via the induction of glucose transporter type 4 (GLUT4) expression by the training (Neufer and Dohm, 1993). Insulin plays important roles in the SM such as protein synthesis and glucose transport, and exercise improves insulin actions, which runs counter to aging (Riedl et al., 2010; JM, 1994; Harris, 2005; Drela et al., 2004). SPARC is known to modulate the interaction of cells with growth factors as well as to interact with AMPK and regulate GLUT4 expression (Francki et al., 2003; Song et al., 2010), which establishes more links between SPARC and muscular energy metabolism.

Based on the links among SPARC, its expression induction by exercise and exercise effects on SM, it seems logic to point SPARC as main factor in the SM remodeling and the exercise-induced SM metabolic changes. The implications of SPARC in heart, which is increased by β- adrenergic stimulation (Masson et al., 1998), seem to be comparable to those in SM. Indeed, following an induced myocardial infarction, prevention of cardiac dysfunction with an amelioration in collagen maturation in gene encoding SPARC overexpressed mice, whereas the SPARC deficiency leads to an immature collagenous ECM and increased cardiac dysfunction (Schellings et al., 2009).

Unlike in the AT, the increased expression of SPARC (rather than its deficiency) is positive for the SM metabolic performance. Indeed, taken together, the previous reported observations show that SPARC is both induced by exercise and also required to mediate some of the exercise-induced benefits both in terms of tissue remodeling (adaptation) and metabolic functions enhancement (enhanced OXPHOS and glucose usages). Therefore, enhance the metabolic ability of the SM. With such SM enhancement properties, SPARC could be used therapeutically for diseases such as sarcopenia as well as disorders involving energy balances deregulation such as obesity. However, herein the missing link seems to be the elucidation of how SPARC affects the SM contractile properties, its mechanic and the SM cytoskeletal properties. Completing such aspect, would give a full image of how SPARC impact the SM both at the metabolic and structural (mechanic properties) levels.

14.6 Applications and therapeutic perspective

The data we have exposed show that SPARC is a multi-functional glycoprotein that has impacts on metabolism which support the theories linking the ECM to the

mitochondrial function and metabolic modifications (Melouane et al., 2018). Furthermore, ECM remodeling has been reported following exercise training in visceral AT (Duarte et al., 2017) as well as with both obesity and exercise in SM as well (Martinez-Huenchullan et al., 2017). This reflects the complex interactions between ECM (impacted by SPARC modifications) and these two key metabolic tissues, AT and SM, that govern most of the energy flow (storage and expenditure, respectively).

Whereas the beneficial effects on AT are seen following SPARC deficiency, the improvement of muscular metabolisms seems to rather be the result of an overexpression of gene encoding SPARC (Figure 14.2). Indeed, the SPARC deficiency would increase the adipocytes extension ability (and fat storage) of the subcutaneous AT more than in the visceral AT which would reduce the negative impacts of visceral adiposity and increase the benefits of a developed subcutaneous AT. However, in the SM it is rather the overexpression of SPARC that would lead to an improved metabolic profile with a better adaptation to exercise, whereas SPARC deficiency would have negative effects on SM such as a disturbed actin cytoskeleton. This indicates that although SPARC expression-induced ECM remodeling follow a similar pattern in muscles and adipocytes (especially in terms of collagen expression/maturation), it leads to different metabolic outcomes in these two tissues. Indeed, the consequences of SPARC deficiency are similar in both AT and SM (limit the development). However, SPARC deficiency leads to divergent phenotype in AT and SM because the functions of these two tissues are different. A developed muscle does increase energy expenditure whereas a developed AT does enhance the energy storage.

SPARC is produced by adipocytes into the circulation with concentrations that are in correlation with the body mass index (BMI) (Kos et al., 2009; Takahashi et al., 2001). In addition, the correlation between AT-derived SPARC with both fat mass and waist circumference (Kos et al., 2009) was also reported. This could also be put in the context of a correcting/balancing metabolic homeostasis via which adipose cells of obese individuals produce SPARC that will migrate ("hormone") to the SM in order to produce its effect of enhancing the metabolism and remodeling of the SM, which would improve muscle dependent energy expenditure and thus, counter the increased caloric intake (beyond the elevated BMI) as an attempt to reestablish the energy balance. Additionally, the SM development is required to carry the excess body weight of obese subjects. This would also explain the AT production of SPARC that will increase SM functions. Following the same line of thoughts, and within the context of "re-establishing" the energy balance, the elevated concentration of SPARC in obese patients would be an attempt to limit the adipogenesis (via the adipogenesis inhibition properties of SPARC (Nie and Sage, 2009a,b)) and would follow similar pattern as leptin which is elevated in obese patients but fails to perform its action as an anti-obesity factor (leptin resistance) (Sainz et al., 2015) (Figure 14.3). Indeed, the increased serum concentration of leptin can also be seen as an attempt to establish the energy balance via the properties leptin to limit food intake and increase energy expenditure (Halaas et al., 1995). However, since leptin fails to balance the energy homeostasis (leptin resistance (Myers et al., 2012)) in obese patient, the raising

question would be whether or not we have a "SPARC resistance" in obesity leading to the loss of the adipogenesis inhibition properties of SPARC. This theory is further supported by the ability of both leptin and insulin (also elevated during obesity (Wang and Liao, 2012)) to enhance SPARC production in AT (Kos et al., 2009), whereas glucose decreased it (Kos et al., 2009). Thus, these observations put SPARC within the pathways involving leptin, insulin (Figure 14.3) and glucose in energy homeostasis regulation especially that SPARC is required for both insulin secretion and glucose homeostasis (Atorrasagasti et al., 2019).

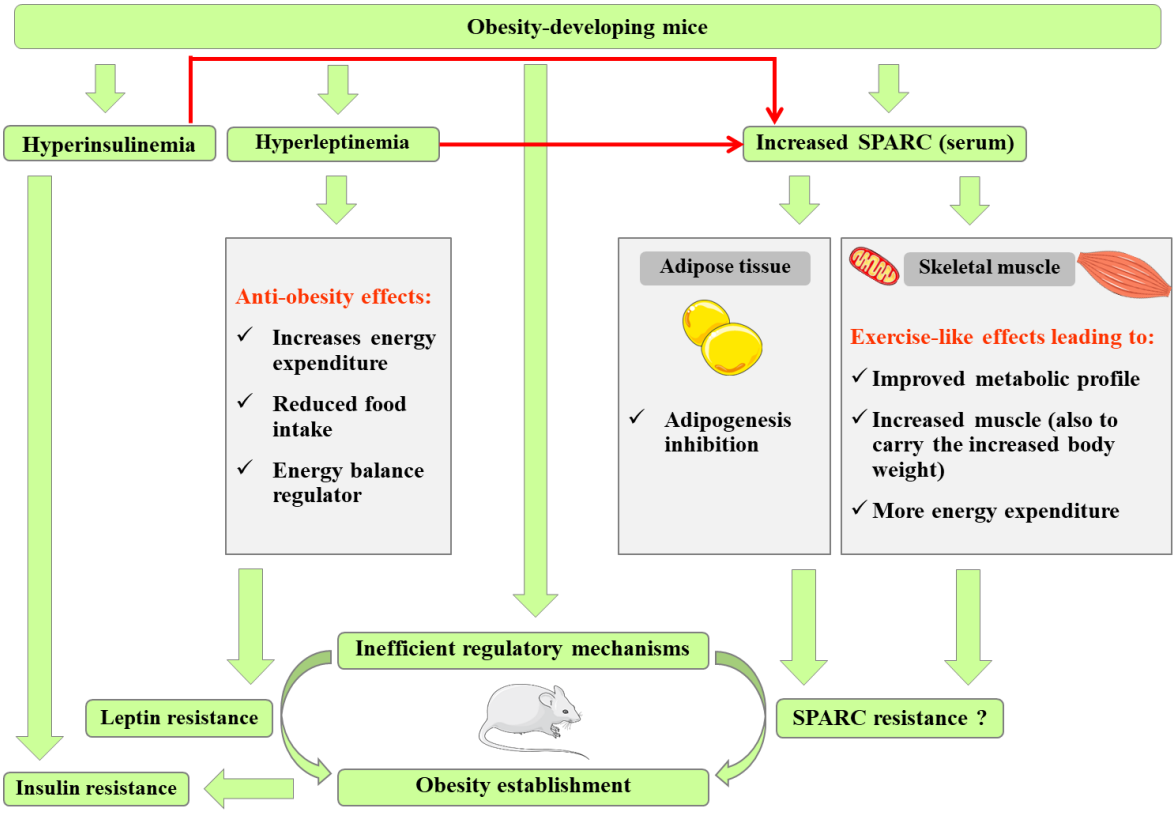


Figure 14.3. SPARC as an obesity-induced factor: An attempt to re-establish energy balance. Abbreviation: SPARC: Secreted protein acidic and rich in cysteine.

This could support the concept defining the role of a factor (hormone, transmitter, etc.) depending on the status. SPARC would not have the same properties in healthy status (balanced energy homeostasis) as it has during broken homeostasis (obesity, diabetes, sarcopenia, etc.)

In addition, the known implications of mitochondria in ageing, especially within AT and SM (Boengler et al., 2017), would justify the need to investigate SPARC roles during the ageing process and its eventual interactions with reactive oxygen species (oxidative stress) (Aseer et al., 2017) that play roles in diabetes (Panigrahy et al., 2017), ageing (Korenevsky et al., 2017), inflammation (Hsu et al., 2018), obesity (McMurray et al., 2016) and sarcopenia (Jackson, 2016; Vasilaki et al., 2017). Indeed, ageing (with the free radicals

accumulation, decreased biological function, etc.) represents a risk factor for many diseases including metabolic disorders.

Studying the functional and, more importantly, the metabolic implications of SPARC in other tissues and organs, especially other key metabolic tissues such as the liver and the brown AT will be the next step to map the metabolic profile of SPARC within the homeostatic balance. The hormonal changes (leptin, insulin, etc.) are also another key step within this path, since leptin and insulin induce SPARC expression in adipocyte (Kos et al., 2009). Importantly, mapping these pathways should take into consideration the different related influencing factors such as the healthy status (obese or lean), the activity level (sedentary or active) and the type of diet (HF, LF, high sucrose, etc.). The effects of SPARC on cells does not only depend on tissues (AT and SM in our examples) but also on the status (and its stage, whether advanced or early), the factors and the environment to which the tissue is exposed (normal, cancer, obesity, development, exercise, etc.).

Therapeutic targeting (gene therapy, pharmacological agents, etc.) of SPARC or gene encoding SPARC-related pathways in obesity for example would require a precise therapy (such as new generations of therapeutic vectors) and an increased pharmacovigilance. Precisely, the targeting would require to increase SPARC expression (or enhance the pathways it activates) in the SM and/or inhibit (or reduce) the expression of these glycoprotein in the AT (preferably the subcutaneous AT so that the adiposity accumulation would be towards the subcutaneous AT rather than the visceral AT). Such approach could provide efficient therapies for obesity, diabetes, sarcopenia and sarcopenic obesity especially that increasing publications describe targeting ECM (Agarwal and Agrawal, 2017; van der Steen et al., 2017; Ito and Ohno, 2018; Islam et al., 2018). Importantly, the described effects of SPARC-modified expressions in both AT and SM leads to discuss the sarcopenic obesity for which age is the main factor and which involves these two tissues and combine the loss of muscle mass with an increase in fat accumulation (Polyzos and Margioris, 2018). Therefore, put a spotlight on SPARC-based therapy (combined with sufficient protein intake) as a good choice to both improve muscle performance and reduce adiposity especially for individuals who are unable to perform the required exercise. In this last case, we would obtain an “exercise pill” that would pharmacologically mimic the exercise benefits at the SM via stimulating the SPARC-induced effects.

Declaration of Competing Interest

None (The authors declare that there is no conflict of interests).

Acknowledgement

Abdelaziz Ghanemi is a recipient of a Merit scholarship for foreign students. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing this merit scholarship program. It was commissioned by the Ministry of Education and Higher Education of Quebec (Canada) which provides the funding. (In

French: Bourses d'excellence pour étudiants étrangers (PBEEE). Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme. Il a été mandaté pour en faire la gestion par le Ministère de l'Éducation et de l'Enseignement supérieur du Québec (MEES) qui en assure le financement). The Figures of this manuscript were created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

14.7 References

Agarwal, R., Agarwal, P., 2017. Targeting extracellular matrix remodeling in disease: Could resveratrol be a potential candidate? Exp. Biol. Med. (Maywood) 242, 374–383.

Aoi, W., Naito, Y., Takagi, T., Tanimura, Y., Takanami, Y., Kawai, Y., Sakuma, K., Hang, L.P., Mizushima, K., Hirai, Y., et al., 2013. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 62, 882–889.

Aratani, Y., Kitagawa, Y., 1988. Enhanced synthesis and secretion of type IV collagen and entactin during adipose conversion of 3T3-L1 cells and production of unorthodox laminin complex. J. Biol. Chem. 263, 16163–16169.

Aseer, K.R., Silvester, A.J., Kumar, A., Choi, M.S., JW, Y., 2017. SPARC paucity alleviates superoxide-mediated oxidative stress, apoptosis, and autophagy in diabetogenic hepatocytes. Free Radic. Biol. Med. 108, 874–895.

Atorrasagasti, C., Onorato, A., Gimeno, M.L., Andreone, L., Garcia, M., Malvicini, M., Fiore, E., Bayo, J., Perone, M.J., Mazzolini, G.D., 2019. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. Clin. Sci. 133, 351–365

Basu, A., Kligman, L.H., Samulewicz, S.J., CC, H., 2001. Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40). BMC Cell Biol. 2, 15.

Berggren, J.R., Tanner, C.J., Koves, T.R., Muoio, D.M., Houmard, J.A., 2005. Glucose uptake in muscle cell cultures from endurance-trained men. Med. Sci. Sports Exerc. 37, 579–584.

Biolo, G., Cederholm, T., Muscaritoli, M., 2014. Muscle contractile and metabolic dysfunction is a common feature of sarcopenia of aging and chronic diseases: from sarcopenic obesity to cachexia. Clin. Nutr. 33, 737–748.

Boengler, K., Kosiol, M., Mayr, M., Schulz, R., Rohrbach, S., 2017. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. J. Cachexia Sarcopenia Muscle 8, 349–369.

Bradshaw, A.D., Sage, E.H., 2001. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J. Clin. Invest. 107, 1049–1054.

Bradshaw, A.D., Reed, M.J., EH, S., 2002. SPARC-null mice exhibit accelerated cutaneous wound closure. J. Histochem. Cytochem. 50, 1–10.

Bradshaw, A.D., Graves, D.C., Motamed, K., EH, S., 2003a. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc. Natl. Acad. Sci. U. S. A. 100, 6045–6050.

Bradshaw, A.D., Graves, D.C., Motamed, K., EH, S., 2003b. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc. Natl. Acad. Sci. U. S. A. 100, 6045–6050.

Brekken, R.A., Sage, E.H., 2000. SPARC, a matricellular protein: at the crossroads of cellmatrix. Matrix Biol. 19, 569–580.

Chavey, C., Boucher, J., Monthouel-Kartmann, M.N., Sage, E.H., Castan-Laurell, I., Valet, P., Tartare-Deckert, S., Van Obberghen, E., 2006. Regulation of secreted protein acidic and rich in cysteine during adipose conversion and adipose tissue hyperplasia. Obesity Silver Spring (Silver Spring) 14, 1890–1897.

Chen, P., Hou, X., Hu, G., Wei, L., Jiao, L., Wang, H., Chen, S., Wu, J., Bao, Y., W, J., 2018. Abdominal subcutaneous adipose tissue: a favorable adipose depot for diabetes? Cardiovasc. Diabetol. 17, 93.

DeFronzo, R.A., Jacot, E., Jequier, E., Maeder, E., Wahren, J., Felber, J.P., 1981. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30, 1000–1007.

Delany, A.M., Kalajzic, I., Bradshaw, A.D., Sage, E.H., Canalis, E., 2003. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. Endocrinology 144, 2588–2596.

Delany, A.M., Amling, M., Priemel, M., Howe, C., Baron, R., Canalis, E., 2000. Osteopenia and decreased bone formation in osteonectin-deficient mice. J. Clin. Invest. 105, 915–923.

Despotovic, S.Z., Milicevic, N.M., Milosevic, D.P., Despotovic, N., Erceg, P., Svorcan, P., Schumacher, U., Ullrich, S., Mihajlovic, G., Kalem, D., et al., 2017. Remodeling of extracellular matrix of the lamina propria in the uninvolved human rectal mucosa 10 and 20 cm away from the malignant tumor. Tumour Biol. 39, 1010428317711654.

Despres, J.P., 1992. Abdominal obesity and the risk of coronary artery disease. Can. J. Cardiol. 8, 561–562.

Després, J.-P., 2012. Body fat distribution and risk of cardiovascular disease. Circulation126, 1301–1313.

Despres, J.P., Lemieux, I., 2006. Abdominal obesity and metabolic syndrome. Nature 444, 881–887.

Despres, J.P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A., Bouchard, C., 1990. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis 10, 497–511.

Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., Raptis, S.A., 2011. Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. 93, S52–S59.

Drela, N., Kozdron, E., Szczypiorski, P., 2004. Moderate exercise may attenuate some aspects of immunosenescence. BMC Geriatr. 4, 8.

Duarte, F.O., Gomes-Gatto, C.D.V., Oishi, J.C., Lino, A.D.S., Stotzer, U.S., Rodrigues, M.F.C., Gatti da Silva, G.H., Selistre-de-Araujo, H.S., 2017. Physical training improves visceral adipose tissue health by remodelling extracellular matrix in rats with estrogen absence: a gene expression analysis. Int. J. Exp. Pathol. 98, 203–213.

Emanuel Anna, L., Meijer Rick, I., Muskiet Marcel, H.A., van Raalte Daniël, H., Eringa Etto, C., Serné Erik, H., 2017. Role of insulin-stimulated adipose tissue perfusion in the development of whole-body insulin resistance. Arterioscler. Thromb. Vasc. Biol. 37, 411–418.

Erdogan, B., Webb, D.J., 2017. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem. Soc. Trans. 45, 229–236.

Ford, A.J., Rajagopalan, P., 2018. Extracellular matrix remodeling in 3D: implications in tissue homeostasis and disease progression. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 10, e1503.

Francki, A., Bradshaw, A.D., Bassuk, J.A., Howe, C.C., Couser, W.G., EH, S., 1999. SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. J. Biol. Chem. 274, 32145–32152.

Francki, A., Motamed, K., McClure, T.D., Kaya, M., Murri, C., Blake, D.J., Carbon, J.G., EH, S., 2003. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. J. Cell. Biochem. 88, 802–811.

Frontera, W.R., Ochala, J., 2015. Skeletal muscle: a brief review of structure and function. Calcif. Tissue Int. 96, 183–195.

Ghanemi, A., St-Amand, J., 2018. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 55, 20–22.

Ghanemi, A., Melouane, A., Mucunguzi, O., Yoshioka, M., St-Amand, J., 2018b. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 215, 190–197.

Ghanemi, A., Yoshioka, M., St-Amand, J., 2018a. Broken energy homeostasis and obesity pathogenesis: the surrounding concepts. J. Clin. Med. 7, 453.

Gilmour, D.T., Lyon, G.J., Carlton, M.B., Sanes, J.R., Cunningham, J.M., Anderson, J.R., Hogan, B.L., Evans, M.J., Colledge, W.H., 1998. Mice deficient for the secreted glycoprotein SPARC/osteonectin/BM40 develop normally but show severe age-onset cataract formation and disruption of the lens. EMBO J. 17, 1860–1870.

Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.

Hardouin, P., Rharass, T., Lucas, S., 2016. Bone marrow adipose tissue: to Be or not to Be a typical adipose tissue? Front. Endocrinol. (Lausanne) 7, 85.

Harris, B.A., 2005. The influence of endurance and resistance exercise on muscle capillarization in the elderly: a review. Acta Physiol. Scand. 185, 89–97.

Health topics, 2019. Obesity. [http://www.who.int/topics/obesity/en/].

Hocking, S.L., Chisholm, D.J., James, D.E., 2008. Studies of regional adipose transplantation reveal a unique and beneficial interaction between subcutaneous adipose tissue and the intra-abdominal compartment. Diabetologia 51, 900–902.

Hsu, C.P., Lin, C.H., CY, K., 2018. Endothelial-cell inflammation and damage by reactive oxygen species are prevented by propofol via ABCA1-mediated cholesterol efflux. Int. J. Med. Sci. 15, 978–985.

Islam, M.S., Ciavattini, A., Petraglia, F., Castellucci, M., Ciarmela, P., 2018. Extracellularmatrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics. Hum. Reprod. Update 24, 59–85.

Ito, M., Ohno, K., 2018. Protein-anchoring therapy to target extracellular matrix proteins to their physiological destinations. Matrix Biol. 68–69, 628–636.

Jackson, M.J., 2016. Reactive oxygen species in sarcopenia: should we focus on excess oxidative damage or defective redox signalling? Mol. Aspects Med. 50, 33–40.

Jager, S., Handschin, C., St-Pierre, J., Spiegelman, B.M., 2007. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 104, 12017–12022.

Jequier, E., 2002. Leptin signaling, adiposity, and energy balance. Ann. N. Y. Acad. Sci. 967, 379–388.

JM, H., 1994. Physical activity, fitness, health and aging. In: Bouchard, C., Shephard, R.J., Stephens, T. (Eds.), Physical Activity, Fitness, and Health: International Proceedings and Consensus Statement. Human Kinetics Publishers Inc, Champaign, pp. 993–1005.

Jorgensen, L.H., Jepsen, P.L., Boysen, A., Dalgaard, L.B., Hvid, L.G., Ortenblad, N., Ravn, D., Sellathurai, J., Moller-Jensen, J., Lochmuller, H., Schroder, H.D., 2017. SPARC interacts with actin in skeletal muscle in vitro and in vivo. Am. J. Pathol. 187, 457–474.

Jorgensen, L.H., Petersson, S.J., Sellathurai, J., Andersen, D.C., Thayssen, S., Sant, D.J., Jensen, C.H., Schroder, H.D., 2009. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. J. Histochem. Cytochem. 57, 29–39.

Kilov, D., G, K., 2018. Philosophical determinants of obesity as a disease. Obes. Rev. 19, 41–48.

Korenevsky, A., Milyutina, Y., Kozina, L., Kerkeshko, G., Arutjunyan, A., 2017. Role of reactive oxygen species in premature ageing of the female reproductive function. Curr. Aging Sci. 10, 26–31.

Kos, K., Wong, S., Tan, B., Gummesson, A., Jernas, M., Franck, N., Kerrigan, D., Nystrom, F.H., Carlsson, L.M., Randeva, H.S., et al., 2009. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. Diabetes 58, 1780–1788.

Lane, T.F., Sage, E.H., 1994. The biology of SPARC, a protein that modulates cell-matrix interactions. FASEB J. 8, 163–173.

Lin, C.T.H., Kang, L., 2016. Adipose extracellular matrix remodelling in obesity and insulin resistance. Biochem. Pharmacol. 119, 8–16.

Lira, V.A., Benton, C.R., Yan, Z., Bonen, A., 2010. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. 299, E145–161.

Mansergh, F.C., Wells, T., Elford, C., Evans, S.L., Perry, M.J., Evans, M.J., Evans, B.A., 2007. Osteopenia in Sparc (osteonectin)-deficient mice: characterization of phenotypic determinants of femoral strength and changes in gene expression. Physiol. Genomics 32, 64–73.

Mariman, E.C., Wang, P., 2010. Adipocyte extracellular matrix composition, dynamics and role in obesity. Cell. Mol. Life Sci. 67, 1277–1292.

Martinez-Huenchullan, S., McLennan, S.V., Verhoeven, A., Twigg, S.M., Tam, C.S., 2017. The emerging role of skeletal muscle extracellular matrix remodelling in obesity and exercise. Obes. Rev. 18, 776–790.

Mason, I.J., Murphy, D., Munke, M., Francke, U., Elliott, R.W., Hogan, B.L., 1986a. Developmental and transformation-sensitive expression of the Sparc gene on mouse chromosome 11. EMBO J. 5, 1831–1837.

Mason, I.J., Taylor, A., Williams, J.G., Sage, H., Hogan, B.L., 1986b. Evidence from molecular cloning that SPARC, a major product of mouse embryo parietal endoderm, is related to an endothelial cell' culture shock' glycoprotein of Mr 43,000. EMBO J. 5, 1465–1472.

Masson, S., Arosio, B., Luvara, G., Gagliano, N., Fiordaliso, F., Santambrogio, D., Vergani, C., Latini, R., Annoni, G., 1998. Remodelling of cardiac extracellular matrix during beta-adrenergic stimulation: upregulation of SPARC in the myocardium of adult rats. J. Mol. Cell. Cardiol. 30, 1505–1514.

McCurdy, S., Baicu, C.F., Heymans, S., Bradshaw, A.D., 2010. Cardiac extracellular matrix remodeling: fibrillar collagens and secreted Protein Acidic and Rich in Cysteine (SPARC). J. Mol. Cell. Cardiol. 48, 544–549.

McMurray, F., Patten, D.A., Harper, M.E., 2016. Reactive oxygen species and oxidative stress in obesity recent findings and empirical approaches. Obesity (Silver Spring) 24, 2301–2310.

Melouane, A., Yoshioka, M., Kanzaki, M., St-Amand, J., 2019. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 229, 277–287.

Melouane, A., Carbonell, A., Yoshioka, M., Puymirat, J., St-Amand, J., 2018. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS One 13, e0192714.

Miyata, S., Kitagawa, H., 2017. Formation and remodeling of the brain extracellular matrix in neural plasticity: roles of chondroitin sulfate and hyaluronan. Biochim. Biophys. Acta Gen. Subj. 1861, 2420–2434.

Mori, S., Kiuchi, S., Ouchi, A., Hase, T., Murase, T., 2014. Characteristic expression of extracellular matrix in subcutaneous adipose tissue development and adipogenesis; comparison with visceral adipose tissue. Int. J. Biol. Sci. 10, 825–833.

Morrissey, M.A., Jayadev, R., Miley, G.R., Blebea, C.A., Chi, Q., Ihara, S., Sherwood, D.R., 2016. SPARC promotes cell invasion in vivo by decreasing type IV collagen levels in the basement membrane. PLoS Genet. 12, e1005905.

Moustafa, P.E., Abdelkader, N.F., El Awdan, S.A., El-Shabrawy, O.A., Zaki, H.F., 2018. Extracellular Matrix Remodeling and Modulation of Inflammation and Oxidative Stress by Sulforaphane in Experimental Diabetic Peripheral Neuropathy. Inflammation 41, 1460–1476.

Mucunguzi, O., Melouane, A., Ghanemi, A., Yoshioka, M., Boivin, A., Calvo, E.L., J, S.-A., 2017. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. (Lond.) 14, 66.

Myers Jr., M.G., Heymsfield, S.B., Haft, C., Kahn, B.B., Laughlin, M., Leibel, R.L., Tschöp, M.H., Yanovski, J.A., 2012. Challenges and opportunities of defining clinical leptin resistance. Cell Metab. 15, 150–156.

Naczki, C., John, B., Patel, C., Lafferty, A., Ghoneum, A., Afify, H., White, M., Davis, A., Jin, G., Kridel, S., Said, N., 2018. SPARC inhibits metabolic plasticity in ovarian cancer. Cancers (Basel) 10.

Nakamura, K., Nakano, S., Miyoshi, T., Yamanouchi, K., Matsuwaki, T., Nishihara, M., 2012. Age-related resistance of skeletal muscle-derived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. Aging (Albany NY) 4, 40–48.

Nakamura, K., Nakano, S., Miyoshi, T., Yamanouchi, K., Nishihara, M., 2013. Loss of SPARC in mouse skeletal muscle causes myofiber atrophy. Muscle Nerve 48, 791–799.

Neeland, I.J., Ross, R., Despres, J.P., Matsuzawa, Y., Yamashita, S., Shai, I., Seidell, J., Magni, P., Santos, R.D., Arsenault, B., et al., 2019. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. Lancet Diabetes Endocrinol. 7 (9), 715–725.

Neufer, P.D., Dohm, G.L., 1993. Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. Am. J. Physiol. 265, C1597–1603.

Neuzillet, C., Tijeras-Raballand, A., Cros, J., Faivre, S., Hammel, P., Raymond, E., 2013. Stromal expression of SPARC in pancreatic adenocarcinoma. Cancer Metastasis Rev. 32, 585–602.

Nie, J., Sage, E.H., 2009a. SPARC functions as an inhibitor of adipogenesis. J. Cell Commun. Signal. 3, 247–254.

Nie, J., Sage, E.H., 2009b. SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling. J. Biol. Chem. 284, 1279–1290.

Nie, J., Bradshaw, A.D., Delany, A.M., Sage, E.H., 2011. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. Connect. Tissue Res. 52, 99–108.

Nishida, Y., Tanaka, H., Tobina, T., Murakami, K., Shono, N., Shindo, M., Ogawa, W., Yoshioka, M., St-Amand, J., 2010. Regulation of muscle genes by moderate exercise. Int. J. Sports Med. 31, 656–670.

Norose, K., Clark, J.I., Syed, N.A., Basu, A., Heber-Katz, E., Sage, E.H., Howe, C.C., 1998. SPARC deficiency leads to early-onset cataractogenesis. Invest. Ophthalmol. Vis. Sci. 39, 2674–2680.

Nuutila, P., Knuuti, M.J., Raitakari, M., Ruotsalainen, U., Teras, M., Voipio-Pulkki, L.M., Haaparanta, M., Solin, O., Wegelius, U., Yki-Jarvinen, H., 1994. Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans. Am. J. Physiol. 267, E941–946.

Panigrahy, S.K., Bhatt, R., Kumar, A., 2017. Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. J. Drug Target. 25, 93–101.

Park, H.S., Lee, K., 2005. Greater beneficial effects of visceral fat reduction compared with subcutaneous fat reduction on parameters of the metabolic syndrome: a study of weight reduction programmes in subjects with visceral and subcutaneous obesity. Diabet. Med. 22, 266–272.

Petersson, S.J., Jorgensen, L.H., Andersen, D.C., Norgaard, R.C., Jensen, C.H., Schroder, H.D., 2013. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 28, 1451–1460.

Polyzos, S.A., Margioris, A.N., 2018. Sarcopenic obesity. Hormones (Athens) 17, 321–331.

Radom-Aizik, S., Hayek, S., Shahar, I., Rechavi, G., Kaminski, N., Ben-Dov, I., 2005. Effects of aerobic training on gene expression in skeletal muscle of elderly men. Med. Sci. Sports Exerc. 37, 1680–1696.

Riedl, I., Yoshioka, M., Nishida, Y., Tobina, T., Paradis, R., Shono, N., Tanaka, H., StAmand, J., 2010. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 45, 896–903.

Rosset, E.M., Bradshaw, A.D., 2016. SPARC/osteonectin in mineralized tissue. Matrix Biol. 52–54, 78–87.

Sage, H., Vernon, R.B., Funk, S.E., Everitt, E.A., Angello, J., 1989. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. J. Cell Biol. 109, 341–356.

Said, N., Socha, M.J., Olearczyk, J.J., Elmarakby, A.A., Imig, J.D., Motamed, K., 2007. Normalization of the ovarian cancer microenvironment by SPARC. Mol. Cancer Res. 5, 1015–1030.

Sainz, N., Barrenetxe, J., Moreno-Aliaga, M.J., Martinez, J.A., 2015. Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism 64, 35–46.

Schellings, M.W., Vanhoutte, D., Swinnen, M., Cleutjens, J.P., Debets, J., van Leeuwen, R.E., d'Hooge, J., Van de Werf, F., Carmeliet, P., Pinto, Y.M., et al., 2009. Absence of SPARC results in increased cardiac rupture and dysfunction after acute myocardial infarction. J. Exp. Med. 206, 113–123.

Scime, A., Desrosiers, J., Trensz, F., Palidwor, G.A., Caron, A.Z., Andrade-Navarro, M.A., Grenier, G., 2010. Transcriptional profiling of skeletal muscle reveals factors that are necessary to maintain satellite cell integrity during ageing. Mech. Ageing Dev. 131, 9–20.

Smas, C.M., Sul, H.S., 1995. Control of adipocyte differentiation. Biochem. J. 309 (Pt 3), 697–710.

Smith, J.D., Borel, A.L., Nazare, J.A., Haffner, S.M., Balkau, B., Ross, R., Massien, C., Almeras, N., Despres, J.P., 2012. Visceral adipose tissue indicates the severity of cardiometabolic risk in patients with and without type 2 diabetes: results from the INSPIRE ME IAA study. J. Clin. Endocrinol. Metab. 97, 1517–1525.

So, B., Kim, H.J., Kim, J., Song, W., 2014. Exercise-induced myokines in health and metabolic diseases. Integr. Med. Res. 3, 172–179.

Song, H., Guan, Y., Zhang, L., Li, K., Dong, C., 2010. SPARC interacts with AMPK and regulates GLUT4 expression. Biochem. Biophys. Res. Commun. 396, 961–966.

St-Amand, J., Yoshioka, M., Nishida, Y., Tobina, T., Shono, N., Tanaka, H., 2012. Effects of mild-exercise training cessation in human skeletal muscle. Eur. J. Appl. Physiol. 112, 853–869.

St-Onge, M.P., Gallagher, D., 2010. Body composition changes with aging: the cause or the result of alterations in metabolic rate and macronutrient oxidation? Nutrition 26, 152–155.

Swaroop, A., Hogan, B.L., Francke, U., 1988. Molecular analysis of the cDNA for human SPARC/osteonectin/BM-40: sequence, expression, and localization of the gene to chromosome 5q31-q33. Genomics 2, 37–47.

Tai, I.T., Tang, M.J., 2008. SPARC in cancer biology: its role in cancer progression and potential for therapy. Drug Resist. Updat. 11, 231–246.

Takahashi, M., Nagaretani, H., Funahashi, T., Nishizawa, H., Maeda, N., Kishida, K., Kuriyama, H., Shimomura, I., Maeda, K., Hotta, K., et al., 2001. The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease. Obes. Res. 9, 388–393.

Tanaka, H.Y., Kitahara, K., Sasaki, N., Nakao, N., Sato, K., Narita, H., Shimoda, H., Matsusaki, M., Nishihara, H., Masamune, A., Kano, M.R., 2019. Pancreatic stellate cells derived from human pancreatic cancer demonstrate aberrant SPARC-dependent ECM remodeling in 3D engineered fibrotic tissue of clinically relevant thickness. Biomaterials 192, 355–367.

Taneda, S., Pippin, J.W., Sage, E.H., Hudkins, K.L., Takeuchi, Y., Couser, W.G., Alpers, C.E., 2003. Amelioration of diabetic nephropathy in SPARC-null mice. J. Am. Soc. Nephrol. 14, 968–980.

Tartare-Deckert, S., Chavey, C., Monthouel, M.N., Gautier, N., Van Obberghen, E., 2001. The matricellular protein SPARC/osteonectin as a newly identified factor up-regulated in obesity. J. Biol. Chem. 276, 22231 22237.

Termine, J.D., Kleinman, H.K., Whitson, S.W., Conn, K.M., McGarvey, M.L., Martin, G.R., 1981. Osteonectin, a bone-specific protein linking mineral to collagen. Cell 26, 99–105.

Theocharis, A.D., Skandalis, S.S., Gialeli, C., Karamanos, N.K., 2016. Extracellular matrix structure. Adv. Drug Deliv. Rev. 97, 4–27.

Reynolds, T.H., Reid, P., Larkin, L.M., Dengel, D.R., 2004. Effects of aerobic exercise training on the protein kinase B (PKB)/mammalian target of rapamycin (mTOR) signaling pathway in aged skeletal muscle. Exp. Gerontol. 39, 379–385.

Tomos, I.P., Tzouvelekis, A., Aidinis, V., Manali, E.D., Bouros, E., Bouros, D., Papiris, S.A., 2017. Extracellular matrix remodeling in idiopathic pulmonary fibrosis. It is the' bed' that counts and not' the sleepers'. Expert Rev. Respir. Med. 11, 299–309.

Tran, T.T., Yamamoto, Y., Gesta, S., Kahn, C.R., 2008a. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 7, 410–420.

Tran, T.T., Yamamoto, Y., Gesta, S., Kahn, C.R., 2008b. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 7, 410–420.

van der Steen, S.C., Raave, R., Langerak, S., van Houdt, L., van Duijnhoven, S.M., van Lith, S.A., Massuger, L.F., Daamen, W.F., Leenders, W.P., van Kuppevelt, T.H., 2017.
Targeting the extracellular matrix of ovarian cancer using functionalized, drug loaded lyophilisomes. Eur. J. Pharm. Biopharm. 113, 229–239.

Vasilaki, A., Richardson, A., Van Remmen, H., Brooks, S.V., Larkin, L., McArdle, A., Jackson, M.J., 2017. Role of nerve-muscle interactions and reactive oxygen species in regulation of muscle proteostasis with ageing. J. Physiol. 595, 6409–6415.

Wang, C.-Y., Liao, J.K., 2012. A mouse model of diet-induced obesity and insulin resistance. Methods Mol. Biol. (Clifton, NJ) 821, 421–433.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C., Spiegelman, B.M., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.

Yoshioka, M., Bolduc, C., Raymond, V., St-Amand, J., 2008. High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity (Silver Spring) 16, 2302–2307.

Chapter 15. Review - Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 15 January 2020; Accepted: 29 March 2020; Published: 1 April 2020

15.1 Résumé (French abstract)

La matrice extracellulaire (ECM) est un réseau de macromolécules qui représente le support structurel cellulaire impliqué dans des biofonctions clés telles que la transduction du signal et l'adhésion cellulaire. Les protéines associées à l'ECM interagissent avec l'ECM et avec d'autres structures et molécules endogènes pour contrôler la croissance cellulaire, les modifications structurelles, la migration cellulaire, etc. Parmi les protéines associées à l'ECM, la secreted protein acidic and rich in cysteine (SPARC) est une protéine connue pour être exprimée durant le remodelage des tissus. Ici, nous mettons l'accent sur des propriétés métaboliques et homéostatiques au-delà des propriétés connues de l'ECM et du SPARC. La synchronisation des implications métaboliques et structurelles de SPARC et de l'ECM indiquerait une adaptation du métabolisme pour répondre aux besoins liés aux changements tissulaires. La mise en évidence de telles propriétés aurait des applications importantes dans divers domaines tels que les thérapies, le métabolisme et la pathogenèse.

15.2 Abstract

An extracellular matrix (ECM) is a network of numerous macromolecules that represents the cellular structural support involved in key biofunctions such as signal transduction and cellular adhesion. In addition, ECM-associated proteins interact with ECM and with other endogenous structures and molecules to control cellular growth, structural modifications, cellular migration, etc. Among the ECM-associated proteins, secreted protein acidic and rich in cysteine (SPARC) is a protein that is known to be expressed when tissues change. Herein, we put a spotlight on selected, metabolic and homeostatic properties beyond the known properties of ECM and SPARC. Importantly, the synchronization of the metabolic and structural implications of SPARC and the ECM would indicate an adaptation of the metabolism to meet the needs of the changes that the tissues undergo. Highlighting

such properties would have important applications in diverse fields that include therapeutics, metabolics, and pathogenesis.

Keywords: secreted protein acidic and rich in cysteine; extracellular matrix; metabolism; homeostasis

15.3 Extracellular Matrix (ECM) and Secreted Protein Acidic and Rich in Cysteine (SPARC)

The increasing number of pathologies involving structural or functional abnormalities, combined with the lack of organ donors, has made developing novel approaches in regenerative medicine a necessity. Recent advances in regenerative medicine have brought a lot of hope for tissue engineering, as shown by clinical trials that use stem cells (both somatic and embryonic and even adipose-derived stem cells) in therapeutic applications [1–3]. Within the context of regenerative medicine, the ECM is implicated in a variety of processes that include cellular repair and regeneration, remodeling [4], and intercellular communication [5] (Figure 15.1). Therefore, ECM products, including hydrogels [5], have been used or suggested in clinical practice [6,7]. Such applications derive from the structural and functional properties of the ECM and its associated proteins. Thus, further understanding these properties could expand the applications in regenerative medicine as well as in fields such as pharmacology and in vitro research.

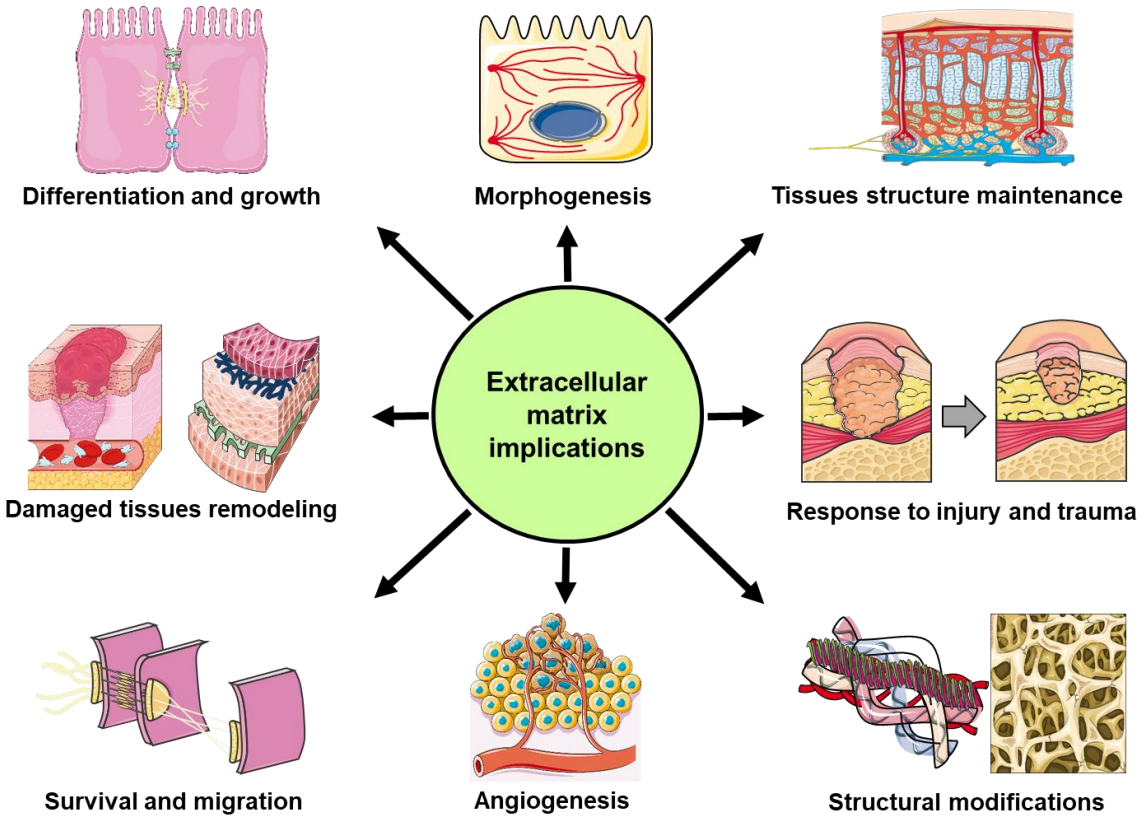


Figure 15.1. Example of extracellular matrix (ECM) implications. The extracellular matrix is involved in a variety of biological functions, mainly but not only during tissue development under physiological and pathological conditions.

ECM is a three-dimensional network surrounding cells, and is made of different macromolecules, including collagens, elastins, proteoglycans/glycosaminoglycans, laminins, and fibronectins [5,8]. It governs a variety of biological functions such as cellular differentiation, growth, survival, migration, homeostasis, and morphogenesis [8]. ECM remodeling through its components is crucial in both physiology and pathology [9–11]. Such remodeling is controlled by epigenetics through the dynamic cellular environment (pH, cytokines, etc. [12]) that induces gene expression modulations [9] towards a modified proteomic profile required for remodeling. On the other hand, the proteins associated with the ECM structurally and functionally complete the molecular network surrounding the cells and governing tissue properties. Herein, we mention the SPARC that represents an example of an ECM-associated protein expressed, mainly when tissues change [13,14], indicating its particular importance during tissue remodeling and suggesting its close interaction with the ECM during such a process. SPARC (also known as BM-40 or osteonectin) is a glycoprotein with a molecular weight of 32 kDa [15] encoded by a highly conserved single gene [16], which reflects the evolutionary importance of this gene. It has a single polypeptide chain [17], and its human-matured version has 286 amino acid residues [18]. This glycoprotein is made of three domains (N-terminal domain, C-terminal domain, and a domain characterized by a follistatin-like domain) [19]. The three domains provide SPARC with its biophysical and biochemical properties such as Ca2+-binding, protease inhibitor, and collagen binding [19]. These properties allow SPARC to bind to both collagen and hydroxyapatite [13]. This binding ability is illustrated by studies showing that collagen I and SPARC are both reduced by valproate treatment in cultured bone cells [20], the implication of SPARC in stromal mineralization, as well as the adhesion of both osteoblasts and platelets to the ECM [21]. In fact, SPARC was first discovered in bones [18] and shown to be highly important in osteogenesis [17]. It is also the most expressed in the bone compared to the other noncollagenous polypeptides [22]. SPARC was initially named osteonectin following its discovery in bones and was even thought to be bone-specific [13,23] before it was shown to be expressed in most tissues.

Beyond the structural implications of SPARC and ECM, it remains important to clarify their metabolic and homeostatic properties under different circumstances. Indeed, the increasing importance of ECM implications and applications in biological research and clinical practice [24–26] makes exploring the ECM metabolic aspects important in order to uncover novel roles or identify new possible applications for ECM. For instance, among the ECM's current applications we mention is the use of ECM bioscaffolds (decellularized) in clinical tissue remodeling [6] to enhance the functional reconstruction of injured tissues (muscle, esophagus, etc.) rather than develop scar tissues at the end of the healing process of these tissues [7]. It is worth mentioning that ischemic injuries have been treated using

ECM hydrogels [26]. Moreover, these hydrogels are also used to study the ECM effects on culture [4] as well as to mimic tumor microenvironments [12].

Elucidating such concepts would both deepen our knowledge of metabolics and uncover unknown effects of regenerative medicine approaches. Understating how ECM and its associated proteins impact aspects beyond structures such as metabolism and cellular biochemistry could lead to the development of novel therapies or optimize those currently available. Furthermore, expanding the field of investigation beyond the ECM to include the biomolecules associated to the ECM would provide a wider understanding of the noncellular entities of tissues within the context of metabolics, biochemical homeostasis, and energy balance. SPARC represents an illustration of this concept. It is not only involved in tissue response to injury and cellular differentiation [27] but can also shape some metabolic patterns, as described below.

15.4 SPARC: Metabolics and Homeostasis

Both ECM and the proteins associated with it have the ability to interact between them as well as with cells, other active molecules, and cell receptors under diverse physiological and pathological conditions [28–31]. The ECM and its associated proteins represent the frame into which cells reside and upon which key tissue properties depend. Indeed, what provides tissue with its nature (hard, soft, etc.) and properties is governed by the cellular adhesion of cells constituting that tissue and their interactions through the ECM and its associated proteins. This explains the need for ECM remodeling and changes to adapt to new physiological or pathological conditions [32]. Furthermore, the content in other elements like calcium does impact the tissue nature and illustrates this concept as well. For instance, bones are known to be of a hard nature, and this is due to their rich content in calcium. Within this context, SPARC binds to both hydroxyapatite and collagen in a bone matrix [13,33,34], which strengthens the bone hardness. Therefore, the bone content in both SPARC and calcium and the binding ability these two components have would govern the strength and hardness of bones depending on how strong the adhesion is between osteocytes, their ECM, hydroxyapatite, and ECM-associated proteins (SPARC in the current example).

Regarding the energy metabolism pattern, the two components of energy balance are energy storage and energy expenditure. The storage is mainly in the form of lipids in adipocytes, whereas the energy expenditure is governed by the muscle's energy usage (both resting metabolic rate and physical activity). As we have described in a recent paper [15], the ability of certain cells like adipocytes to expend would depend on the ECM's "rigidity", which would—in part—depend upon SPARC expression. Indeed, due to the interaction between SPARC and collagen, a deficiency in SPARC (such as in a *Sparc* knock-out organism) would reduce collagen maturity and weaken the ECM structure, thereby reducing the ECM rigidity. ECM rigidity is defined by how strong the adhesion is between ECM and its associated protein and whether this adhesion would limit the cellular

expansion of these cells surrounded by such an “elastic” or a “rigid” ECM [15]. Following this line of thought, how rigid the ECM of adipocyte is would govern whether the adiposity’s development would be towards hyperplasia or hypertrophy. Whereas adipocyte’s expansion would result from an “elastic” ECM, a “rigid” ECM would limit such expansion and rather direct the adiposity’s development towards hyperplasia [15]. This results in different forms of adiposity distribution, which is mainly towards either subcutaneous or visceral fat accumulation. These metabolic outcomes control the metabolic phenotype [35–37], as well as morbidities and pathological complications [38] seen among different individuals with obesity. Studies on SPARC highlight its numerous metabolic implications, including energy metabolism in the skeletal muscle [15] and adiposity control [39] (Figure 15.2).

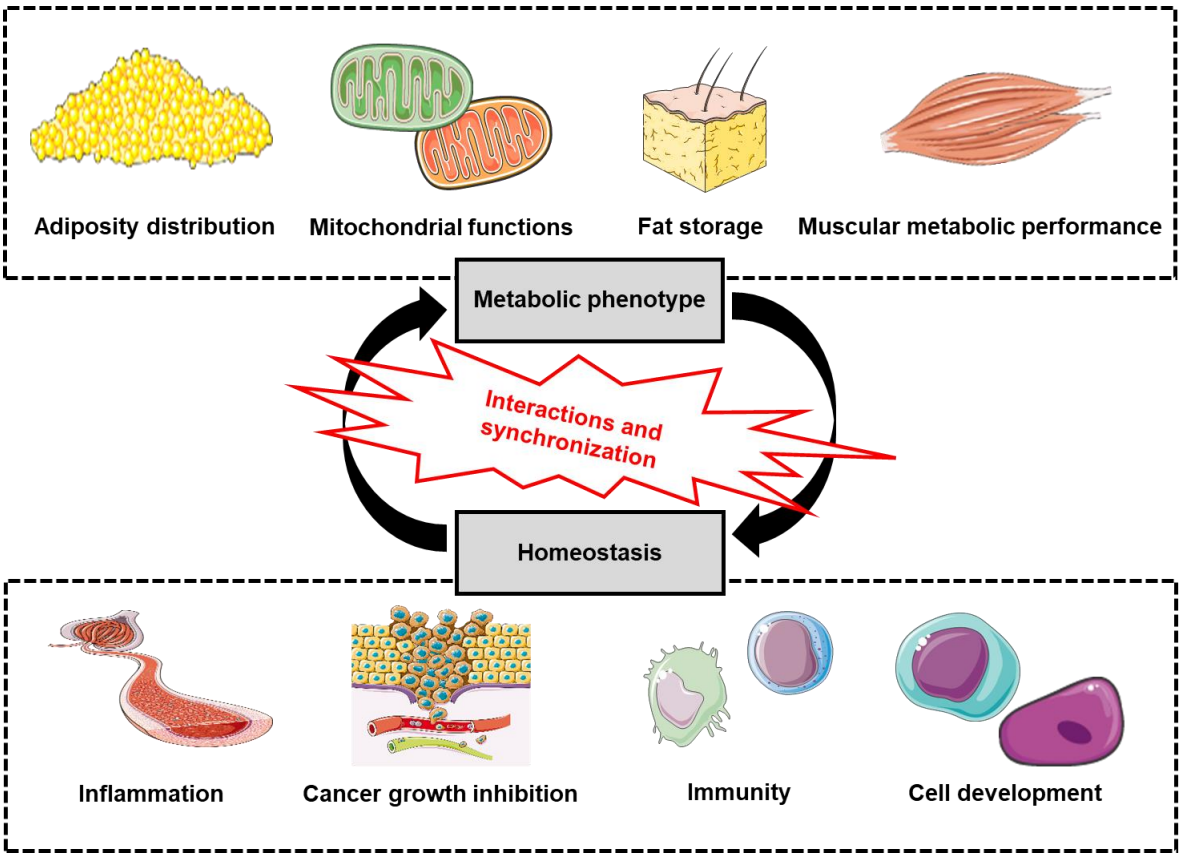


Figure 15.2. Secreted protein acidic and rich in cysteine (SPARC) metabolic and homeostatic implications. SPARC is involved in a variety of metabolic as well as homeostatic processes in diverse tissues, which suggest that SPARC simultaneously interacts with metabolism and homeostasis in order to synchronize with both of them.

Regarding the impact that ECM has on muscles, studies conducted on elderly men have shown that endurance training increased the expression of gene coding for both ECM proteins and SPARC in the skeletal muscles, and also genes related to metabolic functions such as oxidative phosphorylation (OXPHOS) [40]. Such results show a potential

implication of SPARC in exercise-induced metabolic benefits depending on how strongly the *Sparc* gene is expressed or knocked-out as we have previously described [15]. Herein, the differential expression of genes related to the ECM indicates a potential role of the ECM (and eventually ECM-associated proteins such as SPARC) within the muscular metabolic performance seen after training compared to the baseline. In addition, there is a possible link between the ECM and the mitochondrial function [41], which reflects other possible metabolic implications. At the molecular level, studies indicated how ECM (or its remodeling) regulates lipid metabolism [42] and glucose metabolism [43]. Moreover, the ECM-associated protein SPARC has been shown to be implicated in the regulation of the glucose transporter type 4 expression (controlling the glucose uptake) [44]. All these elements show how targeting the ECM or its associated proteins could impact the energy balance and the metabolic paths. Fortunately, cellular and animal models, including those of the knock-out of *Sparc*, shed light on the roles the ECM and its associated proteins (such as collagen) have within different contexts [45–48]. These data will contribute to further mapping the metabolic puzzle related to the ECM and its associated proteins.

More importantly, the implications of SPARC and the ECM in both metabolism and functions like growth, differentiation, and morphogenesis could indicate that the ECM and SPARC might synchronize diverse cellular functions so that the metabolic needs meet the biological changes the tissues undergo (Figures 15.2 and 15.3). This means that the ECM remodeling and SPARC expression would simultaneously impact parameters such as cell growth and differentiation as well as metabolic and other homeostatic patterns. This would result in a cellular profile adapted to the changes that tissues undergo in terms of energy usage and storage and homeostatic needs. Moreover, the implications of the ECM [46] or SPARC [49–52] in other nonstructural functions such as inflammation, immunity, and cell growth would not only emphasize the importance of uncovering more pathways that ECM and SPARC govern but would also strengthen knowledge of metabolics, especially of links that have been established between cytokine (involved in inflammation and immunity) and metabolism [53–55]. Herein, cancer would be an illustrative example of such a “cell development–metabolism synchronization”. Indeed, whereas cancer represents a status in which ECM remodeling is required and observed [56–58], cancer cells and the tumor microenvironment have specific metabolic profiles and unique bioenergetic properties in different form noncancer cells [59,60]. Interestingly, SPARC was suggested to play a homeostasis–regulatory role in cancer. More precisely, while SPARC is overexpressed during cancer development, it inhibits tumor growth without inducing apoptosis in normal cells (selective inhibition), indicating a possible feedback effect towards a homeostatic regulation of cell growth in the context of cancer [61]. This regulatory role does not seem to be limited to development but would also control the metabolic profile of the tumoral cells in order to reach a balance/synchronization of both the structural changes and the metabolic needs.

15.5 Perspective and Implications

These selected examples show numerous metabolic and homeostatic implications of both SPARC and ECM at different levels, from cellular metabolism of glucose and lipids to both energy usage (muscles) and energy storage (adipocytes), as well as fat distribution pattern. It reflects how those biomolecules that form a three-dimensional network could be a starting point either to develop novel therapies or to optimize existing treatments for obesity and other metabolic disorders. Importantly, such concepts would also have applications in tissue engineering and regenerative medicine, including stem cell research [62].

Extracellular matrix changes induce cellular adaptation, which leads to structural and metabolic modifications in the tissues and organs. This metabolic–structural synchronization could suggest an adaptation of the metabolism to meet the energy needs of the changes that the tissues undergo. Elucidating such metabolic–structural synchronization between the metabolism and the structural changes would produce diverse perspectives along with a verity of applications in numerous fields, including therapeutics, cell culture, pathogenesis, and regenerative medicine.

Indeed, putting a spotlight on these nonstructural implications of the ECM and SPARC (as an example of an ECM-associated protein) such as the metabolic properties and growth homeostasis would allow us to predict (and potentially reverse) the “metabolic side effects” and the side effects related to immunity, inflammation, and cell growth of interventions targeting or interacting with matricellular proteins within the context of regenerative medicine [63]. Moreover, since metabolic changes are not limited to energy balance but can also involve drug metabolism, this would expend the implications to the context of pharmacovigilance, among other concepts.

In conclusion, we emphasize the importance of further investigations towards uncovering how SPARC, ECM, and the other ECM-associated proteins can impact (and be influenced by) diverse cellular metabolic, biochemical, and homeostatic pathways. Such discoveries will lead to novel yet important applications in a variety of fields, including therapeutics, pathogenesis, metabolics, and regenerative medicine (Figure 15.3).

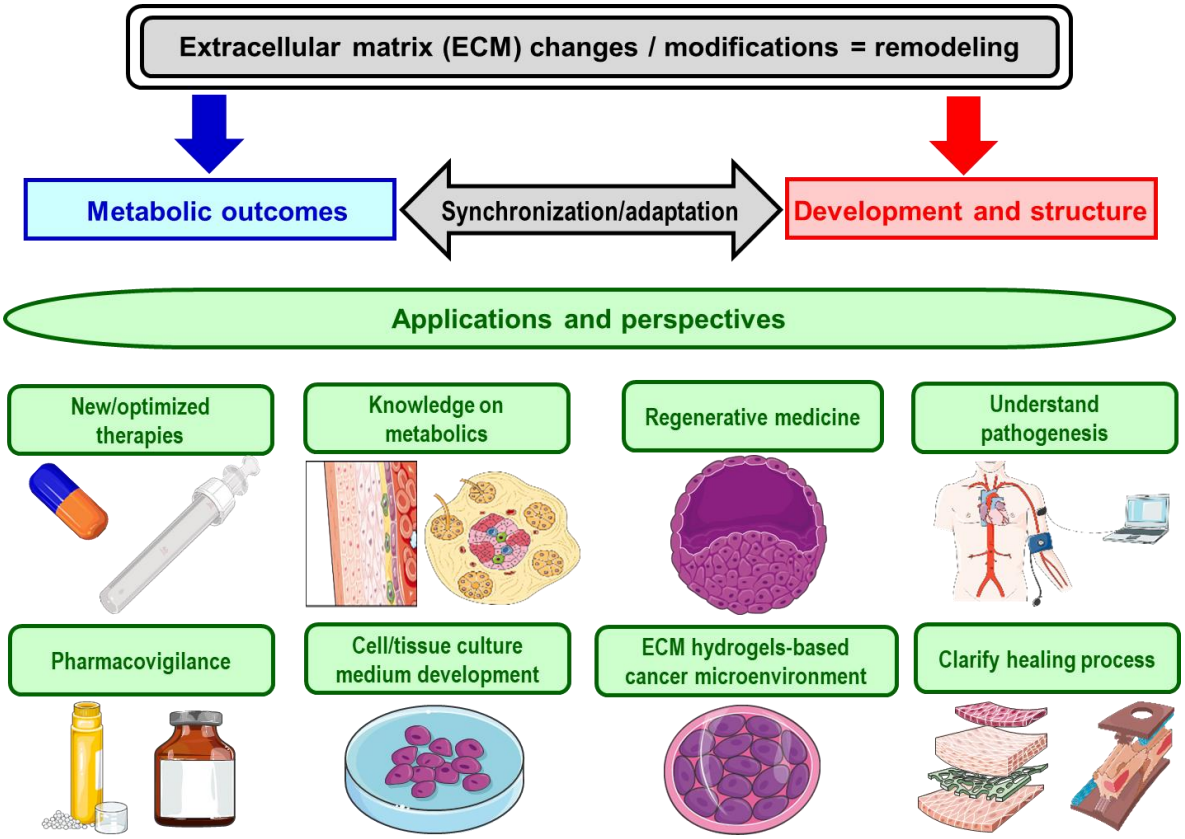


Figure 15.3. Metabolic–structural synchronization of the extracellular matrix properties.

Funding: This research received no external funding.

Acknowledgments : Abdelaziz Ghanemi is a recipient of a Merit scholarship program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec–Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). The figures were created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

15.6 References

1. Riazi, A.M.; Kwon, S.Y.; Stanford, W.L. Stem Cell Sources for Regenerative Medicine. *Adv. Struct. Saf. Stud.* 2009, *482*, 55–90.
2. Mizuno, H. Adipose-derived stem and stromal cells for cell-based therapy: Current status of preclinical studies and clinical trials. *Curr. Opin. Mol. Ther.* 2010, *12*, 442–449.
3. Aboushwareb, T.; Atala, A. Stem cells in urology. *Nat. Clin. Pract. Urol.* 2008, *5*, 621–631.
4. Horton, E.R.; Vallmajo-Martin, Q.; Martin, I.; Snedeker, J.G.; Ehrbar, M.; Blache, U. Extracellular Matrix

Production by Mesenchymal Stromal Cells in Hydrogels Facilitates Cell Spreading and Is Inhibited by FGF-2. *Adv. Heal. Mater.* 2020, 1901669.
5. Geckil, H.; Xu, F.; Zhang, X.; Moon, S.; Demirci, U. Engineering hydrogels as extracellular matrix mimics. Nanomedicine 2010, 5, 469–484.
6. Saldin, L.T.; Cramer, M.C.; Velankar, S.S.; White, L.; Badylak, S.F. Extracellular matrix hydrogels from decellularized tissues: Structure and function. *Acta Biomater.* 2017, *49*, 1–15.
7. Swinehart, I.T.; Badylak, S.F. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. *Dev. Dyn.* 2016, *245*, 351–360
8. Theocharis, A.D.; Skandalis, S.S.; Gialeli, C.; Karamanos, N.K. Extracellular matrix structure. *Adv. Drug Deliv. Rev.* 2016, *97*, 4–27.
9. Vigetti, D.; Viola, M.; Karousou, E.; Deleonibus, S.; Karamanou, K.; De Luca, G.; Passi, A. Epigenetics in extracellular matrix remodeling and hyaluronan metabolism. *FEBS J.* 2014, *281*, 4980–4992.
10. Sonbol, H.S. Extracellular Matrix Remodeling in Human Disease. *J. Microsc. Ultrastruct.* 2018, *6*, 123–128.
11. Rauch, U. Extracellular matrix components associated with remodeling processes in brain. *Cell. Mol. Life Sci.* 2004, *61*, 2031–2045.
12. Park, K.M.; Lewis, D.; Gerecht, S. Bioinspired Hydrogels to Engineer Cancer Microenvironments. *Annu. Rev. Biomed. Eng.* 2017, *19*, 109–133.
13. Termine, J.D.; Kleinman, H.K.; Whitson, S.; Conn, K.M.; McGarvey, M.L.; Martin, G.R. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 1981, *26*, 99–105.
14. Bradshaw, A.D. The role of SPARC in extracellular matrix assembly. *J. Cell Commun. Signal.* 2009, *3*, 239–246.
15. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Boil.* 2019, *117*, 105627.
16. Kelleher, C.M.; McLean, S.; Mecham, R.P. *Vascular Extracellular Matrix and Aortic Development, in Current Topics in Developmental Biology*; Academic Press: Cambridge, MA, USA, 2004; pp. 153–188.
17. Maeda, H.; Wada, N.; Tomokiyo, A.; Monnouchi, S.; Akamine, A. *Chapter Six-Prospective Potency of TGF-β1 on Maintenance and Regeneration of Periodontal Tissue, in International Review of Cell and Molecular Biology*; Jeon, K.W., Ed.; Academic Press: Cambridge, MA, USA, 2013; pp. 283–367.
18. McKee, D.M.; Cole, W.G. *Chapter 2-Bone Matrix and Mineralization, in Pediatric Bone*, 2nd ed.; Glorieux, F.H., Pettifor, J.M., Jüppner, H., Eds.; Academic Press: Cambridge, MA, USA, 2012; pp. 9–37.
19. Workman, G.; Bradshaw, A.D. *Chapter 19-Production and Purification of Recombinant Human SPARC, in Methods in Cell Biology*; Mecham, R.P., Ed.; Academic Press: Cambridge, MA, USA, 2018; pp. 335–345.
20. Humphrey, E.L.; Morris, G.E.; Fuller, H. Valproate reduces collagen and osteonectin in cultured bone cells. *Epilepsy Res.* 2013, *106*, 446–450.
21. Cerilli, A.L.; Wick, M.R. *Chapter 3-Immunohistology of Soft Tissue and Osseous Neoplasms, in Diagnostic Immunohistochemistry*, 2nd ed.; Dabbs, D.J., Ed.; Churchill Livingstone: London, UK, 2006; pp. 65–120.
22. Heinegard, D.; Oldberg, A. Structure and biology of cartilage and bone matrix noncollagenous macromolecules. *Faseb J.* 1989, *3*, 2042–2051.
23. Jundt, G.; Termine, J.D.; Schulz, A. Osteonectin–a differentiation marker of bone cells. *Cell Tissue Res.* 1987, *248*, 409–415.
24. Badylak, S.F. The extracellular matrix as a scaffold for tissue reconstruction. *Semin. Cell Dev. Boil.* 2002, *13*, 377–383.
25. Hodde, J.P. Extracellular matrix as a bioactive material for soft tissue reconstruction. *ANZ J. Surg.* 2006, *76*, 1096–1100.
26. Spang, M.T.; Karen, C. Extracellular matrix hydrogel therapies: In vivo applications and development. *Acta Biomater.* 2018, *68*, 1–14.

27. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J. Clin. Investig.* 2001, *107*, 1049–1054.
28. Brekken, R.A.; Sage, E. SPARC, a matricellular protein: At the crossroads of cell–matrix communication. *Matrix Boil.* 2001, *19*, 815–827.
29. Bella, J.; Hulmes, D.J. Fibrillar Collagens. *Subcell Biochem.* 2017, *82*, 457–490.
30. Mortimer, G.M.; Minchin, R.F. Cryptic epitopes and functional diversity in extracellular proteins. *Int. J. Biochem. Cell Boil.* 2016, *81*, 112–120.
31. Wong, S.L.; Sukkar, M.B. The SPARC protein: An overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease. *Br. J. Pharmacol.* 2016, *174*, 3–14.
32. Bonnans, C.; Chou, J.; Werb, Z. Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Boil.* 2014, *15*, 786–801.
33. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. *Matrix Boil.* 2016, *52*, 78–87.
34. Holland, P.W.H.; Harper, S.J.; McVey, J.H.; Hogan, B.L. In vivo expression of mRNA for the Ca++-binding protein SPARC (osteonectin) revealed by in situ hybridization. *J. Cell Boil.* 1987, *105*, 473–482.
35. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
36. Müller, M.J.; Lagerpusch, M.; Enderle, J.; Schautz, B.; Heller, M.; Bosy-Westphal, A. Beyond the body mass index: Tracking body composition in the pathogenesis of obesity and the metabolic syndrome. *Obes. Rev.* 2012, *13*, 6–13.
37. Goossens, G.H. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. *Obes. Facts* 2017, *10*, 207–215.
38. Després, J.-P.; Fias, F. Body Fat Distribution and Risk of Cardiovascular Disease. *Circulation* 2012, *126*, 1301–1313.
39. Bradshaw, A.D.; Graves, D.C.; Motamed, K.; Sage, E.H. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 6045–6050.
40. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
41. Melouane, A.; Yoshioka, M.; St-Amand, J. Extracellular matrix/mitochondria pathway: A novel potential target for sarcopenia. *Mitochondrion* 2019, *50*, 63–70.
42. Romani, P.; Brian, I.; Santinon, G.; Pocaterra, A.; Audano, M.; Pedretti, S.; Mathieu, S.; Forcato, M.; Bicciato, S.; Manneville, J.-B.; et al. Extracellular matrix mechanical cues regulate lipid metabolism through Lipin-1 and SREBP. *Nature* 2019, *21*, 338–347.
43. Sullivan, W.J.; Mullen, P.J.; Schmid, E.W.; Flores, A.; Momcilovic, M.; Sharpley, M.S.; Jelinek, D.; Whiteley, A.E.; Maxwell, M.B.; Wilde, B.; et al. Extracellular Matrix Remodeling Regulates Glucose Metabolism through TXNIP Destabilization. *Cell* 2018, *175*, 117–132.e21.
44. Song, H.; Guan, Y.; Zhang, L.; Li, K.; Dong, C. SPARC interacts with AMPK and regulates GLUT4 expression. *Biochem. Biophys. Res. Commun.* 2010, *396*, 961–966.
45. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. *J. Veter-Med Sci.* 2019, *81*, 1649–1654.
46. Riley, J.H.; Bradshaw, A.D. *The Influence of the Extracellular Matrix in Inflammation: Findings from the SPARC-Null Mouse*; Anat Rec: Hoboken, NJ, USA, 2019.
47. Gilmour, D.T.; Lyon, G.J.; Carlton, M.B.; Sanes, J.R.; Cunningham, J.M.; Anderson, J.R.; Hogan, B.L.; Evans, M.J.; Colledge, W.H. Mice deficient for the secreted glycoprotein SPARC/osteonectin/BM40 develop normally but show severe age-onset cataract formation and disruption of the lens. *EMBO J.* 1998, *17*, 1860–1870.
48. Swaminathan, S.S.; Oh, D.-J.; Kang, M.H.; Ren, R.; Jin, R.; Gong, H.; Rhee, U.J. Secreted Protein Acidic and Rich in Cysteine (SPARC)-Null Mice Exhibit More Uniform Outflow. *Investig. Opthalmol. Vis. Sci.*

2013, *54*, 2035–2047.
49. Sangaletti, S.; Tripodo, C.; Cappetti, B.; Casalini, P.; Chiodoni, C.; Piconese, S.; Santangelo, A.; Parenza, M.; Arioli, I.; Miotti, S.; et al. SPARC Oppositely Regulates Inflammation and Fibrosis in Bleomycin-Induced Lung Damage. *Am. J. Pathol.* 2011, *179*, 3000–3010.
50. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. *Genes Immun.* 2007, *8*, 262–274.
51. Pichler, R.H.; Bassuk, J.A.; Hugo, C.; Reed, M.J.; Eng, E.; Gordon, K.L.; Pippin, J.; Alpers, C.E.; Couser, W.G.; Sage, E.H.; et al. SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growth-factor-medicated mesangial cell proliferation in vitro. *Am. J. Pathol.* 1996, *148*, 1153–1167.
52. Francki, A.; Motamed, K.; McClure, T.D.; Kaya, M.; Murri, C.; Blake, D.J.; Carbon, J.G.; Sage, E.H. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. *J. Cell. Biochem.* 2003, *88*, 802–811.
53. Ghanemi, A.; St-Amand, J. Interleukin-6 as a "metabolic hormone". *Cytokine* 2018, *112*, 132–136.
54. Xu, Y.; Zhang, Y.; Ye, J. IL-6: A Potential Role in Cardiac Metabolic Homeostasis. *Int. J. Mol. Sci.* 2018, *19*, 2474.
55. Qu, D.; Liu, J.; Lau, C.W.; Huang, Y. IL-6 in diabetes and cardiovascular complications. *Br. J. Pharmacol.* 2014, *171*, 3595–3603.
56. Erdogan, B.; Webb, D.J. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. *Biochem. Soc. Trans.* 2017, *45*, 229–236.
57. Moriggi, M.; Giussani, M.; Torretta, E.; Capitanio, D.; Sandri, M.; Leone, R.; De Palma, S.; Vasso, M.; Vozzi, G.; Tagliabue, E.; et al. ECM Remodeling in Breast Cancer with Different Grade: Contribution of 2D DIGE Proteomics. *Proteom.* 2018, *18*, 1800278.
58. Yuzhalin, A.E.; Lim, S.Y.; Kutikhin, A.; Gordon-Weeks, A.N. Dynamic matrisome: ECM remodeling factors licensing cancer progression and metastasis. *Biochim. Biophys. Acta (BBA) Bioenerg.* 2018, *1870*, 207–228.
59. Yang, M.; Liu, P.; Huang, P. Cancer stem cells, metabolism, and therapeutic significance. *Tumor Boil.* 2016, *37*, 5735–5742.
60. Lyssiotis, C.A.; Kimmelman, A.C. Metabolic Interactions in the Tumor Microenvironment. *Trends Cell Boil.* 2017, *27*, 863–875.
61. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
62. Irminger-Finger, I.; Kargul, J.; Laurent, G.J.; Laurent, G.J. Extra cellular matrix a modular soil for stem cells. *Int. J. Biochem. Cell Boil.* 2016, *81*, 164.
63. Murphy-Ullrich, J.E.; Sage, E.H. Revisiting the matricellular concept. *Matrix Boil.* 2014, *37*, 1–14.

Chapter 16. Special Article - Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property?

Abdelaziz Ghanemi [a,b], Mayumi Yoshioka [a], Jonny St-Amand [a,b]

[a] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, Québec G1V 4G2, Canada

[b] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

Received 9 April 2020; Received in revised form 12 June 2020; Accepted 13 June 2020

16.1 Résumé (French abstract)

La secreted protein acidic and rich in cysteine (SPARC) est exprimée dans diverses situations, y compris celles avec une composante inflammatoire ainsi que lors du remodelage et de la réparation des tissus. Ainsi, SPARC contribuerait à l'inflammation et à la fibrose associée et jouerait un rôle dans la création d'un microenvironnement pour le remodelage et la réparation des tissus. Cependant, si l'inflammation augmente, SPARC aurait un effet anti-inflammatoire. Cet effet anti-inflammatoire de SPARC est probablement une rétroaction (feedback) négative pour contrôler l'inflammation. De tels effets présentent SPARC avec une propriété homéostatique dans les processus inflammatoires par laquelle SPARC limite l'inflammation et contribue à la réparation des tissus. Étant donné que *SPARC* est induit par l'exercice, ces propriétés sont également en corrélation avec les avantages de l'exercice qui seraient médiés par SPARC.

Highlights

- SPARC would contribute to the inflammation and the related fibrosis.
- SPARC play roles to create the microenvironment for tissue remodeling and repair.
- Once inflammation increases, SPARC would have an anti-inflammatory effect.
- This SPARC anti-inflammatory effect is probably a negative feedback.
- These properties present SPARC with a homeostatic pattern in inflammatory processes.

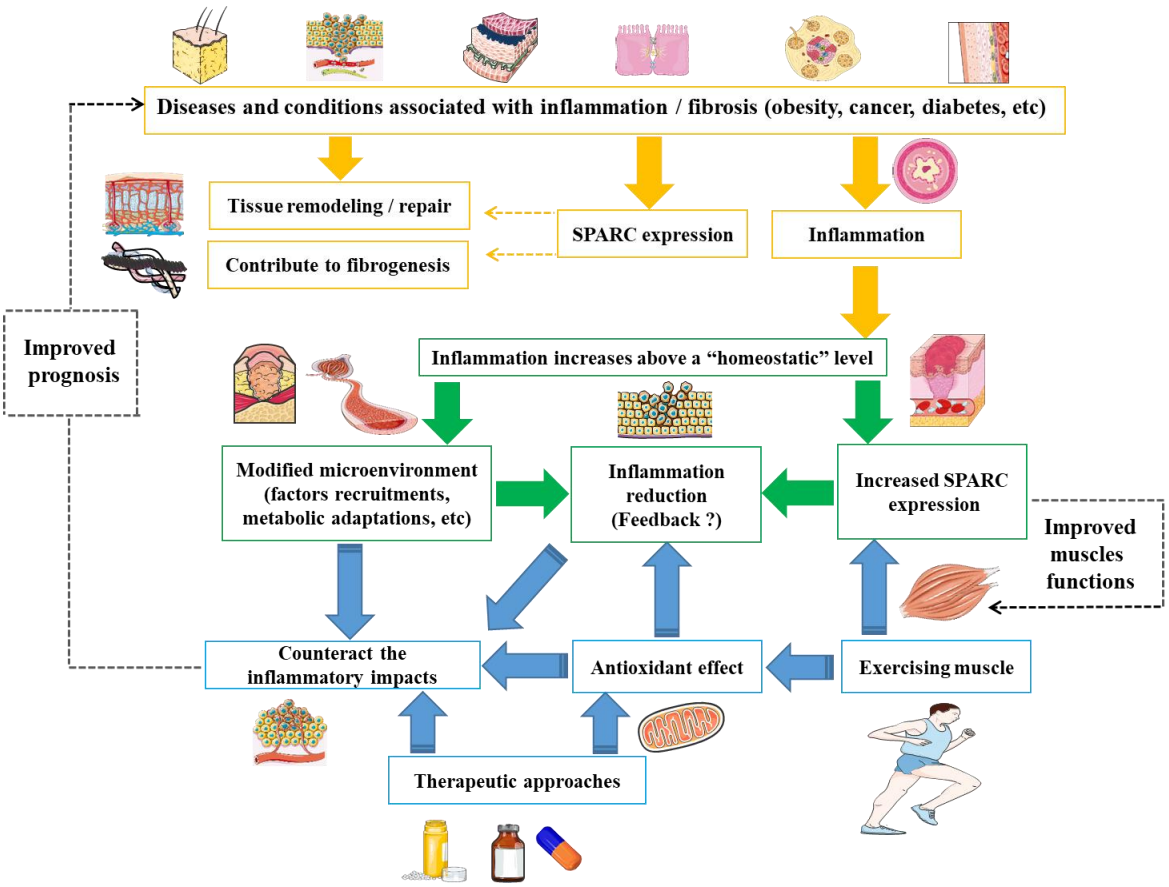
16.2 Abstract

Secreted protein acidic and rich in cysteine (SPARC) is expressed in various situations including those with an inflammatory component as well as during tissue remodelling and repair. Thus, SPARC would contribute to the inflammation and the related fibrosis and plays roles in creating a microenvironment for tissue remodeling and repair. However, once the inflammation

increases, SPARC would have an anti-inflammatory effect. This SPARC anti-inflammatory effect is probably a negative feedback to control the inflammation. Such patterns present SPARC with a homeostatic pattern in inflammatory processes via which SPARC both limits the inflammation and contributes to the tissue repair. Since *SPARC* is induced by exercise, these properties also correlate with the exercise benefits that would be mediated by SPARC.

Keywords: Secreted protein acidic and rich in cysteine; Inflammation; Homeostasis

16.3 Graphical abstract



16.4 Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property?

The inflammation, described since centuries, is a result of different cellular and molecular reactions as a response to stimulus of divers nature including injuries, infections, autoimmune processes or cancer development [1–4]. Inflammation is also related to the pathogenesis and development of numerous diseases [5–9]. Thus, inflammations can be clinically evidenced by numerous inflammatory mediators and indicators that represent tools for diagnosis, treatment evaluation and prognosis of a variety of diseases [10–12]. In addition, the impacts and implications of inflammation on certain health conditions justify the use of anti-inflammatory drugs for which animal models of inflammation have been

developed for screening and tests [5]. Although inflammation is associated to well-known conditions (obesity, cancer, etc), the inflammatory implications of important molecules that have been shown to play key roles in these same conditions remain to be uncovered. Therefore, it remains worth looking into how such molecules impact and are modified by inflammation as well as the conditions involving an inflammatory component.

As an illustration, we put a spotlight on secreted protein acidic and rich in cysteine (SPARC) which is an extracellular-associated glycoprotein. The description of SPARC roles in the contexts of metabolism [13–15], obesity [14,16,17], diabetes [18], immunity [19] and cancer [20–22] makes exploring its inflammatory properties an investigation that would add a piece to the puzzle of this glycoprotein. Indeed, not only SPARC plays roles in these conditions but inflammation is also associated to metabolism [23,24], obesity [25], cancer [26,27], diabetes [28] and other diseases [29] for which inflammation represents a shared pattern as well. More importantly, studies have showed links between SPARC and inflammation. For instance, SPARC is overexpressed in experimental glomerulonephritis [30] and ovarian cancer during which SPARC downregulates the inflammation associated to this cancer [31]. This is supported by the impact of the extracellular matrix on the inflammation depending on SPARC expression as reviewed by Riley and Bradshaw [32] and the fact that Sparc knockout (KO) mice had exacerbated inflammatory cell infiltration following lung damage compared to wild type mice [33].

Following this line of thoughts, it seems reasonable to suppose links between the involvement of SPARC during inflammatory process and the overexpression of SPARC in biological status that involve or lead to inflammation such as obesity and cancer. More precisely, SPARC expression could be either a factor via which the inflammatory pathways and the conditions (diabetes, metabolic syndrome, etc.) are linked and/ or SPARC expression would be an attempt to reestablish homeostasis as a feedback of the inflammatory status, similarly to what we have previously suggested for both cancer [20] and obesity [16]. Therefore, the anti-inflammatory effect of SPARC would be an addition to the puzzle theorizing that SPARC is overexpressed in status like obesity and cancer as an attempt to limit the negative effects of such conditions including inflammation (and the possible resulting fibrosis [33]) as well as cancer growth, adipocytes expansion, metabolic decline, etc. Thus, the expression of SPARC, as a result of obesity, cancer or other inflammation involving mechanisms, would be a feedback aiming to limit or balance the inflammation progress. On the other hand, and for what seems towards a contradictory direction though, some studies indicated that SPARC does play roles in developing and accelerating inflammation [34–36] and that Sparc KO could reduce (rather than exacerbate) inflammation [37–40]. However, such SPARC-induced inflammation could be required in order to repair and remodel tissues via an inflammation-mediated/induced recruitment of factors required for tissues repair and remodeling following injuries or during some pathological conditions (processes in which SPARC would be both required and overexpressed). This theory is supported by the fact that SPARC expression can be

influenced by factors like insulin or leptin [14] that are known to impact several tissues [41,42] as well as inflammation [43].

To reconcile these two directions, reporting both anti-inflammatory and pro inflammatory properties of SPARC, it is essential to attempt to put each property within its appropriate context and the biological step (s) of the tissue modification (injury, disease, tissue remodeling, etc.). Our hypothetic explanation (summarized by the graphical abstract) indicates that at the beginning of the condition development SPARC would contribute to the inflammation and the related fibrosis (especially with the known roles of SPARC in fibrosis [44,45] and collagen interaction with fibroblast [46]), in order to create the suitable microenvironment (factors recruitments, metabolic adaptation, blood flow, etc.) required for the tissue remolding and repair. Following that, and once the inflammation increases above a "homeostatic" level, the effect of SPARC (overexpressed during such inflammatory level) would be shifted towards an anti-inflammatory effect, probably as a negative feedback to rebalance the processes. It is also possible that SPARC has simultaneously both anti inflammatory and pro-inflammatory effects (through different paths). However, it is only after the inflammation increases above a "homeostatic" level that either the anti-inflammatory path predominates or the pro-inflammatory effect decreases. At this point the pro inflammatory effect is not required anymore because the tissue remodeling, for instance, is completed and the inflammatory-induced microenvironment required for the remodeling has no role to play at this biological step. Furthermore, SPARC represents a fibrosis mediator [44] that is overexpressed during hepatic fibrosis [39,47] and its KO attenuates fibrosis [39,40,44]. This fits with both SPARC role in fibrillar content accumulation [48–51] (which increases with fibrosis [51]) and our hypothesized pattern of inflammation progress and the homeostatic role that SPARC plays based on the close links between inflammation and fibrosis in damaged tissues [33,52,53]. These implications could involve, depending on the inflammatory stage, different pathways in which SPARC has been shown to be involved in like platelet-derived growth factor [30] and beta-catenin [51] signaling. These signaling pathways would represent starting points towards a deeper investigation of the molecular mechanisms linking SPARC to the inflammatory processes.

Interestingly, within the examples of both obesity and cancer, inflammation would be the bridge between these two pathologies [54]. This could explain that the impact of obesity on cancer incidence might be, at least, via an inflammatory-mediated process that could also involve SPARC. SPARC is known to be expressed in tissues undergoing modifications and is virtually expressed in all tissues [16]. This allows us to expect more roles of SPARC beyond what literature indicates about its implications in tissues development, metabolism, structure, obesity, cancer and other diseases especially based on what is known about matricellular proteins and inflammation [55,56]. Importantly, the effects of SPARC (also characterized as exercise-induced gene [57]) on muscles would be towards an improved metabolic profile [16,58] that could, via an-exercise related anti-inflammatory and antioxidant effect [59,60], counteract the inflammatory consequences.

This shows one of the indirect mechanisms by which SPARC would counteract the inflammatory-induced damages (antioxidant) in addition to the direct anti-inflammatory effect. This would, for instance, explain some of the benefits of physical activity (including inflammation attenuation) in diseases such as cancer [61] and diabetes [62].

Finally, such involvement of SPARC within the inflammatory process regulation and the ability of selected cells to secret SPARC into the circulation [14,63] further strengthen its classification as hormone, as we have previously suggested [20], or a cytokine, in addition to its classification as a myokine [64] even with an autocrine effect on the skeletal muscle [16]. All these properties highlight SPARC as a therapeutic (for example through pharmacological approaches or exercise-induced) and a prognostic target [65–68] especially for diseases involving both inflammatory and fibrotic components [40,69]. SPARC, the extracellular matrix and the other proteins associated to it are further shown to be worth investigating beyond their structural roles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Abdelaziz Ghanemi is a recipient of a Merit scholarship program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). The graphical abstract was created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

16.5 References

[1] P. Arulselvan, et al., Role of antioxidants and natural products in inflammation, Oxid. Med. Cell Longev. 2016 (2016) 5276130.
[2] G.W. Schmid-Schönbein, Analysis of inflammation, Annu. Rev. Biomed. Eng. 8 (1) (2006) 93–151.
[3] D.V. Kuprash, S.A. Nedospasov, Molecular and cellular mechanisms of inflammation, Biochemistry (Mosc) 81 (11) (2016) 1237–1239.
[4] W.G. Spector, D.A. Willoughby, Vasoactive amines in acute inflammation, Ann. N. Y. Acad. Sci. 116 (1964) 839–846.
[5] K.R. Patil, et al., Animal Models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals, Int. J. Mol. Sci. 20 (18) (2019).
[6] T. Ganz, Anemia of inflammation, N. Engl. J. Med. 381 (12) (2019) 1148–1157.
[7] A. Ghanemi, M. Yoshioka, J. St-Amand, Broken energy homeostasis and obesity pathogenesis: the surrounding concepts, J. Clin. Med. 7 (11) (2018).

[8] A. Ghanemi, J. St-Amand, Interleukin-6 as a “metabolic hormone”, Cytokine 112 (2018) 132–136.
[9] A. Ghanemi, J. St-Amand, Redefining obesity toward classifying as a disease, Eur. J. Intern. Med. 55 (2018) 20–22.
[10] N.V. Zotova, et al., Integral estimation of systemic inflammatory response under sepsis, Gen. Physiol. Biophys. 39 (1) (2020) 13–26.
[11] Y. Zhu, et al., Changes of inflammatory mediators and oxidative stress indicators in children with Henoch-Schonlein purpura and clinical effects of hemoperfusion in the treatment of severe Henoch-Schonlein purpura with gastrointestinal involvement in children, BMC Pediatr 19 (1) (2019) 409.
[12] D.C. McMillan, K. Canna, C.S. McArdle, Systemic inflammatory response predicts survival following curative resection of colorectal cancer, Br. J. Surg. 90 (2) (2003) 215–219.
[13] H. Song, et al., MiR-29 family members interact with SPARC to regulate glucose metabolism, Biochem. Biophys. Res. Commun. 497 (2) (2018) 667–674.
[14] K. Kos, et al., Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose, Diabetes 58 (8) (2009) 1780–1788.
[15] A. Ghanemi, M. Yoshioka, J. St-Amand, Secreted protein acidic and rich in cysteine: metabolic and homeostatic properties beyond the extracellular matrix structure, Appl. Sci. 10 (7) (2020) 2388.
[16] A. Ghanemi, et al., Secreted protein acidic and rich in cysteine and bioenergetics: extracellular matrix, adipocytes remodeling and skeletal muscle metabolism, Int. J. Biochem. Cell Biol. 117 (2019) 105627.
[17] Y. Shen, et al., SPARC is over-expressed in adipose tissues of diet-induced obese rats and causes insulin resistance in 3T3-L1 adipocytes, Acta Histochem. 116 (1) (2014) 158–166.
[18] K. Kos, J.P. Wilding, SPARC: a key player in the pathologies associated with obesity and diabetes, Nat. Rev. Endocrinol. 6 (4) (2010) 225–235.
[19] S.A. Rempel, et al., Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response, Genes Immun. 8 (3) (2007) 262–274.
[20] A. Ghanemi, M. Yoshioka, J. St-Amand, Secreted protein acidic and rich in cysteine and cancer: a homeostatic hormone? Cytokine 127 (2020) 154996.
[21] G.P. Nagaraju, D. Sharma, Anti-cancer role of SPARC, an inhibitor of adipogenesis, Cancer Treat. Rev. 37 (7) (2011) 559–566.
[22] S.L.I. Wong, M.B. Sukkar, The SPARC protein: an overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease, Br. J. Pharmacol. 174 (1) (2017) 3–14.
[23] L. Ali, J.G. Schnitzler, J. Kroon, Metabolism: the road to inflammation and atherosclerosis, Curr. Opin. Lipidol. 29 (6) (2018) 474–480.
[24] J. Saravia, et al., Signaling networks in immunometabolism, Cell Res. (2020).
[25] L. Chen, et al., Mechanisms linking inflammation to insulin resistance, Int. J. Endocrinol. 2015 (2015) 508409.
[26] N. Singh, et al., Inflammation and cancer, Ann. Afr. Med. 18 (3) (2019) 121–126.
[27] M. Murata, Inflammation and cancer, Environ. Health Prev. Med. 23 (1) (2018) 50.
[28] S. Tsalamandris, et al., The role of inflammation in diabetes: current concepts and future perspectives, Eur. Cardiol. 14 (1) (2019) 50–59.
[29] P. Hunter, The inflammation theory of disease. The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment, EMBO Rep. 13 (11) (2012) 968–970.
[30] R.H. Pichler, et al., SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growth-factor-medicated mesangial cell proliferation in vitro, Am. J. Pathol. 148 (4) (1996)
1153–1167.
[31] N.A. Said, et al., SPARC ameliorates ovarian cancer-associated inflammation, Neoplasia 10 (10) (2008) 1092–1104.
[32] H.J. Riley, A.D. Bradshaw, The influence of the extracellular matrix in inflammation: findings from the SPARC-null mouse, Anatom. Rec. n/a (n/a).

[33] S. Sangaletti, et al., SPARC oppositely regulates inflammation and fibrosis in bleomycin-induced lung damage, Am. J. Pathol. 179 (6) (2011) 3000–3010.
[34] Z. Cao, M.E. Cooper, Role of angiotensin II in tubulointerstitial injury, Semin. Nephrol. 21 (6) (2001) 554–562.
[35] H. Toba, et al., Secreted protein acidic and rich in cysteine facilitates age-related cardiac inflammation and macrophage M1 polarization, Am. J. Physiol. Cell Physiol. 308 (12) (2015) C972–C982.
[36] M. Tanaka, et al., Secreted protein acidic and rich in cysteine functions in colitis via IL17A regulation in mucosal CD4(+) T cells, J. Gastroenterol. Hepatol. 33 (3) (2018) 671–680.
[37] M.J. Socha, et al., Secreted protein acidic and rich in cysteine deficiency ameliorates renal inflammation and fibrosis in angiotensin hypertension, Am. J. Pathol. 171 (4) (2007) 1104–1112.
[38] Y.L. Ng, et al., Secreted protein acidic and rich in cysteine (SPARC) exacerbates colonic inflammatory symptoms in dextran sodium sulphate-induced murine colitis, PLoS ONE 8 (10) (2013) e77575.
[39] C. Atorrasagasti, et al., Lack of the matricellular protein SPARC (secreted protein, acidic and rich in cysteine) attenuates liver fibrogenesis in mice, PLoS ONE 8 (2) (2013) e54962.
[40] G. Mazzolini, et al., SPARC expression is associated with hepatic injury in rodents and humans with non-alcoholic fatty liver disease, Sci. Rep. 8 (1) (2018) 725.
[41] G. Dimitriadis, et al., Insulin effects in muscle and adipose tissue, Diabetes Res. Clin. Pract. 93 (2011) S52–S59.
[42] D.S. Edgerton, et al., Insulin's direct effects on the liver dominate the control of hepatic glucose production, J. Clin. Invest. 116 (2) (2006) 521–527.
[43] V. Abella, et al., Leptin in the interplay of inflammation, metabolism and immune system disorders, Nat. Rev. Rheumatol. 13 (2) (2017) 100–109.
[44] J. Trombetta-Esilva, A.D. Bradshaw, The function of SPARC as a mediator of fibrosis, Open Rheumatol. J. 6 (2012) 146–155.
[45] A.D. Bradshaw, The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: does expression of SPARC by macrophages influence outcomes? J. Mol. Cell. Cardiol. 93 (2016) 156–161.
[46] B.S. Harris, et al., SPARC regulates collagen interaction with cardiac fibroblast cell surfaces, Am. J. Physiol. Heart Circ. Physiol. 301 (3) (2011) H841–H847.
[47] E. Frizell, et al., Expression of SPARC in normal and fibrotic livers, Hepatology 21 (3) (1995) 847–854.
[48] S. Omi, et al., Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice, J. Vet. Med. Sci. 81 (11) (2019) 1649–1654.
[49] L.T. McDonald, et al., Increased macrophage-derived SPARC precedes collagen deposition in myocardial fibrosis, Am. J. Physiol. Heart Circ. Physiol. 315 (1) (2018) H92–h100.
[50] A.D. Bradshaw, et al., Age-dependent alterations in fibrillar collagen content and myocardial diastolic function: role of SPARC in post-synthetic procollagen processing, Am. J. Physiol. Heart Circ. Physiol. 298 (2) (2010) H614–H622.
[51] W. Chang, et al., SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin, J. Biol. Chem. 285 (11) (2010) 8196–8206.
[52] M. Mack, Inflammation and fibrosis, Matrix Biol. 68–69 (2018) 106–121.
[53] C.J. Song, et al., Inflammation and fibrosis in polycystic kidney disease, Results Probl. Cell Differ. 60 (2017) 323–344.
[54] R. Kolb, F.S. Sutterwala, W. Zhang, Obesity and cancer: inflammation bridges the two, Curr. Opin. Pharmacol. 29 (2016) 77–89.
[55] C. Chiodoni, M.P. Colombo, S. Sangaletti, Matricellular proteins: from homeostasis to inflammation, cancer, and metastasis, Cancer Metastasis Rev. 29 (2) (2010) 295–307.
[56] A.S. Llera, et al., Matricellular proteins and inflammatory cells: a task force to promote or defeat cancer? Cytokine Growth Factor Rev. 21 (1) (2010) 67–76.
[57] I. Riedl, et al., Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance

training at lactate threshold intensity, Exp. Gerontol. 45 (11) (2010) 896–903.
[58] W. Aoi, et al., Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation, FASEB J. 33 (9) (2019) 10551–10562.
[59] F.A. Galle, D. Martella, G. Bresciani, Antioxidant and anti-inflammatory modulation of exercise during aging, Rev Esp Geriatr Gerontol 53 (5) (2018) 279–284.
[60] C.V. de Sousa, et al., The antioxidant effect of exercise: a systematic review and meta-analysis, Sports Med. 47 (2) (2017) 277–293.
[61] S. Krebs, A. Berling-Ernst, M. Halle, Physical activity and cancer, Sportverletz. Sportschaden 32 (2) (2018) 143–147.
[62] D.W. Jenkins, A. Jenks, Exercise and diabetes: a narrative review, J. Foot Ankle Surg. 56 (5) (2017) 968–974.
[63] M. Takahashi, et al., The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease, Obes. Res. 9 (7) (2001) 388–393.
[64] B. So, et al., Exercise-induced myokines in health and metabolic diseases, Integr. Med. Res 3 (4) (2014) 172–179.
[65] J. Vaz, et al., SPARC: a potential prognostic and therapeutic target in pancreatic cancer, Pancreas 44 (7) (2015) 1024–1035.
[66] M. Sinn, et al., SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study, Ann. Oncol. 25 (5) (2014) 1025–1032.
[67] S. Mukherjee, et al., Secreted protein acidic and rich in cysteine (SPARC) regulates thermogenesis in white and brown adipocytes, Mol. Cell. Endocrinol. 506 (2020) 110757.
[68] A. Munasinghe, et al., Fibronectin acts as a molecular switch to determine SPARC function in pancreatic cancer, Cancer Lett. 477 (2020) 88–96.
[69] T. Carvalheiro, et al., Extracellular SPARC cooperates with TGF-beta signalling to induce pro-fibrotic activation of systemic sclerosis patient dermal fibroblasts, Rheumatology (Oxford) (2019).

Chapter 17. Special Article - Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone?

Abdelaziz Ghanemi [a,b], Mayumi Yoshioka [a], Jonny St-Amand [a,b]

[a] Functional Genomics Laboratory, CREMI, Québec Genome Center, CHUL-CHU de Québec Research Center, Québec, Québec G1V 4G2, Canada

[b] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

Received 20 September 2019; Received in revised form 8 January 2020; Accepted 10 January 2020

17.1 Résumé (French abstract)

La secreted protein acidic and rich in cysteine (SPARC) est surexprimée lors de la croissance des tumeurs. Cette glycoprotéine a une capacité de suppression tumorale mais aucun effet apoptotique sur les cellules normales (spécificité). Ces propriétés inhibitrices de SPARC vers le développement de cancer pourraient être explorées, ce qui augmenterait la sécurité d'un traitement antitumoral basé sur les voies liées au SPARC (amélioration de la pharmacovigilance). Cependant, une possible « résistance au SPARC » et/ou d'autres changements dans les facteurs de croissance liés au cancer pourraient limiter les effets inhibiteurs du SPARC sur les tissus cancéreux.

Highlights

• Secreted protein acidic and rich in cysteine (SPARC) is associated to the extracellular matrix.
• SPARC is overexpressed in cancer.
• SPARC is linked to cancer growth inhibition.
• SPARC would have a homeostatic effect on cancer growth (feedback?).
• SPARC-cancer interactions could be explored to develop antitumor therapies.

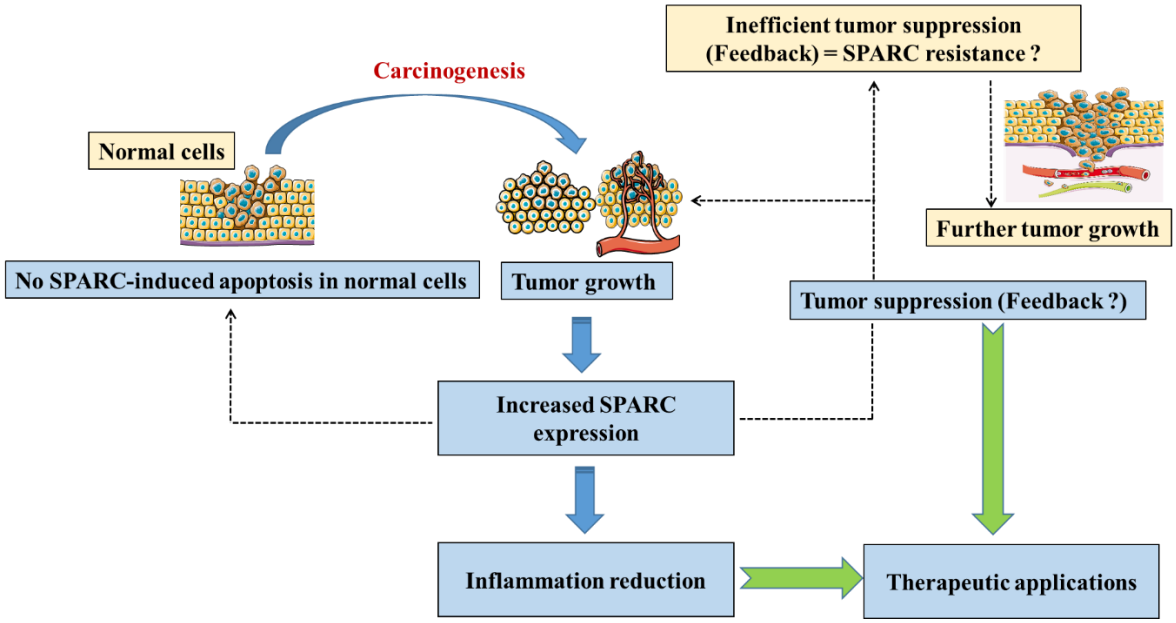
17.2 Abstract

Secreted protein acidic and rich in cysteine (SPARC) is overexpressed during tumoral growth. This glycoprotein has a tumor suppression ability but no apoptotic effect on normal cells (specificity). This inhibitory property of SPARC towards cancer development could be further explored, especially that SPARC induces apoptosis in cancer cells but not in normal cells which would increase the safety of an anti-tumor therapy based on SPARC-related pathways (improved pharmacovigilance). However, a possible "SPARC

resistance" and/or other changes in cancer-related growth factors could limit the inhibitory effects of SPARC towards cancer tissues.

Keywords: Secreted protein acidic and rich in cysteine; Cancer; Hormone; Homeostasis

17.3 Graphical abstract



17.4 Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone?

The implications of the extracellular matrix (ECM) in cell development are critical [1]. Such involvements cover processes, including diseases-related pathways [2], controlling a variety of biological functions such as signals transduction and cellular adhesion [3]. Fibronectin, collagens and glycosaminoglycans are examples of biomolecules involved in the dynamics and structure of the ECM [3]. Among the molecules associated with the ECM, secreted protein acidic and rich in cysteine (SPARC) is a 32-kDa glycoprotein expressed mainly during tissues remodeling [4]. Secreted by different types of cells, SPARC is associated with ECM organization and remodeling [5–8], controls cellular interaction with the ECM, and binds to matrix proteins [9]. Interestingly, gene-encoding SPARC is also induced in muscles by exercise [10] and by electrical pulse stimulation (considered as an in vitro model of exercise) in myoblasts culture [11] suggesting that some exercise-induced modifications in muscles would be mediated by SPARC [12]. These examples of SPARC implications in ECM functions suggest that changes in SPARC expression would have an impact on cellular remodeling especially in tissues for which changes are of the highest importance to accomplish the related functions or to develop the

required biological properties such as adipocytes and skeletal muscles [12] as well as cancer cells, as illustrated below.

Cancer epidemiology makes it the second cause of death worldwide [13]. Therefore, further understanding its biology and processes, especially at the molecular level, is crucial to develop and optimize diagnosis approaches and therapies. Within this context, evidence from SPARC implications in metabolic and growth patterns of cancer cells is of interest. In fact, the developmental properties (proliferation, apoptosis, etc.) and the metabolic patterns that are shaped in cancer cells make SPARC (as a hormone) implications under carcinogenesis conditions worth exploring to further shine the light on the homeostatic effects that SPARC might have on such abnormal cell growth. For instance, both bioenergetics and metabolic plasticity in ovarian cancer were shown to involve SPARC via paracrine effects leading to tumor suppression [14] which correlates with the metastasis inhibitory properties of SPARC shown by Ma et al. [15]. This paracrine inhibition could have the same pattern as the adipogenesis inhibition properties of SPARC reported in the adipose tissue [12,16,17]. Indeed, the shared pattern between adiposity overdevelopment (obesity) [18] and carcinogenesis is that both are towards a pathological status (broken homeostasis). Therefore, the inhibitory properties of SPARC towards both adipogenesis and tumors would highlight this glycoprotein as a homeostasis regulator (paracrine) acting toward limiting an unhealthy cellular development. Within the contexts of the association between SPARC overexpression and tumor growth [19], the SPARC expression could indicate a possible negative feedback aiming to limit the cancer development and metastasis via the tumors inhibitory properties of SPARC.

Following this line of thoughts, the expression of SPARC when tissues undergo changes (repair, renewal and remodeling) might not only be required during such cellular stages of structural and metabolic changes but seems also to be a mechanism to regulate growth and bioenergetics balance. As an illustration, whereas SPARC reduces the associated inflammation [20], inhibits the proliferation [21] and leads to the apoptosis [22] in ovarian cancer cells, inoculation of ovarian cancer cells into the peritoneal cavity of *Sparc* knock-out (KO) mice leads to an increase in tumor proliferation and reduction in survival compared to the wild-type (WT) mice that were also injected with ovarian cancer cells [23]. The authors of this study even suggested SPARC as a therapeutic candidate to treat ovarian cancer [23]. Said et al. considered SPARC as a “normalizer” for the cancer microenvironment [21] acting towards moderating the cancer development, which fits with the increase of SPARC expression associated with an advanced stage of ovarian cancer [24]. It is worth mentioning that these conclusions about the effect of SPARC on tumors result from studies in which tumor cells (WT) were injected into hosts (*Sparc* KO) to clearly observe the effect of SPARC absence (in the host) on tumor cells that are not *Sparc* KO. Indeed, due to the implication of SPARC in the ECM maturation, we need to have cancer cells that are not *Sparc* KO (allow normal development) developing within a host (*Sparc* KO) to confirm such observations. If we had both host and cancer cells that are *Sparc* KO (cancer developing within a *Sparc* KO mice), we would see that the *Sparc* KO

leads to cell proliferation suppression and metastasis inhibition [24]. These results are due to the lack of SPARC in the cancer tissue (*Sparc* KO) leading to a limitation of its development. This is different from the situation of cancer cells (WT) inside a *Sparc* KO host, which will remove the inhibitory effect of SPARC and lead to further cancer invasion compared to having such cancer cells within a microenvironment rich in SPARC. These observations indicate that SPARC acts toward conserving the cell growth homeostasis. This is, in part, illustrated by the fact that the treatment of cell cultures with exogenous SPARC induced apoptosis in cancer cells but not in normal cells [22]. This understating also explains the apparently contradictory SPARC properties of both being overexpressed in several cancers and showing the tumor suppression ability (cell growth homeostasis regulation) in other tumor types [25]. However, the efficiency of such SPARC related regulation (more obviously seen with exogenous SPARC treatment rather than biological changes-induced SPARC overexpression) might be limited by the existence of other pathways counteracting SPARC effects such as inflammatory processes, tumors induced-mediators, hormones, etc. These counteracting factors would limit or suppress the regulatory ability of SPARC. In addition, a SPARC resistance might also be involved and would lead to further cancer development in spite of the SPARC overexpression by tumors tissues. Such resistance would follow the same pattern as what we have previously suggested for adipocytes development and the effect of SPARC on it [12]. It could explain why highly metastatic and most aggressive tumors are associated with increased SPARC levels [26] in the sense that the “disabled” SPARC inhibitory properties towards tumors (resistance) would be interpreted as a lack of SPARC and thus induces further increase in its production.

In conclusion, the effects of SPARC on cells and its expression do not only depend on tissues types but also on the status (healthy, diseases, etc.), its stage, (advanced or early) and on the factors of the microenvironment to which the tissue is exposed (normal, cancer, development, etc.). Therefore, SPARC expression was also proposed as a possible prognostic marker for resectable pancreatic cancer [27] and nonsmall cell lung cancer [28]. The other points that emphasize the importance of SPARC-cancer interactions are the distribution of SPARC among divers tissues and the existence of divers cancer types (liver, lung, stomach, thyroid, pancreas, etc.) [13]. This expends the applications of such interactions. In addition, the inhibitory properties and the effect of SPARC towards cancer cells could be further exploited by exploring cancer pathways related to factors shown to interact with SPARC such as integrin-linked kinase and β-catenin signaling in diverse conditions [11,29,30]. Elucidating molecular pathways linking SPARC and cancer cells could lead to a new generation of anti-tumor therapies. These therapies get their specificities form the fact that SPARC induces apoptosis in cancer cells but not in normal cells [22], which would increase the safety of an anti-tumor therapy based on SPARC-related pathways. Therefore, it would optimize the selectivity toward the therapeutic target, reduce the side effects and improve the pharmacovigilance of such an oncopharmacology.

Credit authorship contribution statement

Abdelaziz Ghanemi: Writing-original draft. Abdelaziz Ghanemi: Writing-review & editing. Mayumi Yoshioka: Writing-review & editing. Jonny St-Amand: Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Abdelaziz Ghanemi is a recipient of a Merit scholarship program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme).

The graphical abstract was created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

17.5 References

[1] P. Lu, et al., Extracellular matrix degradation and remodeling in development and disease, Cold Spring Harb. Perspect. Biol. 3 (12) (2011).
[2] C. Bonnans, J. Chou, Z. Werb, Remodelling the extracellular matrix in development and disease, Nat. Rev. Mol. Cell Biol. 15 (12) (2014) 786–801.
[3] A.D. Theocharis, et al., Extracellular matrix structure, Adv. Drug Deliv. Rev. 97 (2016) 4–27.
[4] J.D. Termine, et al., Osteonectin, a bone-specific protein linking mineral to collagen, Cell 26 (1 Pt 1) (1981) 99–105.
[5] I.T. Tai, M.J. Tang, SPARC in cancer biology: its role in cancer progression and potential for therapy, Drug Resist. Updat. 11 (6) (2008) 231–246.
[6] A.D. Bradshaw, E.H. Sage, SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury, J. Clin. Invest. 107 (9) (2001) 1049–1054.
[7] A. Basu, et al., Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40), BMC Cell Biol. 2 (2001) 15.
[8] E.M. Rosset, A.D. Bradshaw, SPARC/osteonectin in mineralized tissue, Matrix Biol. 52–54 (2016) 78–87.
[9] R.A. Brekken, E.H. Sage, SPARC, a matricellular protein: at the crossroads of cellmatrix, Matrix Biol. 19 (7) (2000) 569–580.
[10] I. Riedl, et al., Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity, Exp. Gerontol. 45 (11) (2010) 896–903.
[11] A. Melouane, et al., Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells, Life Sci. 229 (2019) 277–287.
[12] A. Ghanemi, et al., Secreted protein acidic and rich in cysteine and bioenergetics: extracellular matrix, adipocytes remodeling and skeletal muscle metabolism, Int. J. Biochem. Cell Biol. 117 (2019) 105627.

[13] C. Mattiuzzi, G. Lippi, Current cancer epidemiology, J. Epidemiol. Glob. Health 9 (4) (2019) 217–222.
[14] C. Naczki, et al., SPARC inhibits metabolic plasticity in ovarian cancer, Cancers (Basel) 10 (10) (2018).
[15] J. Ma, et al., SPARC inhibits breast cancer bone metastasis and may be a clinical therapeutic target, Oncol. Lett. 14 (5) (2017) 5876–5882.
[16] J. Nie, E.H. Sage, SPARC functions as an inhibitor of adipogenesis, J. Cell Commun. Signal. 3 (3–4) (2009) 247–254.
[17] J. Nie, E.H. Sage, SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling, J. Biol. Chem. 284 (2) (2009) 1279–1290.
[18] A. Ghanemi, M. Yoshioka, J. St-Amand, Broken energy homeostasis and obesity pathogenesis: the surrounding concepts, J. Clin. Med. (2018) 7(11).
[19] A. Zhu, et al., SPARC overexpression in primary tumors correlates with disease recurrence and overall survival in patients with triple negative breast cancer, Oncotarget 7 (47) (2016) 76628–76634.
[20] N.A. Said, et al., SPARC ameliorates ovarian cancer-associated inflammation, Neoplasia 10 (10) (2008) 1092–1104.
[21] N. Said, et al., Normalization of the ovarian cancer microenvironment by SPARC, Mol. Cancer Res. 5 (10) (2007) 1015–1030.
[22] G.K. Yiu, et al., SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells, Am. J. Pathol. 159 (2) (2001) 609–622.
[23] N. Said, K. Motamed, Absence of host-secreted protein acidic and rich in cysteine (SPARC) augments peritoneal ovarian carcinomatosis, Am. J. Pathol. 167 (6) (2005) 1739–1752.
[24] J. Chen, et al., SPARC is a key regulator of proliferation, apoptosis and invasion in human ovarian cancer, PLoS One 7 (8) (2012) e42413.
[25] C. Neuzillet, et al., Stromal expression of SPARC in pancreatic adenocarcinoma, Cancer Metastasis Rev. 32 (3–4) (2013) 585–602.
[26] O.L. Podhajcer, et al., The role of the matricellular protein SPARC in the dynamic interaction between the tumor and the host, Cancer Metastasis Rev. 27 (4) (2008) 691–705.
[27] M. Murakawa, et al., The impact of SPARC expression on the survival of pancreatic ductal adenocarcinoma patients after curative resection, J Cancer 10 (3) (2019) 627–633.
[28] Y. Huang, et al., SPARC expression and prognostic value in non-small cell lung cancer, Chinese J. Cancer 31 (11) (2012) 541–548.
[29] H. Alachkar, et al., SPARC promotes leukemic cell growth and predicts acute myeloid leukemia outcome, J. Clin. Invest. 124 (4) (2014) 1512–1524.
[30] W. Chang, et al., SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin, J. Biol. Chem. 285 (11) (2010) 8196–8206.

Chapter 18. Opinion - Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 7 December 2020; Accepted: 7 January 2021; Published: 8 January 2021

18.1 Résumé (French abstract)

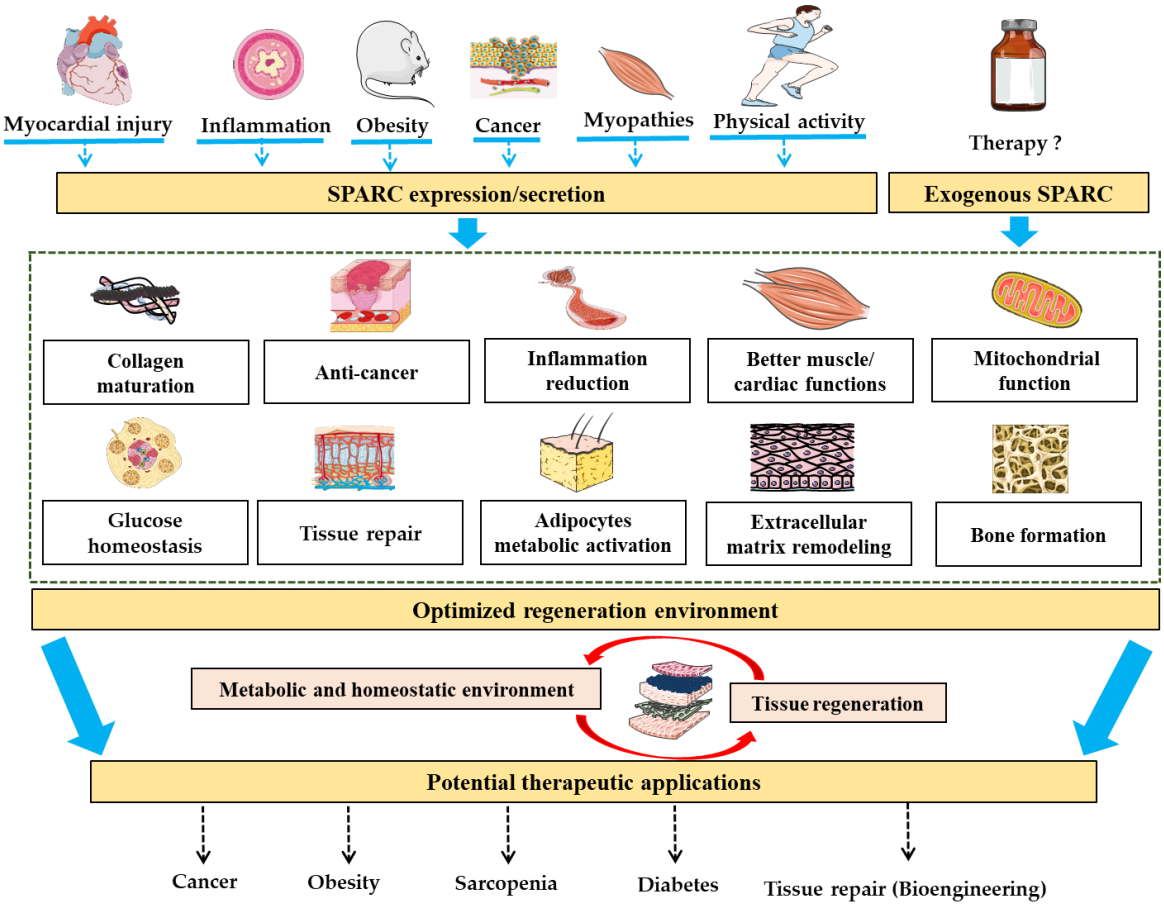
Diverse pathologies (inflammation, lésions tissulaires, cancer, etc.) et conditions physiologiques (obésité, activité physique, etc.) induisent l'expression/sécrétion de la protéine matricellulaire, secreted protein acidic and rich in cysteine (SPARC). SPARC contribue à la création d'un environnement adapté à la régénération des tissus via un nombre de propriétés, y compris l'homéostasie métabolique, la réduction de l'inflammation, le remodelage de la matrice extracellulaire et la maturation du collagène. Un tel environnement homéostatique optimise la régénération des tissus et améliore leur capacité à se réparer. Ces propriétés que SPARC a dans les contextes de la régénération pourraient avoir une variété d'applications, telles que dans l'obésité, le cancer, la sarcopénie, le diabète et le génie biologique.

18.2 Abstract

Diverse pathologies (inflammation, tissues injuries, cancer, etc.) and physiological conditions (obesity, physical activity, etc.) induce the expression/secretion of the matricellular protein, secrete protein acidic and rich in cysteine (SPARC). SPARC contributes to the creation of an environment that is suitable for tissue regeneration through a variety of roles, including metabolic homeostasis, inflammation reduction, extracellular matrix remodeling and collagen maturation. Such a homeostatic environment optimizes tissue regeneration and improves tissues' repair ability. These properties that SPARC has within the regeneration contexts could have a variety of applications, such as in obesity, cancer, sarcopenia, diabetes and bioengineering.

Keywords: secreted protein acidic and rich in cysteine; regeneration; homeostasis

18.3 Graphical abstract



18.4 Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair

Tissue regeneration is a vital process allowing organisms to overcome biological disturbances and adapt to changes and physiological development via the renewal, growth and restoration of diverse cells and tissues. The regeneration ability changes throughout the lifespan, which leads to diverse tissue malfunctions and diseases [1]. The regenerative process could be either normal or limited (abnormal) depending on the biological environment. Indeed, under healthy environmental conditions (stem cells growth ratio [2], growth factors [3], hormones [4,5], pH [6,7], etc.), the regenerative processes are optimized. They allow for regular tissue development and adaptation to the corresponding biological functions. However, under physiological (ageing [8,9]) or pathological (cancer [10], obesity [11], inflammation [12], etc.) conditions, or when impacted by disturbing stimuli or exogenous factors (such as radiations [13]), tissues' regeneration ability and functions could be impaired. To overcome this "negative" regeneration environment, the organism has a variety of tools to compensate or reduce the intensity or the impacts. These correcting or counteracting mechanisms are mediated through what could be considered

regeneration factors. Among these molecules, secreted protein acidic and rich in cysteine (SPARC) has a variety of roles and implications. One of the SPARC properties is its ability to optimize the regeneration environment with an improved cellular regenerative capacity from different perspectives (metabolics, tissue repair, oxidation, inflammation, cancer, etc.), as illustrated below.

SPARC, also known as BM-40 or osteonectin (32 kDa [14]), is a matricellular (extracellular matrix-associated) protein. Unlike its name (osteonectin) might suggest, SPARC expression is not limited to bones, but this glycoprotein is also present in diverse tissues including nonmineralized tissues, in platelets [15] and in muscles [16]. Such wide distribution correlates with SPARC roles during embryogenesis [17] as well as during tissue repair, cell turnover, cellular differentiation and remodeling [18–22], which are key steps in tissue regeneration. Therefore, SPARC expression or levels increase following injuries such as myocardial injury [23], myopathies [24] and in situations (either physiological or pathological) where tissues undergo changes (repair, renewal and remodeling) such as during obesity [18,25], skeletal muscle regeneration [26], cancer [27], systemic sclerosis, hepatic fibrosis [28] and physical exercise. Indeed, SPARC/*Sparc* expression increases in the skeletal muscle during training [29], as well as following electrical pulse stimulation in muscle cells (considered to be the in vitro equivalent of exercise) [30]. Such situations do represent a disturbance of the homeostasis that leads to a "negative" regeneration environment. Therefore, biological processes that overcome such a homeostatic disturbance, restore a suitable environment for regeneration and rescue the affected tissues to allow better developmental patters are required. Interestingly, the situations in which SPARC is overexpressed are mainly those requiring regeneration, either to repair tissues (injury) or adapt to tissue changes (obesity, exercised muscle, etc.). These specific patterns highlight SPARC as a regenerative factor. In addition, the importance of the extracellular matrix in regeneration suggests close interactions between SPARC, the extracellular matrix [31] and matricellular protein components such as thrombospondin-2 [32] during the regeneration process.

Tissue regeneration is a process that requires the implication of numerous cellular organelles and the use of energy. Thus, regeneration has metabolic and biochemical needs to which the cellular machinery has to adapt [33]. In this context, SPARC has been shown to be implicated in a variety of metabolic functions, such as glucose tolerance improvement [34], while it is also required for both glucose homeostasis maintenance and insulin secretion [35]. In the skeletal muscle, SPARC also seems to act towards improved metabolic properties and functions [18,24], including mitochondrial functions [30,36,37], which is of interest knowing the importance of the mitochondria during regeneration [38, 39]. Importantly, our latest study suggests that exercise-induced muscle phenotype changes are SPARC-dependent [40]. These SPARC properties are also completed by their important roles in energy balance and storage. For instance, SPARC inhibits adipogenesis [41] and its inactivation leads to an enhancement of high-fat diet-induced obesity [42]. These patterns correlate with the role of SPARC in brown adipocyte activation and lipid usage

in white adipocytes [43]. Such energy metabolism effects - in addition to optimizing the regeneration (synchronization) - also lead to increased energy usage, thus reducing the risk of obesity through increased energy expenditure. This represents another illustration of how SPARC counteracts the “negative” regeneration environment, since obesity itself represents a status of impaired regeneration [44]. Indeed, during obesity, many factors lead to such a “negative” regeneration environment due to all the conditions induced by or associated with obesity, such as inflammation, insulin resistance, metabolic disorders [45, 46] and even stem cell changes [47,48], that impact regeneration. SPARC is extremely important for bone formation, remodeling and regeneration [14,32,49–51]. This is important as well, not only for the structural homeostasis, but also for both locomotion and, most importantly, the energy metabolism. Indeed, the skeletal muscle that governs most of the energy expenditure [52] is supported by the skeleton with which it forms the locomotor (musculoskeletal) system. Therefore, the good metabolic and contractile function (strength) of muscles would require homeostatic skeleton development due to the close ties between both bones and skeletal muscles, including synchronized development [53].

Furthermore, in addition to such metabolic implications, SPARC is also involved in other growth and homeostasis-related patterns, including cancer homeostasis. SPARC is overexpressed during cancer [27] and has been reported to have anti-cancer properties [54,55]. SPARC has also been shown to have interesting roles within the inflammatory processes [56,57]. It has anti-inflammatory properties [56] and can, for instance, protect from adverse cardiac inflammation during viral myocarditis [58]. These properties of controlling cancer and inflammation development would impact the microenvironment, contributing to an improved homeostasis. Moreover, SPARC is required for the immune system functions [59], which is relevant, for instance, during immune-modulatory therapy to support the regeneration of injured muscles [60] and muscle healing [61]. Importantly, more roles are yet to be explored in terms of SPARC contribution at the physiological levels, such as in cardiomyocyte contraction [23]. This cardiac role would improve the blood circulation for diverse cells, which are vital for tissue regeneration). In addition, the therapeutic practice of cardiac regeneration [2,62] could benefit from SPARC properties in cardiac regeneration [19,63] as well. Beyond the cardiac properties, SPARC has roles in the cardiovascular properties, as suggested by its production by both bone-marrow-derived cells during myocardial fibrosis (in left ventricular pressure overload) [64] and pericytes, with a possible role in postinfarct healing [65], which is supported by the possible classification of SPARC as a marker for vascular complications in pre-diabetics [66].

All these highlighted properties point to SPARC as a regeneration factor. It not only has significant roles in tissue repair or development but contributes directly and indirectly to generating a “positive” biological environment that optimizes regeneration, as summarized in the graphical abstract. Moreover, other factors that work towards reducing the regeneration ability, such as ageing [1,67] and oxidative stress [68,69], are also counteracted - at least indirectly - by SPARC effects. For instance, SPARC-induced

increased muscle functions (including via interactions with actin in skeletal muscle [24]) and metabolism would increase the antioxidant effect induced by exercise [70]. This contributes to the improvement in the regeneration environment by decreasing the oxidative stress. Furthermore, an improved muscular function (including during exercise) would lead to reducing the accumulation of the lactic acid and, therefore, better control of the pH, which both impacts muscle fatigue [71,72] and represents another important factor for different cellular functions [73], including those related to regeneration [74,75]. In addition, ageing-induced collagen loss [76] would be counteracted via the roles of SPARC in collagen properties [77–80]. Moreover, many SPARC effects counteract ageing impacts. In this context, ageing is a factor that decreases the regeneration ability [67], and with which we see an increased risk of obesity [81], sarcopenia [82,83], osteoporosis [84], etc. This points to SPARC not only as a regeneration factor that counteracts the ageing-related decrease in regeneration ability, but also as a factor with key roles against ageing-induced conditions that lead to health problems including sarcopenia, obesity (a health problem that could increase with the ongoing COVID-19 crisis [85]) and osteoporosis, through metabolic, structural and functional roles, and the impacts SPARC has on the corresponding tissues and organs (muscles, adipose tissue, bone, etc.). Therefore, SPARC remains worth exploring in the ageing process and geriatric research. These examples represent additional illustrations of SPARC's contribution to creating the optimal environment for regeneration, and further point to it as a regenerative factor.

These patterns show complimentary roles in terms of the implications of SPARC in tissue repair, and the diverse metabolic and homeostatic effects it mediates [86]. Importantly, the fact that SPARC is overexpressed during pathological situations such as obesity and cancer, as well as during physical activity (physiological adaptation), further indicates that it could represent feedback. Rather than a damaging factor, SPARC would aim to counteract/correct the negative impacts induced by the pathological situations such as inflammation and tissue damage through properties including regeneration ability, as illustrated for the skeletal muscle [87]. Indeed, conditions (pathological and physiological) that lead to impaired regeneration by creating a negative environment are the same conditions under which SPARC overexpression has been reported. Such overexpressed SPARC improves the regeneration ability and reduces the negative environment by inducing functional and metabolic enhancement at different tissues. These actions reverse, correct or reduce the impacts those initial conditions had on regeneration, which will lead to a SPARC-induced corrected regeneration ability.

This paper presents SPARC as a promising therapeutic tool in a variety of health conditions, ranging from metabolism and inflammation to obesity and sarcopenia. Importantly, SPARC could also be an option in the area of tissue engineering based on its involvement in and impacts on the regenerative processes, especially with the known implications of SPARC in the functions of stem cells [88,89], as well as other types of cells such as erythroid progenitors [90]. Thus, SPARC-related pathways also represent a

potential pharmacological target to optimize therapies in regenerative medicine as an adjuvant to optimize the regeneration environment of the targeted tissues and organs.

Author Contributions: A.G. drafted the manuscript; A.G., M.Y. and J.S.-A. critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). The graphical abstract was created using images from: http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

18.5 References

1. Yun, M.H. Changes in Regenerative Capacity through Lifespan. *Int. J. Mol. Sci.* 2015, *16*, 25392–25432.
2. Zhang, Y.; Mignone, J.; MacLellan, W.R. Cardiac Regeneration and Stem Cells. *Physiol. Rev.* 2015, *95*, 1189–1204.
3. Sarker, M.D.; Naghieh, S.; McInnes, A.D.; Schreyer, D.J.; Chen, X.B. Regeneration of peripheral nerves by nerve guidance conduits: Influence of design, biopolymers, cells, growth factors, and physical stimuli. *Prog. Neurobiol.* 2018, *171*, 125–150.
4. Easterling, M.R.; Engbrecht, K.M.; Crespi, E.J. Endocrine Regulation of Epimorphic Regeneration. *Endocrinology* 2019, *160*, 2969–2980.
5. Wojda, S.J.; Donahue, S.W. Parathyroid hormone for bone regeneration. *J. Orthop. Res.* 2018, *36*, 2586–2594.
6. Shen, Y.; Liu, W.C.; Lin, K.; Pan, H.; Darvell, B.; Peng, S.; Wen, C.; Deng, L.; Lu, W.W.; Chang, J. Interfacial pH: A Critical Factor for Osteoporotic Bone Regeneration. *Langmuir* 2011, *27*, 2701–2708.
7. Shen, Y.; Liu, W.; Wen, C.; Pan, H.; Wang, T.; Darvell, B.W.; Lu, W.W.; Huang, W. Bone regeneration: Importance of local pH—Strontium-doped borosilicate scaffold. *J. Mater. Chem.* 2012, *22*, 8662–8670.
8. Pibiri, M. Liver regeneration in aged mice: New insights. *Aging* 2018, *10*, 1801–1824.
9. Varela-Eirin, M.; Loureiro, J.; Fonseca, E.; Corrochano, S.; Caeiro, J.R.; Collado, M.; Mayan, M.D.

Cartilage regeneration and ageing: Targeting cellular plasticity in osteoarthritis. *Ageing Res. Rev.* 2018, *42*, 56–71.
10. Li, H.; Ye, Z.-H. Microenvironment of liver regeneration in liver cancer. *Chin. J. Integr. Med.* 2017, *23*, 555–560.
11. Ambrosi, T.H.; Scialdone, A.; Graja, A.; Gohlke, S.; Jank, A.M.; Bocian, C.; Woelk, L.; Fan, H.; Logan, D.W.; Schürmann, A.; et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. *Cell. Stem. Cell.* 2017, *20*, 771–784.e6.
12. Cooke, J.P. Inflammation and Its Role in Regeneration and Repair. *Circ. Res.* 2019, *124*, 1166–1168.
13. Korolev, Y.N.; Geniatulina, M.S.; Mikhailik, L.V.; Nikulina, L.A. Intracellular regeneration of adrenocorticocytes in response to the prophylactic application of low-intensity electromagnetic radiation under the conditions of radiation (an experimental study). *Vopr. Kurortol. Fizioter. Lech. Fiz. Kul'tury* 2019, *96*, 43–49.
14. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. *Matrix Biol.* 2016, *54*, 78–87.
15. Maillard, C.; Malaval, L.; Delmas, P. Immunological screening of SPARC/Osteonectin in nonmineralized tissues. *Bone* 1992, *13*, 257–264.
16. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
17. Motamed, K. SPARC (osteonectin/BM-40). *Int. J. Biochem. Cell Biol.* 1999, *31*, 1363–1366.
18. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
19. Bradshaw, A.D. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? *J. Mol. Cell. Cardiol.* 2016, *93*, 156–161.
20. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). *J. Mol. Cell. Cardiol.* 2010, *48*, 544–549.
21. Yan, Q.; Sage, E.H. SPARC, a Matricellular Glycoprotein with Important Biological Functions. *J. Histochem. Cytochem.* 1999, *47*, 1495–1505.
22. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J. Clin. Investig.* 2001, *107*, 1049–1054.
23. Deckx, S.; Johnson, D.M.; Rienks, M.; Carai, P.; Van Deel, E.; Van der Velden, J.; Sipido, K.R.; Heymans, S.; Papageorgiou, A.P. Extracellular SPARC increases cardiomyocyte contraction during health and disease. *PLoS ONE* 2019, *14*, e0209534.
24. Jørgensen, L.H.; Jepsen, P.L.; Boysen, A.; Dalgaard, L.B.; Hvid, L.G.; Ørtenblad, N.; Ravn, D.; Sellathurai, J.; Møller-Jensen, J.; Lochmuller, H.; et al. SPARC Interacts with Actin in Skeletal Muscle In Vitro and In Vivo. *Am. J. Pathol.* 2017, *187*, 457–474.
25. Shen, Y.; Zhao, Y.; Yuan, L.; Yi, W.; Zhao, R.; Yi, Q.; Yong, T. SPARC is over-expressed in adipose tissues of diet-induced obese rats and causes insulin resistance in 3T3-L1 adipocytes. *Acta Histochem.* 2014, *116*, 158–166.
26. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schroeder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. *Histol. Histopathol.* 2013, *28*, 1451–1460.
27. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
28. Scavelli, K.; Chatterjee, A.; Rhee, U.J. Secreted Protein Acidic and Rich in Cysteine in Ocular Tissue. *J. Ocul. Pharmacol. Ther.* 2015, *31*, 396–405.
29. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.

30. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.
31. Niklason, L. Understanding the Extracellular Matrix to Enhance Stem Cell-Based Tissue Regeneration. *Cell. Stem. Cell.* 2018, *22*, 302–305.
32. Delany, A.M.; Hankenson, K. Thrombospondin-2 and SPARC/osteonectin are critical regulators of bone remodeling. *J. Cell Commun. Signal.* 2009, *3*, 227–238.
33. Caldez, M.J.; Van Hul, N.; Koh, H.W.; Teo, X.Q.; Fan, J.J.; Tan, P.Y.; Dewhurst, M.R.; Too, P.G.; Talib, S.Z.A.; Chiang, B.E.; et al. Metabolic Remodeling during Liver Regeneration. *Dev. Cell* 2018, *47*, 425–438.e5.
34. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. *FASEB J.* 2019, *33*, 10551–10562.
35. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. *Clin. Sci.* 2019, *133*, 351–365.
36. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
37. Melouane, A.; Yoshioka, M.; St-Amand, J. Extracellular matrix/mitochondria pathway: A novel potential target for sarcopenia. *Mitochondrion* 2020, *50*, 63–70.
38. Smith, G.M.; Gallo, G. The role of mitochondria in axon development and regeneration. *Dev. Neurobiol.* 2018, *78*, 221–237.
39. Han, S.M.; Baig, H.S.; Hammarlund, M. Mitochondria Localize to Injured Axons to Support Regeneration. *Neuron* 2016, *92*, 1308–1323.
40. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine *(Sparc)* KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
41. Nie, J.; Sage, E.H. SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling. *J. Biol. Chem.* 2009, *284*, 1279–1290.
42. Nie, J.; Bradshaw, A.D.; Delany, A.M.; Sage, E.H. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. *Connect. Tissue Res.* 2011, *52*, 99–108.
43. Mukherjee, S.; Choi, M.J.; Kim, S.W.; Yun, J.-W. Secreted protein acidic and rich in cysteine (SPARC) regulates thermogenesis in white and brown adipocytes. *Mol. Cell. Endocrinol.* 2020, *506*, 110757.
44. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
45. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
46. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
47. Hermetet, F.; Buffière, A.; Aznague, A.; De Barros, J.-P.P.; Bastie, J.-N.; Delva, L.; Quéré, R. High-fat diet disturbs lipid raft/TGF-β signaling-mediated maintenance of hematopoietic stem cells in mouse bone marrow. *Nat. Commun.* 2019, *10*, 1–11.
48. Wilkinson, A.C.; Yamazaki, S. The hematopoietic stem cell diet. *Int. J. Hematol.* 2018, *107*, 634–641.
49. Ribeiro, N.; Sousa, S.R.; Brekken, R.A.; Monteiro, F.J. Role of SPARC in bone remodeling and cancer-related bone metastasis. *J. Cell Biochem.* 2014, *115*, 17–26.
50. Baloˇglu, M.; Özkorkmaz, E.G. Biochemical and immunohistochemical investigations on bone formation and remodelling in ovariectomised rats with tamoxifen citrate administration. *Folia Morphol.* 2019, *78*, 789–797.

51. Delany, A.M.; McMahon, D.J.; Powell, J.S.; Greenberg, D.A.; Kurland, E.S. Osteonectin/SPARC polymorphisms in Caucasian men with idiopathic osteoporosis. *Osteoporos. Int.* 2007, *19*, 969–978.
52. Zurlo, F.; Larson, K.; Bogardus, C.; Ravussin, E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J. Clin. Investig.* 1990, *86*, 1423–1427.
53. Di Girolamo, D.J.; Kiel, D.P.; Esser, K.A. Bone and Skeletal Muscle: Neighbors with Close Ties. *J. Bone Miner. Res.* 2013, *28*, 1509–1518.
54. Nagaraju, G.P.; Sharma, D. Anti-cancer role of SPARC, an inhibitor of adipogenesis. *Cancer Treat. Rev.* 2011, *37*, 559–566.
55. Chlenski, A.; Guerrero, L.J.; Peddinti, R.; Spitz, J.A.; Leonhardt, P.T.; Yang, Q.; Tian, Y.; Salwen, H.R.; Cohn, S.L.; Yang, Q. Anti-angiogenic SPARC peptides inhibit progression of neuroblastoma tumors. *Mol. Cancer* 2010, *9*, 138.
56. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
57. Sangaletti, S.; Tripodo, C.; Cappetti, B.; Casalini, P.; Chiodoni, C.; Piconese, S.; Santangelo, A.; Parenza, M.; Arioli, I.; Miotti, S.; et al. SPARC Oppositely Regulates Inflammation and Fibrosis in Bleomycin-Induced Lung Damage. *Am. J. Pathol.* 2011, *179*, 3000–3010.
58. Rienks, M.; Carai, P.; van Teeffelen, J.; Eskens, B.; Verhesen, W.; Hemmeryckx, B.; Johnson, D.M.; van Leeuwen, R.; Jones, E.A.; Heymans, S.; et al. SPARC preserves endothelial glycocalyx integrity, and protects against adverse cardiac inflammation and injury during viral myocarditis. *Matrix Biol.* 2018, *74*, 21–34.
59. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. *Genes Immun.* 2007, *8*, 262–274.
60. Sass, F.A.; Fuchs, M.; Pumberger, M.; Geissler, S.; Duda, G.N.; Perka, C.; Schmidt-Bleek, K. Immunology Guides Skeletal Muscle Regeneration. *Int. J. Mol. Sci.* 2018, *19*, 835.
61. Muire, P.J.; Mangum, L.H.; Wenke, J.C. Time Course of Immune Response and Immunomodulation During Normal and Delayed Healing of Musculoskeletal Wounds. *Front. Immunol.* 2020, *11*.
62. Ahuja, P.; Sdek, P.; MacLellan, W.R. Cardiac Myocyte Cell Cycle Control in Development, Disease, and Regeneration. *Physiol. Rev.* 2007, *87*, 521–544.
63. Stary, M.; Pasteiner, W.; Summer, A.; Hrdina, A.; Eger, A.; Weitzer, G. Parietal endoderm secreted SPARC promotes early cardiomyogenesis in vitro. *Exp. Cell Res.* 2005, *310*, 331–343.
64. Riley, H.J.; Kelly, R.R.; Van Laer, A.O.; Neff, L.S.; Dasgupta, S.; Baicu, C.F.; McDonald, L.T.; LaRue, A.C.; Zile, M.R.; Bradshaw, A.D. SPARC Production by Bone Marrow-Derived Cells Contributes to Myocardial Fibrosis in Pressure-Overload. *Am. J. Physiol. Heart Circ. Physiol.* 2020.
65. Avolio, E.; Mangialardi, G.; Slater, S.C.; Alvino, V.V.; Gu, Y.; Cathery, W.; Beltrami, A.P.; Katare, R.; Heesom, K.; Caputo, M.; et al. Secreted Protein Acidic and Cysteine Rich Matricellular Protein Is Enriched in the Bioactive Fraction of the Human Vascular Pericyte Secretome. *Antioxid. Redox Signal.* 2020.
66. Benincasa, G.; Franzese, M.; Schiano, C.; Marfella, R.; Miceli, M.; Infante, T.; Sardu, C.; Zanfardino, M.; Affinito, O.; Mansueto, G.; et al. DNA methylation profiling of CD04(+)/CD08(+) T cells reveals pathogenic mechanisms in increasing hyperglycemia: PIRAMIDE pilot study. *Annu. Med. Surg.* 2020, *60*, 218–226.
67. West, M.D.; Sternberg, H.; Labat, I.; Janus, J.; Chapman, K.B.; Malik, N.N.; De Grey, A.D.; LaRocca, D. Toward a unified theory of aging and regeneration. *Regen. Med.* 2019, *14*, 867–886.
68. Chen, F.; Liu, Y.; Wong, N.-K.; Xiao, J.; So, K.-F. Oxidative Stress in Stem Cell Aging. *Cell Transplant.* 2017, *26*, 1483–1495.
69. Wang, J.; Wang, H. Oxidative Stress in Pancreatic Beta Cell Regeneration. *Oxidative Med. Cell. Longev.* 2017, *2017*, 1930261.
70. Brioche, T.; Lemoine-Morel, S. Oxidative Stress, Sarcopenia, Antioxidant Strategies and Exercise: Molecular Aspects. *Curr. Pharm. Des.* 2016, *22*, 2664–2678.
71. Hultman, E.; Spriet, L.L.; Söderlund, K. Biochemistry of muscle fatigue. *Biomed. Biochim. Acta* 1986, *45*, S97–S106.

72. Sahlin, K. Muscle fatigue and lactic acid accumulation. *Acta Physiol. Scand. Suppl.* 1986, *556*, 83–91.
73. Capuano, P.; Capasso, G. The importance of intracellular pH in the regulation of cell function. *G Ital. Nfrol.* 2003, *20*, 139–150.
74. Lönnqvist, S.; Emanuelsson, P.; Kratz, G. Influence of acidic pH on keratinocyte function and re-epithelialisation of human in vitro wounds. *J. Plast. Surg. Hand Surg.* 2015, *49*, 346–352.
75. Kruse, C.R.; Singh, M.; Targosinski, S.; Sinha, I.; Sørensen, J.A.; Eriksson, E.; Nuutila, K. The effect of pH on cell viability, cell migration, cell proliferation, wound closure, and wound reepithelialization: In vitro and in vivo study. *Wound Repair Regen.* 2017, *25*, 260–269.
76. Shuster, S. Osteoporosis, like skin ageing, is caused by collagen loss which is reversible. *J. R. Soc. Med.* 2020, *113*, 158–160.
77. Ramu, I.; Buchholz, S.M.; Patzak, M.S.; Goetze, R.G.; Singh, S.K.; Richards, F.M.; Jodrell, D.I.; Sipos, B.; Ströbel, P.; Ellenrieder, V.; et al. SPARC dependent collagen deposition and gemcitabine delivery in a genetically engineered mouse model of pancreas cancer. *EBioMedicine* 2019, *48*, 161–168.
78. Bradshaw, A.D.; Puolakkainen, P.; Wight, T.N.; Helene, S.E.; Dasgupta, J.; Davidson, J.M. SPARC-Null Mice Display Abnormalities in the Dermis Characterized by Decreased Collagen Fibril Diameter and Reduced Tensile Strength. *J. Investig. Dermatol.* 2003, *120*, 949–955.
79. Francki, A.; Bradshaw, A.D.; Bassuk, J.A.; Howe, C.C.; Couser, W.G.; Sage, E.H. SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. *J. Biol. Chem.* 1999, *274*, 32145–32152.
80. Rentz, T.J.; Poobalarahi, F.; Bornstein, P.; Sage, E.H.; Bradshaw, A.D. SPARC regulates processing of procollagen I and collagen fibrillogenesis in dermal fibroblasts. *J. Biol. Chem.* 2007, *282*, 22062–22071.
81. Jura, M.; Kozak, L.P. Obesity and related consequences to ageing. *Age (Dordrecht)* 2016, *38*, 1–18.
82. Marzetti, E.; Calvani, R.; Tosato, M.; Cesari, M.; Di Bari, M.; Cherubini, A.; Collamati, A.; D'Angelo, E.; Pahor, M.; Bernabei, R.; et al. Sarcopenia: An overview. *Aging Clin. Exp. Res.* 2017, *29*, 11–17.
83. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res.* 2019, *781*, 175–185.
84. Aspray, T.J.; Hill, T.R. Osteoporosis and the Ageing Skeleton. *Subcell. Biochem.* 2019, *91*, 453–476.
85. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
86. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
87. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schroeder, H.D. Secreted Protein Acidic and Rich in Cysteine (SPARC) in Human Skeletal Muscle. *J. Histochem. Cytochem.* 2008, *57*, 29–39.
88. Cheng, L.; Sun, X.; Guo, J.; Lu, G. SPARC support the expansion of cord blood stem cells in vitro. *Cell Res.* 2008, *18*, S49.
89. Zhu, J.; Wang, L.-Y.; Li, C.-Y.; Wu, J.-Y.; Zhang, Y.-T.; Pang, K.-P.; Wei, Y.; Du, L.-Q.; Liu, M.; Wu, X.-Y. SPARC promotes self-renewal of limbal epithelial stem cells and ocular surface restoration through JNK and p38-MAPK signaling pathways. *Stem Cells* 2019, *38*, 134–145.
90. Luo, Z.; Luo, P.; Yu, Y.; Zhao, Q.; Zhao, X.; Cheng, L. SPARC promotes the development of erythroid progenitors. *Exp. Hematol.* 2012, *40*, 828–836.

Chapter 19. Opinion - Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

Received: 1 November 2021; Accepted: 12 November 2021; Published: 13 November 2021

19.1 Résumé (French abstract)

La secreted protein acidic and rich in cysteine (SPARC) est exprimée dans divers tissus et joue un rôle dans diverses fonctions et processus biologiques. Une augmentation des taux sériques de SPARC ou de sa surexpression génique a été rapportée à la suite de nombreux changements physiologiques et pathologiques, notamment de blessures, de l'exercice, de la régénération, de l'obésité, du cancer et de l'inflammation. Une telle corrélation entre ces changements biologiques et l'expression/sécrétion de SPARC la désigne comme un biomarqueur. Ces propriétés pourraient conduire à une variété d'applications allant des études mécanistiques et la validation de modèles d'animaux à l'évaluation clinique et thérapeutique du pronostic des maladies ainsi que des agents pharmacologiques.

19.2 Abstract

Secreted protein acidic and rich in cysteine (SPARC) is expressed in diverse tissues and plays roles in various biological functions and processes. Increased serum levels of SPARC or its gene overexpression have been reported following numerous physiological and pathological changes including injuries, exercise, regeneration, obesity, cancer, and inflammation. Such expression pattern interrelation between these biological changes and the SPARC expression/secretion points to it as a biomarker. This property could lead to a variety of potential applications ranging from mechanistic studies and animal model validation to the clinical and therapeutic evaluation of both disease prognosis and pharmacological agents.

Keywords: secreted protein acidic and rich in cysteine; expression; physiology; pathology; biomarker

19.3 Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker

Secreted protein acidic and rich in cysteine (SPARC), also called BM-40 and osteonectin, is a non-collagenous [1] and collagen-binding [2], plays a non-structural role in ECM/bone [3], and has three structural domains with active glycoproteins [4] that was initially reported in bones under another name, osteonectin [1]. Additionally, studies have highlighted its implications in numerous physiological and pathological contexts at different biological levels, including in injuries and wound healing [5–8], exercise and exercise induced muscle changes [9–11], glucose homeostasis and insulin secretion [12,13], metabolism and energy balance [14,15], regeneration [16], inflammation [17–19], cancer [20–25], obesity and diabetes [26], fibrillar collagen assembly and extracellular matrix maintenance and remodelling [17,27,28], lipid metabolism [29], immunity [30], myocardial repair and fibrosis [2], and vascular biology [31].

Importantly, SPARC protein and gene expression or its serum level changes are involved in an increase during a variety of situations. For injuries in the adult rat cerebral cortex, cortical brain injury leads to an increased expression of *Sparc* mRNA in the blood vessels [32]. While serum SPARC increases with obesity [33], its levels are reduced following bariatric surgery for weight loss [34]. In addition, the fat mass also correlates with the human adipose tissue SPARC expression [35], and *SPARC* mRNA expression in the adipose tissue is correlated to body mass index [33], which points to SPARC as a molecular indicator of the adiposity percentage. In oncology, many studies have shown that SPARC is overexpressed in different forms of cancer, including cervical carcinoma [36], colon cancer [37], and hepatocellular carcinoma [38]. Moreover, in patients with cervical carcinoma [36] or ampullary cancer [39], such overexpression is associated with a poor prognosis, and increased serum SPARC levels have been reported in melanoma patients as well [40]. Interestingly, the serum SPARC level has been proposed as a pancreatic cancer marker, as it has also been correlated to tumour size [41]. Serum SPARC levels also correlate with coronary artery lesion severity in type 2 diabetic patients with coronary heart disease [42], and newly diagnosed type 2 diabetes mellitus patients also have high plasma SPARC levels [43]. In addition to these illustrative examples, SPARC/*Sparc* overexpression has also been reported in inflammation [44], following exercise [45], and during skeletal muscle regeneration [46]. All of these elements highlight the molecular importance that SPARC has biologically. Regarding the expression pattern and how SPARC is circulated, we have hypothesized that its secretion would be involved in controlling or reducing the biological damage that is associated with the processes that initially lead to its increase (feedback-like mechanism). This is, for instance, illustrated by the increase of SPARC during both obesity and cancer as well as the SPARC properties to inhibit both adipogenesis and tumor development [14,21]. Furthermore, SPARC has been found both extracellularly and intracellularly [47] in addition to its presence in the blood, a distribution and secretion pattern that support classifying it as a biomarker.

The objective of this piece of writing is to introduce the concept of measuring SPARC protein or gene expression/level in selected biological samples as a biomarker that has potential applications in the diverse fields of biomedical research as well as in clinical practice. Indeed, since SPARC/*Sparc* expression/secretion changes with various diseases and physiological status, measuring the expression levels of SPARC (or its genes, *SPARC*/*Sparc*) or SPARC serum levels could allow the determination of how severely the disease has advanced, how efficient the treatment is, or how the pathogenesis evolves. The biological significance of such status-dependent expression patterns would lead to numerous potential biomedical and clinical applications (Figure 19.1). For instance, whereas high SPARC levels would reflect a disease evolution or a poor prognosis, decreased SPARC levels would be considered as an indicator of positive disease evolution or a reduced severity. The same logic applies to therapeutic evaluation in which reduced SPARC expression/level could indicate treatment efficacy. It seems acceptable to assume that the precision of such an evaluation would be higher in the tissues that express the most SPARC compared to those that express it less. Furthermore, changes during pathological phases could allow SPARC expression to be followed throughout different disease stages as a marker and thus to build a reference library for diagnosis and prognosis evaluation based on SPARC levels. Similarly, it can also be used to evaluate disease treatments since it may change with diseases improvement. Therefore, we suggest the use of SPARC as a biomolecular evaluation tool either during disease progress, treatment, or during studies aiming to evaluate disease pathogenesis. Pathogenesis exploration, mechanism studies, and animal model validations represent other applications that can be achieved through the expression of SPARC within pathway models and as a validation criterion for animal model building.

Importantly, with this expression specificity of SPARC within different pathological contexts, the potential implications of SPARC in pathogenesis are worthy of further exploration in order to identify new therapeutic targets, drugs, or adjuvants for metabolic disorders, inflammation, or cancer, especially because SPARC has been shown to play roles related to the cytotoxic effect of sorafenib against hepatocellular carcinoma cells [48]. To conclude, the interindividual differences in terms of SPARC expression in pathophysiological and therapeutic contexts can contribute to the optimization of a precision medicine supported by advanced methods in screening and sequencing. These perspectives are relevant to various applications ranging from biomolecular medical research to clinical applications. There are some challenges that have yet to be overcome. The first challenge would be the detection method and whether to use the protein level or the mRNA level as a marker. This would mainly depend on the available biological samples (sampling would depend on the patient's physiopathological status) as well as the laboratory equipment/budget. If more than one type of sample is available, then the choice requires further studies in order to first evaluate whether the protein level and mRNA level are equivalently accurate to build standard measurement methods. Overall, we still need more in-depth studies and comparative measures to determine SPARC/*Sparc* measuring

protocols for each physiological or pathological condition in order to determine the more convenient methods for use in a hospital laboratory.

Herein, SPARC/*Sparc* illustrates how identifying biomolecules and elucidating their related expression patterns based on pathophysiological variables could lead to the identification novel, yet specific, biomarkers that could be used as parameters for diagnosis, prognosis, therapeutic tests, and clinical evaluation.

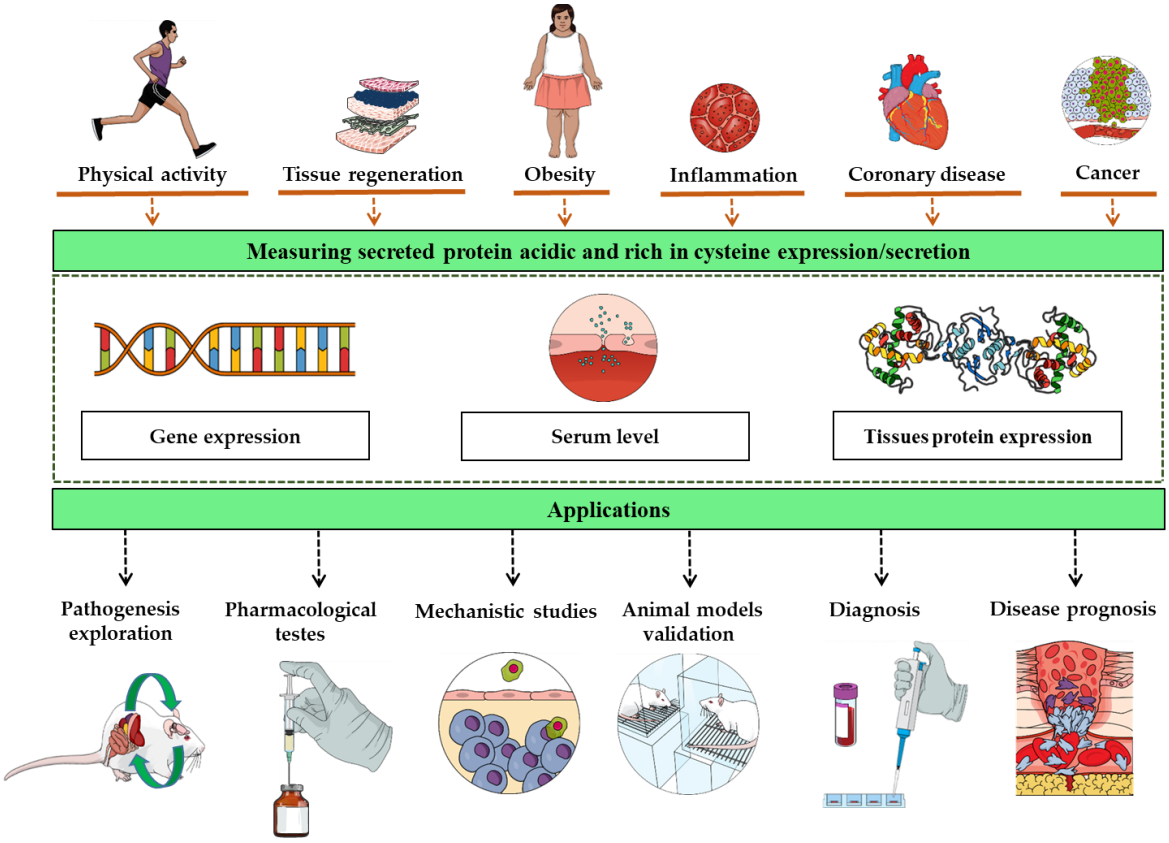


Figure 19.1. The overexpression of the secreted protein acidic and rich in cysteine gene, protein, or its increased blood concentration follow numerous physiological and pathological changes including exercise, obesity, cancer, injuries, and inflammation. Such interrelation between these biological changes and the secreted protein acidic and rich in cysteine expression/secretion points to it as a biomarker with a variety of potential applications, ranging from mechanistic studies to the clinical and therapeutic evaluation of both disease prognosis and pharmacological agents.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y., and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, Figure, references selection, etc.) throughout the writing process, edited, and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship « Bourse Tremplin-Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec–Sante (FRQS), Quebec, Canada. Figure 19.1 was created using images from https:// mindthegraph.com/ (Accessed on 12 September 2021).

Conflicts of Interest: The authors declare that there is no conflict of interest.

19.4 References

1. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. *Matrix Biol.* 2016, *52–54*, 78–87.
2. Bradshaw, A.D. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? *J. Mol. Cell. Cardiol.* 2016, *93*, 156–161.
3. Chlenski, A.; Guerrero, L.J.; Salwen, H.R.; Yang, Q.; Tian, Y.; Morales La Madrid, A.; Mirzoeva, S.; Bouyer, P.G.; Xu, D.; Walker, M.; et al. Secreted protein acidic and rich in cysteine is a matrix scavenger chaperone. *PLoS ONE* 2011, *6*, e23880.
4. Motamed, K. SPARC (osteonectin/BM-40). *Int. J. Biochem. Cell. Biol.* 1999, *31*, 1363–1366.
5. Wang, L.Y.; Zhang, Y.T.; Du, L.Q.; Wu, X.Y.; Zhu, J. The Effect of SPARC on the Proliferation and Migration of Limbal Epithelial
Stem Cells During the Corneal Epithelial Wound Healing. *Stem Cells Dev.* 2021, *30*, 301–308.
6. Basu, A.; Kligman, L.H.; Samulewicz, S.J.; Howe, C.C. Impaired wound healing in mice deficient in a matricellular protein SPARC
(osteonectin, BM-40). *BMC Cell Biol.* 2001, *2*, 15.
7. Mazzolini, G.; Atorrasagasti, C.; Onorato, A.; Peixoto, E.; Schlattjan, M.; Sowa, J.P.; Sydor, S.; Gerken, G.; Canbay, A. SPARC expression is associated with hepatic injury in rodents and humans with non-alcoholic fatty liver disease. *Sci. Rep.* 2018, *8*, 725.
8. Chen, S.; Zou, Q.; Chen, Y.; Kuang, X.; Wu, W.; Guo, M.; Cai, Y.; Li, Q. Regulation of SPARC family proteins in disorders of the central nervous system. *Brain Res. Bull.* 2020, *163*, 178–189.
9. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
10. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
11. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are

SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
12. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G.D. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. *Clin. Sci. (Lond.)* 2019, *133*, 351–365.
13. Hu, L.; He, F.; Huang, M.; Zhao, Q.; Cheng, L.; Said, N.; Zhou, Z.; Liu, F.; Dai, Y.S. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. *Sci. Rep.* 2020, *10*, 17581.
14. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
15. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
16. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
17. Riley, H.J.; Bradshaw, A.D. The Influence of the Extracellular Matrix in Inflammation: Findings from the SPARC-Null Mouse. *Anat. Rec. (Hoboken)* 2020, *303*, 1624–1629.
18. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
19. Tanaka, M.; Takagi, T.; Naito, Y.; Uchiyama, K.; Hotta, Y.; Toyokawa, Y.; Ushiroda, C.; Hirai, Y.; Aoi, W.; Higashimura, Y.; et al. Secreted protein acidic and rich in cysteine functions in colitis via IL17A regulation in mucosal CD4(+) T cells. *J. Gastroenterol. Hepatol.* 2018, *33*, 671–680.
20. Framson, P.E.; Sage, E.H. SPARC and tumor growth: Where the seed meets the soil? *J. Cell Biochem.* 2004, *92*, 679–690.
21. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
22. Wong, S.L.; Sukkar, M.B. The SPARC protein: An overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease. *Br. J. Pharmacol.* 2017, *174*, 3–14.
23. Bao, J.M.; Dang, Q.; Lin, C.J.; Lo, U.G.; Feldkoren, B.; Dang, A.; Hernandez, E.; Li, F.; Panwar, V.; Lee, C.F.; et al. SPARC is a key mediator of TGF-β-induced renal cancer metastasis. *J. Cell. Physiol.* 2021, *236*, 1926–1938.
24. Camacho, D.; Jesus, J.P.; Palma, A.M.; Martins, S.A.; Afonso, A.; Peixoto, M.L.; Pelham, C.J.; Moreno, E.; Gogna, R. SPARC-p53: The double agents of cancer. *Adv. Cancer Res.* 2020, *148*, 171–199.
25. Feng, J.; Tang, L. SPARC in Tumor Pathophysiology and as a Potential Therapeutic Target. *Curr. Pharm. Des.* 2014, *20*, 6182–6190.
26. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. *Nat. Rev. Endocrinol.* 2010, *6*, 225–235.
27. Whittal, M.C.; Molladavoodi, S.; Zwambag, D.P.; Millecamps, M.; Stone, L.S.; Gregory, D.E. Mechanical Consequence of Induced Intervertebral Disc Degeneration in the SPARC-Null Mouse. *J. Biomech. Eng.* 2021, *143*, 024501.
28. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). *J. Mol. Cell. Cardiol.* 2010, *48*, 544–549.
29. Onorato, M.A.; Fiore, E.; Bayo, J.; Casali, C.; Fernandez-Tomé, M.; Rodríguez, M.; Domínguez, L.; Argemi, J.; Hidalgo, F.; Favre, C.; et al. SPARC inhibition accelerates NAFLD-associated hepatocellular carcinoma development by dysregulating hepatic lipid metabolism. *Liver Int.* 2021, *41*, 1677–1693.
30. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. *Genes Immun.*2007, *8*, 262–274.
31. Rivera, L.B.; Bradshaw, A.D.; Brekken, R.A. The regulatory function of SPARC in vascular biology. *Cell Mol. Life Sci.* 2011, *68*, 3165–3173.

32. Mendis, D.B.; Ivy, G.O.; Brown, I.R. SPARC/osteonectin mRNA is induced in blood vessels following injury to the adult rat cerebral cortex. *Neurochem. Res.* 1998, *23*, 1117–1123.
33. Lee, S.H.; Lee, J.A.; Park, H.S.; Song, Y.S.; Jang, Y.J.; Kim, J.H.; Lee, Y.J.; Heo, Y. Associations among SPARC mRNA expression in adipose tissue, serum SPARC concentration and metabolic parameters in Korean women. *Obesity (Silver Spring)* 2013, *21*, 2296–2302.
34. Lee, Y.J.; Heo, Y.S.; Park, H.S.; Lee, S.H.; Lee, S.K.; Jang, Y.J. Serum SPARC and matrix metalloproteinase-2 and metalloproteinase-9 concentrations after bariatric surgery in obese adults. *Obes. Surg.* 2014, *24*, 604–610.
35. Kos, K.; Wong, S.; Tan, B.; Gummesson, A.; Jernas, M.; Franck, N.; Kerrigan, D.; Nystrom, F.H.; Carlsson, L.M.; Randeva, H.S.; et al. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. *Diabetes* 2009, *58*, 1780–1788.
36. Shi, D.; Jiang, K.; Fu, Y.; Fang, R.; Liu, X.I.; Chen, J. Overexpression of SPARC correlates with poor prognosis in patients with cervical carcinoma and regulates cancer cell epithelial-mesenchymal transition. *Oncol. Lett.* 2016, *11*, 3251–3258.
37. Zhong, M.E.; Chen, Y.; Xiao, Y.; Xu, L.; Zhang, G.; Lu, J.; Qiu, H.; Ge, W.; Wu, B. Serum extracellular vesicles contain SPARC and LRG1 as biomarkers of colon cancer and differ by tumour primary location. *EBioMedicine* 2019, *50*, 211–223.
38. Liu, Y.; Feng, Y.; Wang, X.; Yang, X.; Hu, Y.; Li, Y.; Zhang, Q.; Huang, Y.; Shi, K.; Ran, C.; et al. SPARC Negatively Correlates With Prognosis After Transarterial Chemoembolization and Facilitates Proliferation and Metastasis of Hepatocellular Carcinoma via ERK/MMP Signaling Pathways. *Front. Oncol.* 2020, *10*, 813.
39. Bloomston, M.; Ellison, E.C.; Muscarella, P.; Al-Saif, O.; Martin, E.W.; Melvin, W.S.; Frankel, W.L. Stromal osteonectin overexpression is associated with poor outcome in patients with ampullary cancer. *Ann. Surg. Oncol.* 2007, *14*, 211–217.
40. Ikuta, Y.; Nakatsura, T.; Kageshita, T.; Fukushima, S.; Ito, S.; Wakamatsu, K.; Baba, H.; Nishimura, Y. Highly sensitive detection of melanoma at an early stage based on the increased serum secreted protein acidic and rich in cysteine and glypican-3 levels. *Clin. Cancer Res.* 2005, *11*, 8079–8088.
41. Papapanagiotou, A.; Sgourakis, G.; Karkoulias, K.; Raptis, D.; Parkin, E.; Brotzakis, P.; Panchal, S.; Papavassiliou, A.G. Osteonectin as a screening marker for pancreatic cancer: A prospective study. *J. Int. Med. Res.* 2018, *46*, 2769–2779.
42. Wang, Z.; Song, H.Y.; An, M.M.; Zhu, L.L. Association of serum SPARC level with severity of coronary artery lesion in type 2 diabetic patients with coronary heart disease. *Int. J. Clin. Exp. Med.* 2015, *8*, 19290–19296.
43. Wu, D.; Li, L.; Yang, M.; Liu, H.; Yang, G. Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus. *Eur. J. Endocrinol.* 2011, *165*, 597–601.
44. Pichler, R.H.; Bassuk, J.A.; Hugo, C.; Reed, M.J.; Eng, E.; Gordon, K.L.; Pippin, J.; Alpers, C.E.; Couser, W.G.; Sage, E.H.; et al. SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growthfactor-medicated mesangial cell proliferation in vitro. *Am. J. Pathol.* 1996, *148*, 1153–1167.
45. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
46. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. *Histol. Histopathol.* 2013, *28*, 1451–1460.
47. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix communication. *Matrix Biol.* 2001, *19*, 816–827.
48. Hua, H.W.; Jiang, H.S.; Jia, L.; Jia, Y.P.; Yao, Y.L.; Chen, Y.W.; Jiang, F.; Lu, D.Q.; Zhou, Q.; Jiang,

M.W.; et al. SPARC regulates ferroptosis induced by sorafenib in human hepatocellular carcinoma. *Cancer Biomark.* 2021, 1–9.

Chapter 20. Research Article - Exercise Training of Secreted Protein Acidic and Rich in Cysteine *(Sparc)* KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent

Abdelaziz Ghanemi [1,2], Aicha Melouane [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 1 December 2020; Accepted: 15 December 2020; Published: 20 December 2020

20.1 Résumé (French abstract)

Le gène secreted protein acidic and rich in cysteine (*SPARC*) est induit par l'exercice. Pour identifier certaines implications de SPARC, des souris ont été divisées en huit groupes en fonction de trois variables (âge, génotype et exercice): Âgées ou jeunes× *Sparc* knock-out ou sauvages × sédentaires ou exercice et des paramètres métabolique et fonctionnelles ont été mesurés. Les effets du *Sparc* KO sur la composition corporelle, l'adiposité et les paramètres métaboliques vont dans le sens d'une réduction du tissue adipeux et du poids corporel, mais avec un phénotype métabolique et fonctionnel négatif du muscle. Alors que ces effets négatifs sur le muscle sont aggravés avec le vieillissement, ils sont relativement améliorés par l'exercice. Nos données suggèrent particulièrement que les changements induits par l'exercice en termes d'augmentation des performances musculaires (métabolisme, force et développement), y compris les changements induits par le lactate, dépendent de SPARC.

20.2 Abstract

We previously identified secreted protein acidic and rich in cysteine (*SPARC)* as an exercise-induced gene in young and elderly individuals. Via this animal experiment, we aim to identify selected implications of SPARC mainly within the muscle in the contexts of exercise. Mice were divided into eight groups based on three variables (age, genotype and exercise): Old (O) or young (Y) × *Sparc* knock-out (KO) or wild-type (WT) × sedentary (Sed) or exercise (Ex). The exercised groups were trained for 12 weeks at the lactate threshold (LT) speed (including 4 weeks of adaptation period) and all mice were sacrificed

afterwards. Body and selected tissues were weighed, and lactate levels in different conditions measured. Expression of skeletal muscle (SM) collagen type I alpha 1 chain (COL1A1) and mitochondrially encoded cytochrome c oxidase I (MT-CO1) in addition to SM strength (grip power) were also measured. Ageing increased the body and white adipose tissue (WAT) weights but decreased SM weight percentage (to body weight) and MT-CO1 expression (in WT). Exercise increased SM COL1A1 in WT mice and MT-CO1 expression, as well as weight percentage of the tibialis anterior muscle, and decreased WAT weight (trend). Compared to WT mice, *Sparc* KO mice had lower body, muscle and WAT weights, with a decrease in SM MT-CO1 and COL1A1 expression with no genotype effect on lactate levels in all our blood lactate measures. *Sparc* KO effects on body composition, adiposity and metabolic patterns are toward a reduced WAT and body weight, but with a negative metabolic and functional phenotype of SM. Whereas such negative effects on SM are worsened with ageing, they are relatively improved by exercise. Importantly, our data suggest that the exercise-induced changes in the SM phenotype, in terms of increased performance (metabolic, strength and development), including lactate-induced changes, are SPARC-dependent.

Featured Application: This work highlights secreted protein acidic and rich in cysteine (SPARC) and its pathways as pharmacological targets/tools for conditions and diseases in which muscle properties enhancement would provide therapeutic benefits.

Keywords: secreted protein acidic and rich in cysteine (*Sparc*); exercise; muscle performance; metabolic phenotype; lactate; ageing

20.3 Secreted Protein Acidic and Rich in Cysteine as an Exercise-Induced Gene

The modern lifestyle, characterized by the lack of physical activity combined with an unhealthy diet, leads to an increase in health problems typical of our era such as obesity and diabetes. In addition, the improvement of health care systems increased the life expectancy and, therefore, geriatric health problems, such as sarcopenia, are also increasing. Interestingly, exercise is considered as a “panacea” for many of these problems [1]. Whereas exercise benefits have been widely documented, many exercise-related molecular mechanisms are yet to be fully elucidated. In addition to its direct metabolic implications, skeletal muscles (SM) represent secretary organs producing myokine, such as secreted protein acidic and rich in cysteine (SPARC) [2]. Within the exercise context, functional genomics studies (mainly but not only in the energy metabolism context [3]) have identified genes related to physical activity among which we have *SPARC*/*Sparc*. Indeed, beyond the known implications of SPARC in wound healing and tissue repair [4,5], this gene was characterized (for the first time) as an exercise-induced gene [6] and also as an electrical pulse stimulation (considered as an in vitro model of exercise)-induced gene in

muscular cells [7]. Moreover, SPARC secretion is induced by exercise [8–10] after which the concentrations of myokines (including SPARC, interleukin 6 and fibroblast growth factor) increase in the circulation [11]. Therefore, we hypothesize that at least some of the exercise benefits and biological consequences, mainly the muscular phenotype adaptation to exercise, would be mediated by SPARC or the pathways it controls. Therefore, in this study we aim to explore the implication of *Sparc* (via it knock-out (KO)) in mice with a focus on exercise effects on muscles. In addition, age was also introduced as a variable in this study. Therefore, we would find out the combinatory impacts of *Sparc* KO, exercise and age on selected patterns related to SM physiological properties and metabolic performance. We explore the lactate levels and their implications with the SM phenotype changes (both structural and metabolic) in a SPARC-dependent way.

20.4 Animal Experimental Design, Material and Methods

Our study was carried out on male mice and involved both wild-type (WT) mice (C57BL/6J, the most commonly used strain for genetic and/or transgenic study that also consistently showed the highest level of voluntary wheel-running [12]) and *Sparc* KO mice (129/Sv-C57BL/6J) fed with chow diet (Teklad global 18% protein rodent diets [13]). Mice had access to food and water ad libitum during the whole experimental period (except for fasting periods during which they had access to water only). WT mice were from the Jackson Laboratory (https://www.jax.org/) and *Sparc* KO mice were generated via in vitro fertilization using *Sparc* KO mice sperm generously provided by Dr. Amy D. Bradshaw. *Sparc* KO mice of Dr. Amy D. Bradshaw were generated as previously described [14,15]. Each age-group of mice (young (Y) and old (O)) was divided based on the genotype (KO or WT) to obtain 4 groups: Y-KO, Y-WT, O-KO, O-WT. Finally, each of these 4 groups was further subdivided into two groups according to whether they were exercising (Ex) or sedentary (Sed) mice. Therefore, our experimental design included 8 groups: Y-WT-Sed, Y-WT-Ex, Y-KO-Sed, Y-KO-Ex, O-WT-Sed, O-WT-Ex, O-KO-Sed and O-KO-Ex. Each group had 11 to 12 mice (n). Mice were housed at the animal facility of the CHU de Québec Université Laval Research Center (12-h light/dark cycle) and periodically checked by animal care technicians for health and wellness. The exercise groups were trained during the dark phase.

The exercising mice were trained during 12 weeks (starting at the age of 9 weeks for Y mice and 66 weeks for O mice) on running wheels (Lafayette instrument Co, Lafayette, IN, USA) placed horizontally (no angle adjustment). Whereas Y mice were sacrificed at the age of 21 weeks, old mice were sacrificed at the age of 78 weeks. Mice were sacrificed following a 12-h fasting (postprandial period) by cardiac puncture following isoflurane inhalation anesthesia. The coming sub-sections detail the measures performed before, during and after the training, as well as on and after the sacrifice day.

All animal experimentation was conducted in accord with the guidelines of the Canadian Council on Animal Care and approved by the Animal Protection Committee of Laval University (Identifications: 2014165 and 2014168). Mice with any type of illness were immediately euthanized by cervical dislocation and excluded from the study. Mice found with anatomical abnormalities (during the sacrifice) were also excluded from the study.

20.4.1 Mice Exercise Protocol and Running Speed Determination

At the beginning of the training, mice had an adaptation period of 4 weeks. During those 4 weeks, mice performed the incremental exercise. They were trained through a progressive (gradual) increase in both running speed and duration (up to their maximum endurance) throughout this adaptation step, at the end of which we determined the speed at the lactate threshold (LT). The LT level [16] is a parameter indicating the level of physical activity corresponding to the metabolic point at which the muscle production of lactate starts to increase and overcome the blood clearance of lactate. This indicates that the energy produced via oxidative phosphorylation is insufficient to meet energetic needs and, therefore, the muscles trigger anaerobic energy production that generates lactate at a level superior to its blood clearance. At the end of the adaptation period, LT levels were determined following a measure of running speed-dependent blood lactate level curves, based on previously reported protocols [12,17]. Briefly, the mouse run at a determined speed for 4 min, after which we immediately measure the blood lactate level (within 1 min), after that it ran at the next speed (higher) and again the blood lactate speed was measured. We repeated this procedure until the mouse was not able to run (cannot maintain the speed). At the end, we obtained the curve representing blood lactate levels corresponding to the different running speeds, based on which we obtained the speed at the LT. The blood lactate levels were measured, as described in the next section (20.4.2). The LT speed was chosen as a parameter for our study, based on evidence showing that exercise at LT generates metabolic and functional benefits, including improved insulin sensitivity, peripheral glucose effectiveness, lipid profile, blood pressure, physiological fitness [18–21] and body fat weight percentage decrease [6]. The LT was determined for each mouse of the exercise groups. After that, for each set of mice trained at the same period, the running speed of the 8 remaining weeks of training was a value chosen among the average values (range) of all the mice of that set.

The training was at those values close to the LT levels (LT speed) because it was the LT level that was the speed used during the study in which *SPARC* has been characterised as an exercise-induced gene [6]. In addition, the exercise frequency of our study (60 min/day, five times/week) was also similar to the same study [6]. However, we extended the duration from 6 weeks to 12 weeks to be able to easily see the impacts with significant differences between groups. The literature reported studies exploring the effect of exercise in which mice were both trained for longer periods (over 12 weeks) with the same frequency (60 min/day, five times/week) [22] and also at least as young and as old (3

and 19 months of age) [23] as the mice in this study. In addition, the life span for C57BL/6 mice is around 104 weeks (26 months) [24,25]. Therefore, our choices of mice ages, exercise speed and frequency were within a range of the mice's abilities and did not damage their muscles, nor were they limited by physiological parameters. Importantly, since LT speed was chosen, based on evidence showing that exercise at LT generates metabolic and functional benefits, our study will provide additional data for molecular and biochemical explanations of such training benefits.

During mouse training, and unlike other protocols, no electrical [17] or any potentially harmful stimulations were used to force the mice to run. Only a light air stimulation (using a small hand air pump) was applied for mice in some cases to ensure, as much as possible, that all mice ran during the same period and at the same speed throughout the same training period (optimize the protocol for similar exercise amounts). Moreover, mice were always handled gently when taken from the cage to the training device and vice versa. In addition, sedentary mice were also transported to the exercise training room and kept in their cages, while exercising mice were trained so that all mice received a similar environmental (light, noise, etc.) stimuli. Thus, any possible impacts of stress or environmental stimuli on the performed measures were reduced to minimum.

The last training session was 48 h prior to the sacrifice so that the measures we obtained during the sacrifice and those performed on tissues afterwards did not reflect any possible acute effect of exercise, such as dehydration or neuroendocrine changes that could impact gene expression, post-exercise energy intake or expenditure, etc. Our study aimed to investigate the effects of the 12 weeks of exercise (chronic).

20.4.2 Fasting Lactate and Oral Glucose Gavage-Dependant Lactate Levels

As a resting metabolic indicator in the muscle, blood lactate levels were measured before and after glucose (prepared from 45% solution, Sigma-Aldrich Canada Co., Oakville, ON, Canada) oral gavage (2 mg of glucose per 1 g of body weight). Mice were fasted for 6 h prior to the glucose gavage. Each measure had 5 time points (0, 15, 30, 60 and 120 min after glucose gavage) allowing us to obtain a curve and calculate the area under the curve (AUC). This test was performed three times (a total of three AUCs) before the training, at week 5 and the end of week 12 of the training. In addition, we also measured blood lactate (single measure) at the sacrifice day (following 12 h fasting) and at the end of the last training session. Blood lactate levels were measured via tail pricking with a needle to collect blood samples on lactate test strips that were then inserted into the lactate meter (Lactate scout, Sports Resource Group, Inc., Minneapolis, MN, USA).

20.4.3 Grip Power Test

At the end of the training period, the muscle strengths of all the mice were evaluated through performing a grip power test with a grip strength meter (Columbus Instruments International, Columbus, OH, USA). The grip strength was measured by allowing a mouse to grab (with four limbs) pull bar assemblies attached to the force transducer while the

mouse was pulled horizontally by the tail away from the bars, similar to what has been previously described [26,27]. The peak force applied by the mouse (g) was then shown on a digital display.

This test was conducted five times (5 min apart) for each mouse, after which the forces (both mean and maximum) were calculated both as absolute values as well as being normalized to the body weights of the corresponding mice.

20.4.4 Body and Tissue Weights

Body and selected tissues were weighted at the sacrifice day. The selected tissues were the brain, pituitary gland, hypothalamus, liver, heart, aorta, white adipose tissue (WAT), SMs (gastrocnemius, soleus, tibialis anterior and extensor digitorum longus muscles). The values are reported both as tissue weights as well as weight percentages (normalized) to the body weights of the corresponding mice.

20.4.5 Western Blotting

We measured the SM (tibialis anterior muscle) expression of two proteins: Collagen alpha 1 type I (COL1A1) and mitochondrially encoded cytochrome c oxidase I (MT-CO1). Whereas COL1A1 is important in the structure of the muscle [28], MT-CO1 is an indicator of mitochondrial oxidative phosphorylation [29]. At the day of sacrifice, the tibialis anterior muscle was removed and quickly put in liquid nitrogen (snap frozen) then moved to -80 ◦C and kept until the protein extraction procedure. To measure the expression of both COL1A1 and MT-CO1, total proteins were extracted from the tibialis anterior muscle, using a radio immunoprecipitation assay (RIPA) buffer and protease inhibitors cocktail (Sigma-Aldrich Canada Co., Oakville, ON, Canada) and followed by a protein quantification of each protein extract using Bio-Rad protein assay (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). Fifteen (MT-CO1) or ten (COL1A1) micrograms of proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the TGX Stain-Free FastCast acrylamide solutions (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada), and the trihalo compound in the gels was activated under UV light. Then, total proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada), and gels (before and after the transfer) and membranes were visualized under UV light by using the AlphaImager TM 1220 (Alpha Innotech Co., San Leandro, CA, USA).

Membranes were blocked using the Pierce™ Protein-Free (TBS) blocking buffer (Life Technologies Inc., Burlington, ON, Canada), incubated with 1/400 (sc-8784R for COL1A1 and sc-48143 for MT-CO1) dilution of primary antibodies (Santa Cruz Biotechnology Inc., Dallas, TX, USA) and secondary antibodies (sc-2004 for COL1A1 and sc-2350 for MT-CO1, 1/10000 dilution: Santa Cruz Biotechnology Inc., Dallas, TX, USA), and finally visualized with the Clarity™ Western ECL Blotting Substrate on a film (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). The visualized total proteins on the membranes and target proteins on the films were quantified using ImageJ software (ImageJ

bundled with 64-bit Java 1.8.0_172, U. S. National Institutes of Health, Bethesda, MD, USA) [30]. The methodology of lane and band quantifications, followed by expression evaluations, was performed according to Taylor et al. [31,32] as we have detailed in one of our previous works [33].

20.4.6 Statistical Analyses and Sample Size Determination

The data were analyzed by three-way (age, genotype and exercise) and the four-way (for the lactate AUC) ANOVA. When the ANOVA revealed a significant interaction between two or three variables, the Tukey Kramer post hoc test was performed to identify the significant difference between the groups ($p < 0.05$). A trend corresponds to $0.05 \leq p < 0.1$. In the results section, all the effects are significant ($p < 0.05$), unless mentioned as a trend.

The number of mice (11–12 mice per experimental condition) was based on the results of power analysis by setting the statistical power at 80% ($\alpha = 0.05$ and $\beta = 0.2$) with our previous study, which used the same strain of WT mice [34].

20.5 Results

20.5.1 Exercise Patterns, Running Speed and Lactate Concentrations

Tables 20.1 and 20.2 report the data collected during the 4 weeks of incremental exercise, the weeks of the LT speed training and the day of sacrifice. During the 4 weeks of incremental exercise, we have the effect of age on both LT speed (Y > O) and blood lactate level at rest (O > Y). However, for lactate at rest, this genotype effect is attributed to the *Sparc* KO mice since, for the WT, there is no difference between Y and O mice, but for the *Sparc* KO mice, O mice have a higher lactate concentration at rest than Y mice (significant effects of genotype×age interaction). We also have the effect of age (Y > O) for both the mean exercise speed as well as the total exercise distance during the 12 weeks of training. The same effect of age (Y > O) is observed during the last training session for both speed and lactate concentration, measured at the end of that last running hour (Table 20.1).

Table 20.1. Summary of wheel exercise training.

		Young		Old		2-way ANOVA		
		Wild-type	Knockout	Wild-type	Knockout	A	G	A×G
During an incremental exercise test (wk 4)								
LT speed	m/min	8.7 ± 0.4	7.8 ± 0.5	5.5 ± 0.6	5.4 ± 0.4	Y>O	-	-
Lactate at rest	mM	2.8 ± 0.2	2.6 ± 0.2	3.1 ± 0.4	3.8 ± 0.2	O>Y	-	KO: O>Y
Lactate at LT	mM	3.2 ± 0.2	3.1 ± 0.3	3.4 ± 0.4	3.3 ± 0.5	-	-	-
During LT training (wk 1-12)								
Mean exercise speed	m/min	7.5 ± 0.2	7.6 ± 0.2	5.5 ± 0.0	5.3 ± 0.0	Y>O	-	-
Total exercise time	min	3332 ± 52	3353 ± 45	3334 ± 1	3335 ± 3	-	-	-
Total exercise distance	m	24903 ± 763	25445 ± 670	18219 ± 41	17811 ± 156	Y>O	-	-
During the last LT training (wk 12)								
Speed	m/min	7.8 ± 0.2	7.9 ± 0.2	5.5 ± 0.0	5.3 ± 0.1	Y>O	-	-
Lactate	mM	2.7 ± 0.3	2.9 ± 0.3	2.1 ± 0.2	2.5 ± 0.2	Y>O	-	-

Data are mean ± SEM. Number of mice: 11-12 mice per experimental condition. Abbreviations: A, age; G, genotype; KO, knockout; LT, lactate threshold; m, meter; min, minute; mM, millimolar; O, old; wk, week; Y, young. -: No effect.

In Table 20.2, we notice effect of age (Y > O) for blood lactate level (trend) on the sacrifice day (measured after 12 h of fasting). For the curve of post glucose–gavage lactate concentrations at different time points (0, 15, 30, 60 and 120 min), we only have an effect of the age (Y > O). The value of the AUC was measured three times—before the training and at week 5 and the end of the week 12 of the training. Each time, mice had a 6 h fasting period prior to glucose gavage.

Table 20.2. Fasting and post glucose-gavage blood lactate levels

		Young				Old				ANOVA						
		Wild-type		Knockout		Wild-type		Knockout								
		Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	A	G	Ex	A×G	A×Ex	G×Ex	A×G×Ex
At sacrifice (12 h fast)													3-way			
Blood lactate	mM	1.09 ± 0.12	0.88 ± 0.08	1.28 ± 0.38	1.24 ± 0.15	0.89 ± 0.07	0.99 ± 0.13	0.98 ± 0.08	0.75 ± 0.04	Y>O*	-	-	-	-	-	-
Post glucose gavage (6 h fast)													4-way			
Blood lactate	AUC									Y>O	-	-	-	-	-	-
Pre		615 ± 35	498 ± 63	479 ± 29	533 ± 44	429 ± 29	424 ± 24	473 ± 39	461 ± 54							
At wk 5		529 ± 32	509 ± 34	503 ± 41	519 ± 38	422 ± 27	450 ± 42	479 ± 44	392 ± 32							
After 12 wks		465 ± 34	391 ± 27	452 ± 22	486 ± 45	440 ± 56	381 ± 20	414 ± 35	371 ± 28							

Data are mean ± SEM. Number of mice: 11-12 mice per experimental condition. Abbreviations: A, age; AUC, area under the curve; Ex, Exercise; G, genotype; h, hour; KO, knockout; mM, millimolar; O, old; wk, week; Y, young. *: Trend ($0.05 \leq p < 0.1$). -: No effect.

20.5.2 Body and Tissue Weights

Mice were weighed the morning of the sacrifice. During the sacrifice, tissues were removed and weighed as well (Table 20.3). Analyzed data are both as absolute values (weight) and percentages of the tissues weights to the body weight.

We found the effect of age (O > Y) on body weight as well as on the weights of pituitary gland, hypothalamus, liver, heart, and WAT, in addition to the weight percentage of WAT; the opposite effect of age (Y > O) on the weight percentages (to the body weight) of the brain, heart, aorta, SM and tibialis anterior muscle. We also found an effect (trend) of age (Y > O) on the weights of both SM and the tibialis anterior muscle.

We found an effect of genotype (WT > KO) on the body, aorta, WAT, SM and tibialis anterior muscle weights, and another effect (KO > WT) on the brain weight and weight percentage, liver and heart (both weight percentage). Coming to the last variable, exercise, we also report these exercise effects-Sed > Ex (trend) for body weight and liver weight percentage and WAT weight. Ex > Sed (trend) for weight percentages of both the brain and the hypothalamus. Ex > Sed for tibialis anterior muscle weight and Sed > Ex for liver weight.

Table 20.3. Body and tissue weights.

		Young				Old				3-way ANOVA						
		Wild-type		Knockout		Wild-type		Knockout								
		Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	A	G	Ex	A×G	A×Ex	G×Ex	A×G×Ex
Body weight	g	29.5 ± 0.7	28.9 ± 1.0	27.8 ± 0.6	25.0 ± 0.8	37.4 ± 2.1	35.2 ± 1.0	31.3 ± 1.5	30.4 ± 0.7	O>Y	WT>KO	Sed>Ex*	-	-	-	-
Tissues weights																
Brain	mg	430 ± 2	430 ± 6	448 ± 4	445 ± 3	432 ± 7	436 ± 2	451 ± 4	446 ± 4	-	KO>WT	-	-	-	-	-
	%	1.47 ± 0.03	1.50 ± 0.05	1.62 ± 0.04	1.80 ± 0.05	1.18 ± 0.05	1.25 ± 0.03	1.48 ± 0.07	1.47 ± 0.02	Y>O	KO>WT	Ex>Sed*	-	-	-	WT-Sed: Y>O, WT-Ex: Y>O, KO-Ex: Y>>O
Pituitary gland	mg	1.52 ± 0.20	1.49 ± 0.10	1.47 ± 0.10	1.33 ± 0.10	1.77 ± 0.16	1.76 ± 0.13	1.78 ± 0.04	1.65 ± 0.12	O>Y	-	-	-	-	-	-
	%	0.0052 ± 0.0007	0.0052 ± 0.0003	0.0053 ± 0.0004	0.0054 ± 0.0004	0.0049 ± 0.0006	0.0050 ± 0.0004	0.0059 ± 0.0003	0.0055 ± 0.0005	-	-	-	-	-	-	-
Hypothalamus	mg	8.7 ± 0.7	9.1 ± 0.6	8.0 ± 0.5	8.9 ± 0.8	10.7 ± 0.9	10.8 ± 0.8	9.4 ± 0.8	11.0 ± 0.5	O>Y	-	-	-	-	-	-
	%	0.030 ± 0.003	0.032 ± 0.003	0.029 ± 0.002	0.036 ± 0.003	0.030 ± 0.003	0.031 ± 0.003	0.030 ± 0.003	0.036 ± 0.002	-	-	Ex>Sed*	-	-	-	-
Liver	mg	984 ± 27	954 ± 33	984 ± 35	899 ± 30	1370 ± 136	1114 ± 28	1174 ± 82	1101 ± 24	O>Y	-	Sed>Ex	-	-	-	-
	%	3.34 ± 0.04	3.31 ± 0.09	3.54 ± 0.09	3.60 ± 0.04	3.59 ± 0.14	3.17 ± 0.04	3.71 ± 0.12	3.62 ± 0.05	-	KO>WT	Sed>Ex*	-	Sed: O>Y*	Ex: KO>WT	-
Heart	mg	138 ± 3	149 ± 7	146 ± 6	131 ± 4	159 ± 6	135 ± 4	152 ± 5	159 ± 7	O>Y	-	-	KO: O>Y	-	-	WT-Sed: O>Y, WT-Ex: Y>O*, KO-Ex: O>Y
	%	0.47 ± 0.01	0.51 ± 0.01	0.53 ± 0.02	0.53 ± 0.01	0.43 ± 0.01	0.38 ± 0.01	0.50 ± 0.02	0.52 ± 0.02	Y>O	KO>WT	-	WT: Y>>O	-	-	WT-Sed: Y>O, WT-Ex: Y>>O
Aorta	mg	12.8 ± 1.5	12.0 ± 1.5	12.9 ± 1.3	9.0 ± 0.6	13.0 ± 1.5	12.3 ± 1.0	9.7 ± 1.0	9.9 ± 0.7	-	WT>KO	-	-	-	-	-
	%	0.044 ± 0.006	0.042 ± 0.006	0.047 ± 0.005	0.037 ± 0.003	0.034 ± 0.003	0.035 ± 0.003	0.031 ± 0.003	0.032 ± 0.002	Y>O	-	-	-	-	-	-
White adipose tissue**	mg	1021 ± 206	812 ± 126	955 ± 138	684 ± 125	2913 ± 362	2649 ± 175	1804 ± 226	1505 ± 164	O>Y	WT>KO	Sed>Ex*	WT: O>>Y, KO: O>Y	-	-	-
	%	3.36 ± 0.58	2.72 ± 0.36	3.36 ± 0.44	2.63 ± 0.38	7.52 ± 0.65	7.44 ± 0.32	5.51 ± 0.50	4.85 ± 0.43	O>Y	WT>KO	-	WT: O>>Y, KO: O>Y	-	-	-
Skeletal muscle***	mg	532 ± 11	524 ± 15	432 ± 8	413 ± 7	508 ± 11	510 ± 6	416 ± 8	417 ± 7	Y>O*	WT>KO	-		-	-	-
	%	1.81 ± 0.04	1.82 ± 0.05	1.56 ± 0.04	1.67 ± 0.05	1.39 ± 0.05	1.46 ± 0.05	1.36 ± 0.06	1.38 ± 0.03	Y>O	WT>KO	-	WT: Y>>O, K: Y>O	-	-	-
Tibialis anterior muscle	mg	133 ± 3	138 ± 4	117 ± 3	114 ± 2	133 ± 6	127 ± 3	110 ± 4	118 ± 3	Y>O*	WT>KO	-	-	-	-	WT-Ex: Y>O
	%	0.45 ± 0.01	0.48 ± 0.01	0.42 ± 0.01	0.46 ± 0.01	0.36 ± 0.02	0.36 ± 0.01	0.36 ± 0.01	0.39 ± 0.02	Y>O	-	Ex>Sed	WT: Y>>O, KO: Y>O	-	-	-

Data are mean ± SEM. Number of mice: 11–12 mice per experimental condition. Abbreviations: A, age; Ex, exercise; G, genotype; g, gram; KO, knockout; mg, milligram; O, old; Sed, sedentary; WT, wild-type; Y, young. *: Trend ($0.05 \leq p < 0.1$); **: Inguinal and abdominal adipose tissues; ***: Gastrocnemius, soleus, tibialis anterior and extensor digitorum longus muscles. %: percentage to the body weight. -: No effect.

20.5.3 Muscle Strength (Grip Power Tests)

As a measure of muscle strength for the four limbs (simultaneously), the grip power tests results (Table 20.4) show effect of the age (Y > O) for the both the mean and the maximum grip power as well as for the percentage of each of these two values (mean and the maximum grip power) on body weight. We also have an effect of the genotype (WT > KO) for both mean and the maximum grip powers. For the effect of exercise, we have a trend (Ex > Sed) for the percentage of the maximum grip power to the body weight.

Table 20.4. Grip Power at the End of Week 12.

		Young				Old				3-way ANOVA						
		Wild-type		Knockout		Wild-type		Knockout								
		Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	A	G	Ex	A×G	A×Ex	G×Ex	A×G×Ex
Grip power																
Mean	g	240 ± 12	248 ± 11	226 ± 10	208 ± 11	216 ± 8	220 ± 8	182 ± 8	203 ± 9	Y>O	WT>KO	-	-	-	-	-
	g/BW	8.2 ± 0.5	8.6 ± 0.4	8.1 ± 0.4	8.4 ± 0.4	5.8 ± 0.4	6.1 ± 0.2	5.9 ± 0.5	6.6 ± 0.3	Y>O	-	-	-	-		-
Max	g	308 ± 12	301 ± 11	277 ± 11	250 ± 9	250 ± 8	257 ± 9	210 ± 8	235 ± 10	Y>O	WT>KO	-	-	-	-	-
	g/BW	10.5 ± 0.4	10.5 ± 0.4	10.0 ± 0.4	10.1 ± 0.4	6.7 ± 0.5	7.2 ± 0.3	6.8 ± 0.5	7.6 ± 0.3	Y>O	-	Ex>Sed*	-	-	-	-

Data are mean ± SEM. Number of mice: 11–12 mice per experimental condition. Abbreviations: A, age; BW, body weight; Ex, exercise; G, genotype; g, gram; KO, knockout; O, old; Sed, sedentary; WT, wild-type; Y, young. *: Trend ($0.05 \leq p < 0.1$). -: No effect.

20.5.4 COL1A1 and MT-CO1 Expressions in Tibialis Anterior Muscle

Protein expression of both COL1A1 and MT-CO1 was measured in the SM tibialis anterior muscle. The results (Figure 20.1) indicate an effect of genotype (WT > KO) on both proteins and an effect of exercise (Ex > Sed) on COL1A1 (trend) and MT-CO1. For the interactions, we found one between genotype and exercise for COL1A1 (Ex > Sed in WT) and one between age and genotype for MT-CO1 (high in Y-WT).

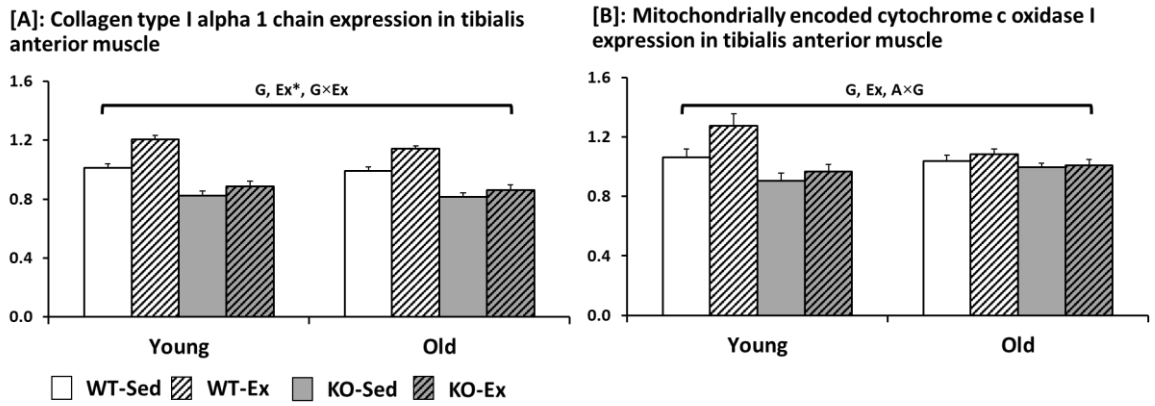


Figure 20.1. Expression of both collagen type I alpha 1 chain (COL1A1) (A) and mitochondrially encoded cytochrome c oxidase I (MT-CO1) (B) in the tibialis anterior muscle. The results indicate an effect of genotype^{G} (WT > KO) for both proteins and an effect of exercise^{Ex} (Ex > Sed) for COL1A1 (trend) and MT-CO1. For the interactions, we have one between genotype and exercise$^{G\times Ex}$ for COL1A1 (Ex > Sed in WT) and one between age and genotype$^{A\times G}$ for MT-CO1 (high in Y-WT). All data are mean ± SEM. The number of mice: 11–12 mice per experimental condition. Abbreviations: A, age; Ex, exercise; G, genotype; KO, knockout; O, old; Sed, sedentary; WT, wild-type; Y, young. *: Trend ($0.05 \leq p < 0.1$)

20.6 Discussion and Interpretation

As per Table 20.1, there is no effect in the genotype for the LT speed (in all the performed measures both during the 4 weeks of adaptation and during the 8 weeks of LT training), exercise speed, exercise time, exercise distance and even lactate concentrations during exercise. This has a key importance, since it means that mice of the two different genotypes (KO and WT) had equal amounts of exercise training (speed, distance, time and frequency) and blood lactate levels. Therefore, genotypes effects seen for the other measures will be, indeed, due to the genotype itself (consequence of *Sparc* KO) rather than difference in the exercise amount.

SPARC (osteonectin or BM-40) is a three-modular-domain [35,36] calcium binding extracellular matrix-associated glycoprotein [37,38]. The *Sparc* gene localized to the central region of chromosome 11 in mice [39] and in the chromosomal site at 5q31–q33 in

humans [40]. It is well known for its roles in extracellular matrix (ECM) organization, growth, cellular differentiation, cell–matrix communication, wound healing, cell cycle and tissue response to injury [35,36,41–43]. SPARC is also implicated in metabolism [44,45], cancer [46] and inflammatory [47] homeostasis. Importantly, for the SM, a key metabolic tissue and the key organ for the exercise performance, SPARC represents an important element for its development [28] and function [7].

Indeed, SPARC is known for its importance is SM development and regeneration (satellite cells/myoblasts. myotubes and muscle fibers) [48]. Moreover, whereas during embryogenesis SPARC is highly expressed, its expression is mainly restricted to tissues undergoing changes and remodeling during adulthood [35,49–51] which indicates its importance for exercising muscle; which does undergo remodeling as an adaptation to exercise [52,53]. Importantly, SPARC modulates actin cytoskeleton within the SM structure which results in defective force recovery following in vitro fatigue stimulation in muscle from *Sparc* KO mice [54]; but in normal and uninjured muscles, SPARC is not detectable [48]. This further indicates the importance of SPARC in the context of healing, repair, remodeling and development, especially that the ECM (important for the cellular remodeling, for instance) repair, disassembly and degradation is mediated by SPARC [55]. These impacts on regeneration and during embryogenesis suggest that SPARC deficiency could impact some tissue development and growth, as illustrated by the loss of bone mass (osteopenia) in *Sparc* KO mice [56].

Another structural importance for SPARC in SM derives from its ability to interact with collagens. It interacts with collagen I and procollagen I [57], binds to fibrillar collagens [58], maintains SM stiffness (collagen accumulation regulation) [59] and specifically binds several molecules, including collagen types I, III and IV [60]. SPARC deficiency has also been shown to reduce the expression of different types of collagen such as collagen type I in mesangial cells [61], collagen in skin [62] and fibrillar collagen accumulation in tibialis anterior muscle [59].

Moving from SPARC-related structural muscle properties to metabolic implications, SPARC has been shown to be required for the expression of the exercise-induced (in vitro model of) mitochondrial enzymes (oxidative phosphorylation) [28] and is suggested to enhance the muscle mitochondrial biogenesis [63] as supported by the fact that small interfering RNA (siRNA) of *SPARC* reduces 5-aminoimidazole-4-carboxamide-1-β-Dribofuranoside (AICAR)-stimulated adenosine monophosphate-activated protein kinase (AMPK) phosphorylation [64], which is known to induce mitochondrial biogenesis via the activation/induction of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A, also known as PGC1α) [65–67], a master regulator of mitochondrial biogenesis. Importantly, knowing the importance of the mitochondria during regeneration [68,69], SPARC would impact regeneration. In addition, SPARC regulates glucose transporter type 4 expression [64] and improves glucose tolerance [70]. These are selected illustrations of SPARC importance and implications for the metabolism, mainly for the SM that we focus on in our study.

For other tissues, the implication of SPARC in tissue regeneration and development (including tissue repair, cell turnover, cellular differentiation and remodeling) [35,38,44,71,72], especially with the known implications of SPARC in the functions of stem cells [73,74] and other types of cells such as erythroid progenitors [74], could indicate that SPARC-deficient mice could exhibit impairments in terms of development for certain tissues under selected conditions.

20.6.1 Lactate Concentrations among the Indicators of Muscles Metabolic Performance

Lactate is not just produced by SM and WAT [75] but it is also consumed by muscles [76] with special metabolic patterns [77] and serves as a gluconeogenic precursor [78]. Therefore, the blood lactate levels represent the outcome of the balance between the production and the consumption (clearance) of lactate [79] mainly (but not only) by SM [80]. The production of lactate by SM does not always mean insufficient energy production through oxidative phosphorylation, but could also be due to the lack of oxygen [81], as illustrated by the production of lactate in the adipose tissue of obese subjects as a consequence of hypoxia in this adipose tissue [75]. Importantly, exercise-produced lactate both upregulates the expression cytochrome oxidase gene and protein expression and is a mitochondrial biogenesis activation signal [79]. All these changes seem to result from negative feedback, aiming to increase the oxidative phosphorylation ability and, therefore, reduce lactate production and increase its clearance (usage). The liver and heart also contribute to lactate clearance and, whereas myocardia oxidases lactate as a fuel, the brain also takes it when its levels increase in the blood and the liver uptakes it to form glucose [80]. The fact that no genotype effect has been seen for lactate levels, at similar amount of exercise indicates that *Sparc* KO mice are able to maintain the lactate concentrations at a homeostatic level (similar to that of WT mice) in spite of the impaired muscular functions (compared to WT mice), suggesting a compensatory effect of other tissues to re-balance blood lactate (as we detail below). This compensatory pathway highlights the importance of lactate blood homeostasis.

20.6.2 Body and Tissue Weights (Table 20.3)

The importance of SPARC is tissue development, embryogenesis, regeneration, its interaction with collagen and ECM, in addition to its role in collagen accumulation [59] would explain why *Sparc* KO reduces body weight and SM weights, including the tibialis anterior muscle (correlated with what Omi et al. reported [59]) in addition to other tissues (aorta and WAT), as a result of regeneration and development deficiency, similar to the decrease in bone mass (osteopenia) as a result of SPARC deficiency [56]. However, the observed increased weights or weight percentages of other tissues, such as the brain, liver and heart in *Sparc* KO mice, could result from feedback signals. Indeed, the reduced development and metabolic deficiency in *Sparc* KO mice would lead to the production of signals aiming to correct this developmental and metabolic deficiency (resulting from

muscle low oxidation capacity, myokines secretion reduction, etc). Such signal effects would target selected tissues (those increased with *Sparc* KO, such as the brain, which is the center of numerous neuroendocrine signals and in which Compolongo et al., have shown that the neuronal activity levels of *Sparc* KO mice are increased in the brain region dentate gyrus [82] which could support the hypothesis of such signals in *Sparc* KO mice) and either be nonspecific or with insufficient impacts on other tissues (those for which *Sparc* KO does not reduce the weights). For instance, the increased heart weight percentage in *Sparc* KO mice could be adaptive to the fact that these mice have reduced oxidative phosphorylation ability (as shown by the low MT-CO1 expression) and would have more muscle-produced lactate. The developed heart could be an adaptation to the increased lactate production in order to increase the circulation and, therefore, increase lactate clearance, which could be taken by the liver to form glucose [80], which could also explain the increased weight (percentage) of the liver in *Sparc* KO mice. Therefore, although *Sparc* KO mice SM produced more lactate (weak oxidation capacity), they have the same blood lactate levels as WT mice because they would compensate via increased lactate blood clearance through enhanced blood circulation (increased heart weight percentage) combined to an increased intake by the liver (increased weight percentage), the brain (increased weight and weight percentage) and probably other tissues leading to that weight/weight percentage increase in those tissues in *Sparc* KO mice. This correlates with the liver weight percentage for which we have Ex-KO>Ex-WT, meaning that, in the exercised groups, the liver (weight percentage) of *Sparc* KO mice is superior to the liver (weight percentage) of WT mice (even though both had similar amount of training). This could indicate more tissue glycogen storage [83] (in a hydrated form that adds more water weight to the liver [84]) built from glucose made of the taken lactate because the *Sparc* KO mice SM would produce more lactate (weak oxidative phosphorylation reflected by the decrease in MT-CO1 expression in the *Sparc* KO mice) but clear it better through an increased blood circulation (increased heart weight percentage in *Sparc* KO) combined with lactate uptake (clearance) by the liver [80] and also by the brain [80] (that also increased in weight in *Sparc* KO mice) to compensate the low oxidation ability of the SM (supposed to contribute to lactate clearance but remains insufficient in terms of lactate clearance in *Sparc* KO mice). Overall, there is no genotype-related difference in lactate level because there would be compensation. Indeed, whereas WT mice have good muscle lactate clearance (with low lactate production), *Sparc* KO mice (although they have higher lactate production) have increased lactate clearance via the liver, brain, heart (that have increased weight percentage, compared to those in WT mice), etc.

Furthermore, the known implications of SPARC in the functions of erythroid progenitors [74] could suggest that *Sparc* KO mice would have reduced hemoglobin (low blood cells cancer) and, therefore, reduced oxygen transport ability. This would require one to increase the blood supply to different tissues to compensate low blood oxygenation via increased blood circulation that would require a developed cardiac pump and, thus, explains the increased weight (percentage) of the heart in *Sparc* KO mice; such low oxygenation

further worsens the weak oxidative phosphorylation capacity in SM that *Sparc* KO mice already have.

The other tissues patterns (age- and exercise-dependant) are in accordance with the known effects of both ageing and exercise on diverse tissues. For instance, the increased brain and hypothalamus weight percentages (trend) with exercise fits with the ability of exercise to enhance neurogenesis [23,85,86], the exercise also reduces (trend) both body weight and liver weight percentage and WAT weight, whereas it increases the tibialis anterior muscle weight percentage. All these elements correlate with the ability of exercise to increase energy usage (WAT lipids and liver glycogen) as well as muscle weight. Regarding the tibialis anterior muscle, in addition to its increase (weight percentage) with exercise, it decreases with both age (weight percentage) and *Sparc* KO (weight). It is for these patterns in changes according to genotype, age and exercise that we have chosen the tibialis anterior muscle to measure the expression of COL1A1 and MT-CO1; which allowed us to make a correlation between the genotype-dependent changes in muscle weight and power and the corresponding changes in the expression of these two proteins, depending on SPARC expression. Additionally, the decrease in brain weight percentage with ageing correlates with age-related neurodegeneration and related diseases [87,88], which are improved by exercise [89–91] and that, also, correlate with our data, showing an increase (trend) in the brain weight percentage with exercise.

Interleukin 6 (among other myokine) is produced by the muscles during exercise [92,93], which reduces appetite [94] and WAT [93]. This correlates with our results, indicating an effect (trend) of exercise on reducing the body weight and WAT weight percentage, but without any interaction effect on genotype and exercise. This indicates that SPARC absence would not impact the ability of exercise to reduce adiposity. The possibilities could be whether the effect of SPARC is partial since (in *Sparc* KO mice) the WAT weight is lower in Y mice compare to O mice, or there are other SPARC-independent pathways linking myokine to adiposity reduction, such as IL-6 or also because both WT and *Sparc* KO mice spent similar amounts of exercise, leading to similar exercise-induced energy expenditure (would have similar impacts of reducing the WAT).

For the WAT, both for the age and age × genotype (both in WT and *Sparc* KO mice), we always found an increase in adiposity (weight and weight percentage) with ageing. In addition, there is also a reduction in the muscle mass (percentage) with age and for both WT and *Sparc* KO mice, which corresponds to the classical ageing profile (decreased muscle mass and increased adiposity) along with increased body weight with ageing [95–98], as our data show. It is worth noting that, while looking into the effects of genotype × age, both for the decrease in SM mass percentage and the increase in WAT weight (as well as weight percentage), we notice that these ageing-induced changes (musculature decrease and adiposity decrease), are more important in O mice than in Y mice. This could be explained by the implication of SPARC in these changes. Indeed, since *SPARC* expression is downregulated by ageing [8], the consequences of its KO would

be more important in Y mice compared to O mice, where its expression is already reduced by ageing.

This ageing effect on SM explains the results of Table 20.1, showing that ageing reduces the LT speed (adaptation phase), mean exercise speed, total exercise distance (12 weeks of training) and both running speed and the lactate concentration of the last running session at the end of the 12 weeks training. However, the lactate at rest level (week 4 of the adaptation phase), which increases with age, indicates a reduced aerobic metabolic performance of the muscle. Importantly, the effect of genotype × age interaction reveals that the age effect comes from the *Sparc* KO mice rather than WT mice, meaning that it is the *Sparc* KO in O mice that leads to an increase in the resting lactate compared to both WT mice and KO-Y mice. This also explains, in part, how ageing is both a risk factor for numerous diseases and health conditions [99–103].

Since Norose et al. reported that, when handled, *Sparc* KO mice reduced physical activity [104], we deduce that our *Sparc* KO mice had reduced energy expenditure (compared to WT mice), but with a lower body and WAT weights they most probably had less food intake compared to the WT mice. This could indicate an effect (direct or indirect) of *Sparc* KO on appetite. This appetite (in addition to the physical activity patter), both impacting the body weight, could be explained by the increased levels of anxiety and reduced depression-related behaviors in *Sparc* KO mice [82]. Such variations in mood states would impact food intake and energy balance [105,106] and, therefore, body and tissue weights.

20.6.3 Protein Expressions (Figure 20.1) and Muscle Strength (Table 20.4)

The reduced expression of COL1A1 in *Sparc* KO mice fits with what Omi et al. reported [59] and confirms the importance of SPARC for COL1A1 expression, as we have previously shown [28], and as reported by Norose et al. [104] and Bradshaw et al. [107].

In addition, MT-CO1 decreased expression with *Sparc* KO highlights the implication of SPARC in mitochondrial enzyme expression [28] and mitochondrial regeneration [63], whereas MT-CO1 increases expression with exercise, which fits with our previous gene expression studies, showing an increase in oxidative phosphorylation genes with training at LT intensity [6], which further validates the choice of the exercise speed in this study.

Sparc KO mice have been reported as passive and with reduced physical activity compared to WT mice [104], this would indicate weak muscles and correlates with reduced grip power (both mean and maximum) in *Sparc* KO mice compared to WT mice of our study. The importance of SPARC in myoblast fusion [28] and, more important, the interaction of SPARC with actin in SM (actin cytoskeleton modulator) [54] also support our data, indicating a decrease in muscle strength with SPARC deficiency. This SPARC deficiency also reduced COL1A1 expression; indicating an impact on muscle structure (for which collage is a key element) and correlated with the *Sparc* KO-induced muscle strength decrease.

The effect of age (Y > O) on the muscle strength and the effect of exercise (Ex > Sed, trend of the percentage of maximum grip power) is an additional illustration of how ageing worsened the effects of SPARC deficiency (reduce the muscle power) while exercise improved it (increase in both SM strength and expression of both COL1A1 and MT-CO1 with exercise represents muscle adaptation to exercise).

20.7 Conclusions and Hypothetical Mechanisms

Sparc KO effects are toward a reduced body and WAT weights with a negative SM phenotype (metabolism and strength). Such negative effects worsen with ageing but relatively improve through exercise (Figure 20.2). While exercise reduces risk factor for many diseases, ageing increases those risks [108].

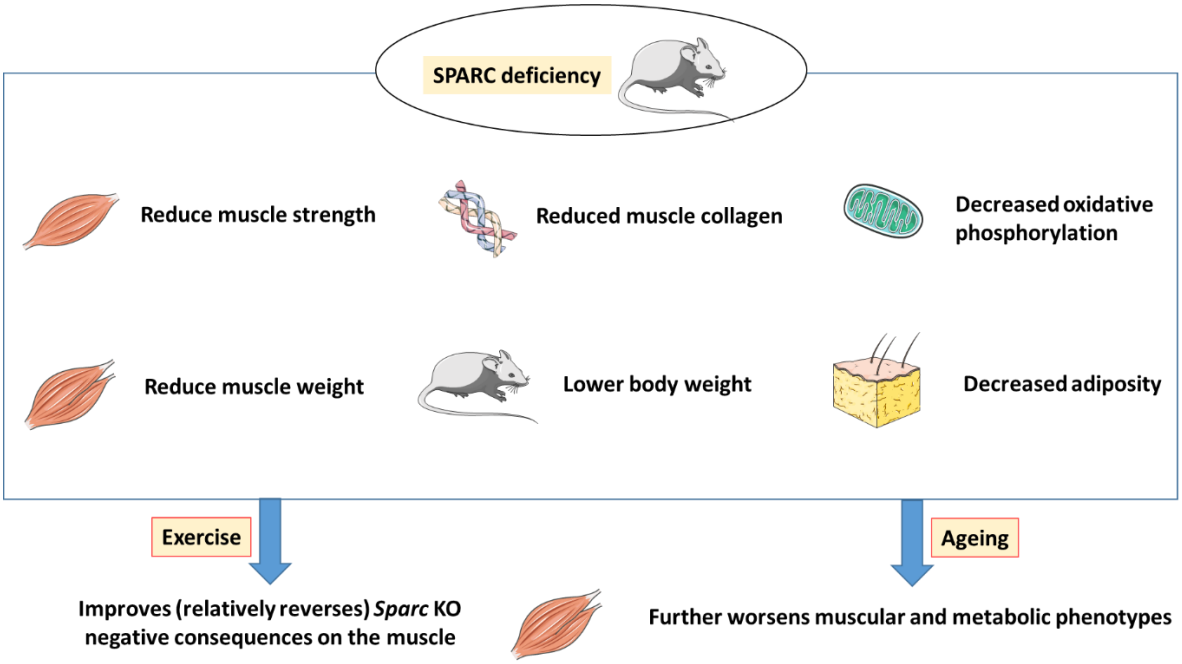


Figure 20.2. SPARC-deficiency impacts. Our data highlight that *Sparc* KO effects are toward a reduced body and white adipose tissue weights with a negative skeletal muscle phenotype (metabolic and strength). Such negative effects worsen with ageing but relatively improve through exercise. Abbreviations: KO, knockout; SPARC, secreted protein acidic and rich in cysteine.

Within this context, Aoi et al. reported 24 genes (including *SPARC*) that are both upregulated by exercise and downregulated by ageing [8] suggesting, once more, that some of the exercise-induced benefits such as mitochondrial biogenesis [109] could be SPARC-dependent or partially mediated by SPARC. It is within this perspective that we developed our hypothesis.

The exercise-produced lactate induces both an up-regulation of the expression of the cytochrome oxidase gene and protein, as well as a mitochondrial biogenesis activation signal [79], and since *Sparc* KO did not induce any genotype effect on the different lactate

levels we measured (both WT and *Sparc* KO mice had statistically similar lactate levels) but reduced the expression of the MT-CO1 (WT and *Sparc* KO mice have similar lactate levels but WT mice express more MT-CO1 than *Sparc* KO mice), we hypothesize that the exercise-produced lactate-induced cytochrome oxidase upregulation and mitochondrial biogenesis activation do require SPARC (Figure 20.3A). This would, at least in part, explain why SPARC deficiency reduces tumor growth. Indeed, cancer cells require glycolytic energy and produce lactate, leading to lower pH, compared to normal tissue extracellular pH [110,111], and lactate would be an attempt to increase oxidative phosphorylation capacity via improving mitochondrial biogenesis. However, in the absence of SPARC this lactate-induced mitochondrial biogenesis remains limited, which worsens the tumor bioenvironment and results in tumor progress inhibition. This concept of lactate-related signaling correlates with the theory presenting lactate as a signaling molecule "lactormone" in the context of lactate shuttle [112,113] of lactate formation, utilization and exchange between tissues [114].

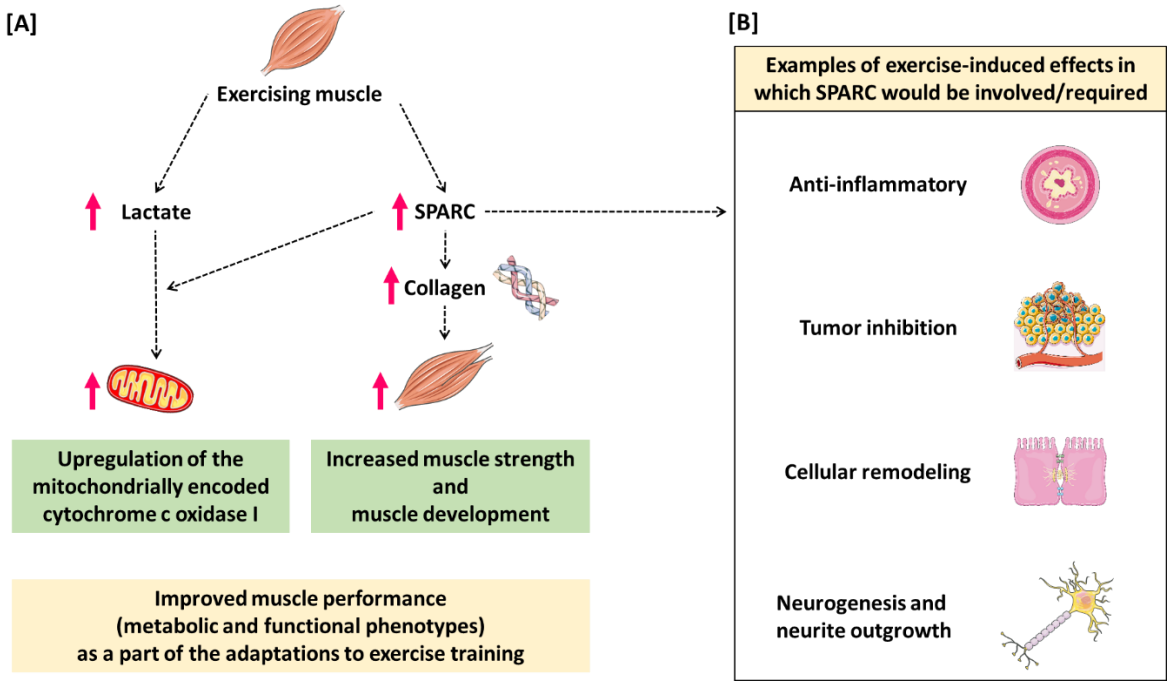


Figure 20.3. Hypothetical mechanisms linking SPARC to the exercise-induced SPARC-mediated changes in the skeletal muscle phenotype. [**A**] Exercise induces the secretion and expression of SPARC as well as the production of lactate. Our data suggest that, whereas SPARC enhances collagen expression and muscle strength, the lactate increases mitochondrial enzyme expression in a SPARC-dependant manner (probably via mitochondrial biogenesis induction) and, therefore, the oxidative phosphorylation capacity. [**B**] The similarities between exercise benefits and effects shown to be regulated or modulated by SPARC, such as inflammation, cancer growth, metabolic and structural remodeling of the skeletal muscle, and even neurite outgrowth and neurogenesis, all suggest that part of exercise benefits would be mediated by or dependent on SPARC expression. Abbreviation: SPARC, secreted protein acidic and rich in cysteine.

The results of Figure 20.1B further support our hypothesis that SPARC-dependent exercise impacts SM. Indeed, whereas there is a significant effect of exercise (increase in COL1A1 expression) in WT mice (WT-Ex > WT-Sed), there is no such effect in *Sparc* KO mice (both KO-Ex and KO-Sed mice have statistically similar expression of COL1A1). This also correlates with the genotype-induced decrease in COL1A1 expression (WT > KO) although both WT and *Sparc* KO mice had equal amounts of exercise (as detailed in the introduction of Section 20.6), therefore, indicating that exercise-induced COL1A1 is SPARC-dependent (Figure 20.3A). Moreover, since exercise-induced COL1A1 seems SPARC-dependent, and based on the importance of collagens in the SM structure (and fibrillar collagen I is reported to bind SPARC [115]) and development [116], this could also explain, in part, the low tibialis anterior muscle weight in *Sparc* KO mice compared to WT mice, even though all mice had similar amounts of exercise. Thus, this also suggests that SM development (tibialis anterior weight percentage increase; Table 20.3), as a part to the adaptation to exercise, would also be SPARC-dependent (Figure 20.3A).

These conclusions are based on the fact that, as per Table 20.1, there is no effect of the genotype for LT speed, exercise speed, exercise time, exercise distance and lactate concentrations during exercise. This means that mice of two different genotypes (*Sparc* KO and WT) had equal amounts of training (similar speed, distance, time and frequency). Therefore, genotype effects seen for MT-CO1, COL1A1 (Figure 20.1) and grip power (Table 20.4) are, indeed, due to the genotype itself (*Sparc* KO) rather than the difference in the exercise amount; suggesting that these exercise-induced changes are SPARC-dependent/mediated. Such exercise-induced effects in the muscle represent a part of the adaptation via increasing the respiratory capacity, mitochondrial content [52] and contractile properties of the SM [53].

In addition to these SPARC-mediated effects, the similarity of exercise benefits and the effects shown to be regulated or modulated by SPARC, such as inflammation [22,47], cancer growth [8,46], metabolic and structural remodeling of the SM [53], and even neurite outgrowth [117,118] and neurogenesis [85,119], all suggest that some of the exercise benefits would indeed be mediated by or dependent on SPARC expression (Figure 20.3B).

20.8 Implications and Perspectives

Our data suggest that the benefits of exercise would also be reduced in *Sparc* KO mice, not only because some of exercise benefits are directly mediated via SPARC, but also because physical performance and muscle performance is reduced as a result of *Sparc* KO (indirect effects). As an illustration, *Sparc* KO increases tumor growth [120,121], loss of SPARC increases cancer progression [122] and the tumorigenesis is prevented and suppressed by exercise-induced SPARC [8,123,124]. More generally, since SPARC is also a myokine secreted during exercise [2,8], exercise benefits including metabolic benefits and inflammation regulation would also be reduced with the *Sparc* KO since SPARC has been shown to play roles in metabolism [44], inflammation regulation [47] and cancer

homeostasis [46]. Thus, its absence from circulation would impact tissues other than the SM. For instance, even some of the mechanisms underlying the beneficial effects of exercise that have been shown to involve factors other than SPARC, such as tumor growth suppression through interleukin 6 and epinephrine [92], would also be deficient in *Sparc* KO mice, since the absence of SPARC would reduce the ability of SM to correctly secrete the other myokines involved in the related pathways.

Overall, SM, both as a secretory organ [2] and a metabolic engine, represents the key tissue upon which exercise benefits depend. SPARC represents a "booster" of the SM, with beneficial effects on some other tissues as well. Therefore, SPARC and the pathways it governs would represent good targets to pharmacologically mimic the effects of SPARC, including improved muscle strength and metabolic performances. This is of a particular importance for individuals suffering from health problems, such as heart failure or physical handicap and, therefore, are unable to perform the required physical activity although they need it (reduce obesity, treat lipid disorders, etc.). In such a scenario we could imagine an "exercise pill", targeting SPARC-related pathways and inducing exercise-like effects that would also be of a high therapeutic importance for diseases, such as sarcopenia. Such a therapeutic goal still requires further investigations into the implications of SPARC, not only within the SM but also on other tissues and in the diverse aspects of homeostasis. This will both extend the expected benefits of such an "exercise pill" and anticipate the side effects to decide whether it would be better given as a systemic drug or rather target a specific tissue (that would be the SM based on this study) to optimize clinical efficiency. Importantly, the results obtained from studying SPARC/*Sparc* in mice would be expected to be valid in humans, due the high homology between mouse and human SPARC [104], which would reduce the bridge between results in animals and future clinical studies. Perspectives of the future studies on SPARC implications in SM metabolism and contractile properties, both for sedentary and exercise individuals, would be of great clinical importance not only for SM diseases but also for ageing-related health deterioration and, due to the importance of the SM in the energy homeostasis, energy balance-related pathologies such as obesity and diabetes.

Our study was conducted only on male mice. Therefore, we acknowledge the limitation of sex-determined factors. Similar studies in female subjects, as well as studies comparing male and female subjects, will add significant data of clinical importance, especially with the sex-related difference effects on SM and exercise patterns, such as exercise capacity [125], pinch force reproduction [126], maximal oxygen uptake [127], cardiac adaptation to exercise [125], as well as metabolism, including lactate levels [128,129], aerobic oxidation and anaerobic glycolysis [130]. The parameters illustrated by patterns, including differences between men/male animals and women/female animals in red pepper-induced metabolic phenotype (carbohydrate oxidation Vs lipid oxidation) [131,132], beta-oxidation [130], type I fiber percentage [133] and enzyme activities [134], explain beyond such sex-related differences in exercise and SM properties. Based on such sex-related differences, our results could also indicate that SPARC involvement in exercise-

induced muscle phenotype changes could also be sex-dependent and points to a possible interaction between SPARC activities and sexual hormones based on the known impacts of sexual hormones on exercise patterns and the adaptation to exercise [135–137].

Our data indicate that the impacts of *Sparc* KO on body composition, adiposity and metabolic patterns point toward a reduced WAT and body weight, but with negative metabolic and functional phenotypes of SM. Whereas such negative effects on SM worsen with ageing, they are relatively improved by exercise. Importantly, we report, for the first time, evidence suggesting that the exercise-induced changes in the SM phenotype in terms of increased performance (metabolic, strength and development), including lactate-induced changes, are SPARC-dependent. Such important implications of SPARC highlight SPARC and its pathways as pharmacological targets/tools for conditions and diseases in which muscle properties enhancement would provide therapeutic benefits.

Author Contributions: Data curation, A.G., A.M., M.Y. and J.S.-A.; Formal analysis, A.G., A.M. and M.Y.; Funding acquisition, J.S.-A.; Investigation, A.G., A.M., M.Y. and J.S.-A.; Methodology, A.G., A.M., M.Y. and J.S.-A.; Project administration, M.Y. and J.S. A.; Supervision, M.Y. and J.S.-A.; Validation, M.Y. and J.S.-A.; Visualization, A.G., A.M., M.Y. and J.S.-A.; Writing-original draft, A.G.; Writing-review & editing, A.G., A.M., M.Y. and J.S.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Canadian Institutes of Health Research (CIHR), Number: 201309MOP-311306-BCA-CFBA-40425.

Acknowledgments: We thank Amy D. Bradshaw for providing our research group with sperm of *Sparc* KO mice. Abdelaziz Ghanemi received a Merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Figures 20.2 and 20.3 were created using images from http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

20.9 References

1. Pedersen, B.K. The physiology of optimizing health with a focus on exercise as medicine. *Annu. Rev. Physiol.* 2019, *81*, 607–627.
2. Aoi, W.; Sakuma, K. Skeletal muscle: Novel and intriguing characteristics as a secretory organ. *BioDiscovery* 2013, *7*, e8942.

3. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and high-fat diet in obesity: Functional genomics perspectives of two energy homeostasis pillars. *Genes* 2020, *11*, 875.
4. Berryhill, B.L.; Kane, B.; Stramer, B.M.; Fini, M.E.; Hassell, J.R. Increased SPARC accumulation during corneal repair. *Exp. Eye Res.* 2003, *77*, 85–92.
5. Phan, E.; Ahluwalia, A.; Tarnawski, A.S. Role of SPARC—Matricellular protein in pathophysiology and tissue injury healing. Implications for gastritis and gastric ulcers. *Med. Sci. Monit.* 2007, *13*, RA25–RA30.
6. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
7. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.
8. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
9. Matsuo, K.; Sato, K.; Suemoto, K.; Miyamoto-Mikami, E.; Fuku, N.; Higashida, K.; Tsuji, K.; Xu, Y.; Liu, X.; Iemitsu, M.; et al. A mechanism underlying preventive effect of high-intensity training on colon cancer. *Med. Sci. Sports Exerc.* 2017, *49*, 1805–1816.
10. Norheim, F.; Raastad, T.; Thiede, B.; Rustan, A.C.; Drevon, C.A.; Haugen, F. Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. *Am. J. Physiol. Endocrinol. Metab.* 2011, *301*, E1013–E1021.
11. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma myokine concentrations after acute exercise in non-obese and obese sedentary women. *Front. Physiol.* 2020, *11*, 18.
12. Billat, V.L.; Mouisel, E.; Roblot, N.; Melki, J. Inter- and intrastrain variation in mouse critical running speed. *J. Appl. Physiol. (1985)* 2005, *98*, 1258–1263.
13. Available online: https://insights.envigo.com/hubfs/resources/data-sheets/2018s-datasheet-0915.pdf (accessed on 9 August 2020).
14. Norose, K.; Lo, W.K.; Clark, J.I.; Sage, E.H.; Howe, C.C. Lenses of SPARC-null mice exhibit an abnormal cell surface-basement membrane interface. *Exp. Eye Res.* 2000, *71*, 295–307.
15. Nie, J.; Bradshaw, A.D.; Delany, A.M.; Sage, E.H. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. *Connect. Tissue Res.* 2011, *52*, 99–108.
16. Faude, O.; Kindermann, W.; Meyer, T. Lactate threshold concepts: How valid are they? *Sports Med.* 2009, *39*, 469–490.
17. Schefer, V.; Talan, M.I. Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity. *Exp. Gerontol.* 1996, *31*, 387–392.
18. Nishida, Y.; Tokuyama, K.; Nagasaka, S.; Higaki, Y.; Shirai, Y.; Kiyonaga, A.; Shindo, M.; Kusaka, I.; Nakamura, T.; Ishibashi, S.; et al. Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartment labeled minimal model. *Diabetes* 2004, *53*, 315–320.
19. Tanaka, H.; Shindo, M. The benefits of the low intensity training. *Ann. Physiol. Anthr.* 1992, *11*, 365–368.
20. Motoyama, M.; Sunami, Y.; Kinoshita, F.; Kiyonaga, A.; Tanaka, H.; Shindo, M.; Irie, T.; Urata, H.; Sasaki, J.; Arakawa, K. Blood pressure lowering effect of low intensity aerobic training in elderly hypertensive patients. *Med. Sci. Sports Exerc.* 1998, *30*, 818–823.
21. Sunami, Y.; Motoyama, M.; Kinoshita, F.; Mizooka, Y.; Sueta, K.; Matsunaga, A.; Sasaki, J.; Tanaka, H.; Shindo, M. Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. *Metabolism* 1999, *48*, 984–988.
22. Kawanishi, N.; Yano, H.; Mizokami, T.; Takahashi, M.; Oyanagi, E.; Suzuki, K. Exercise training attenuates hepatic inflammation, fibrosis and macrophage infiltration during diet induced-obesity in mice. *Brain Behav. Immun.* 2012, *26*, 931–941.

23. Van Praag, H.; Shubert, T.; Zhao, C.; Gage, F.H. Exercise enhances learning and hippocampal neurogenesis in aged mice. *J. Neurosci.* 2005, *25*, 8680–8685.
24. Rowlatt, C.; Chesterman, F.C.; Sheriff, M.U. Lifespan, age changes and tumour incidence in an ageing C57BL mouse colony. *Lab. Anim.* 1976, *10*, 419–442.
25. Kunstyr, I.; Leuenberger, H.G. Gerontological data of C57BL/6J mice. I. Sex differences in survival curves. *J. Gerontol.* 1975, *30*, 157–162.
26. Smith, J.P.; Hicks, P.S.; Ortiz, L.R.; Martinez, M.J.; Mandler, R.N. Quantitative measurement of muscle strength in the mouse. *J. Neurosci. Methods* 1995, *62*, 15–19.
27. Takeshita, H.; Yamamoto, K.; Nozato, S.; Inagaki, T.; Tsuchimochi, H.; Shirai, M.; Yamamoto, R.; Imaizumi, Y.; Hongyo, K.; Yokoyama, S.; et al. Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice. *Sci. Rep.* 2017, *7*, 42323.
28. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
29. Zong, S.; Wu, M.; Gu, J.; Liu, T.; Guo, R.; Yang, M. Structure of the intact 14 subunit human cytochrome c oxidase. *Cell Res.* 2018, *28*, 1026–1034.
30. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 2012, *9*, 671–675.
31. Taylor, S.C.; Berkelman, T.; Yadav, G.; Hammond, M. A defined methodology for reliable quantification of Western blot data. *Mol. Biotechnol.* 2013, *55*, 217–226.
32. Taylor, S.C.; Posch, A. The design of a quantitative western blot experiment. *Biomed. Res. Int.* 2014, *2014*, 361590.
33. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
34. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; StAmand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity (Silver Spring)* 2013, *21*, 1389–1395.
35. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J. Clin. Investig.* 2001, *107*, 1049–1054.
36. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix communication. *Matrix Biol.* 2001, *19*, 816–827.
37. Lane, T.F.; Sage, E.H. The biology of SPARC, a protein that modulates cell-matrix interactions. *FASEB J.* 1994, *8*, 163–173.
38. Yan, Q.; Sage, E.H. SPARC, a matricellular glycoprotein with important biological functions. *J. Histochem. Cytochem.* 1999, *47*, 1495–1506.
39. Mason, I.J.; Murphy, D.; Münke, M.; Francke, U.; Elliott, R.W.; Hogan, B.L. Developmental and transformation-sensitive expression of the Sparc gene on mouse chromosome 11. *EMBO J.* 1986, *5*, 1831 1837.
40. Swaroop, A.; Hogan, B.L.; Francke, U. Molecular analysis of the cDNA for human SPARC/osteonectin/BM-40: Sequence, expression, and localization of the gene to chromosome 5q31-q33. *Genomics* 1988, *2*, 37–47.
41. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix. *Matrix Biol.* 2000, *19*, 569–580.
42. Francki, A.; Motamed, K.; McClure, T.D.; Kaya, M.; Murri, C.; Blake, D.J.; Carbon, J.G.; Sage, E.H. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. *J. Cell. Biochem.* 2003, *88*, 802–811.
43. Basu, A.; Kligman, L.H.; Samulewicz, S.J.; Howe, C.C. Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40). *BMC Cell Biol.* 2001, *2*, 15.

44. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
45. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine: Metabolic and homeostatic properties beyond the extracellular matrix structure. *Appl. Sci.* 2020, *10*, 2388.
46. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
47. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
48. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schrøder, H.D. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. *J. Histochem. Cytochem.* 2009, *57*, 29–39.
49. Holland, P.W.; Harper, S.J.; McVey, J.H.; Hogan, B.L. In vivo expression of mRNA for the Ca++ binding protein SPARC (osteonectin) revealed by in situ hybridization. *J. Cell Biol.* 1987, *105*, 473–482.
50. Sage, H.; Vernon, R.B.; Decker, J.; Funk, S.; Iruela-Arispe, M.L. Distribution of the calcium-binding protein SPARC in tissues of embryonic and adult mice. *J. Histochem. Cytochem.* 1989, *37*, 819–829.
51. Framson, P.E.; Sage, E.H. SPARC and tumor growth: Where the seed meets the soil? *J. Cell. Biochem.* 2004, *92*, 679–690.
52. Holloszy, J.O. Muscle metabolism during exercise. *Arch. Phys. Med. Rehabil.* 1982, *63*, 231–234.
53. Ferraro, E.; Giammarioli, A.M.; Chiandotto, S.; Spoletini, I.; Rosano, G. Exercise-induced skeletal muscle remodeling and metabolic adaptation: Redox signaling and role of autophagy. *Antioxid. Redox Signal.* 2014, *21*, 154–176.
54. Jørgensen, L.H.; Jepsen, P.L.; Boysen, A.; Dalgaard, L.B.; Hvid, L.G.; Ørtenblad, N.; Ravn, D.; Sellathurai, J.; Møller-Jensen, J.; Lochmüller, H.; et al. SPARC interacts with actin in skeletal muscle in vitro and in vivo. *Am. J. Pathol.* 2017, *187*, 457–474.
55. Chlenski, A.; Guerrero, L.J.; Salwen, H.R.; Yang, Q.; Tian, Y.; La Madrid, A.M.; Mirzoeva, S.; Bouyer, P.G.; Xu, D.; Walker, M.; et al. Secreted protein acidic and rich in cysteine is a matrix scavenger chaperone. *PLoS ONE* 2011, *6*, e23880.
56. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectin-deficient mice. *J. Clin. Investig.* 2000, *105*, 915–923.
57. Wang, H.; Fertala, A.; Ratner, B.D.; Sage, E.H.; Jiang, S. Identifying the SPARC binding sites on collagen I and procollagen I by atomic force microscopy. *Anal. Chem.* 2005, *77*, 6765–6771.
58. Giudici, C.; Raynal, N.; Wiedemann, H.; Cabral, W.A.; Marini, J.C.; Timpl, R.; Bächinger, H.P.; Farndale, R.W.; Sasaki, T.; Tenni, R. Mapping of SPARC/BM-40/osteonectin-binding sites on fibrillar collagens. *J. Biol. Chem.* 2008, *283*, 19551–19560.
59. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. *J. Vet. Med. Sci.* 2019, *81*, 1649–1654.
60. Sage, H.; Vernon, R.B.; Funk, S.E.; Everitt, E.A.; Angello, J. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. *J. Cell Biol.* 1989, *109*, 341–356.
61. Francki, A.; Bradshaw, A.D.; Bassuk, J.A.; Howe, C.C.; Couser, W.G.; Sage, E.H. SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. *J. Biol. Chem.* 1999, *274*, 32145–32152.
62. Bradshaw, A.D.; Reed, M.J.; Sage, E.H. SPARC-null mice exhibit accelerated cutaneous wound closure. *J. Histochem. Cytochem.* 2002, *50*, 1–10.
63. Melouane, A.; Yoshioka, M.; St-Amand, J. Extracellular matrix/mitochondria pathway: A novel potential target for sarcopenia. *Mitochondrion* 2020, *50*, 63–70.
64. Song, H.; Guan, Y.; Zhang, L.; Li, K.; Dong, C. SPARC interacts with AMPK and regulates GLUT4

expression. *Biochem. Biophys. Res. Commun.* 2010, *396*, 961–966.
65. Lira, V.A.; Benton, C.R.; Yan, Z.; Bonen, A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. *Am. J. Physiol. Endocrinol. Metab.* 2010, *299*, E145–E161.
66. Jäger, S.; Handschin, C.; St-Pierre, J.; Spiegelman, B.M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc. Natl. Acad. Sci. USA* 2007, *104*, 12017–12022.
67. Wu, Z.; Puigserver, P.; Andersson, U.; Zhang, C.; Adelmant, G.; Mootha, V.; Troy, A.; Cinti, S.; Lowell, B.; Scarpulla, R.C.; et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999, *98*, 115–124.
68. Smith, G.M.; Gallo, G. The role of mitochondria in axon development and regeneration. *Dev. Neurobiol.* 2018, *78*, 221–237.
69. Han, S.M.; Baig, H.S.; Hammarlund, M. Mitochondria localize to injured axons to support regeneration. *Neuron* 2016, *92*, 1308–1323.
70. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. *FASEB J.* 2019, *33*, 10551–10562.
71. Bradshaw, A.D. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? *J. Mol. Cell. Cardiol.* 2016, *93*, 156–161.
72. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and secreted protein acidic and rich in cysteine (SPARC). *J. Mol. Cell. Cardiol.* 2010, *48*, 544–549.
73. Cheng, L.; Sun, X.; Guo, J.; Lu, G. SPARC support the expansion of cord blood stem cells in vitro. *Cell Res.* 2008, *18*, S49.
74. Zhu, J.; Wang, L.Y.; Li, C.Y.; Wu, J.Y.; Zhang, Y.T.; Pang, K.P.; Wei, Y.; Du, L.Q.; Liu, M.; Wu, X.Y. SPARC promotes self-renewal of limbal epithelial stem cells and ocular surface restoration through JNK and p38-MAPK signaling pathways. *Stem Cells* 2020, *38*, 134–145.
75. Rooney, K.; Trayhurn, P. Lactate and the GPR81 receptor in metabolic regulation: Implications for adipose tissue function and fatty acid utilisation by muscle during exercise. *Br. J. Nutr.* 2011, *106*, 1310–1316.
76. Gladden, L.B. Muscle as a consumer of lactate. *Med. Sci. Sports Exerc.* 2000, *32*, 764–771.
77. Gladden, L.B. Lactate metabolism: A new paradigm for the third millennium. *J. Physiol.* 2004, *558*, 5–30.
78. Hashimoto, T.; Hussien, R.; Oommen, S.; Gohil, K.; Brooks, G.A. Lactate sensitive transcription factor network in L6 cells: Activation of MCT1 and mitochondrial biogenesis. *FASEB J.* 2007, *21*, 2602–2612.
79. Hashimoto, T.; Brooks, G.A. Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Med. Sci. Sports Exerc.* 2008, *40*, 486–494.
80. Gladden, L.B. A lactatic perspective on metabolism. *Med. Sci. Sports Exerc.* 2008, *40*, 477–485.
81. Jacobs, I. Blood lactate. *Sports Med.* 1986, *3*, 10–25.
82. Campolongo, M.; Benedetti, L.; Podhajcer, O.L.; Pitossi, F.; Depino, A.M. Hippocampal SPARC regulates depression-related behavior. *Genes Brain Behav.* 2012, *11*, 966–976.
83. Leveille, G.A.; Chakrabarty, K. Diurnal variations in tissue glycogen and liver weight of meal-fed rats. *J. Nutr.* 1967, *93*, 546–554.
84. Kreitzman, S.N.; Coxon, A.Y.; Szaz, K.F. Glycogen storage: Illusions of easy weight loss, excessive weight regain, and distortions in estimates of body composition. *Am. J. Clin. Nutr.* 1992, *56* (Suppl. 1), 292s–293s.
85. Ma, C.L.; Ma, X.T.; Wang, J.J.; Liu, H.; Chen, Y.F.; Yang, Y. Physical exercise induces hippocampal neurogenesis and prevents cognitive decline. *Behav. Brain Res.* 2017, *317*, 332–339.
86. Yuan, T.F.; Paes, F.; Arias-Carrión, O.; Ferreira Rocha, N.B.; de Sá Filho, A.S.; Machado, S. Neural

mechanisms of exercise: Anti-depression, neurogenesis, and serotonin signaling. *CNS Neurol. Disord. Drug Targets* 2015, *14*, 1307–1311.
87. Uddin, M.S.; Hossain, M.F.; Mamun, A.A.; Shah, M.A.; Hasana, S.; Bulbul, I.J.; Sarwar, M.S.; Mansouri, R.A.; Ashraf, G.M.; Rauf, A.; et al. Exploring the multimodal role of phytochemicals in the modulation of cellular signaling pathways to combat age-related neurodegeneration. *Sci. Total Environ.* 2020, *725*, 138313.
88. Hung, C.W.; Chen, Y.C.; Hsieh, W.L.; Chiou, S.H.; Kao, C.L. Ageing and neurodegenerative diseases. *Ageing Res. Rev.* 2010, *9* (Suppl. 1), S36–S46.
89. Mahalakshmi, B.; Maurya, N.; Lee, S.-D.; Bharath Kumar, V. Possible neuroprotective mechanisms of physical exercise in neurodegeneration. *Int. J. Mol. Sci.* 2020, *21*, 5895.
90. Ang, E.-T.; Tai, Y.-K.; Lo, S.-Q.; Seet, R.; Soong, T.-W. Neurodegenerative diseases: Exercising toward neurogenesis and neuroregeneration. *Front. Aging Neurosci.* 2010, *2*, 25.
91. Liu, Y.; Yan, T.; Chu, J.M.; Chen, Y.; Dunnett, S.; Ho, Y.S.; Wong, G.T.; Chang, R.C. The beneficial effects of physical exercise in the brain and related pathophysiological mechanisms in neurodegenerative diseases. *Lab. Investig.* 2019, *99*, 943–957.
92. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. *Cell Metab.* 2016, *23*, 554–562.
93. Wedell-Neergaard, A.S.; Lang Lehrskov, L.; Christensen, R.H.; Legaard, G.E.; Dorph, E.; Larsen, M.K.; Launbo, N.; Fagerlind, S.R.; Seide, S.K.; Nymand, S.; et al. Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: A randomized controlled trial. *Cell Metab.* 2019, *29*, 844–855.e3.
94. Ellingsgaard, H.; Hojman, P.; Pedersen, B.K. Exercise and health—Emerging roles of IL-6. *Curr. Opin. Physiol.* 2019, *10*, 49–54.
95. Sakuma, K.; Yamaguchi, A. Sarcopenic obesity and endocrinal adaptation with age. *Int. J. Endocrinol.* 2013, *2013*, 204164.
96. Candow, D.G.; Chilibeck, P.D. Differences in size, strength, and power of upper and lower body muscle groups in young and older men. *J. Gerontol. A Biol. Sci. Med. Sci.* 2005, *60*, 148–156.
97. Kalyani, R.R.; Corriere, M.; Ferrucci, L. Age-related and disease-related muscle loss: The effect of diabetes, obesity, and other diseases. *Lancet Diabetes Endocrinol.* 2014, *2*, 819–829.
98. Kim, T.N.; Choi, K.M. The implications of sarcopenia and sarcopenic obesity on cardiometabolic disease. *J. Cell. Biochem.* 2015, *116*, 1171–1178.
99. Dhingra, R.; Vasan, R.S. Age as a risk factor. *Med. Clin. N. Am.* 2012, *96*, 87–91.
100. Keenan, C.R.; White, R.H. Age as a risk factor for venous thromboembolism after major surgery. *Curr. Opin. Pulm. Med.* 2005, *11*, 398–402.
101. Koshiyama, M.; Tamaki, K.; Ohsawa, M. Age-specific incidence rates of atrial fibrillation and risk factors for the future development of atrial fibrillation in the Japanese general population. *J. Cardiol.* 2020, *77*, 88–92.
102. Zhang, H.; Rogers, K.; Sukkar, L.; Jun, M.; Kang, A.; Young, T.; Campain, A.; Cass, A.; Chow, C.K.; Comino, E.; et al. Prevalence, incidence and risk factors of diabetes in Australian adults aged ≥ 45 years: A cohort study using linked routinely-collected data. *J. Clin. Transl. Endocrinol.* 2020, *22*, 100240.
103. Mosher, C.L.; Weber, J.M.; Frankel, C.W.; Neely, M.L.; Palmer, S.M. Risk factors for mortality in lung transplant recipients aged ≥ 65 years: A retrospective cohort study of 5815 patients in the scientific registry of transplant recipients. *J. Heart Lung Transplant.* 2020.
104. Norose, K.; Clark, J.I.; Syed, N.A.; Basu, A.; Heber-Katz, E.; Sage, E.H.; Howe, C.C. SPARC deficiency leads to early-onset cataractogenesis. *Investig. Ophthalmol. Vis. Sci.* 1998, *39*, 2674–2680.
105. Singh, M. Mood, food, and obesity. *Front. Psychol.* 2014, *5*, 925.
106. Ortolani, D.; Oyama, L.M.; Ferrari, E.M.; Melo, L.L.; Spadari-Bratfisch, R.C. Effects of comfort food on food intake, anxiety-like behavior and the stress response in rats. *Physiol. Behav.* 2011, *103*, 487–492.
107. Bradshaw, A.D.; Graves, D.C.; Motamed, K.; Sage, E.H. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 6045–6050.

108. Sinclair, A.J.; Abdelhafiz, A.H. Cardiometabolic disease in the older person: Prediction and prevention for the generalist physician. *Cardiovasc. Endocrinol. Metab.* 2020, *9*, 90–95.
109. Islam, H.; Hood, D.A.; Gurd, B.J. Looking beyond PGC-1α: Emerging regulators of exercise-induced skeletal muscle mitochondrial biogenesis and their activation by dietary compounds. *Appl. Physiol. Nutr. Metab.* 2020, *45*, 11–23.
110. Su, J.; Chen, X.; Kanekura, T. A CD147-targeting siRNA inhibits the proliferation, invasiveness, and VEGF production of human malignant melanoma cells by down-regulating glycolysis. *Cancer Lett.* 2009, *273*, 140–147.
111. Gillies, R.J.; Raghunand, N.; Karczmar, G.S.; Bhujwalla, Z.M. MRI of the tumor microenvironment. *J. Magn. Reason. Imaging* 2002, *16*, 430–450.
112. Gladden, L.B. Current trends in lactate metabolism: Introduction. *Med. Sci. Sports Exerc.* 2008, *40*, 475 476.
113. Brooks, G.A. Lactate shuttles in nature. *Biochem. Soc. Trans.* 2002, *30*, 258–264.
114. Brooks, G.A. Current concepts in lactate exchange. *Med. Sci. Sports Exerc.* 1991, *23*, 895–906.
115. Sasaki, T.; Hohenester, E.; Göhring, W.; Timpl, R. Crystal structure and mapping by site-directed mutagenesis of the collagen-binding epitope of an activated form of BM-40/SPARC/osteonectin. *EMBO J.* 1998, *17*, 1625–1634.
116. Bailey, A.J. The role of collagen in the development of muscle and its relationship to eating quality. *J. Anim. Sci.* 1985, *60*, 1580–1587.
117. Au, E.; Richter, M.W.; Vincent, A.J.; Tetzlaff, W.; Aebersold, R.; Sage, E.H.; Roskams, A.J. SPARC from olfactory ensheathing cells stimulates Schwann cells to promote neurite outgrowth and enhances spinal cord repair. *J. Neurosci.* 2007, *27*, 7208–7221.
118. Lee, M.H.; Amin, N.D.; Venkatesan, A.; Wang, T.; Tyagi, R.; Pant, H.C.; Nath, A. Impaired neurogenesis and neurite outgrowth in an HIV-gp120 transgenic model is reversed by exercise via BDNF production and Cdk5 regulation. *J. Neurovirol.* 2013, *19*, 418–431.
119. Vincent, A.J.; Lau, P.W.; Roskams, A.J. SPARC is expressed by macroglia and microglia in the developing and mature nervous system. *Dev. Dyn.* 2008, *237*, 1449–1462.
120. Brekken, R.A.; Puolakkainen, P.; Graves, D.C.; Workman, G.; Lubkin, S.R.; Sage, E.H. Enhanced growth of tumors in SPARC null mice is associated with changes in the ECM. *J. Clin. Investig.* 2003, *111*, 487–495.
121. Puolakkainen, P.A.; Brekken, R.A.; Muneer, S.; Sage, E.H. Enhanced growth of pancreatic tumors in SPARC-null mice is associated with decreased deposition of extracellular matrix and reduced tumor cell apoptosis. *Mol. Cancer Res.* 2004, *2*, 215–224.
122. Said, N.; Frierson, H.F.; Sanchez-Carbayo, M.; Brekken, R.A.; Theodorescu, D. Loss of SPARC in bladder cancer enhances carcinogenesis and progression. *J. Clin. Investig.* 2013, *123*, 751–766.
123. Liu, Y.P.; Hsiao, M. Exercise-induced SPARC prevents tumorigenesis of colon cancer. *Gut* 2013, *62*, 810–811.
124. Aoi, W. Possibility of the novel myokine SPARC: A mechanistic approach to colon cancer prevention by physical exercise. *Jpn. J. Phys. Fit. Sports Med.* 2013, *62*, 263–271.
125. Konhilas, J.P.; Maass, A.H.; Luckey, S.W.; Stauffer, B.L.; Olson, E.N.; Leinwand, L.A. Sex modifies exercise and cardiac adaptation in mice. *Am. J. Physiol. Heart Circ. Physiol.* 2004, *287*, H2768–H2776.
126. Li, L.; Li, Y.; Wang, H.; Chen, W.; Liu, X. Effect of force level and gender on pinch force perception in healthy adults. *Iperception* 2020, *11*, 2041669520927043.
127. Sundberg, S. Maximal oxygen uptake in relation to age in blind and normal boys and girls. *Acta Paediatr. Scand.* 1982, *71*, 603–608.
128. Hafen, P.S.; Vehrs, P.R. Sex-related differences in the maximal lactate steady state. *Sports* 2018, *6*, 154.
129. Zhang, J.; Ji, L. Gender differences in peak blood lactate concentration and lactate removal. *Ann. Sports Med. Res.* 2016, *3*, 1088.
130. Green, H.J.; Fraser, I.G.; Ranney, D.A. Male and female differences in enzyme activities of energy

metabolism in vastus lateralis muscle. *J. Neurol. Sci.* 1984, *65*, 323–331.
131. Yoshioka, M.; St-Pierre, S.; Suzuki, M.; Tremblay, A. Effects of red pepper added to high-fat and high carbohydrate meals on energy metabolism and substrate utilization in Japanese women. *Br. J. Nutr.* 1998, *80*, 503–510.
132. Yoshioka, M.; Lim, K.; Kikuzato, S.; Kiyonaga, A.; Tanaka, H.; Shindo, M.; Suzuki, M. Effects of red pepper diet on the energy metabolism in men. *J. Nutr. Sci. Vitam. (Tokyo)* 1995, *41*, 647–656.
133. Simoneau, J.A.; Bouchard, C. Human variation in skeletal muscle fiber-type proportion and enzyme activities. *Am. J. Physiol.* 1989, *257*, E567–E572.
134. Simoneau, J.A.; Lortie, G.; Boulay, M.R.; Thibault, M.C.; Thériault, G.; Bouchard, C. Skeletal muscle histochemical and biochemical characteristics in sedentary male and female subjects. *Can. J. Physiol. Pharm.* 1985, *63*, 30–35.
135. Di Luigi, L.; Romanelli, F.; Sgrò, P.; Lenzi, A. Andrological aspects of physical exercise and sport medicine. *Endocrine* 2012, *42*, 278–284.
136. Isacco, L.; Duché, P.; Boisseau, N. Influence of hormonal status on substrate utilization at rest and during exercise in the female population. *Sports Med.* 2012, *42*, 327–342.
137. Amelink, G.J.; Bär, P.R. Exercise-induced muscle protein leakage in the rat. Effects of hormonal manipulation. *J. Neurol. Sci.* 1986, *76*, 61–68.

Chapter 21. Opinion - Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec City, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec City, QC G1V 0A6, Canada

Received: 29 October 2021; Accepted: 28 November 2021; Published: 29 November 2021

21.1 Résumé (French abstract)

Le vieillissement et l'obésité représentent des défis médicaux pour les professionnels de la santé. L'obésité et le vieillissement partagent des caractéristiques communes, y compris des voies cellulaires et moléculaires connexes ainsi que les impacts qu'elles ont en tant que facteurs de risque pour une variété de maladies et de problèmes de santé. Le vieillissement et l'obésité partagent aussi l'exercice et un mode de vie sain comme les meilleures options thérapeutiques. Il est important de noter que le vieillissement et l'obésité ont également des changements épigénétiques communs qui sont affectés par l'exercice. Cela suggère que les voies épigénétiques font partie des mécanismes par lesquels l'exercice induit ses avantages, y compris les améliorations du vieillissement et de l'obésité. L'exploration de ces interrelations conduirait à optimiser les approches thérapeutiques disponibles pour améliorer la gestion de l'obésité et du vieillissement.

21.2 Abstract

In modern societies, ageing and obesity represent medical challenges for healthcare professionals and caregivers. Obesity and ageing share common features including the related cellular and molecular pathways as well as the impacts they have as risk factors for a variety of diseases and health problems. Both of these health problems also share exercise and a healthy lifestyle as the best therapeutic options. Importantly, ageing and obesity also have common epigenetic changes (histone modification, DNA methylation, noncoding RNAs, and chromatin remodeling) that are also impacted by exercise. This suggests that epigenetic pathways are among the mechanisms via which exercise induces its benefits, including ageing and obesity improvements. Exploring these interrelations and based on the fact that both ageing and obesity represent risk factors for each other, would lead to optimizing the available therapeutic approaches towards improved obesity management and healthy ageing.

Keywords: obesity; ageing; epigenetics; pathogenesis; exercise

21.3 Biological Similarities between Ageing and Obesity

Ageing and obesity are major topics in biomedical studies, mainly because both represent risk factors for numerous diseases and health conditions [1]. The modern lifestyle and industrial development have increased obesity rates as well as the aged population percentage worldwide. Obesity is specifically increasing among the elderly [2,3], which contributes to sarcopenic obesity, a chronic, age-related class of obesity [4,5]. These interactions between two important risk factors strengthen the need to further deepen our biological and clinical understanding of the interrelation and correlations between ageing and obesity. Such mechanistic elucidation would allow to develop the medical care including geriatrics and obesity management, among other chronic diseases. Within this piece of writing, we aim to elucidate selected links between ageing and obesity through different illustrations starting from pathogenesis and molecular pathways towards epigenetics, supported by evidence from exercise being a therapeutic tool for both.

Obesity is defined as an abnormal accumulation of adiposity resulting from a disturbed energy balance in which energy intake is higher than energy expenditure [6] with a modified metabolic phenotype [7], complex neuroendocrine changes [8], and pathogenic implications [9]. Obesity has even been classified as a disease [10] and associated with health problems including impaired fertility [11,12], neurodegenerative disease [13], cognitive decline (in mid-life) [14], coronavirus disease 2019 (COVID-19) severity and resulting health problems [15–18], type 2 diabetes [19], cancer [20], cardiovascular diseases [21], pulmonary diseases [22], insulin resistance [23], atherosclerosis [24], mitochondrial dysfunction [25], dyslipidemia [26], liver disease [27], impaired immunity [28,29], and impaired regeneration [30]. Ageing, on the other hand, represents the progressive decline of the biological functions with time [31]. It also represents a risk factor for numerous diseases and health conditions, many of which are similar to those associated with obesity. These include neurodegenerative disease [32], cognitive decline [33], COVID-19 severity [34], type 2 diabetes [35], skeletal muscle loss [36], cancer [37], cardiovascular disease [38], pulmonary disease [39], insulin resistance [40], atherosclerosis [41], mitochondrial dysfunction [42], dyslipidemia [43], liver disease [44], fertility alteration [45,46] immunity alteration [47], and declined regeneration [48].

Although the risks related to obesity are independent from ageing and those related to ageing are independent from obesity, such similarities between ageing and obesity as risk factors suggest common patterns and share underlying mechanisms of both ageing and obesity. Early epidemiologic data approved the prevalence of obesity increases by ageing, especially in women. Therefore, the ongoing step is to know more about how ageing and obesity could be related at the molecular level. Within this context, obesity and ageing have been described as sharing common pathways at the molecular and cellular

levels. For instance, in both, we have increased inflammation [49,50], free radicals, and oxidative stress [51,52] as well as microbiota changes [53,54]. In addition, healthy diet and physical activity are prescribed to manage obesity [55,56] and also optimize healthy ageing [57,58]. While the main goal of prescribing the physical activity in obesity is to increase the energy expenditure and, thus, reduce the adiposity and lose weight [59,60], in ageing, the physical activity aims mostly to improve muscular and metabolic performance [57,61,62]. Importantly, physical activity as a common therapy for both ageing and obesity has significant impacts on reducing the risk factors mediated by ageing and obesity and also improves numerous biomolecular markers and pathological profiles. As illustrations, physical activity improves and optimizes treatment/prevention or reduces the risk of metabolic disorders [63], cancer [64], cardiovascular disease [65–67], immune functions [68], insulin resistance [69], oxidative stress [70], liver disease [71], regeneration [72,73], pulmonary disease [74,75], atherosclerosis [76], and mitochondrial remodeling [77]. These evidence add up on those of functional genomics [78–81] as illustrated by the secreted protein acidic and rich in cysteine (SPARC). Indeed, SPARC expression changes during obesity [82] and with ageing [83] and *Sparc/SPARC* represents an exercise-induced gene upon which exercise-induced muscle phenotype changes would depend [84,85]. In addition, SPARC is involved in diverse biological activities [86] related to those described above in the context of obesity, ageing, and exercise. These include metabolic and homeostatic properties [87], inflammation [88], cancer [89], regeneration [90], and metabolism [91]. This exercise-induced key myokine with obesity and age-related expression patterns further points to molecular links between obesity and ageing.

21.4 Epigenetics: An Additional Link between Ageing and Obesity

Furthermore, epigenetics studies provide additional evidence of similar patterns shared by obesity and ageing. Therefore, epigenetics represents a field worth exploring to reveal further links between obesity and ageing. This is reflected by the changes such as histone modification, DNA methylation, noncoding RNAs, and chromatin remodeling that have been associated with both ageing [31,92–97] and obesity [98–103]. These changes can follow diverse patterns. For instance, region-specific DNA hypermethylation [104] and proliferation-dependent alterations of the DNA methylation [105] have been reported in ageing during which we talk about epigenetic clocks [106]. The possible use of DNA methylation-based measures as a tool to evaluate the accelerated biological ageing [107–110] represents a potential application of the DNA methylation age (DNAmAge), which would contribute to several diseases such as obesity. Similarly, obesity-related DNA methylation can be site-specific [111] and with specific methylation signatures [112]. Other related features such as telomere attrition are also shared between ageing [31] and obesity [113,114].

Importantly, exercise - prescribed for both elderly and obese patients - has impacts on the epigenetics patterns related to both ageing and obesity including DNA methylation

[115], histone modification [116], chromatin modifications [117], and noncoding RNAs [118]. These exercise-related properties suggest that epigenetics pathways are among the mechanisms via which exercise induces its benefits-as it has been shown, for instance, for exercise-mediated heart protection [119]. They further support targeting epigenetic pathways as a therapy [120,121] as well; potentially, to treat obesity and improve ageing. These observations also suggest correlations between epigenetics changes and obesity/ageing-related pathologic phenotypes. In addition, these molecular and clinical evidence, from genetics to epigenetics and pathogenesis, further present obesity as a risk factor for ageing and, at the same time, highlight ageing as a risk factor for obesity [1]. This would explain why losing weight "rejuvenates". Moreover, dietary restriction (that has both antiageing and antiobesity effects) also impacts epigenetics towards significant health benefits [122,123] bringing an additional correlation between ageing and obesity.

21.5 Perspectives

These introduced concepts would have important applications in the medical fields, especially that both ageing and obesity are among what medically characterize the epidemiological and pathological profiles of most modern societies. Although a healthy ageing is the optimum target of geriatrics, we have limited options to manage ageing (irreversible time effects). However, obesity, on the other hand, has realistically more management options since it remains relatively reversible. Therefore, managing obesity toward healthy ageing remains more practical than targeting healthy ageing to manage obesity. It is worth noting, however, that treating obesity would optimize ageing and healthy ageing would decrease obesity risk. Nevertheless, the key approach remains to target a healthy lifestyle including exercise, diet, sleeping, and psychological well-being to manage obesity, optimize healthy ageing, and control most diseases' risk factors.

We would like to introduce a new concept via which there is a potential to combine the age-related and the obesity-related epigenetics measures to obtain a full evaluation of how deep both the age and obesity worsen the other as well as the various diseases and risk factors for which either ageing or obesity represent a risk factor. The need to actualize this idea nowadays comes from the urgent epidemiological situations related to ageing and obesity in the modern societies both in developed and in developing countries. To expand this vision, the advances and added value of this work is that it puts epigenetics along with pathological phenotype, molecular patterns, and lifestyle impacts as a set that regroups the elements shared between obesity and ageing (Figure 21.1). Such approaches would allow for optimizing therapies and lifestyle management choices.

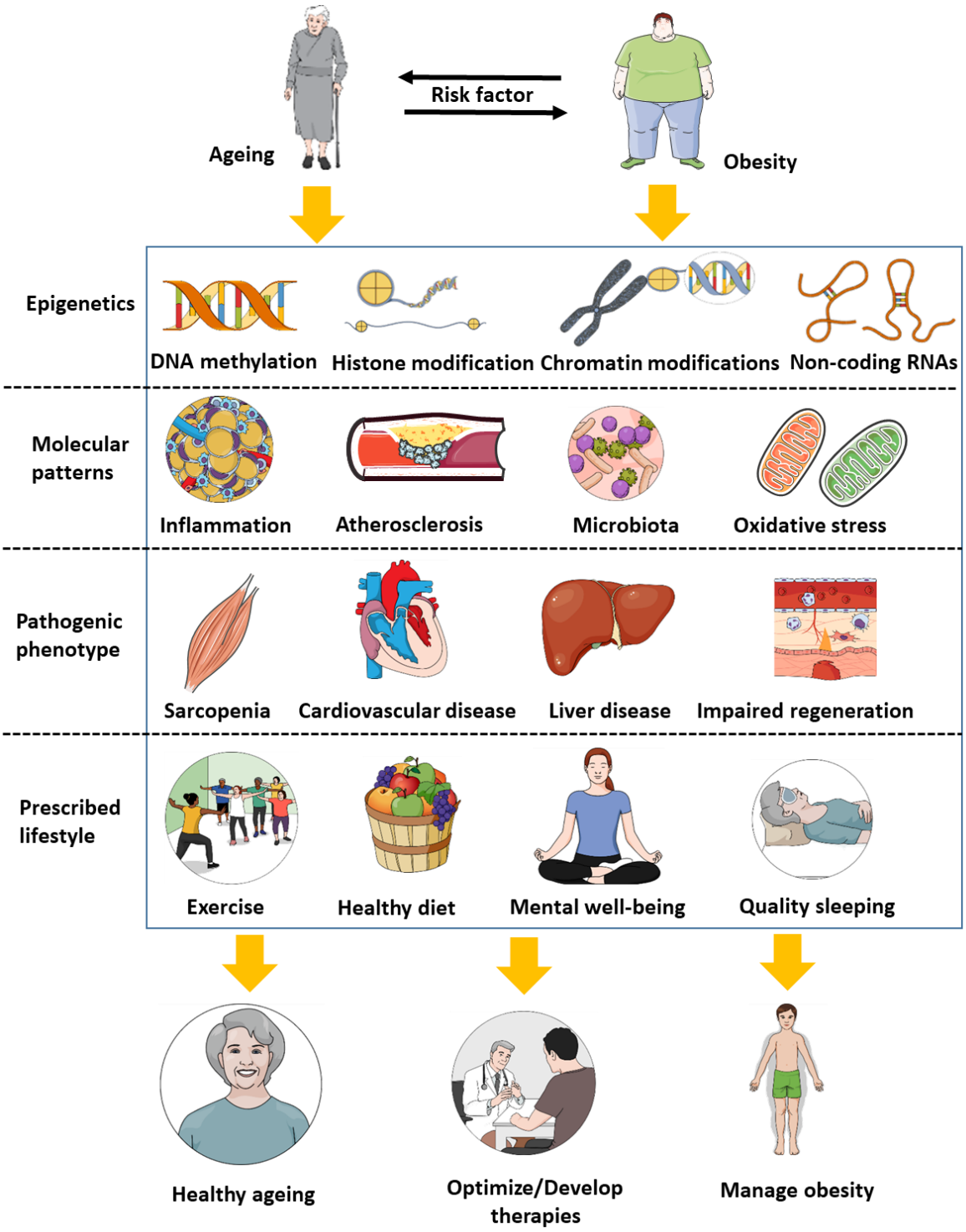


Figure 21.1. Examples of ageing and obesity shared patterns. Both ageing and obesity represent a risk factor for each other. Elucidating the patterns shared between ageing and obesity, from epigenetics to molecular pathogenesis, would allow to both optimize healthy ageing and manage obesity.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y., and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add,

the figure, references selection, etc.) throughout the writing process, edited, and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi is the received the scholarship « Bourse Tremplin -Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec-Sante (FRQS), Quebec, Canada. Figure 21.1 was created using images from https://mindthegraph.com/ and http://smart.servier.com. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (accessed on 26 October.2021).

Conflicts of Interest: The authors declare no conflict of interest.

21.6 References

1. Jura, M.; Kozak, L.P. Obesity and related consequences to ageing. *Age* 2016, *38*, 23.
2. Han, T.S.; Tajar, A.; Lean, M.E. Obesity and weight management in the elderly. *Br. Med. Bull.* 2011, *97*, 169–196.
3. Han, T.S.; Wu, F.C.; Lean, M.E. Obesity and weight management in the elderly: A focus on men. *Best Pract. Res. Clin. Endocrinol. Metab.* 2013, *27*, 509–525.
4. Batsis, J.A.; Villareal, D.T. Sarcopenic obesity in older adults: Aetiology, epidemiology and treatment strategies. *Nat. Rev. Endocrinol.* 2018, *14*, 513–537.
5. Polyzos, S.A.; Margioris, A.N. Sarcopenic obesity. *Hormones* 2018, *17*, 321–331.
6. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
7. Goossens, G.H. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. *Obes. Facts* 2017, *10*, 207–215.
8. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.
9. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages

and Applications. *Medicina* 2021, *57*, 999.
10. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
11. Škurla, M.; Rybáˇr, R. Obesity and reduced fertility of men. *Ceska Gynekol.* 2018, *83*, 212–217.
12. Best, D.; Bhattacharya, S. Obesity and fertility. *Horm. Mol. Biol. Clin. Investig.* 2015, *24*, 5–10.
13. Saad, M.J.; Santos, A.; Prada, P.O. Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance. *Physiology* 2016, *31*, 283–293.
14. Dye, L.; Boyle, N.B.; Champ, C.; Lawton, C. The relationship between obesity and cognitive health and decline. *Proc. Nutr. Soc.* 2017, *76*, 443–454.
15. Petrakis, D.; Margină, D.; Tsarouhas, K.; Tekos, F.; Stan, M.; Nikitovic, D.; Kouretas, D.; Spandidos, D.A.; Tsatsakis, A. Obesity—A risk factor for increased COVID-19 prevalence, severity and lethality (Review). *Mol. Med. Rep.* 2020, *22*, 9–19.
16. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
17. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
18. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Post-Coronavirus Disease-2019 (COVID-19): Toward a Severe Multi-Level Health Crisis? *Med. Sci.* 2021, *9*, 68.
19. Rubio-Almanza, M.; Cámara-Gómez, R.; Merino-Torres, J.F. Obesity and type 2 diabetes: Also linked in therapeutic options. *Endocrinol. Diabetes Nutr.* 2019, *66*, 140–149.
20. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms andperspectives. *Metabolism* 2019, *92*, 121–135.
21. Koliaki, C.; Liatis, S.; Kokkinos, A. Obesity and cardiovascular disease: Revisiting an old relationship. *Metabolism* 2019, *92*, 98–107.
22. Dixon, A.E.; Peters, U. The effect of obesity on lung function. *Expert Rev. Respir. Med.* 2018, *12*, 755 767.
23. Barazzoni, R.; Gortan Cappellari, G.; Ragni, M.; Nisoli, E. Insulin resistance in obesity: An overview of fundamental alterations. *Eat Weight Disord.* 2018, *23*, 149–157.
24. Csige, I.; Ujvárosy, D.; Szabó, Z.; L″orincz, I.; Paragh, G.; Harangi, M.; Somodi, S. The Impact of Obesity on the Cardiovascular System. *J. Diabetes Res.* 2018, *2018*, 3407306.
25. De Mello, A.H.; Costa, A.B.; Engel, J.D.G.; Rezin, G.T. Mitochondrial dysfunction in obesity. *Life Sci.* 2018, *192*, 26–32.
26. Vekic, J.; Zeljkovic, A.; Stefanovic, A.; Jelic-Ivanovic, Z.; Spasojevic-Kalimanovska, V. Obesity and dyslipidemia. *Metabolism* 2019, *92*, 71–81.
27. Polyzos, S.A.; Kountouras, J.; Mantzoros, C.S. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism* 2019, *92*, 82–97.
28. Andersen, C.J.; Murphy, K.E.; Fernandez, M.L. Impact of Obesity and Metabolic Syndrome on Immunity. *Adv. Nutr.* 2016, *7*, 66–75.
29. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. *Medicines* 2021, *8*, 57.
30. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
31. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* 2013, *153*, 1194–1217.
32. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* 2019, *15*, 565–581.
33. Bramorska, A.; Zarzycka, W.; Podolecka, W.; Kuc, K.; Brzezicka, A. Age-Related Cognitive Decline May Be Moderated by Frequency of Specific Food Products Consumption. *Nutrients* 2021, *13*, 2504.
34. Gallo Marin, B.; Aghagoli, G.; Lavine, K.; Yang, L.; Siff, E.J.; Chiang, S.S.; Salazar-Mather, T.P.;

Dumenco, L.; Savaria, M.C.; Aung, S.N.; et al. Predictors of COVID-19 severity: A literature review. *Rev. Med. Virol.* 2021, *31*, 1–10.
35. Al-Sofiani, M.E.; Ganji, S.S.; Kalyani, R.R. Body composition changes in diabetes and aging. *J. Diabetes Complicat.* 2019, *33*, 451–459.
36. Park, S.W.; Goodpaster, B.H.; Lee, J.S.; Kuller, L.H.; Boudreau, R.; de Rekeneire, N.; Harris, T.B.; Kritchevsky, S.; Tylavsky, F.A.; Nevitt, M.; et al. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. *Diabetes Care* 2009, *32*, 1993–1997.
37. Fane, M.; Weeraratna, A.T. How the ageing microenvironment influences tumour progression. *Nat. Rev. Cancer* 2020, *20*, 89–106.
38. Costantino, S.; Paneni, F.; Cosentino, F. Ageing, metabolism and cardiovascular disease. *J. Physiol.* 2016, *594*, 2061–2073.
39. Barnes, P.J. Pulmonary Diseases and Ageing. *Subcell. Biochem.* 2019, *91*, 45–74.
40. Consitt, L.A.; Dudley, C.; Saxena, G. Impact of Endurance and Resistance Training on Skeletal Muscle Glucose Metabolism in Older Adults. *Nutrients* 2019, *11*, 2636.
41. Tyrrell, D.J.; Goldstein, D.R. Ageing and atherosclerosis: Vascular intrinsic and extrinsic factors and potential role of IL-6. *Nat. Rev. Cardiol.* 2021, *18*, 58–68.
42. Tyrrell, D.J.; Blin, M.G.; Song, J.; Wood, S.C.; Zhang, M.; Beard, D.A.; Goldstein, D.R. Age-Associated Mitochondrial Dysfunction Accelerates Atherogenesis. *Circ. Res.* 2020, *126*, 298–314.
43. Liu, H.H.; Li, J.J. Aging and dyslipidemia: A review of potential mechanisms. *Ageing Res. Rev.* 2015, *19*, 43–52.
44. Stahl, E.C.; Haschak, M.J.; Popovic, B.; Brown, B.N. Macrophages in the Aging Liver and Age-Related Liver Disease. *Front. Immunol.* 2018, *9*, 2795.
45. Almeida, S.; Rato, L.; Sousa, M.; Alves, M.G.; Oliveira, P.F. Fertility and Sperm Quality in the Aging Male. *Curr. Pharm. Des.* 2017, *23*, 4429–4437.
46. American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee. Female age-related fertility decline. Committee Opinion No. 589. *Fertil. Steril.* 2014, *101*, 633–634.
47. Sadighi Akha, A.A. Aging and the immune system: An overview. *J. Immunol. Methods* 2018, *463*, 21–26.
48. Sousounis, K.; Baddour, J.A.; Tsonis, P.A. Aging and regeneration in vertebrates. *Curr. Top Dev. Biol.* 2014, *108*, 217–246.
49. Cox, A.J.; West, N.P.; Cripps, A.W. Obesity, inflammation, and the gut microbiota. *Lancet Diabetes Endocrinol.* 2015, *3*, 207–215.
50. Sendama, W. The effect of ageing on the resolution of inflammation. *Ageing Res. Rev.* 2020, *57*, 101000.
51. Pomatto, L.C.D.; Davies, K.J.A. Adaptive homeostasis and the free radical theory of ageing. *Free Radic. Biol Med.* 2018, *124*, 420–430.
52. Rani, V.; Deep, G.; Singh, R.K.; Palle, K.; Yadav, U.C. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sci.* 2016, *148*, 183–193.
53. Torres-Fuentes, C.; Schellekens, H.; Dinan, T.G.; Cryan, J.F. The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol. Hepatol.* 2017, *2*, 747–756.
54. Dinan, T.G.; Cryan, J.F. Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. *J. Physiol.* 2017, *595*, 489–503.
55. Fock, K.M.; Khoo, J. Diet and exercise in management of obesity and overweight. *J. Gastroenterol. Hepatol.* 2013, *28* (Suppl. S4), 59–63.
56. Swift, D.L.; McGee, J.E.; Earnest, C.P.; Carlisle, E.; Nygard, M.; Johannsen, N.M. The Effects of Exercise and Physical Activity on Weight Loss and Maintenance. *Prog. Cardiovasc. Dis.* 2018, *61*, 206–213.
57. Papaioannou, K.G.; Nilsson, A.; Nilsson, L.M.; Kadi, F. Healthy Eating Is Associated with Sarcopenia Risk in Physically Active Older Adults. *Nutrients* 2021, *13*, 2813.
58. Mora, J.C.; Valencia, W.M. Exercise and Older Adults. *Clin. Geriatr. Med.* 2018, *34*, 145–162.
59. Stoner, L.; Beets, M.W.; Brazendale, K.; Moore, J.B.; Weaver, R.G. Exercise Dose and Weight Loss in

Adolescents with Overweight Obesity: A Meta-Regression. *Sports Med.* 2019, *49*, 83–94.
60. Swift, D.L.; Johannsen, N.M.; Lavie, C.J.; Earnest, C.P.; Church, T.S. The role of exercise and physical activity in weight loss and maintenance. *Prog. Cardiovasc. Dis.* 2014, *56*, 441–447.
61. Elia, M. Obesity in the elderly. *Obes. Res.* 2001, *9* (Suppl. S4), 244s–248s.
62. Fleg, J.L. Aerobic exercise in the elderly: A key to successful aging. *Discov. Med.* 2012, *13*, 223–228.
63. Ghanemi, A.; St-Amand, J. Interleukin-6 as a "metabolic hormone". *Cytokine* 2018, *112*, 132–136.
64. Idorn, M.; Thor Straten, P. Exercise and cancer: From "healthy" to "therapeutic"? *Cancer Immunol. Immunother.* 2017, *66*, 667–671.
65. Cattadori, G.; Segurini, C.; Picozzi, A.; Padeletti, L.; Anzà, C. Exercise and heart failure: An update. *ESC Heart Fail.* 2018, *5*, 222–232.
66. Fiuza-Luces, C.; Santos-Lozano, A.; Joyner, M.; Carrera-Bastos, P.; Picazo, O.; Zugaza, J.L.; Izquierdo, M.; Ruilope, L.M.; Lucia, A. Exercise benefits in cardiovascular disease: Beyond attenuation of traditional risk factors. *Nat. Rev. Cardiol.* 2018, *15*, 731–743.
67. Hansen, D.; Niebauer, J.; Cornelissen, V.; Barna, O.; Neunhäuserer, D.; Stettler, C.; Tonoli, C.; Greco, E.; Fagard, R.; Coninx, K.; et al. Exercise Prescription in Patients with Different Combinations of Cardiovascular Disease Risk Factors: A Consensus Statement from the EXPERT Working Group. *Sports Med.* 2018, *48*, 1781–1797.
68. Wang, J.; Liu, S.; Li, G.; Xiao, J. Exercise Regulates the Immune System. *Adv. Exp. Med. Biol.* 2020, *1228*, 395–408.
69. Whillier, S. Exercise and Insulin Resistance. *Adv. Exp. Med. Biol.* 2020, *1228*, 137–150.
70. De Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. *Sports Med.* 2017, *47*, 277–293.
71. Van der Windt, D.J.; Sud, V.; Zhang, H.; Tsung, A.; Huang, H. The Effects of Physical Exercise on Fatty Liver Disease. *Gene Expr.* 2018, *18*, 89–101.
72. Saito, Y.; Chikenji, T.S.; Matsumura, T.; Nakano, M.; Fujimiya, M. Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors. *Nat. Commun.* 2020, *11*, 889.
73. Schüttler, D.; Clauss, S.; Weckbach, L.T.; Brunner, S. Molecular Mechanisms of Cardiac Remodeling and Regeneration in Physical Exercise. *Cells* 2019, *8*, 1128.
74. Paneroni, M.; Simonelli, C.; Vitacca, M.; Ambrosino, N. Aerobic Exercise Training in Very Severe Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. *Am. J. Phys. Med. Rehabil.* 2017, *96*, 541–548.
75. Nolan, C.M.; Rochester, C.L. Exercise Training Modalities for People with Chronic Obstructive Pulmonary Disease. *COPD* 2019, *16*, 378–389.
76. Yang, J.; Cao, R.Y.; Gao, R.; Mi, Q.; Dai, Q.; Zhu, F. Physical Exercise Is a Potential "Medicine" for Atherosclerosis. *Adv. Exp. Med. Biol.* 2017, *999*, 269–286.
77. Gan, Z.; Fu, T.; Kelly, D.P.; Vega, R.B. Skeletal muscle mitochondrial remodeling in exercise and diseases. *Cell Res.* 2018, *28*, 969–980.
78. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
79. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res. Rev. Mutat. Res.* 2019, *781*, 175–185.
80. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
81. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
82. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G.D. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. *Clin. Sci.* 2019, *133*, 351–365.

83. Kwon, J.H.; Moon, K.M.; Min, K.W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. *Healthcare* 2020, *8*, 378.
84. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
85. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. *Genes* 2021, *12*, 1832.
86. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. *Biomolecules* 2021, *11*, 1689.
87. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
88. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
89. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
90. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
91. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
92. Morgan, A.E.; Davies, T.J.; Mc Auley, M.T. The role of DNA methylation in ageing and cancer. *Proc. Nutr. Soc.* 2018, *77*, 412–422.
93. Zhang, W.; Qu, J.; Liu, G.H.; Belmonte, J.C.I. The ageing epigenome and its rejuvenation. *Nat. Rev. Mol. Cell Biol.* 2020, *21*, 137–150.
94. Covarrubias, A.J.; Perrone, R.; Grozio, A.; Verdin, E. NAD(+) metabolism and its roles in cellular processes during ageing. *Nat. Rev. Mol. Cell Biol.* 2021, *22*, 119–141.
95. Booth, L.N.; Brunet, A. The Aging Epigenome. *Mol. Cell.* 2016, *62*, 728–744.
96. Thum, T. Non-coding RNAs in ageing. *Ageing Res. Rev.* 2014, *17*, 1–2.
97. Gupta, S.K.; Piccoli, M.T.; Thum, T. Non-coding RNAs in cardiovascular ageing. *Ageing Res. Rev.* 2014, *17*, 79–85.
98. Samblas, M.; Milagro, F.I.; Martínez, A. DNA methylation markers in obesity, metabolic syndrome, and weight loss. *Epigenetics* 2019, *14*, 421–444.
99. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. *Cell Metab.* 2019, *29*, 1028–1044.
100. Horikoshi, M.; Beaumont, R.N.; Day, F.R.; Warrington, N.M.; Kooijman, M.N.; Fernandez-Tajes, J.; Feenstra, B.; van Zuydam, N.R.; Gaulton, K.J.; Grarup, N.; et al. Genome-wide associations for birth weight and correlations with adult disease. *Nature* 2016, *538*, 248–252.
101. Eeckhoute, J.; Oger, F.; Staels, B.; Lefebvre, P. Coordinated Regulation of PPARγ Expression and Activity through Control of Chromatin Structure in Adipogenesis and Obesity. *PPAR Res.* 2012, *2012*, 164140.
102. Shi, Y.; Qu, J.; Gai, L.; Yuan, D.; Yuan, C. Long Non-coding RNAs in Metabolic and Inflammatory Pathways in Obesity. *Curr. Pharm. Des.* 2020, *26*, 3317–3325.
103. Ouni, M.; Schürmann, A. Epigenetic contribution to obesity. *Mamm. Genome.* 2020, *31*, 134–145.
104. Jung, M.; Pfeifer, G.P. Aging and DNA methylation. *BMC Biol.* 2015, *13*, 7.
105. Beerman, I.; Bock, C.; Garrison, B.S.; Smith, Z.D.; Gu, H.; Meissner, A.; Rossi, D.J. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem. Cell* 2013, *12*, 413–425.
106. Unnikrishnan, A.; Freeman, W.M.; Jackson, J.; Wren, J.D.; Porter, H.; Richardson, A. The role of DNA methylation in epigenetics of aging. *Pharmacol. Ther.* 2019, *195*, 172–185.
107. Sibbett, R.A.; Altschul, D.M.; Marioni, R.E.; Deary, I.J.; Starr, J.M.; Russ, T.C. DNA methylation-based

measures of accelerated biological ageing and the risk of dementia in the oldest-old: A study of the Lothian Birth Cohort 1921. *BMC Psychiatry* 2020,
20, 91.
108. Matías-García, P.R.; Ward-Caviness, C.K.; Raffield, L.M.; Gao, X.; Zhang, Y.; Wilson, R.; Gào, X.; Nano, J.; Bostom, A.; Colicino, E.; et al. DNAm-based signatures of accelerated aging and mortality in blood are associated with low renal function. *Clin. Epigenetics* 2021, *13*, 121.
109. Ambatipudi, S.; Horvath, S.; Perrier, F.; Cuenin, C.; Hernandez-Vargas, H.; Le Calvez-Kelm, F.; Durand, G.; Byrnes, G.; Ferrari, P.; Bouaoun, L.; et al. DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility. *Eur. J. Cancer* 2017, *75*, 299–307.
110. Peng, C.; Cardenas, A.; Rifas-Shiman, S.L.; Hivert, M.F.; Gold, D.R.; Platts-Mills, T.A.; Lin, X.; Oken, E.; Avila, L.; Celedón, J.C.; et al. Epigenetic age acceleration is associated with allergy and asthma in children in Project Viva. *J. Allergy Clin. Immunol.* 2019, *143*, 2263–2270.e2214.
111. Van Dijk, S.J.; Molloy, P.L.; Varinli, H.; Morrison, J.L.; Muhlhausler, B.S. Epigenetics and human obesity. *Int. J. Obes.* 2015, *39*, 85–97.
112. Ahrens, M.; Ammerpohl, O.; von Schönfels, W.; Kolarova, J.; Bens, S.; Itzel, T.; Teufel, A.; Herrmann, A.; Brosch, M.; Hinrichsen, H.; et al. DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. *Cell Metab.* 2013, *18*, 296–302.
113. Zhou, Y.; Hambly, B.D.; McLachlan, C.S. FTO associations with obesity and telomere length. *J. Biomed. Sci.* 2017, *24*, 65.
114. Gurung, R.L.; Liu, S.; Liu, J.J.; Chan, S.M.; Moh, M.C.; Ang, K.; Tang, W.E.; Sum, C.F.; Tavintharan, S.; Lim, S.C. Ethnic disparities in relationships of obesity indices with telomere length in Asians with type 2 diabetes. *J. Diabetes* 2019, *11*, 386–393.
115. Voisin, S.; Eynon, N.; Yan, X.; Bishop, D.J. Exercise training and DNA methylation in humans. *Acta Physiol.* 2015, *213*, 39–59.
116. Fernandes, J.; Arida, R.M.; Gomez-Pinilla, F. Physical exercise as an epigenetic modulator of brain plasticity and cognition. *Neurosci. Biobehav. Rev.* 2017, *80*, 443–456.
117. Solagna, F.; Nogara, L.; Dyar, K.A.; Greulich, F.; Mir, A.A.; Türk, C.; Bock, T.; Geremia, A.; Baraldo, M.; Sartori, R.; et al. Exercisedependent increases in protein synthesis are accompanied by chromatin modifications and increased MRTF-SRF signalling. *Acta Physiol.* 2020, *230*, e13496.
118. Bonilauri, B.; Dallagiovanna, B. Long Non-coding RNAs Are Differentially Expressed after Different Exercise Training Programs. *Front. Physiol.* 2020, *11*, 567614.
119. Zhang, Y.; He, N.; Feng, B.; Ye, H. Exercise Mediates Heart Protection via Non-coding RNAs. *Front. Cell Dev. Biol.* 2020, *8*, 182.
120. Dawson, M.A.; Kouzarides, T. Cancer epigenetics: From mechanism to therapy. *Cell* 2012, *150*, 12–27.
121. Miranda Furtado, C.L.; Dos Santos Luciano, M.C.; Silva Santos, R.D.; Furtado, G.P.; Moraes, M.O.; Pessoa, C. Epidrugs: Targeting epigenetic marks in cancer treatment. *Epigenetics* 2019, *14*, 1164–1176.
122. Yong-Quan Ng, G.; Yang-Wei Fann, D.; Jo, D.G.; Sobey, C.G.; Arumugam, T.V. Dietary Restriction and Epigenetics: Part I. *Cond. Med.* 2019, *2*, 284–299.
123. Yong-Quan Ng, G.; Fann, D.Y.; Jo, D.G.; Sobey, C.G.; Arumugam, T.V. Epigenetic Regulation by Dietary Restriction: Part II. *Cond. Med.* 2019, *2*, 300–310.

Chapter 22. Review - Exercise, Diet and Sleeping as Regenerative Medicine Adjuvants: Obesity and Ageing as Illustrations

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

Received: 25 November 2021; Accepted: 13 January 2022; Published: 14 January 2022

22.1 Résumé (French abstract)

La médecine régénérative utilise les connaissances biologiques et médicales sur la façon dont les cellules et les tissus se régénèrent et évoluent afin de développer de nouvelles thérapies. Les problèmes de santé tels que l'obésité et le cancer, ainsi que le vieillissement, entraînent une altération de la capacité de régénération. L'exercice, les choix alimentaires et les habitudes de sommeil ont des impacts significatifs sur la biologie de la régénération par diverses voies, y compris la réduction des composants inflammatoires et oxydatifs. Ainsi, l'exercice, l'alimentation et la gestion du sommeil peuvent être optimisés vers des applications thérapeutiques en médecine régénérative. Ils pourraient permettre de prévenir la dégénérescence, d'optimiser la régénération biologique et également de fournir des adjuvants pour la médecine régénérative.

22.2 Abstract

Regenerative medicine uses the biological and medical knowledge on how the cells and tissue regenerate and evolve in order to develop novel therapies. Health conditions such as ageing, obesity and cancer lead to an impaired regeneration ability. Exercise, diet choices and sleeping pattern have significant impacts on regeneration biology via diverse pathways including reducing the inflammatory and oxidative components. Thus, exercise, diet and sleeping management can be optimized towards therapeutic applications in regenerative medicine. It could allow to prevent degeneration, optimize the biological regeneration and also provide adjuvants for regenerative medicine.

Keywords: regeneration; exercise; diet; sleeping; oxidative stress; inflammation; obesity; ageing

22.3 Regeneration and Medicine

Regeneration can be defined as the biological processes allowing the cells, organs and tissues to renew and proliferate. These processes also allow normal growth and development, maintenance of a healthy body [1] as well as the recovery from injuries [2] or from other external perturbations [3]. It involves various underlying pathways such as cytoprotective mechanisms induction [2], cellular plasticity [4], tissue remodeling [5] and biophysical aspects [6]. Impaired regeneration can have pathological impacts such as degenerative diseases in different tissues [7–10]. These diseases result from the loss of the regenerative ability leading to a status where cellular loss is superior to cellular regeneration. Regeneration processes might also be impaired or disturbed in various status including degenerative diseases [11–13], cancer [14], obesity [15], ageing [16,17], diabetes mellitus [18] and cholestatic liver [19]. Regenerative medicine comes as a branch that aims to understand the regenerative pathways and degenerative processes, both in biology and physiology, to develop methodologies and approaches aiming to correct regeneration-related health challenges and the impaired functions [20] or at least limit the impacts of the variables that can impair regeneration. The regenerative medicine is a medical field based on regeneration, used biomaterials [21], biochemistry [22], stem cells [23,24] and tissue engineering [25,26] and applies them in surgery [27], transplantation [28], ophthalmology [29] and cancer [23] among diverse applications [30–35].

Regenerative medicine, based on regenerative biology [36], aims to elucidate the mechanistic pathways underlying cellular and tissular regeneration along with the endogenous and exogenous factors that influence the regenerative processes and use that knowledge to develop novel therapeutic options. Such therapies target the correction or the optimization of an impaired regeneration resulting from a disease, a physiological adaptation or even therapeutic side effects. Regenerative medicine research involves multiple areas from stem cells [31], gene editing [37], nuclear transfer [38], proteomics, pharmacology, nanotechnology [39], tissue, engineering and three-dimensional printing [40]. Beside the various adjuvant used in regenerative medicine, mainly pharmacological (regenerative pharmacology) [41–43] or bioengineering [28,44], we aim to highlight the importance of lifestyle and how it impacts regeneration. In this piece of writing, we would like to provide illustrative examples on how lifestyle patterns-specifically exercise, diet and sleeping influence regeneration and the related biological processes. We also present potential clinical and biomedical applications.

22.4 Exercise, Diet, Sleeping and Regeneration

The three main lifestyle pillars (exercise, diet and sleeping) represent key factors in regeneration and, thus, in regenerative medicine as we illustrate below. The facts that they impact regeneration and also influence the statuses (obesity, ageing, etc.) in which regeneration patterns change, support that exercise, diet and sleeping as key factors worth

exploring to optimize regeneration medicine applications. In addition, exercise, diet and sleeping are also involved in different physiological changes and pathological prognoses.

Exercise is known for numerous health benefits including metabolic enhancement [45–47] and regenerative induction. Indeed, exercise represents a cardiomyocyte regeneration inducer [48,49], a therapeutic cartilage regeneration adjuvant [50], a skeletal muscle regeneration enhancer [51], and a cardiac remodeling inducer [52]. Exercise might/can also slow down [53] or reverse muscle atrophy [54], improve the post-injury skeletal muscle regeneration [55], prevent stem cells senescence [56], promote peripheral nerve regeneration [57], and rejuvenate muscle stem cells [58,59]. Within the context of the mechanisms underlying the exercise-induced regenerative benefits, secreted protein acidic and rich in cysteine (SPARC) is at the center of a key theory. SPARC is both induced by exercise and has been hypothesized as a regeneration factor [60–62]. Such implication in regeneration enhancement would be explained by the various properties and roles it has [63] including anti-inflammatory [64], antitumor [61,65,66], extracellular matrix structure [67] and metabolism [68,69] in addition to studies linking SPARC to regeneration [70,71] as well as potential applications in personalized medicine [72]. This suggested that at least a part of the benefits induced by exercise are mediated by SPARC. Indeed, we have already presented data suggesting that that exercise-induced muscle phenotype changes are SPARC-dependent [73] which is in accordance with the theory linking myokines to the physical activity effects [66,74]. The positive impacts of exercise on regeneration could be explained by the properties that have been associated to exercise, such as anti-inflammatory [75], antioxidant [76], anticancer [77] and anti-ageing [78] properties, that lead to suitable outcomes for regeneration and bio-homeostasis in general.

Diet, an important determinant of health, has been studied in a variety of contexts including obesity, metabolism and cancer [79]. However, and although diet and nutrition have been exploited for tissue regeneration [80], many details of the molecular mechanistic pathways seem still emerging to light. The choice of diet quality as well as fasting (calorie restriction) [81] do impact regeneration. Diverse examples illustrate how dietary choices could impact regeneration. Supplemented nutrition diet affects regeneration in liver [82], high-fat and high-glucose microenvironment inhibits bone regeneration [83], proliferation and migration of human gingival fibroblasts is impaired by high glucose-induced oxidative stress [84] but following lidocaine-induced injury normal glucose enhances neuronal regeneration [85]. Such links between glucose and regeneration could be behind parts of the regeneration patterns seen during diabetes [86].

Another illustration in the same context is that fasting promotes stem cell-based regeneration [87], promotes intestinal regeneration [88,89], promote hematopoietic-stemcell based regeneration [90] and β-cell regeneration [91,92]. Such fasting benefits on regeneration would involve metabolic and body composition changes [87,93], anti-inflammatory effects [89], stem cell number increase [88], oxidative stress decrease and ageing delaying [94,95]. The dietary choices represent an important player as well. The rationale behind the dietary choice is to generate a biological microenvironment that can

promote regeneration. This could be achieved, for instance, by reducing the intake of the high-fat diet since high-fat diet leads to inflammation [96,97] and cancer progress [96,98]. The other way to improve regeneration environment via diet is to create biological conditions that would optimize the regenerative abilities. This can be achieved with diets that provide properties such as antioxidant [99,100], anti-inflammatory [101,102], omega-3 fatty acids [103], protein intake [104] and microbiota composition change [105,106]. For instance, fasting-mimicking diet promotes intestinal regeneration [88], reduces intestinal inflammation [89] and reduce inflammatory bowel disease pathology [88].

The other pillar of lifestyle is represented by the sleeping wish is neuroprotective [107]. Impact of sleep on stem cell regenerative capacity is shown by the correlation between circadian rhythm and an improved stem cells proliferation microenvironment [108] leading to stem cell maintenance and division control [109,110]. This fits with the melatonin anti-inflammatory, antioxidant and neuroprotective properties [111,112] along with its free radical scavenger function [113], among others, that would be behind its role in regeneration of tissues [111,113]. In addition, protein and pre-sleep are also contributors to regeneration [114,115]. Following the same line of thoughts, sleep deprivation impairs muscle regeneration [116] and delays healing process [117] which supports the importance of sleeping for the regenerative processes.

These examples of the implications of exercise, diet and sleeping at various levels in regeneration and its variables clearly show their importance within any intervention aiming to stimulate or modulate regeneration.

22.5 Obesity and Ageing as Selected Illustrations

Beside the known diseases related to regeneration changes, obesity [118] and ageing [119] represent topics of concern and are health conditions worth exploring in the context of regeneration. They both share common biological and pathological features [120,121] including regeneration-related [122]. The focus on obesity and ageing, that have common patterns [120,123], derives from their globally increasing epidemiological profile along with the diseases and health problems related to them, including those impacting regeneration homeostasis.

Obesity, as a neuroendocrine reprogramming [124], represents a status of a broken energy balance [125] that has been classified as a disease [126–128]. It has been associated with various health problems and diseases [129]. In the ongoing COVID-19 pandemic, it is worth pointing that obesity both increases vulnerability to COVID-19 (vicious cycle [130]) and reduces the immunity [131,132]. Ageing, on the other hand, can be defined as the timedependent biological and functional declines of living entities. It represents a risk factor for various diseases too [133,134]. Ageing has a genetic component [135] and is due—at least in part—to hormonal and metabolic changes [136]. At the molecular levels, epigenetic changes such as DNA methylation [137,138] are involved in age-related changes. Whereas obesity is a status in which regeneration is impaired [15,139], ageing is

also accompanied by a decline in regeneration [59,140–142]. Studies and hypotheses have pointed various age-related underlying mechanism such as the loss of biological plasticity [143] and the changes in the regenerative environment [141].

The prescription of exercise for both obese [144,145] and elderly population [146–148] is well documented. Such prescription is based on the numerous benefits exercise has; among which we cite glycemic control [149,150], weight management [145], antioxidant [76], anti-inflammatory [151,152], cardiovascular risks improvement [153], immune system regulation [154] and anti-inflammatory milieu promotion [49]. All these benefits improve the negative consequences induced by ageing and obesity and, importantly, improve regeneration bioenvironment.

Exercise is prescribed in obesity and ageing for reasons initially independent from regeneration (weight and adiposity loss, muscle function improvement, etc.). However, the above examples clearly reflect how exercise would be important for regeneration, including in the contexts of obesity and ageing. Exercise would both improve regeneration and reduce the negative impacts that obesity and ageing have on regeneration. The dietary choices and sleeping patterns described above would also contribute to reduce the impacts of obesity and ageing as well. Therefore, indirectly improve the biological regeneration ability. Importantly, targeting regeneration-related pathways in both obesity and ageing represents an additional shared pattern between obesity and ageing.

22.6 Perspectives

The above illustrative examples point to the importance of exercise, diet and sleeping within the regenerative context and points the important of combining all these factors to reach the optimum regenerative outcome. This would have two key implications (Figure 22.1). First, developing an unhealthy lifestyle could lead to both regenerative problems and a possible therapeutic failure of the regenerative medical therapies. The second implication is the importance of introducing medically supervised choices for exercise, diet and sleeping patterns as regenerative adjuvants either during regenerative therapies or for individuals suffering from conditions impacting the regenerative abilities such as obese and elderly patients, knowing the shared features between ageing and obesity [123].

However, further studies are required in order to identify the quality and the quantity of each of these three elements and their combination for each specific case. Indeed, the choice of exercise patterns (types of exercise, duration, timing [150], etc.), diet (quantity, composition and timing) as well as sleeping (duration, timing and tissue-specific impacts [155]) are parameters for which additional optimization could improve the use and application of exercise, diet and sleeping as therapeutic adjuvants or even as stimulators for regeneration. This is encouraging considering the recent advances in biology, such as the possible regenerative ability of the adult heart [156]. Molecular tools such as functional genomics [157–163] and metabolics would allow the characterization of diverse genes,

proteins and other molecular and biochemical changes related to exercise, diet and sleeping patterns, along with their implications in regeneration as well understanding regeneration via proteomics [164]. This would elucidate the molecular links and, thus, identify potential novel pharmacological targets based on advances in signaling in regeneration [165]. These targets are of a specific importance since they would allow, for instance, to mimic exercise effects via pharmacological agents without the need of performing exercise. Such an approach is important for individuals who have a limited ability to complete the prescribed physical activities, such as elderly patients and those with physical disabilities.

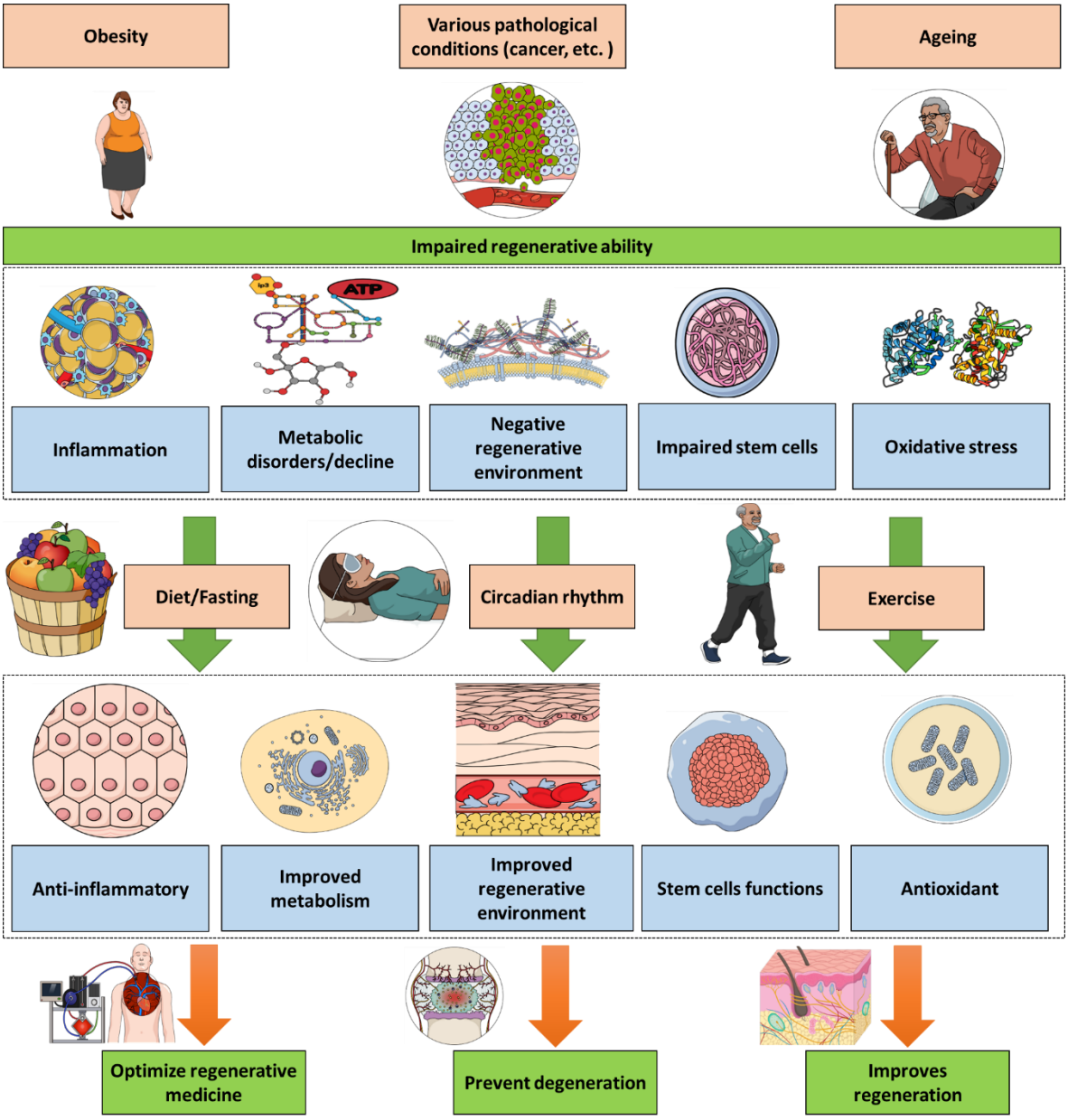


Figure 22.1. Health conditions such as ageing, obesity and cancer lead to an impaired regenerative ability. Exercise, diet and sleeping have significant impacts on regeneration biology via diverse pathways

including reducing the inflammatory and oxidative components. Thus, exercise, diet and sleeping management can be optimized towards therapeutic applications in regenerative medicine.

Overall, the effects of a healthy lifestyle (exercise, diet and sleeping) all contribute towards an improved regeneration ability, which is required to improve healthy ageing, especially with regard to the increased human lifespan [166], in addition to all the known benefits of a healthy lifestyle for a limitless number of health problems including diabetes [167], cancer [168], mental health [169], pediatric asthma [170] and reproductive health [171]. Although we have focused on the impacts exercise, diet and sleeping would have on the biology of regeneration in vivo, we can also extrapolate the concept towards possible in vitro applications. Within this context, the cytokines and factors induced by exercise (ex. SPARC or use the in vitro model of exercise [172]) and sleeping (ex. melatonin) as well as the diet chemical components (ex. antioxidant) could be supplemented during the bioengineering of cellular and tissular cultures (adding to the cells and tissues medium) to enhance the growth and optimize the regenerative abilities (ex. stem cells therapy) prior of their introduction in the organism as a regenerative medical therapy.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, Figure 22.1, references selection, etc.) throughout the writing process, edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Acknowledgments: A.G. received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec Nature et technologies (FRQNT) est responsable de la gestion du programme). A.G. received the scholarship « Bourse Tremplin -Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec-Santé (FRQS), Quebec, Canada. Figure 22.1 was created using images from https://mindthegraph.com/ (accessed on 8 November 2021).

Conflicts of Interest: The authors declare no conflict of interest.

22.7 References

1. Gray, N.; Le Bot, N.; Heemels, M.T. Regeneration. *Nature* 2018, *557*, 321.
2. Diehl, A.M. Liver regeneration. *Front. Biosci.* 2002, *7*, e301–e314.

3. Lin, A.; Makushok, T.; Diaz, U.; Marshall, W.F. Methods for the Study of Regeneration in Stentor. *J. Vis. Exp.* 2018, *136*, 57759.
4. Odelberg, S.J. Cellular plasticity in vertebrate regeneration. *Anat. Rec. B New Anat.* 2005, *287*, 25–35.
5. Majidinia, M.; Sadeghpour, A.; Yousefi, B. The roles of signaling pathways in bone repair and regeneration. *J. Cell Physiol.* 2018, *233*, 2937–2948.
6. Galliot, B.; Crescenzi, M.; Jacinto, A.; Tajbakhsh, S. Trends in tissue repair and regeneration. *Development* 2017, *144*, 357–364.
7. Wooff, Y.; Man, S.M.; Aggio-Bruce, R.; Natoli, R.; Fernando, N. IL-1 Family Members Mediate Cell Death, Inflammation and Angiogenesis in Retinal Degenerative Diseases. *Front. Immunol.* 2019, *10*, 1618.
8. Dugger, B.N.; Dickson, D.W. Pathology of Neurodegenerative Diseases. *Cold Spring Harb. Perspect. Biol.* 2017, *9*, a028035.
9. Vajkoczy, P.; Weidner, N. Degenerative spinal diseases. *Der Nervenarzt* 2018, *89*, 619.
10. Nandi, A.; Yan, L.J.; Jana, C.K.; Das, N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. *Oxid. Med. Cell Longev.* 2019, *2019*, 9613090.
11. Wu, C.L.; Harasymowicz, N.S.; Klimak, M.A.; Collins, K.H.; Guilak, F. The role of macrophages in osteoarthritis and cartilage repair. *Osteoarthr. Cartil.* 2020, *28*, 544–554.
12. Murphy, M.P.; Koepke, L.S.; Lopez, M.T.; Tong, X.; Ambrosi, T.H.; Gulati, G.S.; Marecic, O.; Wang, Y.; Ransom, R.C.; Hoover, M.Y.; et al. Articular cartilage regeneration by activated skeletal stem cells. *Nat. Med.* 2020, *26*, 1583–1592.
13. Jeon, S.; Oh, I.H. Regeneration of the retina: Toward stem cell therapy for degenerative retinal diseases. *BMB Rep.* 2015, *48*, 193–199.
14. Wang, J.; Li, X.; Chen, H. Organoid models in lung regeneration and cancer. *Cancer Lett.* 2020, *475*, 129–135.
15. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
16. Roberts, S.; Colombier, P.; Sowman, A.; Mennan, C.; Rölfing, J.H.; Guicheux, J.; Edwards, J.R. Ageing in the musculoskeletal system. *Acta Orthop.* 2016, *87*, 15–25.
17. Yamakawa, H.; Kusumoto, D.; Hashimoto, H.; Yuasa, S. Stem Cell Aging in Skeletal Muscle Regeneration and Disease. *Int. J. Mol. Sci.* 2020, *21*, 1830.
18. Kennedy, J.M.; Zochodne, D.W. Impaired peripheral nerve regeneration in diabetes mellitus. *J. Peripher. Nerv. Syst.* 2005, *10*, 144–157.
19. Yokoyama, Y.; Nagino, M.; Nimura, Y. Mechanism of impaired hepatic regeneration in cholestatic liver. *J. Hepatobiliary Pancreat Surg.* 2007, *14*, 159–166.
20. Greenwood, H.L.; Singer, P.A.; Downey, G.P.; Martin, D.K.; Thorsteinsdóttir, H.; Daar, A.S. Regenerative medicine and the developing world. *PLoS Med.* 2006, *3*, e381.
21. Brokesh, A.M.; Gaharwar, A.K. Inorganic Biomaterials for Regenerative Medicine. *ACS Appl. Mater. Interfaces* 2020, *12*, 5319–5344.
22. Makarevich, P.I.; Efimenko, A.Y.; Tkachuk, V.A. Biochemical Regulation of Regenerative Processes by Growth Factors and Cytokines: Basic Mechanisms and Relevance for Regenerative Medicine. *Biochemistry* 2020, *85*, 11–26.
23. Cedar, S.H. The function of stem cells and their future roles in healthcare. *Br. J. Nurs.* 2006, *15*, 104–107.
24. Forbes, S.J. Recent advances in stem cells and regenerative medicine. *Qjm* 2014, *107*, 251–252.
25. Raspa, A.; Pugliese, R.; Maleki, M.; Gelain, F. Recent therapeutic approaches for spinal cord injury. *Biotechnol. Bioeng.* 2016, *113*, 253–259.
26. Salgado, A.J.; Oliveira, J.M.; Martins, A.; Teixeira, F.G.; Silva, N.A.; Neves, N.M.; Sousa, N.; Reis, R.L. Tissue engineering and regenerative medicine: Past, present, and future. *Int. Rev. NeuroBiol.* 2013, *108*, 1–33.
27. McPhail, M.J.; Janus, J.R.; Lott, D.G. Advances in regenerative medicine for otolaryngology/head and neck surgery. *BMJ* 2020, *369*, m718.
28. Edgar, L.; Pu, T.; Porter, B.; Aziz, J.M.; La Pointe, C.; Asthana, A.; Orlando, G. Regenerative medicine,

organ bioengineering and transplantation. *Br. J. Surg.* 2020, *107*, 793–800.
29. Sahle, F.F.; Kim, S.; Niloy, K.K.; Tahia, F.; Fili, C.V.; Cooper, E.; Hamilton, D.J.; Lowe, T.L. Nanotechnology in regenerative ophthalmology. *Adv. Drug Deliv. Rev.* 2019, *148*, 290–307.
30. Jahangirian, H.; Azizi, S.; Rafiee-Moghaddam, R.; Baratvand, B.; Webster, T.J. Status of Plant Protein-Based Green Scaffolds for Regenerative Medicine Applications. *Biomolecules* 2019, *9*, 619.
31. Cable, J.; Fuchs, E.; Weissman, I.; Jasper, H.; Glass, D.; Rando, T.A.; Blau, H.; Debnath, S.; Oliva, A.; Park, S.; et al. Adult stem cells and regenerative medicine-a symposium report. *Ann. N. Y. Acad. Sci.* 2020, *1462*, 27–36.
32. Hosseini, S.; Taghiyar, L.; Safari, F.; Baghaban Eslaminejad, M. Regenerative Medicine Applications of Mesenchymal Stem Cells. *Adv. Exp. Med. Biol.* 2018, *1089*, 115–141.
33. Khan, A.; Wang, B.; Ni, Y. Chitosan-Nanocellulose Composites for Regenerative Medicine Applications. *Curr. Med. Chem.* 2020, *27*, 4584–4592.
34. Suman, S.; Domingues, A.; Ratajczak, J.; Ratajczak, M.Z. Potential Clinical Applications of Stem Cells in Regenerative Medicine. *Adv. Exp. Med. Biol.* 2019, *1201*, 1–22.
35. Nilforoushzadeh, M.A.; Sisakht, M.M.; Seifalian, A.M.; Amirkhani, M.A.; Banafshe, H.R.; Verdi, J.; Sharifzad, F.; Taghiabadi, E. Regenerative Medicine Applications in Wound Care. *Curr. Stem Cell Res. Ther.* 2017, *12*, 658–674.
36. Stocum, D.L. Regenerative biology and medicine. *J. Musculoskelet. Neuronal. Interact.* 2002, *2*, 270–273.
37. Hsu, M.N.; Chang, Y.H.; Truong, V.A.; Lai, P.L.; Nguyen, T.K.N.; Hu, Y.C. CRISPR technologies for stem cell engineering and regenerative medicine. *Biotechnol. Adv.* 2019, *37*, 107447.
38. Gouveia, C.; Huyser, C.; Egli, D.; Pepper, M.S. Lessons Learned from Somatic Cell Nuclear Transfer. *Int. J. Mol. Sci.* 2020, *21*, 2314.
39. Lowe, T.L.; Agrahari, V.; Kannan, R.M.; Kannan, S. Nanotechnology enabled regenerative medicine for neurological disorders. *Adv. Drug Deliv. Rev.* 2019, *148*, 1–2.
40. Fricain, J.C.; De Olivera, H.; Devillard, R.; Kalisky, J.; Remy, M.; Kériquel, V.; Le Nihounen, D.; Grémare, A.; Guduric, V.; Plaud, A.; et al. 3D bioprinting in regenerative medicine and tissue engineering. *Med. Sci.* 2017, *33*, 52–59.
41. Christ, G.J.; Saul, J.M.; Furth, M.E.; Andersson, K.E. The pharmacology of regenerative medicine. *Pharmacol. Rev.* 2013, *65*, 1091–1133.
42. Andersson, K.E.; Christ, G.J. Regenerative pharmacology: The future is now. *Mol. Interv.* 2007, *7*, 79–86.
43. Williams, J.K.; Andersson, K.E. Regenerative pharmacology: Recent developments and future perspectives. *Regen. Med.* 2016, *11*, 859–870.
44. Mahdavi, S.S.; Abdekhodaie, M.J.; Mashayekhan, S.; Baradaran-Rafii, A.; Djalilian, A.R. Bioengineering Approaches for Corneal Regenerative Medicine. *Tissue Eng. Regen. Med.* 2020, *17*, 567–593.
45. Craig, D.M.; Ashcroft, S.P.; Belew, M.Y.; Stocks, B.; Currell, K.; Baar, K.; Philp, A. Utilizing small nutrient compounds as enhancers of exercise-induced mitochondrial biogenesis. *Front. Physiol.* 2015, *6*, 296.
46. Booth, F.W.; Ruegsegger, G.N.; Toedebusch, R.G.; Yan, Z. Endurance Exercise and the Regulation of Skeletal Muscle Metabolism. *Prog. Mol. Biol. Transl. Sci.* 2015, *135*, 129–151.
47. McGee, S.L.; Hargreaves, M. Exercise and skeletal muscle glucose transporter 4 expression: Molecular mechanisms. *Clin. Exp. Pharmacol. Physiol.* 2006, *33*, 395–399.
48. Bo, B.; Zhou, Y.; Zheng, Q.; Wang, G.; Zhou, K.; Wei, J. The Molecular Mechanisms Associated with Aerobic Exercise-Induced Cardiac Regeneration. *Biomolecules* 2020, *11*, 19.
49. Fiuza-Luces, C.; Santos-Lozano, A.; Joyner, M.; Carrera-Bastos, P.; Picazo, O.; Zugaza, J.L.; Izquierdo, M.; Ruilope, L.M.; Lucia, A. Exercise benefits in cardiovascular disease: Beyond attenuation of traditional risk factors. *Nat. Rev. Cardiol.* 2018, *15*, 731–743.
50. Smith, J.K. Exercise as an Adjuvant to Cartilage Regeneration Therapy. *Int. J. Mol. Sci.* 2020, *21*, 9471.
51. Saito, Y.; Chikenji, T.S.; Matsumura, T.; Nakano, M.; Fujimiya, M. Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors. *Nat. Commun.* 2020, *11*, 889.
52. Schüttler, D.; Clauss, S.; Weckbach, L.T.; Brunner, S. Molecular Mechanisms of Cardiac Remodeling and

Regeneration in Physical Exercise. *Cells* 2019, *8*, 1128.
53. He, N.; Ye, H. Exercise and Muscle Atrophy. *Adv. Exp. Med. Biol.* 2020, *1228*, 255–267.
54. Nalbandian, A.; Nguyen, C.; Katheria, V.; Llewellyn, K.J.; Badadani, M.; Caiozzo, V.; Kimonis, V.E. Exercise training reverses skeletal muscle atrophy in an experimental model of VCP disease. *PLoS ONE* 2013, *8*, e76187.
55. Perandini, L.A.; Chimin, P.; Lutkemeyer, D.D.S.; Câmara, N.O.S. Chronic inflammation in skeletal muscle impairs satellite cells function during regeneration: Can physical exercise restore the satellite cell niche? *FEBS J.* 2018, *285*, 1973–1984.
56. Valenti, M.T.; Dalle Carbonare, L.; Dorelli, G.; Mottes, M. Effects of physical exercise on the prevention of stem cells senescence. *Stem Cell Rev. Rep.* 2020, *16*, 33–40.
57. Gordon, T.; English, A.W. Strategies to promote peripheral nerve regeneration: Electrical stimulation and/or exercise. *Eur. J. Neurosci.* 2016, *43*, 336–350.
58. Larrick, J.W.; Mendelsohn, A.R. Exercise Partially Rejuvenates Muscle Stem Cells. *Rejuvenation Res.* 2020, *23*, 262–265.
59. Brett, J.O.; Arjona, M.; Ikeda, M.; Quarta, M.; de Morrée, A.; Egner, I.M.; Perandini, L.A.; Ishak, H.D.; Goshayeshi, A.; Benjamin, D.I.; et al. Exercise rejuvenates quiescent skeletal muscle stem cells in old mice through restoration of Cyclin D1. *Nat. Metab.* 2020, *2*, 307–317.
60. Son, J.S.; Chae, S.A.; Testroet, E.D.; Du, M.; Jun, H.P. Exercise-induced myokines: A brief review of controversial issues of this decade. *Expert. Rev. Endocrinol. Metab.* 2018, *13*, 51–58.
61. Liu, Y.P.; Hsiao, M. Exercise-induced SPARC prevents tumorigenesis of colon cancer. *Gut* 2013, *62*, 810–811.
62. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
63. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. *Biomolecules* 2021, *11*, 1689.
64. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
65. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
66. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
67. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
68. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. BioChem. Cell Biol.* 2019, *117*, 105627.
69. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. *FASEB J.* 2019, *33*, 10551–10562.
70. Ribeiro, N.; Sousa, S.R.; Brekken, R.A.; Monteiro, F.J. Role of SPARC in bone remodeling and cancer-related bone metastasis. *J. Cell BioChem.* 2014, *115*, 17–26.
71. Chimal-Monroy, J.; Bravo-Ruíz, T.; Furuzawa-Carballeda, G.J.; Lira, J.M.; de la Cruz, J.C.; Almazán, A.; Krötzsch-Gómez, F.E.; Arrellín, G.; Díaz de León, L. Collagen-PVP accelerates new bone formation of experimentally induced bone defects in rat skull and promotes the expression of osteopontin and SPARC during bone repair of rat femora fractures. *Ann. N. Y. Acad. Sci.* 1998, *857*, 232–236.
72. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. *Genes* 2021, *12*, 1832.
73. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic

and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
74. Kwon, J.H.; Moon, K.M.; Min, K.W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. *Healthcare* 2020, *8*, 378.
75. Nilsson, M.I.; Bourgeois, J.M.; Nederveen, J.P.; Leite, M.R.; Hettinga, B.P.; Bujak, A.L.; May, L.; Lin, E.; Crozier, M.; Rusiecki, D.R.; et al. Lifelong aerobic exercise protects against inflammaging and cancer. *PLoS ONE* 2019, *14*, e0210863.
76. de Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. *Sports Med.* 2017, *47*, 277–293.
77. Hojman, P.; Gehl, J.; Christensen, J.F.; Pedersen, B.K. Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. *Cell Metab.* 2018, *27*, 10–21.
78. Garatachea, N.; Pareja-Galeano, H.; Sanchis-Gomar, F.; Santos-Lozano, A.; Fiuza-Luces, C.; Morán, M.; Emanuele, E.; Joyner, M.J.; Lucia, A. Exercise attenuates the major hallmarks of aging. *Rejuvenation Res.* 2015, *18*, 57–89.
79. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Diet Impact on Obesity beyond Calories and Trefoil Factor Family 2 (TFF2) as an Illustration: Metabolic Implications and Potential Applications. *Biomolecules* 2021, *11*, 1830.
80. Cheng, C.W.; Yilmaz, Ö.H. 100 Years of Exploiting Diet and Nutrition for Tissue Regeneration. *Cell Stem Cell* 2021, *28*, 370–373.
81. Nencioni, A.; Caffa, I.; Cortellino, S.; Longo, V.D. Fasting and cancer: Molecular mechanisms and clinical application. *Nat. Rev. Cancer* 2018, *18*, 707–719.
82. Cornide-Petronio, M.E.; Álvarez-Mercado, A.I.; Jiménez-Castro, M.B.; Peralta, C. Current Knowledge about the Effect of Nutritional Status, Supplemented Nutrition Diet, and Gut Microbiota on Hepatic Ischemia-Reperfusion and Regeneration in Liver Surgery. *Nutrients* 2020, *12*, 284.
83. Wu, X.; Zhang, Y.; Xing, Y.; Zhao, B.; Zhou, C.; Wen, Y.; Xu, X. High-fat and high-glucose microenvironment decreases Runx2 and TAZ expression and inhibits bone regeneration in the mouse. *J. Orthop. Surg. Res.* 2019, *14*, 55.
84. Buranasin, P.; Mizutani, K.; Iwasaki, K.; Pawaputanon Na Mahasarakham, C.; Kido, D.; Takeda, K.; Izumi, Y. High glucose-induced oxidative stress impairs proliferation and migration of human gingival fibroblasts. *PLoS ONE* 2018, *13*, e0201855.
85. Abdel Nazeer, A.; Saito, S.; Sayed, S.; Hassan, L.; Askar, F.; Al-Jahdari, W.; Seki, T.; Hideaki, O. Normal glucose enhances neuronal regeneration after lidocaine-induced injury. *Br. J. Anaesth.* 2010, *104*, 482–486.
86. Camargo, W.A.; de Vries, R.; van Luijk, J.; Hoekstra, J.W.; Bronkhorst, E.M.; Jansen, J.A.; van den Beucken, J. Diabetes Mellitus and Bone Regeneration: A Systematic Review and Meta-Analysis of Animal Studies. *Tissue Eng. Part B Rev.* 2017, *23*, 471–479.
87. Mattson, M.P.; Longo, V.D.; Harvie, M. Impact of intermittent fasting on health and disease processes. *Ageing Res. Rev.* 2017, *39*, 46–58.
88. Rangan, P.; Choi, I.; Wei, M.; Navarrete, G.; Guen, E.; Brandhorst, S.; Enyati, N.; Pasia, G.; Maesincee, D.; Ocon, V.; et al. Fasting-Mimicking Diet Modulates Microbiota and Promotes Intestinal Regeneration to Reduce Inflammatory Bowel Disease Pathology. *Cell Rep.* 2019, *26*, 2704–2719.e2706.
89. Song, S.; Bai, M.; Ling, Z.; Lin, Y.; Wang, S.; Chen, Y. Intermittent administration of a fasting-mimicking diet reduces intestinal inflammation and promotes repair to ameliorate inflammatory bowel disease in mice. *J. Nutr. Biochem.* 2021, *96*, 108785.
90. Cheng, C.W.; Adams, G.B.; Perin, L.; Wei, M.; Zhou, X.; Lam, B.S.; Da Sacco, S.; Mirisola, M.; Quinn, D.I.; Dorff, T.B.; et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. *Cell Stem Cell* 2014, *14*, 810–823.
91. Cheng, C.W.; Villani, V.; Buono, R.; Wei, M.; Kumar, S.; Yilmaz, O.H.; Cohen, P.; Sneddon, J.B.; Perin, L.; Longo, V.D. FastingMimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes.

Cell 2017, *168*, 775–788.e712.
92. Bradley, C.A. Diabetes: Fasting and β cell regeneration. *Nat. Rev. Endocrinol.* 2017, *13*, 252.
93. Arciero, P.J.; Edmonds, R.; He, F.; Ward, E.; Gumpricht, E.; Mohr, A.; Ormsbee, M.J.; Astrup, A. Protein-Pacing Caloric-Restriction Enhances Body Composition Similarly in Obese Men and Women during Weight Loss and Sustains Efficacy during Long-Term Weight Maintenance. *Nutrients* 2016, *8*, 476.
94. Ma, S.; Sun, S.; Geng, L.; Song, M.; Wang, W.; Ye, Y.; Ji, Q.; Zou, Z.; Wang, S.; He, X.; et al. Caloric Restriction Reprograms the Single-Cell Transcriptional Landscape of Rattus Norvegicus Aging. *Cell* 2020, *180*, 984–1001.e1022.
95. He, F.; Zuo, L.; Ward, E.; Arciero, P.J. Serum Polychlorinated Biphenyls Increase and Oxidative Stress Decreases with a ProteinPacing Caloric Restriction Diet in Obese Men and Women. *Int. J. Environ. Res. Public Health* 2017, *14*, 59.
96. Hayashi, T.; Fujita, K.; Nojima, S.; Hayashi, Y.; Nakano, K.; Ishizuya, Y.; Wang, C.; Yamamoto, Y.; Kinouchi, T.; Matsuzaki, K.; et al. High-Fat Diet-Induced Inflammation Accelerates Prostate Cancer Growth via IL6 Signaling. *Clin. Cancer Res.* 2018, *24*, 4309–4318.
97. Kim, J.D.; Yoon, N.A.; Jin, S.; Diano, S. Microglial UCP2 Mediates Inflammation and Obesity Induced by High-Fat Feeding. *Cell Metab.* 2019, *30*, 952–962.e955.
98. Fujita, K.; Hayashi, T.; Matsushita, M.; Uemura, M.; Nonomura, N. Obesity, Inflammation, and Prostate Cancer. *J. Clin. Med.* 2019, *8*, 201.
99. Cömert, E.D.; Gökmen, V. Evolution of food antioxidants as a core topic of food science for a century. *Food Res. Int.* 2018, *105*, 76–93.
100. Benzie, I.F.; Choi, S.W. Antioxidants in food: Content, measurement, significance, action, cautions, caveats, and research needs. *Adv. Food Nutr. Res.* 2014, *71*, 1–53.
101. Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Crit. Rev. Food Sci. Nutr.* 2018, *58*, 1260–1270.
102. Mohd Sahardi, N.F.N.; Makpol, S. Ginger (*Zingiber officinale* Roscoe) in the Prevention of Ageing and Degenerative Diseases: Review of Current Evidence. *Evid.-Based Complement. Alternat. Med.* 2019, *2019*, 5054395.
103. Marsman, H.A.; de Graaf, W.; Heger, M.; van Golen, R.F.; Ten Kate, F.J.; Bennink, R.; van Gulik, T.M. Hepatic regeneration and functional recovery following partial liver resection in an experimental model of hepatic steatosis treated with omega-3 fatty acids. *Br. J. Surg.* 2013, *100*, 674–683.
104. Sharples, A.P.; Hughes, D.C.; Deane, C.S.; Saini, A.; Selman, C.; Stewart, C.E. Longevity and skeletal muscle mass: The role of IGF signalling, the sirtuins, dietary restriction and protein intake. *Aging Cell* 2015, *14*, 511–523.
105. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. *Eur. Rev. Med. Pharmacol. Sci.* 2016, *20*, 4742–4749.
106. Shavandi, A.; Saeedi, P.; Gérard, P.; Jalalvandi, E.; Cannella, D.; Bekhit, A.E. The role of microbiota in tissue repair and regeneration. *J. Tissue Eng. Regen. Med.* 2020, *14*, 539–555.
107. Eugene, A.R.; Masiak, J. The Neuroprotective Aspects of Sleep. *MEDtube Sci.* 2015, *3*, 35–40.
108. Elkhenany, H.; AlOkda, A.; El-Badawy, A.; El-Badri, N. Tissue regeneration: Impact of sleep on stem cell regenerative capacity. *Life Sci.* 2018, *214*, 51–61.
109. Dierickx, P.; Van Laake, L.W.; Geijsen, N. Circadian clocks: From stem cells to tissue homeostasis and regeneration. *EMBO Rep.* 2018, *19*, 18–28.
110. Brown, S.A. Circadian clock-mediated control of stem cell division and differentiation: Beyond night and day. *Development* 2014, *141*, 3105–3111.
111. Majidinia, M.; Reiter, R.J.; Shakouri, S.K.; Mohebbi, I.; Rastegar, M.; Kaviani, M.; Darband, S.G.; Jahanban-Esfahlan, R.; Nabavi, S.M.; Yousefi, B. The multiple functions of melatonin in regenerative medicine. *Ageing Res. Rev.* 2018, *45*, 33–52.
112. Pandi-Perumal, S.R.; BaHammam, A.S.; Brown, G.M.; Spence, D.W.; Bharti, V.K.; Kaur, C.; Hardeland, R.; Cardinali, D.P. Melatonin antioxidative defense: Therapeutical implications for aging and

neurodegenerative processes. *Neurotox. Res.* 2013, *23*, 267–300.
113. Chen, B.; You, W.; Shan, T. The regulatory role of melatonin in skeletal muscle. *J. Muscle Res. Cell Motil.* 2020, *41*, 191–198.
114. Pourabbas, M.; Bagheri, R.; Hooshmand Moghadam, B.; Willoughby, D.S.; Candow, D.G.; Elliott, B.T.; Forbes, S.C.; Ashtary-Larky, D.; Eskandari, M.; Wong, A.; et al. Strategic Ingestion of High-Protein Dairy Milk during a Resistance Training Program Increases Lean Mass, Strength, and Power in Trained Young Males. *Nutrients* 2021, *13*, 948
115. Saracino, P.G.; Saylor, H.E.; Hanna, B.R.; Hickner, R.C.; Kim, J.S.; Ormsbee, M.J. Effects of Pre-Sleep Whey vs. Plant-Based Protein Consumption on Muscle Recovery Following Damaging Morning Exercise. *Nutrients* 2020, *12*, 2049.
116. Mônico-Neto, M.; Dáttilo, M.; Ribeiro, D.A.; Lee, K.S.; de Mello, M.T.; Tufik, S.; Antunes, H.K.M. REM sleep deprivation impairs muscle regeneration in rats. *Growth Factors* 2017, *35*, 12–18.
117. Chen, P.; Yao, H.; Su, W.; He, Y.; Cheng, K.; Wang, Y.; Peng, W.; Li, P. Sleep deprivation worsened oral ulcers and delayed healing process in an experimental rat model. *Life Sci.* 2019, *232*, 116594.
118. Apovian, C.M. Obesity: Definition, comorbidities, causes, and burden. *Am. J. Manag. Care* 2016, *22*, s176–s185.
119. Steptoe, A.; Deaton, A.; Stone, A.A. Subjective wellbeing, health, and ageing. *Lancet* 2015, *385*, 640–648.
120. Tam, B.T.; Morais, J.A.; Santosa, S. Obesity and ageing: Two sides of the same coin. *Obes. Rev.* 2020, *21*, e12991.
121. Ghosh, S.; Sinha, J.K.; Raghunath, M. 'Obesageing': Linking obesity & ageing. *Indian J. Med. Res.* 2019, *149*, 610–615.
122. Ambrosi, T.H.; Scialdone, A.; Graja, A.; Gohlke, S.; Jank, A.M.; Bocian, C.; Woelk, L.; Fan, H.; Logan, D.W.; Schürmann, A.; et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. *Cell Stem Cell* 2017, *20*, 771–784.e776.
123. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. *Diseases* 2021, *9*, 87.
124. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.
125. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
126. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
127. Conway, B.; Rene, A. Obesity as a disease: No lightweight matter. *Obes. Rev.* 2004, *5*, 145–151.
128. Christensen, S. Recognizing obesity as a disease. *J. Am. Assoc. Nurse Pract.* 2020, *32*, 497–503.
129. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.
130. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
131. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
132. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. *Medicines* 2021, *8*, 57.
133. Aunan, J.R.; Watson, M.M.; Hagland, H.R.; Søreide, K. Molecular and biological hallmarks of ageing. *Br. J. Surg.* 2016, *103*, e29–e46.
134. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* 2019, *15*, 565–581.
135. Melzer, D.; Pilling, L.C.; Ferrucci, L. The genetics of human ageing. *Nat. Rev. Genet.* 2020, *21*, 88–101.

136. van den Beld, A.W.; Kaufman, J.M.; Zillikens, M.C.; Lamberts, S.W.J.; Egan, J.M.; van der Lely, A.J. The physiology of endocrine systems with ageing. *Lancet Diabetes Endocrinol.* 2018, *6*, 647–658.
137. Horvath, S.; Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat. Rev. Genet.* 2018, *19*, 371–384.
138. Bacalini, M.G.; D'Aquila, P.; Marasco, E.; Nardini, C.; Montesanto, A.; Franceschi, C.; Passarino, G.; Garagnani, P.; Bellizzi, D. The methylation of nuclear and mitochondrial DNA in ageing phenotypes and longevity. *Mech. Ageing Dev.* 2017, *165*, 156–161.
139. Geiger, A.E.; Daughtry, M.R.; Yen, C.N.; Kirkpatrick, L.T.; Shi, H.; Gerrard, D.E. Dual effects of obesity on satellite cells and muscle regeneration. *Physiol. Rep.* 2020, *8*, e14511.
140. Conboy, I.M.; Rando, T.A. Aging, stem cells and tissue regeneration: Lessons from muscle. *Cell Cycle* 2005, *4*, 407–410.
141. Yun, M.H. Changes in Regenerative Capacity through Lifespan. *Int. J. Mol. Sci.* 2015, *16*, 25392–25432.
142. Blau, H.M.; Cosgrove, B.D.; Ho, A.T. The central role of muscle stem cells in regenerative failure with aging. *Nat. Med.* 2015, *21*, 854–862.
143. Walsh, S.K.; Schneider, S.E.; Amundson, L.A.; Neu, C.P.; Henak, C.R. Maturity-dependent cartilage cell plasticity and sensitivity to external perturbation. *J. Mech. Behav. BioMed. Mater.* 2020, *106*, 103732.
144. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. *Metabolism* 2019, *92*, 163–169.
145. Swift, D.L.; McGee, J.E.; Earnest, C.P.; Carlisle, E.; Nygard, M.; Johannsen, N.M. The Effects of Exercise and Physical Activity on Weight Loss and Maintenance. *Prog. Cardiovasc. Dis.* 2018, *61*, 206–213.
146. Pedersen, B.K. Which type of exercise keeps you young? *Curr. Opin. Clin. Nutr. Metab. Care* 2019, *22*, 167–173.
147. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. *Phys. Med. Rehabil. Clin. N. Am.* 2017, *28*, 659–669.
148. Mora, J.C.; Valencia, W.M. Exercise and Older Adults. *Clin. Geriatr. Med.* 2018, *34*, 145–162.
149. Reddy, R.; Wittenberg, A.; Castle, J.R.; El Youssef, J.; Winters-Stone, K.; Gillingham, M.; Jacobs, P.G. Effect of Aerobic and Resistance Exercise on Glycemic Control in Adults With Type 1 Diabetes. *Can. J. Diabetes* 2019, *43*, 406–414.e401.
150. Savikj, M.; Gabriel, B.M.; Alm, P.S.; Smith, J.; Caidahl, K.; Björnholm, M.; Fritz, T.; Krook, A.; Zierath, J.R.; Wallberg-Henriksson, H. Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: A randomised crossover trial. *Diabetologia* 2019, *62*, 233–237.
151. Gleeson, M.; Bishop, N.C.; Stensel, D.J.; Lindley, M.R.; Mastana, S.S.; Nimmo, M.A. The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. *Nat. Rev. Immunol.* 2011, *11*, 607–615.
152. Collao, N.; Rada, I.; Francaux, M.; Deldicque, L.; Zbinden-Foncea, H. Anti-Inflammatory Effect of Exercise Mediated by Toll-Like Receptor Regulation in Innate Immune Cells—A Review. *Int. Rev. Immunol.* 2020, *39*, 39–52.
153. Ho, S.S.; Dhaliwal, S.S.; Hills, A.P.; Pal, S. The effect of 12 weeks of aerobic, resistance or combination exercise training on cardiovascular risk factors in the overweight and obese in a randomized trial. *BMC Public Health* 2012, *12*, 704.
154. Wang, J.; Liu, S.; Li, G.; Xiao, J. Exercise Regulates the Immune System. *Adv. Exp. Med. Biol.* 2020, *1228*, 395–408.
155. Plikus, M.V.; Van Spyk, E.N.; Pham, K.; Geyfman, M.; Kumar, V.; Takahashi, J.S.; Andersen, B. The circadian clock in skin: Implications for adult stem cells, tissue regeneration, cancer, aging, and immunity. *J. Biol. Rhythm.* 2015, *30*, 163–182.
156. Zhang, Y.; Mignone, J.; MacLellan, W.R. Cardiac Regeneration and Stem Cells. *Physiol. Rev.* 2015, *95*, 1189–1204.
157. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity:

Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
158. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
159. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res. Rev. Mutat. Res.* 2019, *781*, 175–185.
160. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
161. Swindell, W.R. Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. *BMC Genom.* 2009, *10*, 585.
162. Linford, N.J.; Beyer, R.P.; Gollahon, K.; Krajcik, R.A.; Malloy, V.L.; Demas, V.; Burmer, G.C.; Rabinovitch, P.S. Transcriptional response to aging and caloric restriction in heart and adipose tissue. *Aging Cell* 2007, *6*, 673–688.
163. Wanders, D.; Ghosh, S.; Stone, K.P.; Van, N.T.; Gettys, T.W. Transcriptional impact of dietary methionine restriction on systemic inflammation: Relevance to biomarkers of metabolic disease during aging. *Biofactors* 2014, *40*, 13–26.
164. Franco, C.; Soares, R.; Pires, E.; Koci, K.; Almeida, A.M.; Santos, R.; Coelho, A.V. Understanding regeneration through proteomics. *Proteomics* 2013, *13*, 686–709.
165. Stoick-Cooper, C.L.; Moon, R.T.; Weidinger, G. Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. *Genes Dev.* 2007, *21*, 1292–1315.
166. Partridge, L.; Deelen, J.; Slagboom, P.E. Facing up to the global challenges of ageing. *Nature* 2018, *561*, 45–56.
167. Lifestyle Management: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018, *41*, S38–S50.
168. El-Sherif, A.; El-Sherif, S.; Taylor, A.H.; Ayakannu, T. Ovarian Cancer: Lifestyle, Diet and Nutrition. *Nutr. Cancer* 2021, *73*, 1092–1107.
169. Walsh, R. Lifestyle and mental health. *Am. Psychol.* 2011, *66*, 579–592.
170. Lu, K.D.; Forno, E. Exercise and lifestyle changes in pediatric asthma. *Curr. Opin. Pulm. Med.* 2020, *26*, 103–111.
171. Sharma, R.; Biedenharn, K.R.; Fedor, J.M.; Agarwal, A. Lifestyle factors and reproductive health: Taking control of your fertility. *Reprod. Biol. Endocrinol.* 2013, *11*, 66.
172. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.

Chapter 23. Opinion - Impact of Adiposity and Fat Distribution, Rather than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Quebec, QC G1V 0A6, Canada

Received: 8 September 2021; Accepted: 2 October 2021; Published: 8 October 2021

23.1 Résumé (French abstract)

L'obésité représente un facteur de risque pour une variété de maladies en raison de sa composante inflammatoire, entre autres propriétés biologiques. Récemment, avec la crise actuelle de la COVID-19, un accent particulier a été mis sur l'obésité en tant que situation dans lequel la production d'anticorps, entre autres fonctions immunitaires, est altérée, ce qui aurait un impact à la fois sur la pathogenèse de la maladie et sur l'efficacité des vaccins. Dans cet article, nous illustrons que de tels impacts seraient dus à l'augmentation de l'adiposité et du modèle de distribution des graisses plutôt qu'à l'obésité (telle que définie par l'indice de masse corporelle) elle-même. Dans ce contexte, nous soulignons également l'importance des effets de l'exercice qui sont indépendants de la perte de poids.

23.2 Abstract

Obesity represents a risk factor for a variety of diseases because of its inflammatory component, among other biological patterns. Recently, with the ongoing COVID-19 crisis, a special focus has been put on obesity as a status in which antibody production, among other immune functions, is impaired, which would impact both disease pathogenesis and vaccine efficacy. Within this piece of writing, we illustrate that such patterns would be due to the increased adiposity and fat distribution pattern rather than obesity (as defined by the body mass index) itself. Within this context, we also highlight the importance of the weight-loss- independent effects of exercise.

Keywords: obesity; adiposity; antibodies; immunity

23.3 Impact of Adiposity and Fat Distribution, Rather than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits

Obesity is one of most challenging health problems for the modern medicine and therapeutic research [1,2]. The main pattern that makes obesity challenging is that, once established, it is hard to reverse, probably because the new "set up" of the biological reference of body weight and adiposity as neuroendocrine adaptation changes with a broken energy homeostasis [3,4]. The current ongoing coronavirus disease 2019 (COVID-19) pandemic could worsen the obesity pandemic, which would negatively impact the development of this COVID-19 crisis [5,6], especially with the impact that the measures imposed by governments might have on immunity [7]. Therefore, it is of high importance to understand how obesity and adiposity impact the immunity and more specifically antibodies production and function. This is because vaccine-induced antibodies represent the best shot we have to end this pandemic.

Antibodies represent an important mediator and factor of the immune system [8]. On the other hand, obesity represents a status in which different biological and homeostatic processes, such as regeneration [9], energy balance [4] and neuroendocrine factors [3], are impaired or impacted. Within this context, we would like to put a spotlight on selected consequences and impacts obesity and adiposity have on antibody patterns in order to explain some immunological specificities reported in obese patients. Obesity is defined by an abnormal fat accumulation usually as a result of an unhealthy lifestyle that increases the energy intake to more than the energy expenditure [1,4], leading to a variety of health consequences [10,11] with increased impacts [5].

Regarding obesity-related antibody patterns, numerous results reflect the impacts obesity has on antibody properties. For instance, adaptive immune response to influenza virus is impaired during obesity [12], innate and adaptive immune responses against influenza are delayed in obese patient [13] and obesity was suggested to decline influenza antibody titers following influenza vaccination [14] as well as reduce vaccine efficacy [15] with poor vaccine immunogenicity [16]. Similarly, lower COVID-19 mRNA vaccine-induced antibody titers have been associated with central obesity [17] and severe acute respiratory syndrome corona Virus-2 IgG antibodies negatively correlate with body mass index in COVID-19 patients. This is important in the current pandemic context with the vaccination efforts aiming to end this global health crisis. Furthermore, one key concept in obesity is that obesity is an "autoinflammatory" disease characterized by a chronic and low-grade inflammation [18,19], with several immune alterations including altered cell-mediated immune responses and leucocyte counts [20], principally in adipose tissue [21], where we have a localized inflammation [22]. Mechanisms beyond this are based on the links between obesity and both adipose tissue remodeling [23] and regulatory T cells [24]. Macrophage polarization [25], among other obesity-induced changes to macrophages [26],

specifically due to adipocyte–macrophage interaction [27], are also involved within the inflammatory component of obesity.

The impacts obesity has on regeneration [9] could also explain, in part, such reduced antibody production due to the impaired regeneration immunity cells could have. Such observations would explain the reduced efficacy of vaccination in obese patients [28] as illustrated by the impaired immune response to influenza vaccination in obese humans [14] which could lead to recommend additional immunological stimulation (vaccination) for obese patients.

Exercise (combined or not with diet and/or pharmacological therapies) is among the most widely accepted approaches to controlling body weight and managing obesity [29–31]. Exercise has known benefits and effects on the immunity system [32,33] including antibodies [34], B lymphocytes [35], cytokines such as Interleukin-6 [36], antioxidant effects [37], regeneration adjuvants [38–40], and improved immunosurveillance and immunocompetence with an anti-inflammatory effect [41] via macrophage infiltration suppression [42]. Importantly, as illustrated above, the antibody-related immunity decline with obesity would be associated with the adiposity and its distribution rather than body weight [17]. This suggests that the benefits of exercise on antibodies for obese patients can be achieved even without weight loss, as illustrated by the reduced hepatic and visceral lipids following exercise training without weight loss [43]. The adiposity and fat distribution correlations, rather than body weight, with antibodies and immunity-related functions have been shown in other contexts such as inflammatory profiles [44,45] and IgG N-glycosylation [46]. Furthermore, central adiposity has been highlighted in correlation with other diseases [47,48] and health problems as well [49,50]. In addition, acute exercise (and therefore independent of weight lost) has a broad impact on immune functions, including granulocytosis, lymphocytosis (antibody-producing cells) and monocytosis [51], increased natural killer cells [52], which are very responsive to acute exercise [53], increased lymphokine-activated killer cells activity [54] and enhanced T cell activity [55]. Importantly, acute exercise might promote a redistribution in lymphocyte subsets [56] including B cells that produce the antibodies [57,58] and which are affected by obesity [59,60] via diverse pathways including leptin-induced reduction in B cells function [61] as well. These benefits reverse most of those induced by adiposity described earlier (Figure 23.1).

Such concepts indicate and support the importance of exercise even without weight loss so that an interrelation between exercise and immunity regulation has been described [62]. The absence of weight loss does not mean the absence of fat loss or fat redistribution. Indeed, with exercise, body composition can improve toward increased muscle development and/or a new fat distribution but without body weight loss. This pattern could explain the benefits of exercise that does not lead to weight loss, which is of a particular importance since among the anti-obesity therapies (diet, pharmacology, etc.), exercise represents the one with the ability to shift the body composition as well as fat distribution beyond weight loss [63,64]. Moreover, indirect weight-loss-independent

benefits of exercise can improve immunity, for instance by reducing hypertension [65] that is associated with lower post COVID-19 vaccination antibodies titers [17].

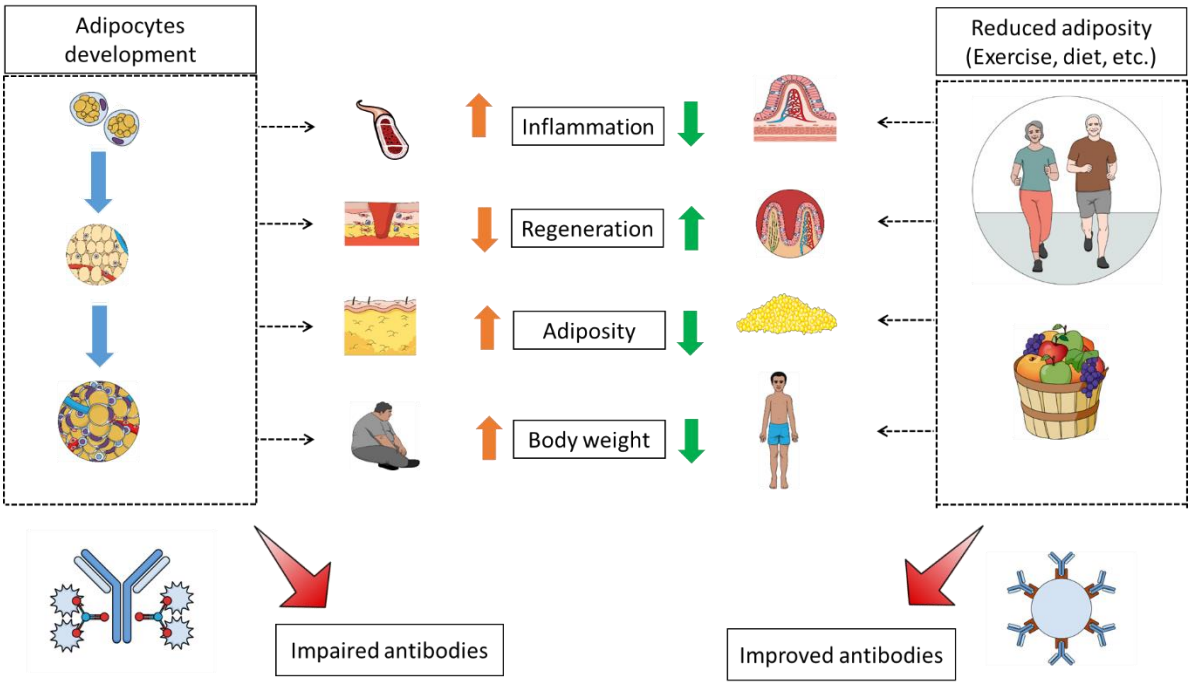


Figure 23.1. Antibody patterns and immunity performance between increased adiposity and exercise. Immunity functions and antibody-related patterns such as inflammation and regeneration are negatively impacted by adiposity development but corrected/improved by exercise and other adiposity-reducing approaches.

The benefits of exercise in the context of obesity are well documented in the context of energy balance, glucose metabolism, adiposity, muscles development, cardiorespiratory fitness and lipids profile [66–69]. However, within this piece of writing, we also illustrate the beneficial effects of exercise on obesity from an immunological perspective that focuses on antibodies. The interesting point is that the exercise effects are seen even with the absence of body weight loss. Therefore, this indicates that a focus on adiposity loss and fat distribution patterns [70] should replace the use of body weight as a medical parameter which correlates with the need to further focus, for instance, on waist circumference, which reflects to some extent visceral obesity, in clinical practice [71]. The concept of fat distribution and adiposity vs. overweight would also explain the concept “metabolically healthy obesity” [72,73], defined by body mass index that could lead to the concept of “immunologically healthy obesity”.

We hope our work could represent an additional encouragement of physical activity and a healthy diet towards a better lifestyle for obese patients even if it does not necessarily lead to weight loss, especially that the benefits shown without weight loss are various and include decreased circulating interleukin-6 [74], reduced hepatic and visceral lipids [43], increased insulin sensitivity [75] and improved endothelium-dependent vasodilation [76].

The possible application of such concepts would be the prescription of exercise to improve the antibody properties of obese patients even if it does not lead to weight loss since, for the COVID-19 mRNA vaccine for instance, low antibody titers have been associated with a higher waist circumference rather than high body weights [17], suggesting, once more, that the impact would be due to the fat distribution (central vs. peripheral obesity) [4,10] rather than increased body weight or even body fat percentage. Indeed, exercise can impact the body composition and fat distribution independently from body weight. This area of interaction between adiposity, fat distribution and immunology is worth further exploring in diverse contexts to develop new therapies, optimize the existing treatments and increase the awareness of how important weight-loss-independent effects of exercise are.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, references selection, etc.) throughout the writing process, edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a Merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship « Bourse Tremplin -Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec–Sante (FRQS), Quebec, Canada. Figure 23.1 was created using images from https://mindthegraph.com/ (accessed on 24 September 2021) and http://smart.servier.com (accessed on 25 September 2021). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare no conflict of interest.

23.4 References

1. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
2. Williams, E.P.; Mesidor, M.; Winters, K.; Dubbert, P.M.; Wyatt, S.B. Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. *Curr. Obes. Rep.* 2015, *4*, 363–370.
3. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.
4. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
5. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
6. Albashir, A.A.D. The potential impacts of obesity on COVID-19. *Clin. Med.* 2020, *20*, e109–e113.
7. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
8. Chaplin, D.D. Overview of the immune response. *J. Allergy Clin. Immunol.* 2010, *125*, S3–S23.
9. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
10. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
11. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.
12. Green, W.D.; Beck, M.A. Obesity Impairs the Adaptive Immune Response to Influenza Virus. *Ann. Am. Thorac. Soc.* 2017, *14*, S406–S409.
13. Honce, R.; Schultz-Cherry, S. Influenza in obese travellers: Increased risk and complications, decreased vaccine effectiveness. *J. Travel Med.* 2019, *26*, taz020.
14. Sheridan, P.A.; Paich, H.A.; Handy, J.; Karlsson, E.A.; Hudgens, M.G.; Sammon, A.B.; Holland, L.A.; Weir, S.; Noah, T.L.; Beck, M.A. Obesity is associated with impaired immune response to influenza vaccination in humans. *Int. J. Obes.* 2012, *36*, 1072–1077.
15. Honce, R.; Schultz-Cherry, S. Impact of Obesity on Influenza A Virus Pathogenesis, Immune Response, and Evolution. *Front. Immunol.* 2019, *10*, 1071.
16. Ovsyannikova, I.G.; White, S.J.; Larrabee, B.; Grill, D.E.; Jacobson, R.M.; Poland, G.A. Leptin and leptin-related gene polymorphisms, obesity, and influenza A/H1N1 vaccine-induced immune responses in older individuals. *Vaccine* 2013, *32*, 881–887.
17. Watanabe, M.; Balena, A.; Tuccinardi, D.; Tozzi, R.; Risi, R.; Masi, D.; Caputi, A.; Rossetti, R.; Spoltore, M.E.; Filippi, V.; et al. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. *Diabetes Metab. Res. Rev.* 2021, e3465.
18. Esser, N.; Legrand-Poels, S.; Piette, J.; Scheen, A.J.; Paquot, N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res. Clin. Pract.* 2014, *105*, 141–150.
19. Cox, A.J.; West, N.P.; Cripps, A.W. Obesity, inflammation, and the gut microbiota. *Lancet Diabetes Endocrinol.* 2015, *3*, 207–215.
20. de Heredia, F.P.; Gómez-Martínez, S.; Marcos, A. Obesity, inflammation and the immune system. *Proc. Nutr. Soc.* 2012, *71*, 332–338.
21. Lee, B.C.; Lee, J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. *Biochim. Biophys. Acta* 2014, *1842*, 446–462.
22. Karczewski, J.; Sledzi´nska, E.; Baturo, A.; Jo´nczyk, I.; Maleszko, A.; Samborski, P.; Begier-Krasi´nska, B.; Dobrowolska, A. Obesity and inflammation. *Eur. Cytokine. Netw.* 2018, *29*, 83–94.
23. Sun, K.; Kusminski, C.M.; Scherer, P.E. Adipose tissue remodeling and obesity. *J. Clin. Investig.* 2011, *121*, 2094–2101.

24. Kucharska, A.M.; Pyrzak, B.; Demkow, U. Regulatory T Cells in Obesity. ˙ *Adv. Exp. Med. Biol.* 2015, *866*, 35–40.
25. Li, C.; Xu, M.M.; Wang, K.; Adler, A.J.; Vella, A.T.; Zhou, B. Macrophage polarization and meta-inflammation. *Transl. Res.* 2018, *191*, 29–44.
26. Thomas, D.; Apovian, C. Macrophage functions in lean and obese adipose tissue. *Metabolism* 2017, *72*, 120–143.
27. Engin, A.B. Adipocyte-Macrophage Cross-Talk in Obesity. *Adv. Exp. Med. Biol.* 2017, *960*, 327–343.
28. Beck, M.A. Influenza and obesity: Will vaccines and antivirals protect? *J. Infect. Dis.* 2012, *205*, 172–173.
29. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. *Metabolism* 2019, *92*, 163–169.
30. Hsu, K.J.; Liao, C.D.; Tsai, M.W.; Chen, C.N. Effects of Exercise and Nutritional Intervention on Body Composition, Metabolic Health, and Physical Performance in Adults with Sarcopenic Obesity: A Meta-Analysis. *Nutrients* 2019, *11*, 2163.
31. Bray, G.A.; Frühbeck, G.; Ryan, D.H.; Wilding, J.P. Management of obesity. *Lancet* 2016, *387*, 1947–1956.
32. da Silveira, M.P.; da Silva Fagundes, K.K.; Bizuti, M.R.; Starck, É.; Rossi, R.C.; de Resende, E.S.D.T. Physical exercise as a tool to help the immune system against COVID-19: An integrative review of the current literature. *Clin. Exp. Med.* 2021, *21*, 15–28.
33. Alawna, M.; Amro, M.; Mohamed, A.A. Aerobic exercises recommendations and specifications for patients with COVID-19: A systematic review. *Eur. Rev. Med. Pharmacol. Sci.* 2020, *24*, 13049–13055.
34. Suzuki, K.; Tagami, K. Voluntary wheel-running exercise enhances antigen-specific antibody-producing splenic B cell response and prolongs IgG half-life in the blood. *Eur. J. Appl. Physiol.* 2005, *94*, 514–519.
35. Fragala, M.S.; Kraemer, W.J.; Mastro, A.M.; Denegar, C.R.; Volek, J.S.; Kupchak, B.R.; Häkkinen, K.; Anderson, J.M.; Maresh, C.M. Glucocorticoid receptor expression on human B cells in response to acute heavy resistance exercise. *Neuroimmunomodulation* 2011, *18*, 156–164.
36. Ghanemi, A.; St-Amand, J. Interleukin-6 as a "metabolic hormone". *Cytokine* 2018, *112*, 132–136.
37. de Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. *Sports Med.* 2017, *47*, 277–293.
38. Smith, J.K. Exercise as an Adjuvant to Cartilage Regeneration Therapy. *Int. J. Mol. Sci.* 2020, *21*, 9471.
39. Schüttler, D.; Clauss, S.; Weckbach, L.T.; Brunner, S. Molecular Mechanisms of Cardiac Remodeling and Regeneration in Physical Exercise. *Cells* 2019, *8*, 1128.
40. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
41. Scheffer, D.D.L.; Latini, A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. *Biochim. Biophys. Acta Mol. Basis Dis.* 2020, *1866*, 165823.
42. Kawanishi, N.; Yano, H.; Yokogawa, Y.; Suzuki, K. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. *Exerc. Immunol. Rev.* 2010, *16*, 105–118.
43. Johnson, N.A.; Sachinwalla, T.; Walton, D.W.; Smith, K.; Armstrong, A.; Thompson, M.W.; George, J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 2009, *50*, 1105–1112.
44. Samouda, H.; de Beaufort, C.; Stranges, S.; Guinhouya, B.C.; Gilson, G.; Hirsch, M.; Jacobs, J.; Leite, S.; Vaillant, M.; Dadoun, F. Adding anthropometric measures of regional adiposity to BMI improves prediction of cardiometabolic, inflammatory and adipokines profiles in youths: A cross-sectional study. *BMC Pediatr.* 2015, *15*, 168.
45. Singh, S.; Sharma, A.N.; Murad, M.H.; Buttar, N.S.; El-Serag, H.B.; Katzka, D.A.; Iyer, P.G. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: A systematic review and meta-analysis. *Clin. Gastroenterol. Hepatol.* 2013, *11*, 1399–1412.

46. Liu, D.; Li, Q.; Dong, J.; Li, D.; Xu, X.; Xing, W.; Zhang, X.; Cao, W.; Hou, H.; Wang, H.; et al. The Association Between Normal BMI With Central Adiposity and Proinflammatory Potential Immunoglobulin G N-Glycosylation. *Diabetes Metab. Syndr. Obesity Targets Ther.* 2019, *12*, 2373–2385.
47. Ali, O.; Cerjak, D.; Kent, J.W.; James, R.; Blangero, J.; Zhang, Y. Obesity, central adiposity and cardiometabolic risk factors in children and adolescents: A family-based study. *Pediatr. Obes.* 2014, *9*, e58–e62.
48. Shang, X.; Scott, D.; Hodge, A.; Khan, B.; Khan, N.; English, D.R.; Giles, G.G.; Ebeling, P.R.; Sanders, K.M. Adiposity assessed by anthropometric measures has a similar or greater predictive ability than dual-energy X-ray absorptiometry measures for abdominal aortic calcification in community-dwelling older adults. *Int. J. Cardiovasc. Imaging* 2016, *32*, 1451–1460.
49. Zimta, A.A.; Tigu, A.B.; Muntean, M.; Cenariu, D.; Slaby, O.; Berindan-Neagoe, I. Molecular Links between Central Obesity and Breast Cancer. *Int. J. Mol. Sci.* 2019, *20*, 5364.
50. Lin, T.Y.; Hung, S.C.; Lim, P.S. Central obesity and incident atherosclerotic cardiovascular disease events in hemodialysis patients. *Nutr. Metab. Cardiovasc. Dis.* 2020, *30*, 500–507.
51. Shinkai, S.; Shore, S.; Shek, P.N.; Shephard, R.J. Acute exercise and immune function. Relationship between lymphocyte activity and changes in subset counts. *Int. J. Sports Med.* 1992, *13*, 452–461.
52. Perna, F.M.; Schneiderman, N.; LaPerriere, A. Psychological stress, exercise and immunity. *Int. J. Sports Med.* 1997, *18*, S78–S83.
53. Timmons, B.W.; Cieslak, T. Human natural killer cell subsets and acute exercise: A brief review. *Exerc. Immunol. Rev.* 2008, *14*, 8–23.
54. Horohov, D.W.; Dimock, A.; Guirnalda, P.; Folsom, R.W.; McKeever, K.H.; Malinowski, K. Effect of exercise on the immune response of young and old horses. *Am. J. Vet. Res.* 1999, *60*, 643–647.
55. Lin, Y.S.; Jan, M.S.; Chen, H.I. The effect of chronic and acute exercise on immunity in rats. *Int. J. Sports Med.* 1993, *14*, 86–92.
56. Frisina, J.P.; Gaudieri, S.; Cable, T.; Keast, D.; Palmer, T.N. Effects of acute exercise on lymphocyte subsets and metabolic activity. *Int. J. Sports Med.* 1994, *15*, 36–41.
57. Tiller, T. Single B cell antibody technologies. *N. Biotechnol.* 2011, *28*, 453–457.
58. Rashidian, J.; Lloyd, J. Single B Cell Cloning and Production of Rabbit Monoclonal Antibodies. *Methods Mol. Biol.* 2020, *2070*, 423–441.
59. Nicholas, D.A.; Nikolajczyk, B.S. B cells shed light on diminished vaccine responses in obesity. *Obesity* 2016, *24*, 551.
60. Shaikh, S.R.; Haas, K.M.; Beck, M.A.; Teague, H. The effects of diet-induced obesity on B cell function. *Clin. Exp. Immunol.* 2015, *179*, 90–99.
61. Frasca, D.; Ferracci, F.; Diaz, A.; Romero, M.; Lechner, S.; Blomberg, B.B. Obesity decreases B cell responses in young and elderly individuals. *Obesity* 2016, *24*, 615–625.
62. Wang, J.; Liu, S.; Li, G.; Xiao, J. Exercise Regulates the Immune System. *Adv. Exp. Med. Biol.* 2020, *1228*, 395–408.
63. Goedecke, J.H.; Micklesfield, L.K. The effect of exercise on obesity, body fat distribution and risk for type 2 diabetes. *Med. Sport Sci.* 2014, *60*, 82–93.
64. Lee, S.; Kuk, J.L.; Davidson, L.E.; Hudson, R.; Kilpatrick, K.; Graham, T.E.; Ross, R. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. *J. Appl. Physiol.* 2005, *99*, 1220–1225.
65. Alpsoy, ¸S. Exercise and Hypertension. *Adv. Exp. Med. Biol.* 2020, *1228*, 153–167.
66. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
67. Kirwan, J.P.; Sacks, J.; Nieuwoudt, S. The essential role of exercise in the management of type 2 diabetes. *Cleve Clin. J. Med.* 2017, *84*, S15–S21.
68. Keating, S.E.; Hackett, D.A.; Parker, H.M.; O'Connor, H.T.; Gerofi, J.A.; Sainsbury, A.; Baker, M.K.;

Chuter, V.H.; Caterson, I.D.; George, J.; et al. Effect of aerobic exercise training dose on liver fat and visceral adiposity. *J. Hepatol.* 2015, *63*, 174–182.
69. Lemura, L.M.; Von Duvillard, S.P.; Andreacci, J.; Klebez, J.M.; Chelland, S.A.; Russo, J. Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic and combination training in young women. *Graefe's Arch. Clin. Exp. Ophthalmol.* 2000, *82*, 451–458.
70. Piché, M.E.; Tchernof, A.; Després, J.P. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. *Circ. Res.* 2020, *126*, 1477–1500.
71. Ross, R.; Neeland, I.J.; Yamashita, S.; Shai, I.; Seidell, J.; Magni, P.; Santos, R.D.; Arsenault, B.; Cuevas, A.; Hu, F.B.; et al. Waist circumference as a vital sign in clinical practice: A Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. *Nat. Rev. Endocrinol.* 2020, *16*, 177–189.
72. Blüher, M. Metabolically Healthy Obesity. *Endocr. Rev.* 2020, *41*, 405–420.
73. Iacobini, C.; Pugliese, G.; Blasetti Fantauzzi, C.; Federici, M.; Menini, S. Metabolically healthy versus metabolically unhealthy obesity. *Metabolism* 2019, *92*, 51–60.
74. Dekker, M.J.; Lee, S.; Hudson, R.; Kilpatrick, K.; Graham, T.E.; Ross, R.; Robinson, L.E. An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. *Metabolism* 2007, *56*, 332–338.
75. Duncan, G.E.; Perri, M.G.; Theriaque, U.W.; Hutson, A.D.; Eckel, R.H.; Stacpoole, P.W. Exercise Training, Without Weight Loss, Increases Insulin Sensitivity and Postheparin Plasma Lipase Activity in Previously Sedentary Adults. *Diabetes Care* 2003, *26*, 557–562.
76. Mestek, M.L.; Westby, C.M.; Van Guilder, G.P.; Greiner, J.J.; Stauffer, B.; DeSouza, C.A. Regular Aerobic Exercise, Without Weight Loss, Improves Endothelium-dependent Vasodilation in Overweight and Obese Adults. *Obesity* 2010, *18*, 1667–1669.

Chapter 24. Research Article - Secreted Protein Acidic and Rich in Cysteine (*Sparc*) KO Leads to an Accelerated Ageing Phenotype Which Is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice

Abdelaziz Ghanemi [1,2], Aicha Melouane [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

Received: 3 January 2022; Accepted: 24 January 2022; Published: 28 January 2022

24.1 Résumé (French abstract)

Secreted protein acidic and rich in cysteine (SPARC/*Sparc*) diminue avec le vieillissement mais augmente avec l'exercice. Nous explorons les conséquences du *Sparc* Knockout (KO) et les comparons aux effets du vieillissement. Nous observons également les effets de l'exercice. Nous étudions aussi les effets de la surexpression de SPARC et les comparons aux effets de l'exercice. Nous résultats confirme que le déficit en SPARC conduit à un phénotype semblable au vieillissement et que la surexpression de SPARC imiterait l'exercice, contrecarrerait le vieillissement et améliorerait les changements liés à l'âge. Les applications potentielles de ces résultats sont de construire/optimiser des modèles d'animaux basés sur *Sparc* KO de divers problèmes de santé et, d'autre part, de développer des thérapies basées sur l'introduction de SPARC ou le ciblage des voies liées au SPARC pour imiter l'exercice contre les troubles métaboliques et ceux liés à l'âge.

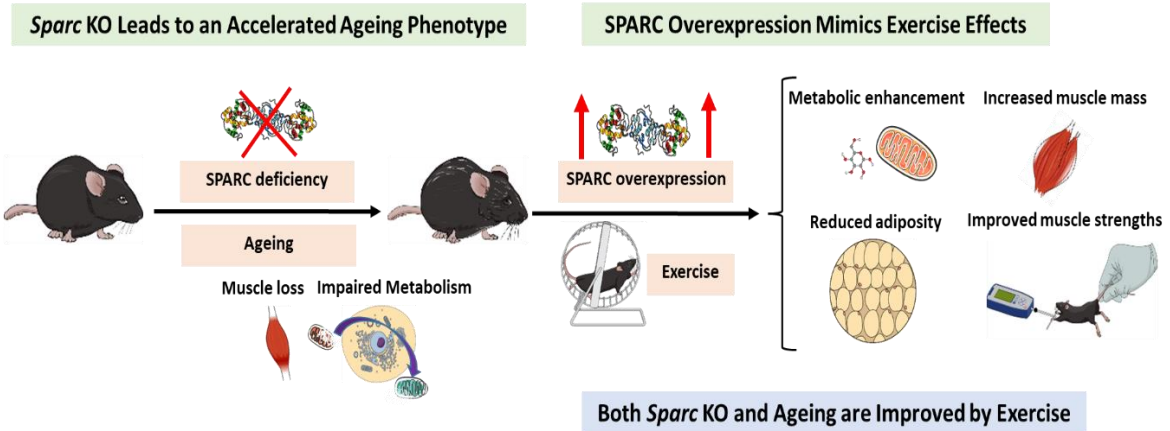
24.2 Abstract

Secreted protein acidic and rich in cysteine (SPARC) is a matricellular glycoprotein implicated in various functions, including metabolism, tissue regeneration, and functional homeostasis. SPARC/*Sparc* declines with ageing but increases with exercise. We aim to verify two hypotheses: (1) SPARC deficiency leads to an ageing-like phenotype (metabolic decline, muscle loss, etc.), and (2) SPARC overexpression would mimic exercise, counteract ageing, and improve age-related changes. Our mice experiments are divided into two parts. First, we explore the consequences of *Sparc* knockout (KO) and compare them to

the ageing effects. We also observe the effects of exercise. In the second part, we study the effects of SPARC overexpression and compare them to the exercise benefits. At the end, we make an analysis of the results to point out the analogies between *Sparc* KO and the ageing-like phenotype on the one hand and make comparisons between SPARC overexpression and exercise in the context of exercise counteracting ageing. The measurements were mainly related to tissue weights, adiposity, metabolism, and muscle strength. The main findings are that *Sparc* KO reduced glucose tolerance, muscle glucose transporter expression, and abdominal adipose tissue weight but increased glycogen content in the muscle. SPARC overexpression increased muscle strength, muscle mass, and expressions of the muscle glucose transporter and mitochondrial oxidative phosphorylation but lowered the glycemia and the adiposity, especially in males. Collectively, these findings, and the data we have previously reported, show that *Sparc* KO mice manifest an ageing-like phenotype, whereas SPARC overexpression and exercise generate similar benefits. The benefits are towards counteracting both the SPARC deficiency-induced ageing-like phenotype as well as reversing the age-related changes. The potential applications of these findings are to build/optimize *Sparc* KO-based animal models of various health conditions and, on the other hand, to develop therapies based on introducing SPARC or targeting SPARC-related pathways to mimic exercise against age-related and metabolic disorders.

Keywords: secreted protein acidic and rich in cysteine (SPARC); exercise; ageing; metabolism

24.3 Graphical abstract



KO: knockout
***Sparc*/SPARC**: Secreted protein acidic and rich in cysteine

24.4 Introduction

With the improvement of the healthcare system and the decline of infectious diseases (vaccines, therapies, etc.), life expectancy has increased significantly over the past 50 years [1], which has enhanced the elderly population. This makes ageing and age-associated metabolic and functional decline important health challenges as they represent risk factors for various health problems, including metabolic disorders and obesity. Geriatrics aims to develop the best medical approaches to face such challenges. Within this context, exercise is the best anti-ageing approach as it minimizes several age-related changes [2,3] and has been prescribed for the older population [4–6]. In addition, exercise has been medically prescribed for a variety of other health conditions, such as obesity and sarcopenia, for which ageing could be a risk factor as well. Therefore, elucidating the molecular pathways of both ageing and exercise and their mechanistic links would significantly contribute to optimizing both the available studying methods (animal models of ageing, cell cultures, etc.) and the available therapeutic tools.

In order to better elucidate the exercise molecular patterns and the mechanism by which exercise leads to its known benefits, we have already characterized secreted protein acidic and rich in cysteine (*SPARC*) as an exercise-induced gene in vivo [7] and in vitro (cellular model of exercise) [8]. SPARC serum levels also increase with exercise [9], both in humans and in mice [10]. We have suggested that the exercise-induced muscle phenotype changes are SPARC-dependent [11], and exercise-induced *Sparc* expression could be an indicator of the therapeutic efficacy of exercise and the response to exercise [12]. In addition, adding SPARC to C2C12 (muscle cells) increased their differentiation [13]. On the other hand, *Sparc* expression is downregulated by ageing [10].

As *Sparc* decreases with ageing, and exercise both increases *Sparc*/SPARC [10,14] and counteracts ageing, we suggest that the ageing results and exercise benefits (including anti-ageing) are mediated, at least in part, by SPARC, especially in the way that age-related alterations in skeletal muscle progenitor cells are linked to SPARC, for instance [15]. These correlate with SPARC implications in different biological and metabolic functions. Indeed, SPARC, also known as osteonectin or basement membrane-40 (BM-40) [16,17], is a matricellular glycoprotein which comprises three distinct structural domains [18] and binds to calcium, collagen [19], and vitronectin (structural matrix proteins) [20]. It is expressed in most tissues, especially when the tissues undergo changes or active remodeling (healing, embryogenesis, cancer, obesity, etc.), which reflects its importance in tissue development and growth. To achieve such functions, SPARC is, for instance, involved in tissue repair [21], cell turnover [22], cell renewal and growth [23,24], maintenance of bone mass [25], osteoblast maturation [16], angiogenesis regulation [25], extracellular matrix organization [26], collagen maturation [27], glucose and lipid metabolism [28–30], remodeling [31,32], regeneration [33], differentiation [34], and adipose tissue regulation [35]. Such distribution and implications also allowed us to suggest SPARC as a molecular physiological and pathological biomarker [36].

Elucidating the interplay between ageing, SPARC, and exercise can represent a breakthrough for a deeper understanding of ageing and metabolic disorders towards novel SPARC-based molecular therapies for the related health conditions. Within this context, the roles and properties of SPARC allow us to formulate two hypotheses. Whereas SPARC deficiency leads to an ageing-like phenotype (metabolic decline, muscle loss, etc.), SPARC overexpression would mimic exercise, counteract ageing, and improve age-related changes (boost metabolism, strengthen muscles, reduce adiposity, etc.).

We have designed two independent, yet correlated, studies to verify our hypotheses. Our key molecular and functional measures focus on exploring skeletal muscle for specific reasons. First, the studies which revealed that *SPARC*/*Sparc* is overexpressed in response to exercise were conducted in muscles (in vivo) and muscle cell culture (in vitro). In addition, muscle is the main tissue implicated in the metabolic and mechanic functions and is modified by exercise; SPARC represents a myokine secreted by the muscle in response to exercise and represents a secretory organ. This is in addition to the role of muscles in generating force and power (mechanical property [37]). Indeed, the skeletal muscle implication in homeostasis is not limited to its metabolic activity (mainly energy expenditure); it is also an organ that secrets bioactive proteins, including interleukins and growth factors [9] which allow the muscle's communication with diverse tissues [38], including key tissues such as the adipose tissue, the liver, and the brain [39]. Finally, the metabolic and contractile performance of the muscles changes with ageing (muscle weakness and atrophy [37]), which is a key variable that we focus on in this work. However, as adiposity and tissue weights are also important in the context of ageing and exercise, we have also added related measures to complete the investigation.

24.5 Results

24.5.1. *Sparc* Knockout (KO) Mice, Exercise, and Ageing

Each age group of the mice, young (Y) and old (O), was divided based on the genotypes of knockout (KO) or wild-type (WT) to obtain four groups: Y-KO, Y-WT, O-KO and O-WT. Finally, each of these four groups was further subdivided into two groups according to whether they were exercising (Ex) or sedentary (Sed) mice. Therefore, our experimental design included eight groups.

24.5.1.1. Tissue Weights and Sizes

The mice tissues were weighed immediately after they were removed, following the sacrifice. The analyzed data (Table 24.1) are in the form of both the absolute values (weight) and the weight percentages of the tissues (to the body weight). The tissues were the Achilles tendon, brown adipose tissue (BAT), inguinal adipose tissue (IngAT), abdominal adipose tissue (AbdAT), retroperitoneal adipose tissue (RetAT), epididymal adipose tissue (EpiAT), and Mesenteric adipose tissue (MesAT) and the three muscles,

gastrocnemius (GC), soleus (Sol), and extensor digitorum longus (EDL). We also provide inguinal and epididymal adipocytes size (measured) and number (estimated).

The data show a genotype effect (WT > KO) for all the tissues for either the weights or the weight percentages or for both, except for the BAT, for which we had an opposite genotype effect (KO > WT) for its weight percentage. In addition, a genotype effect (WT > KO) was seen on the estimated number of EpiAT adipocytes.

We found the effect of age (O > Y) on the weights of the Achilles tendon, BAT, IngAT, AbdAT, RetAT, EpiAT, and MesAT, as well as the weight percentages of the BAT (trends), AbdAT, RetAT, EpiAT, and MesAT, in addition to the adipocytes size of EpiAT and IngAT and the estimated adipocytes number of EpiAT. We had the opposite effect of age (Y > O) on the weights of GC and the weight percentages of GC, Sol, and EDL (trend), as well as the estimated adipocytes number of IngAT.

For the exercise effects, exercise increased the weight percentage of BAT but decreased the weights of AbdAT (trend), RetAT (trend), and MesAT and the weight percentage of MesAT.

The statistical analysis also revealed an interaction between genotype and age. Indeed, whereas the age effect (O > Y) was more pronounced in the WT mice than in the *Sparc* KO mice in the weight and weight percentages of AbdAT, RetAT, EpiAT and MesAT, it was only seen in the WT mice (not the *Sparc* KO mice) in the adipocytes size of IngAT and the estimated adipocytes number of EpiAT. On the other hand, only in the Achilles tendon was the age effect (O > Y) on weight more pronounced in the *Sparc* KO mice, and the age effect (O > Y) on weight percentage was only seen in the *Sparc* KO mice.

Table 24.1. Tissues weights and sizes

		Young				Old				3-way ANOVA				
		Wild-type		*Sparc* knockout		Wild-type		*Sparc* knockout						
		Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Sedentary	Exercise	Age	Genotype	Exercise	Age × Genotype	Genotype × Exercise
Achilles tendon	mg	4.3 ± 0.6	4.0 ± 0.2	3.1 ± 0.2	2.8 ± 0.2	5.0 ± 0.2	5.0 ± 0.4	4.8 ± 0.2	5.1 ± 0.5	O > Y	WT > KO	-	WT: O>Y*, KO: O>>Y	-
	%	0.015 ± 0.002	0.014 ± 0.001	0.011 ± 0.001	0.011 ± 0.001	0.013 ± 0.001	0.014 ± 0.001	0.016 ± 0.001	0.017 ± 0.002	-	-	-	KO: O>>Y	-
Brown adipose tissue	mg	93 ± 13	103 ± 16	103 ± 8	130 ± 33	159 ± 27	142 ± 13	135 ± 12	192 ± 32	O > Y	-	-	-	-
	%	0.31 ± 0.04	0.35 ± 0.05	0.37 ± 0.03	0.50 ± 0.10	0.41 ± 0.04	0.40 ± 0.03	0.42 ± 0.02	0.63 ± 0.10	O > Y*	KO > WT	Ex > Sed	-	Ex: KO>WT, Sed: WT=KO
Inguinal adipose tissue	mg	19 ± 4	16 ± 4	18 ± 6	17 ± 3	41 ± 11	23 ± 1	22 ± 3	21 ± 2	O > Y	-	-	-	-
	%	0.065 ± 0.014	0.054 ± 0.014	0.063 ± 0.023	0.068 ± 0.015	0.100 ± 0.020	0.065 ± 0.004	0.069 ± 0.008	0.069 ± 0.007	-	-	-	-	-
	μm^2	1076 ± 109	1020 ± 126	1157 ± 87	1201 ± 102	3335 ± 646	3262 ± 542	2949 ± 613	1834 ± 215	O > Y	-	-	WT: O>Y, K: O=Y	-
	N	844 ± 140	471 ± 134	793 ± 206	411 ± 173	616 ± 144	332 ± 67	619 ± 161	578 ± 130	Y > O	-	-	-	-
Abdominal adipose tissue	mg	982 ± 204	781 ± 126	919 ± 133	651 ± 125	2831 ± 347	2603 ± 175	1760 ± 221	1463 ± 162	O > Y	WT > KO	Sed > Ex*	WT: O>>Y, KO: O>Y	-
	%	3.23 ± 0.58	2.61 ± 0.36	3.24 ± 0.43	2.49 ± 0.38	7.32 ± 0.63	7.31 ± 0.33	5.38 ± 0.49	4.71 ± 0.43	O > Y	WT > KO	-	WT: O>>Y, KO: O>Y	-
Retroperitoneal adipose tissue	mg	177 ± 38	125 ± 19	182 ± 34	119 ± 23	435 ± 40	410 ± 27	247 ± 30	230 ± 25	O > Y	WT > KO	Sed > Ex*	WT: O>>Y, KO: O>Y	-
	%	0.58 ± 0.11	0.42 ± 0.05	0.64 ± 0.11	0.46 ± 0.07	1.15 ± 0.08	1.15 ± 0.05	0.76 ± 0.07	0.74 ± 0.07	O > Y	WT > KO	-	WT: O>>Y, KO: O>Y	-
Epididymal adipose tissue	mg	305 ± 67	246 ± 42	293 ± 41	205 ± 39	870 ± 101	838 ± 54	556 ± 70	502 ± 58	O > Y	WT > KO	-	WT: O>>Y, KO: O>Y	-
	%	1.00 ± 0.19	0.82 ± 0.12	1.03 ± 0.13	0.78 ± 0.12	2.27 ± 0.21	2.36 ± 0.10	1.71 ± 0.17	1.61 ± 0.16	O > Y	WT > KO	-	WT: O>>Y, KO: O>Y	-
	μm^2	2501 ± 196	2417 ± 226	3007 ± 230	2456 ± 255	4739 ± 635	4722 ± 512	4686 ± 562	4115 ± 287	O > Y	-	-	-	-
	N	3397 ± 437	7037 ± 2648	2924 ± 291	6037 ± 2164	2553 ± 250	2856 ± 463	2392 ± 219	2805 ± 314	O > Y	WT>KO	-	WT: O>Y, KO: O=Y	-
Mesenteric adipose tissue	mg	195 ± 35	164 ± 25	152 ± 21	122 ± 25	656 ± 126	518 ± 46	400 ± 63	230 ± 31	O > Y	WT > KO	Sed > Ex	WT: O>>Y, KO: O>Y	-
	%	0.64 ± 0.10	0.55 ± 0.07	0.54 ± 0.07	0.46 ± 0.08	1.63 ± 0.22	1.44 ± 0.10	1.19 ± 0.14	0.74 ± 0.09	O > Y	WT > KO	Sed > Ex	WT: O>>Y, KO: O>Y	-
Gastrocnemius	mg	325 ± 9	316 ± 10	258 ± 8	244 ± 7	308 ± 7	307 ± 4	242 ± 5	237 ± 6	Y > O	WT > KO	-	-	-
	%	1.11 ± 0.04	1.10 ± 0.03	0.93 ± 0.04	0.99 ± 0.04	0.84 ± 0.04	0.88 ± 0.03	0.80 ± 0.04	0.78 ± 0.02	Y > O	WT > KO	-	-	-
Soleus	mg	11.5 ± 1.8	9.9 ± 0.2	9.1 ± 0.2	9.0 ± 0.4	10.7 ± 0.3	10.7 ± 0.3	9.0 ± 0.6	10.0 ± 0.6	-	WT > KO	-	-	-
	%	0.039 ± 0.005	0.034 ± 0.001	0.033 ± 0.001	0.036 ± 0.001	0.029 ± 0.001	0.031 ± 0.001	0.029 ± 0.001	0.033 ± 0.002	Y > O	-	-	-	Sed: WT>KO, Ex: KO>WT
Extensor digitorum longus	mg	25 ± 2	25 ± 2	20 ± 2	19 ± 1	23 ± 2	27 ± 1	23 ± 1	21 ± 1	-	WT > KO	-	-	-
	%	0.086 ± 0.005	0.089 ± 0.007	0.070 ± 0.007	0.075 ± 0.006	0.065 ± 0.004	0.078 ± 0.003	0.078 ± 0.007	0.069 ± 0.003	Y > O*	-	-	WT: Y>O, KO: Y=O	-

Data are mean ± SEM. The number of mice: 11–12 per experimental condition. *: Trend ($0.05 \leq p < 0.1$). μm^2: mean adipocyte size. N: estimated number of adipcytes ($\times 10^3$). %: percentage to the body weight.
-: No effect. Abbreviations: Ex, exercise; KO, knockout; O, old; Sed, sedentary; *Sparc*, secreted protein acidic and rich in cysteine; WT, wild-type; Y, young.
Note: No effect for the interactions Age × Exercise and Age × Genotype × Exercise (The two corresponding lines have been removed).

24.5.1.2. Oral Glucose Tolerance Test (OGTT)

Figure 24.1 shows that the glucose tolerance decreased with the *Sparc* KO and increased with exercise. The ageing also decreased the glucose tolerance only in the WT mice (not in the *Sparc* KO mice). The 4-way ANOVA analysis also revealed an interaction (age × genotype × exercise) in which ageing decreased the tolerance only in the WT-Ex mice (not the WT-Sed mice) and increased the glucose tolerance in the KO-Ex mice (not the KO-Sed mice).

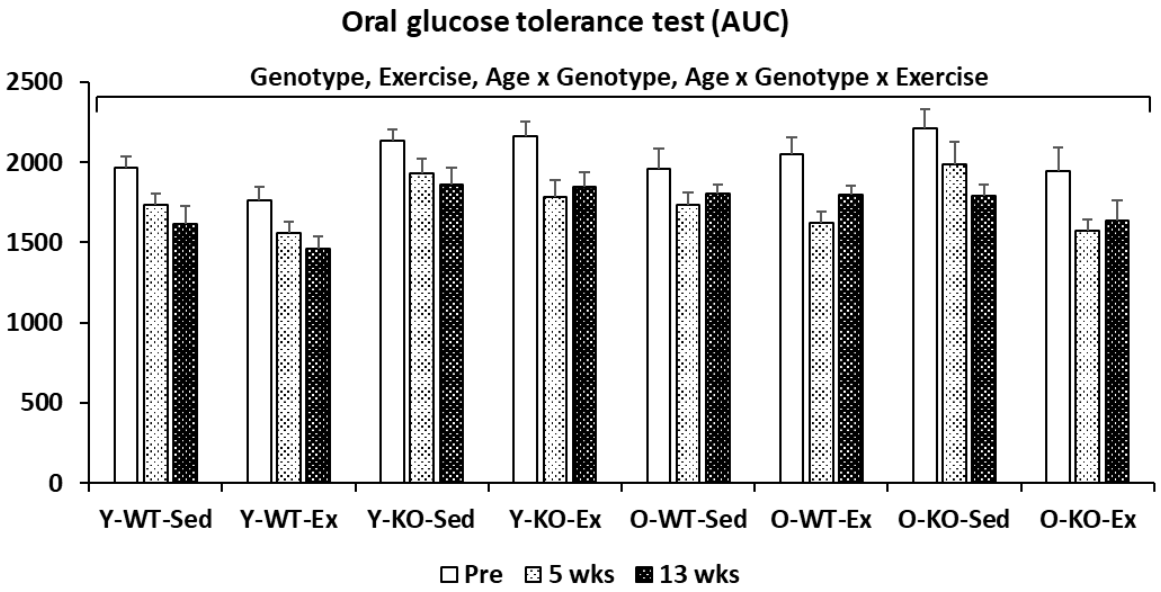


Figure 24.1. Oral Glucose Tolerance Test. Glucose tolerance decreased with *Sparc* KO and increased with exercise. The ageing also decreased the glucose tolerance only in WT mice (not in *Sparc* KO mice). The 4-way ANOVA analysis also revealed an interaction (age × genotype × exercise) in which ageing decreased the tolerance only in WT-Ex mice (not WT-Sed mice) and increased the glucose tolerance in KO-Ex mice (not KO-Sed mice). All data are mean ± SEM. The number of mice: 11–12 mice per experimental condition. Abbreviations: AUC, area under the curve; Ex, exercise; KO, knockout; O, old; Sed, sedentary; *Sparc*, secreted protein acidic and rich in cysteine; WT, wild-type; Y, young.

24.5.1.3. Glycogen Content and Glucose Transporter Type 4 (GLUT4) Expression in the Muscle

For the muscular expression of GLUT4 (Figure 24.2A), ageing and the *Sparc* KO decreased it, but exercise increased it. The interaction (age × genotype) revealed that the ageing effect was only seen in the WT mice.

Among the three variables, only the genotype had an effect on the glycogen content (Figure 24.2B). The *Sparc* KO increased the muscular glycogen.

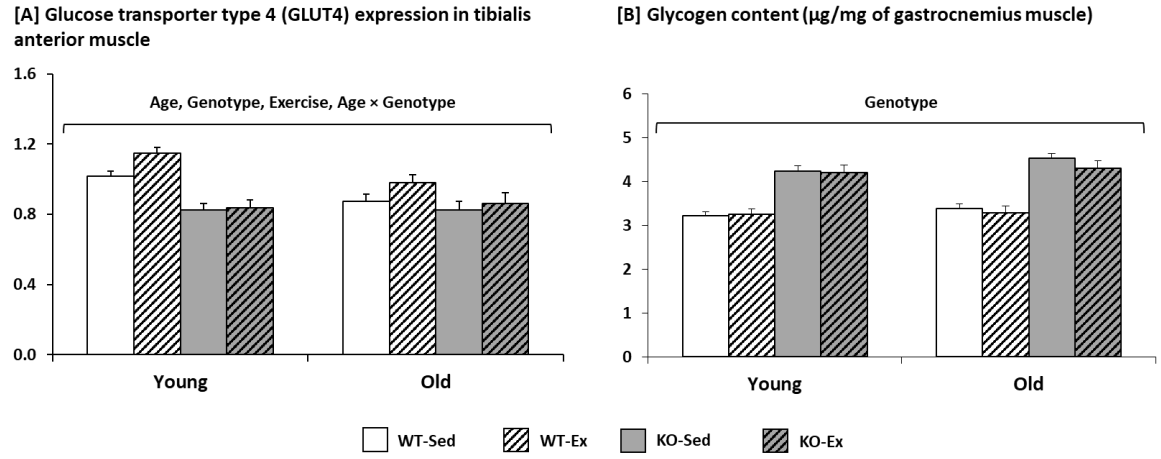


Figure 24.2. Glucose transporter type 4 (GLUT4) expression and glycogen content in the muscle. (**A**) Ageing and *Sparc* KO decreased muscular expression of GLUT4, but exercise increased it. The interaction (age × genotype) revealed that the effect of ageing was only seen in WT mice. (**B**) *Sparc* KO increased the muscular glycogen. All data are mean ± SEM. The number of mice: 11–12 mice per experimental condition. Abbreviations: Ex, exercise; KO, knockout; Sed, sedentary; *Sparc*, secreted protein acidic and rich in cysteine; WT, wild-type.

24.5.2. *Sparc* Transgeneic (Tg) Mice

The study involved 32 mice. They were divided into four groups depending on the genotype, Tg (*Sparc* overexpression) or WT, and sex, male (M) or female (F). Therefore, we had four groups: Tg-M (n = 9), WT-M (n = 7), Tg-F (n = 11), and WT-F (n = 5).

24.5.2.1. Tissues Weights

The mice overexpressing *Sparc* tended to have larger total skeletal muscle mass (GC, Sol, TA, and EDL) (Figure 24.3A). In addition, the muscle mass was greater in the male mice than in the female mice. For the IngAT mass (Figure 24.3B), the decreased (trend) weight was only seen in the male mice (by 23%), whereas the male mice had more IngAT than the female mice.

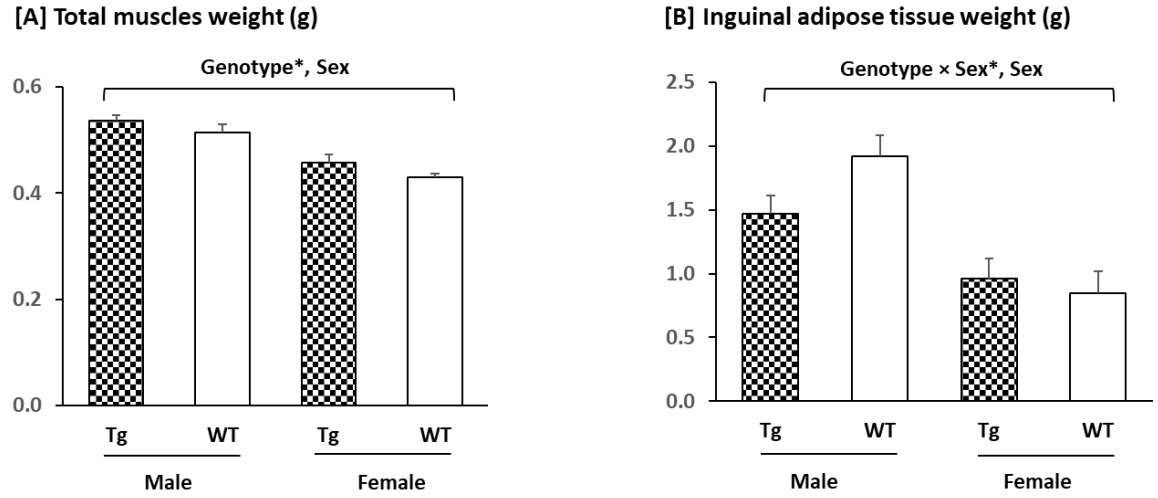


Figure 24.3. Total muscle and inguinal adipose tissue weights. (**A**) *Sparc* overexpression tended to increase the total skeletal muscles (gastrocnemius, soleus, tibialis anterior, and extensor digitorum longus) weight. Male mice had a heavier muscle weight than female mice. For the inguinal adipose tissue (**B**), the *Sparc* overexpression decreased its weight in male mice by 23%. Male mice had more inguinal adipose tissue than female mice. All data are mean ± SEM. The number of mice: 5–11 mice per experimental condition. *: Trend ($0.05 \leq p \leq 0.1$). Abbreviations: g, gram; *Sparc*, secreted protein acidic and rich in cysteine; Tg, transgenic (*Sparc* overexpression); WT, wild-type.

24.5.2.2. Muscle Strength (Grip Power Test)

The mice overexpressing *Sparc* had a greater grip power, and the female mice had a higher grip power than the male mice (Figure 24.4). The grip power is given as a muscle strength divided by the body weight.

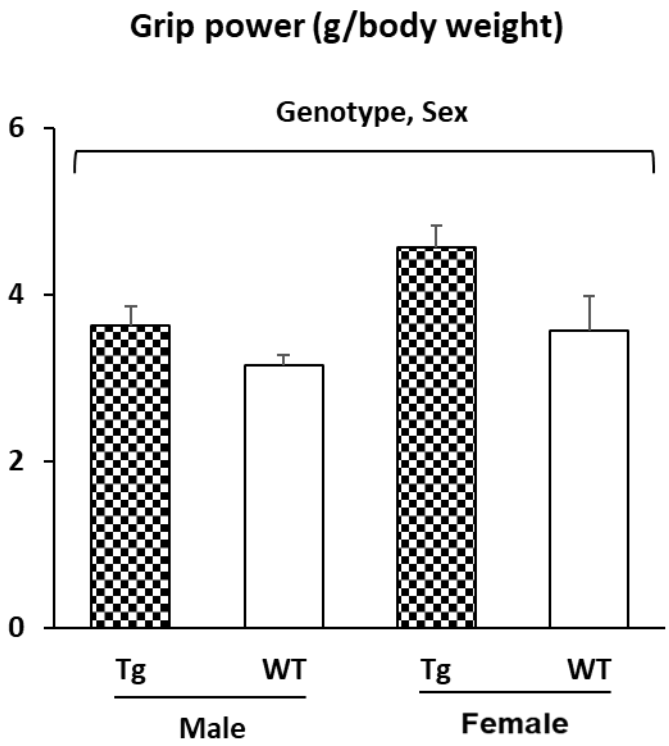


Figure 24.4. Muscle strength (grip power test). *Sparc* overexpression increased the grip power. Female mice had a higher grip power per body weight than male mice. All data are mean ± SEM. The number of mice: 5–11 mice per experimental condition. Abbreviations: g, gram; *Sparc*, secreted protein acidic and rich in cysteine; Tg, transgenic (*Sparc* overexpression); WT, wild-type.

24.5.2.3. Glycemia, Glucose Transporter Type 4 (GLUT4), and Mitochondrially Encoded Cytochrome c Oxidase II (MT-CO2) Expression in the Muscle

We measured the fasting glycemia along with the protein expression of both the GLUT4 and the MT-CO2. These measures are a part of a set of parameters that - together with our designed context - support our conclusions.

The *Sparc* overexpressed mice had a lower fasting blood glucose level along with higher expressions of both the GLUT4 and the MT-CO2 (Figure 24.5). The female mice had a lower fasting glucose level than the male mice. We also confirmed that SPARC is constantly overexpressed in the *Sparc* Tg mice, regardless of generation or sex. The SPARC expression also correlated with the GLUT4 and MT-CO2 expression, which confirms the genotype effect.

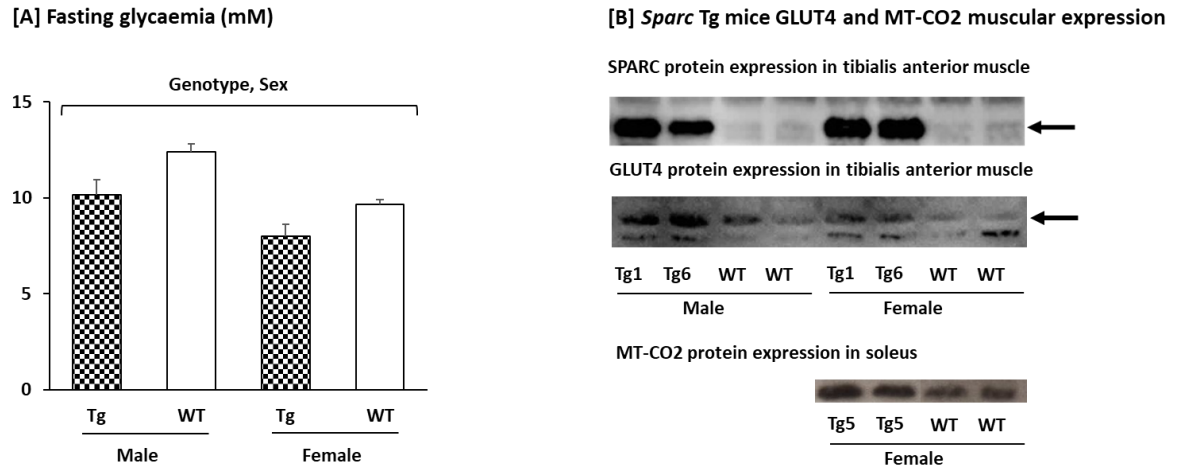


Figure 24.5. Glycemia as well as glucose transporter type 4 (GLUT4) and mitochondrially encoded cytochrome c oxidase II (MT-CO2) expressions in the muscle of F1 and F2 mice. As metabolic indicators, our measures showed that *Sparc* overexpression leads to a lower blood glucose (**A**) along with higher expressions of both GLUT4 and MT-CO2 (**B**). Glucose is lower in females compared to males as well (**A**). We also confirmed that SPARC is constantly overexpressed in *Sparc* Tg mice regardless of generation or sex (**B**). For the glycemia, all data are mean ± SEM, and the number of mice: 5 -11 mice per experimental condition. Abbreviations: SPARC/*Sparc*, secreted protein acidic and rich in cysteine; Tg, transgenic (*Sparc* overexpression); WT, wild type; Tg1, *Sparc* Tg line 1; Tg5, *Sparc* Tg line 5; Tg6, *Sparc* Tg line 6.

24.6 Discussion

For the *Sparc* Tg experiment, the male mice had a higher body weight than the female mice (Figure 24.S1). This could have impacted the statistical analyses of IngAT and muscle (higher in males) (Figure 24.3) as well as the grip power (lower in males) as the grip power was divided by the body weight (Figure 24.4). Thus, the sex effect will not be discussed because we already have a sex effect on the body weight. We will focus, rather, on the genotype (*Sparc* overexpression) effect in the discussions. For the *Sparc* KO experiment, the discussions will include the three variables (genotype, age and exercise).

The roles that SPARC plays at various levels, as shown by the published evidence, allowed us to predicate some of the impacts that SPARC deficiency and SPARC overexpression would have. These impacts are to be explored to build/optimize animal models of health conditions or diseases as well as to be starting points towards novel molecular therapies. Our data, compiled with the previous evidence, allow us to suggest new SPARC-related models and applications. As illustrated below, we highlight that *Sparc* KO mice would represent (accelerated) ageing animal models. After that, we also give other examples of how *Sparc* KO mice could be models, or a starting point to build models, of other diseases or health conditions as well.

24.6.1. *Sparc* KO to Optimize (Accelerated) Ageing Animal Models

Ageing is the biological process of the progressive loss of homeostatic and metabolic functions, along with compromised physical performance and physiological integrity [40,41], such as a decline in aerobic capacity [3], reduced muscle oxidative capacity [2], and imbalanced glucose utilization [6]. It leads to new cellular, metabolic, and molecular (including transcriptional [42]) patterns, with a pathophysiological predisposition for diseases [43,44]. Therefore, understanding the underlying mechanisms of ageing and the related metabolic and structural properties is very important to optimize healthcare. In this context, various (accelerated) ageing animal models have been developed to study ageing [45], especially mouse models [46,47]. They represent animals that have been modified (such as with chemicals [48]) in order to biologically mimic what is seen during ageing.

On the other hand, our previous [11] and current in vivo results showed that ageing increased adiposity and body weight but decreased muscle mass, strength, glucose tolerance (in WT mice), and glucose muscular uptake (GLUT4). To highlight the similarities between ageing effects (either shown from our data or from the literature) and *Sparc* KO, our data, as well as the results we previously reported from the same set of *Sparc* KO experiment mice [11], showed that *Sparc* KO led to an ageing-like phenotype, including muscle loss, grip power reduction, lower oxidative phosphorylation, glucose intolerance, and reduced glucose transport into muscles.

A part of the ageing-like phenotype is the similarity between the *Sparc* KO phenotype and sarcopenia. Sarcopenia, for which ageing represents a risk factor [37], is characterized by a generalized loss of muscle mass and strength [49]. This clinical definition fits with the *Sparc* KO phenotype (low muscle mass, reduced muscle metabolic performance, and reduced muscle strength) as we reported previously [11] on the signs that are also related to both ageing and a sedentary lifestyle [39], which are impacted by myokines, including SPARC [39]. The *Sparc* KO consequences on muscle are probably due, in part, to the interaction between SPARC and actin, which are important for muscle function [50]. Regarding the *Sparc* KO mice, we saw an increased glycogen content in the muscles in addition to the muscle loss. Thus, not only did the *Sparc* KO mice have lower muscle weight, but a part of that weight was glycogen, which suggests further reduced mechanical properties, as shown by the reduced muscle strength in *Sparc* KO mice [11]. Moreover, the impact of the SPARC deficiency could also contribute to the reduced grip-power performance of the *Sparc* KO mice. Indeed, the extracellular matrix collagen fibrils are important in tendons [51,52]. Thus, the interaction of SPARC with both the extracellular matrix and the collagen would contribute to the impact *Sparc* KO had on the Achilles tendon (reduced weight that could reflect a lack of development) and eventually even impact the grip power (also decreased in the *Sparc* KO mice [11]). In particular, SPARC is required for tendon mechanobiology, and its deficiency impairs tendon maturation [53].

The declined mitochondrial functions (MT-CO1 [11]) in the *Sparc* KO mice (considered as an ageing model), as well as the anti-SPARC antibody-induced decrease in the expression of mitochondrial proteins, ubiquinol-cytochrome c reductase core protein II (UQCRC2) and succinate dehydrogenase iron-sulfur subunit (SDHB) in muscle cells [13], support the mitochondrial theory of ageing [54] and further highlight SPARC as an important factor in the age-related changes, as illustrated by the decreased myogenesis and myogenin expression in the C2C12 muscle cells following the treatment with the anti-SPARC antibody [13]. The glucose intolerance (revealed by the OGTT) in *Sparc* KO mice also mimics ageing-related glucose intolerance [55].

For the adiposity, our data show that the *Sparc* KO reduced the AT weight and the adipocyte number only in the visceral AT (EpiAT) and not the subcutaneous AT (IngAT), a pattern that can be explained by the suggestion that SPARC expression in the AT is predominant in the subcutaneous AT [56], as shown by the SPARC mRNA expression that was significantly higher in the subcutaneous abdominal adipose tissue compared to the visceral adipose tissue [57]. The property SPARC had on white AT, and also on BAT, towards improving their metabolic performance would also have consequences. Indeed, whereas SPARC induced lipolysis, fat oxidation, and browning in the white adipocytes and BAT activation, its knockdown reduced the markers of these metabolic pathways [58]. Thus, *Sparc* KO would be towards adiposity development resulting from a poor local metabolic rate of the adipose tissues. In our *Sparc* KO mice, we have an increased BAT weight percentage that would rather be a white AT depot inside the BAT areas (infiltration) that could reflect a *Sparc* KO-dependent fat distribution. For the BAT, we still have the effect of the exercise that increased (percentage weight). Together (BAT increase with exercise and decrease with *Sparc* KO), these elements support the fact that our BAT was infiltrated with white AT (two tissues together in the BAT location) and that the exercise-induced increase is due to BAT, whereas the *Sparc* KO-induced increase is due to the infiltrating of white AT into BAT location. This is confirmed by the interaction between the genotype and exercise effects that reveal that the *Sparc* KO increased BAT only in the exercised mice and not in the sedentary mice. On the other hand, although ageing tends towards body fat increase [59], the *Sparc* KO reduced visceral adiposity (AbdAT). This can be explained by the fact that the *Sparc* KO mice have a higher glycogen content, suggesting that the energy storage takes the form of muscular glycogen (suggested explanation in Section 24.6.2) rather than fat storage in adipocytes. Although AT is heterogenic in male C57BL/6J mice (that we have used) [60] and its growth has a dynamic between hypertrophy and hyperplasia [61], we have seen no genotype effect on the EpiAT and IngAT adipocytes size. This supports our hypothesis, which states that to see a SPARC deficiency-related difference in the adipocyte, we need to induce obesity as HFD-induced obesity is enhanced in the absence of SPARC [62]. The high muscle mass (storage ability) percentage (in humans, it is 40% of total body weight [37]) compared to the body fat also supports this energy storage redistribution from adiposity to muscle glycogen seen in *Sparc* KO, especially given that our mice were fed a chow diet and not a high-fat diet

(HFD). Indeed, HFD-induced obesity is enhanced in the absence of SPARC [62]. Therefore, non-similarity between the age-related changes and the *Sparc* KO impacts of adiposity in our study can be overcome by feeding mice HFD and building a new age-related animal model of sarcopenic obesity (Section 24.6.3).

Moreover, SPARC declines with ageing would also be implicated in the age-related decline in muscle cell regeneration [63,64] as SPARC has been pointed out as a regeneration factor [65] and, whereas the anti-SPARC antibody prevented the differentiation of C2C12 myoblasts, adding SPARC increased the differentiation [13]. Importantly, for the properties in which we had an interaction between age and genotype, the *Sparc* KO effects are more significant in the young mice compared to the old mice. This is explained by the fact that SPARC declines with ageing and, thus, SPARC deficiency will have more impacts in young mice compared to old/aged mice that already have an age-related reduced SPARC expression. This is seen, for instance, in AbdAT, RetAT, EpiAT, and MesAT, where the difference in AT weight between old and young is more important in the WT mice (KO reduces the age-dependent difference).

Moreover, other data of the literature report other ageing-like changes that result from SPARC deficiency, such as reduced bone formation and osteoblast number [16], osteopenia [25,66], diminished levels of collagen [62], early onset of cataracts [21,67], immune alterations [68], and accelerated degeneration [69,70]. Thus, this further highlights the similarities between ageing biological patterns and SPARC deficiency and supports the classification of *Sparc* KO mice as an accelerated ageing animal model.

24.6.2. *Sparc* KO as Type 1 Diabetes Model?

As impaired glucose tolerance has been reported in SPARC-deficient mice [62,71] and Atorrasagasti et al. showed evidence that SPARC deficiency produced diabetic mice [71]. However, they also showed that the mice had an impaired insulin-secretion capacity rather than insulin resistance (on HFD) [71]. Such findings point to a type 1 diabetes-like phenotype (rather than type 2 diabetes), especially as it has been suggested that SPARC plays a role in insulin secretion [72] and promotes insulin secretion in pancreatic β cells [73]. Therefore, SPARC deficiency would reduce insulin secretion (independently of insulin resistance) [72].

As glucose intolerance characterises diabetes [74] and OGTT is one of the most widely used methods to characterise diabetes mouse models [75], we performed an OGTT, along with other metabolic measures, during our *Sparc* KO study. Our data support the findings of Atorrasagasti et al. Indeed, we have found that *Sparc* KO reduced both glucose tolerance as well as glucose uptake (GLUT4). The analysis of the interaction between the age, genotype, and exercise shows that there is no difference between the young and old mice of the *Sparc* KO sedentary mice, but in the *Sparc* KO exercise mice, the old mice had a better glucose tolerance than the young mice. This is explained by the higher expression of SPARC in the young mice compared to the old mice. Thus, SPARC deficiency would have a greater impact on the younger mice. The exploration of the mitochondrial

metabolism revealed that *Sparc* KO reduced the expression of MT-CO1 (mitochiondrial oxidative phosphorylation) [11] and also increased glycogen storage in muscle. This *Sparc* KO-induced increase in the glycogen content in the muscle suggests that, overall, *Sparc* KO shifts the metabolic homeostasis from glucose utilization/AT fat storage towards muscle glycogen storage. It might indicate that SPARC is required for the glycogen breakdown and/or that SPARC deficiency would increase glycogenesis and/or reduce glycogenolysis. The *Sparc* KO induced increase in muscle glycogen requires further investigation, which could lead to the elucidation of metabolic patterns that would further add to the mechanistic explanations of how SPARC deficiency impacts glucose metabolism (decreased glucose tolerance, mitochondrial metabolism [11], and glucose GLUT4 uptake) and lead to both abnormal glucose metabolism [71] and glycogen usage, especially after researchers started investigating the links between SPARC and skeletal muscle glycogen breakdown [76]. It is worth noting that the increased glucose intolerance in our *Sparc* KO mice correlates with the decreased GLUT4 expression in the same mice and the previously reported impaired insulin secretion in SPARC-deficient mice [71]. The declined metabolic performance of the muscle, combined with the diabetes-like phenotype in the *Sparc* KO mice, would limit their need for glycogenolysis-released glucose and, thus, might conserve the glycogen storage more than in the WT mice. This could also explain the increased glycogen content in the muscles. In addition, *Sparc* KO amplified the HFD-induced obesity [62]. Therefore, a combination of *Sparc* KO and a HFD could further optimize such an impaired-glucose homeostasis model (add insulin resistance), knowing the links between obesity and diabetes through either HFD induced or obesity-related insulin resistance.

24.6.3. *Sparc* KO to Optimize Sarcopenic Obesity

Sarcopenic obesity is an age-related health problem in which we see a combination of obesity with a reduced muscle mass and strength [77]. Above, we have detailed similarities between the *Sparc* KO muscle phenotype and sarcopenia. The enhanced HFD-induced obesity in *Sparc* KO mice [62] would be a good example of how *Sparc* KO mice can be a starting model from which to build an optimized model on it. It indicates that we can apply the diverse method used to generate obesity models [78] on the *Sparc* KO mice to create an obesity model with enhanced obesity features. The model of the *Sparc* KO will manifest reduced muscle mass and muscle power [11] combined with the enhancement of HFD induced obesity [62]. Therefore, combining the KO (and even a knockdown) of *Sparc* and an HFD would build a sarcopenic obesity animal model. In addition, such models would be further optimized in aged animals (compared to young animals), especially given that ageing and obesity have shared patterns [79]. The advantages of such an animal model are that it would have the two features of the disease that are enhanced. Indeed, whereas the ageing would be accelerated with the *Sparc* KO, the HFD-induced obesity will also be enhanced by the *Sparc* KO. This is of particular importance because we can create two conditions with one approach, *Sparc* KO (instead of using one method to generate

sarcopenia and another one to enhance obesity), which will reduce the variabilities and the limitations of such an animal model.

24.6.4. SPARC Overexpression Mimics Exercise Benefits

In our *Sparc* Tg mice that overexpress SPARC, we did not observe any side effects of the *Sparc* Tg, even up to 10 months old. In addition, for the body weight as well as the key metabolic and functional tissues, including the heart, liver, kidney, pancreas, spleen, and femur, there is no impact of the SPARC overexpression on tissue weights (data not shown), which would reflect no abnormal growth and the safety of overexpressing SPARC in terms of possible carcinogenesis. In fact, we have previously reviewed data that even suggested an antitumor character of SPARC [80].

It has already been suggested that SPARC treatment mimics the exercise effects by enhancing the glucose uptake (and glucose tolerance) in skeletal muscle [81]. This correlates with the increase in GLUT4 expression and the lower glycemia seen in our *Sparc* Tg mice. It also fits with the exercise-induced GLUT4-increased expression [82] and the SPARC-induced increase in glucose uptake and GLUT4 levels combined with the SPARC knockdown-induced GLUT4 protein level reduction [30]. The overexpression of SPARC in our Tg mice also leads to an increased muscle weight (trend), muscle strength, and mitochondrial oxidative phosphorylation (MT-CO2) expression, along with reduced IngAT in male mice (trend). Moreover, our previous in vitro study showed that adding or inducing SPARC in C2C12 muscle cells increased their differentiation, myogenin expression, collagen expression, and the expression of two mitochondrial proteins (UQCRC2 and SDHB) [13] and a mitochondrial biogenesis master regulator (PGC-1α) [8]. The reduced adiposity in Tg mice correlated with the property SPARC had on white AT and also on BAT towards improving their metabolic performance (lipolysis, fat oxidation, and browning in white adipocytes and BAT activation [58]). Similarly, our previous [11] and current in vivo results indicated that whereas exercise improved muscle (TA) mass, grip power (trend), glucose tolerance, collagen expression (trend), glucose uptake (GLUT4 expression), and mitochondrial oxidative phosphorylation (MT-CO1 expression), it reduced adiposity and body weight (trend). In addition, it has already been documented that exercise increased muscle mass [83], muscle oxidative capacity [2], muscle strength [84], and mitochondrial oxidative phosphorylation [11]. Exercise also reduced adiposity [85] and increased muscle differentiation [86], mitochondrial biogenesis [87] and, in general, attenuated age-related decreases in muscle properties (regeneration, metabolism, strength, and mass) [41]. All together, these properties show key similarities between SPARC overexpression and exercise benefits and exemplify how exercise-induced myokines (including SPARC) can explain the impacts of exercise, especially in the aged population [88] and on metabolism [89]. In addition, SPARC has been pointed to in the mediation of other effects that are also produced by exercise, such as tumor growth suppression [80,90] and the anti-inflammatory effect [91].

Such observations have two main implications. First, they further support the implication of SPARC as a key biomolecular mediator of the exercise benefits and place SPARC as the mechanistic link between exercise and its benefits. This leads us to the second implication, which is the possible therapeutic use of SPARC or its related pathways to induce exercise-like effects without performing exercise. The benefits would also include other exercise-related benefits such as tumor growth suppression [90].

24.7 Materials and Methods

Our work is divided into two parts. First, we explore the effects of *Sparc* knockout (KO) and compare them to the ageing consequences. We also observe the effects of exercise. In the second part, we study the effects of *Sparc* overexpression and compare them to the exercise benefits. At the end, we make combinatory analyses of the results to point out the analogies between the *Sparc* KO and the ageing-like phenotype on the one hand and make comparisons between *Sparc* overexpression and exercise in the context of exercise counteracting ageing.

To optimize the results of both parts towards the combinatory conclusions, the mice of both studies had the same genetic background, C57BL/6J. The C57BL/6J mouse, with a lifespan of around 104 weeks (26 months) [92,93], is the most commonly used strain for genetic and/or transgenic study that also consistently shows the highest level of voluntary wheel-running (suitable for exercise) [94], and in this strain, exercise capacity declines with age [95]. These properties make C57BL/6J mouse strain suitable for our studies, which both use transgenic mice and explore exercise effects. Furthermore, the mice of both studies were housed at the same animal facility of the CHU de Québec-Université Laval Research Center (12 h light/dark cycle), under the same conditions and fed with the same chow diet (Teklad global 18% protein rodent diets [96]) and had access to food and water ad libitum during the whole experimental period (except for fasting periods, during which they had access to water only). All the mice were sacrificed by cardiac puncture following isoflurane inhalation anesthesia. Figure 24.6 summarizes the key experimental designs that we detail below.

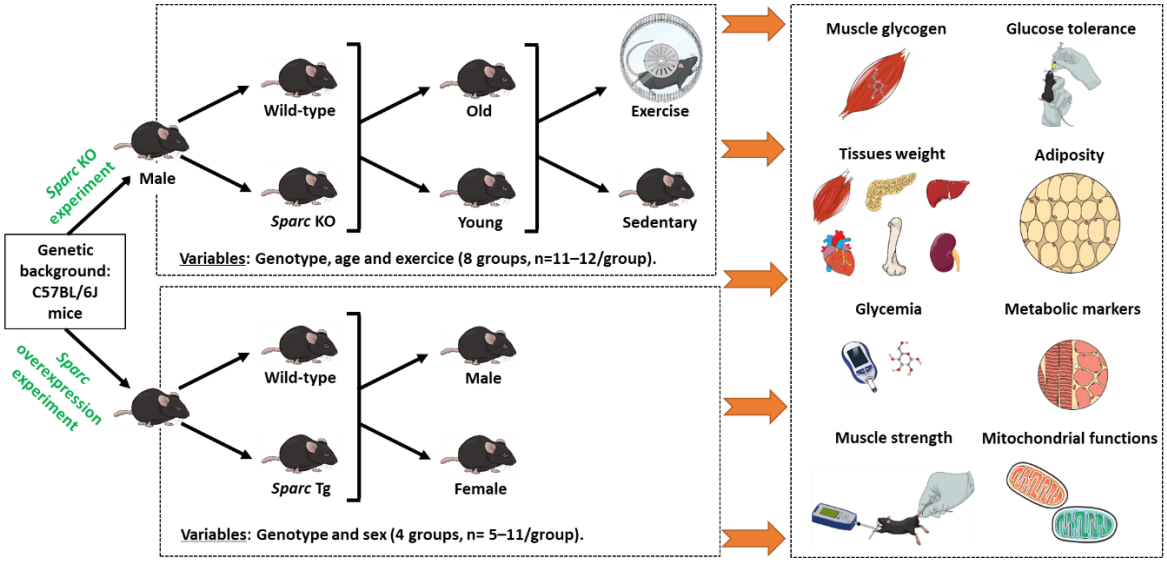


Figure 24.6. Experimental designs to explore the impacts of *Sparc* KO (in the context of ageing and exercise) as well as the impacts of *Sparc* overexpression in both male and female mice. For each experiment, a number of parameters have been conducted. Abbreviations: KO, knockout; *Sparc*, secreted protein acidic and rich in cysteine; Tg, transgenic (*Sparc* overexpression).

24.7.1. *Sparc* KO Experiment

The first study was carried out on biological samples from the mice used in our previous study. The previous study we conducted mainly focused on the effects of exercise patterns and suggested that exercise-induced muscle phenotype changes are SPARC dependent [11]. In this continuation, we aim to rather identify the potential similarities between the *Sparc KO* phenotype and ageing. Therefore, we performed additional measures towards a set of data to validate our hypothesis linking *Sparc* KO to an ageing-like phenotype. Briefly, the study was carried out on male mice and involved both WT mice (C57BL/6J) and *Sparc* KO mice (129/Sv-C57BL/6J). Whereas the WT mice were from the Jackson Laboratory (https://www.jax.org/, Accessed date: 31 December 2021), the *Sparc* KO mice were generated via in vitro fertilization using *Sparc* KO mice sperm generously provided by Dr. Amy D. Bradshaw, who generated the *Sparc* KO mice, as previously described [62,97]. Each age-group of mice (young (Y) and old (O)) was divided based on the genotype (KO or WT) to obtain 4 groups: Y-KO, Y-WT, O-KO, and O-WT. Finally, each of these 4 groups was further subdivided into two groups according to whether they were exercising (Ex) or sedentary (Sed) mice. Therefore, our experimental design included 8 groups: Y-WT-Sed, Y-WT-Ex, Y-KO-Sed, Y-KO-Ex, O-WT-Sed, O-WT-Ex, O-KO-Sed, and O-KO-Ex. Each group had 11 to 12 mice (Figure 24.6).

The exercising mice were trained (starting at the age of 9 weeks for the young mice and 66 weeks for the old mice) on running wheels (Lafayette instrument Co, Lafayette, IN, USA) placed horizontally. The exercise groups were trained during the dark phase. The exercising mice were trained for a total of 12 weeks. Based on previously reported

protocols, also applied to C57BL/6J mice [94,95], we first determined the speed at the lactate threshold (LT), at which the mice were trained afterwards on running wheels. The training speed was fixed as the LT intensity based on the benefits and effects that such an exercise pattern has been shown to produce, including those on glucose effectiveness, body fat percentage, insulin sensitivity, blood pressure, physical fitness, and lipid profile [7,98–101]. The LT level was also the speed used during the study during which *SPARC* has been characterised as an exercise-induced gene [7]. Moreover, as this part mainly focuses on exploring the potential similarities between the *Sparc* KO phenotype and ageing, the LT speed was selected for the exercise as its intensity can be prescribed for an older person [99].

To optimize our design, the sedentary mice were also taken to the training room to experiment with similar stimuli (auditory, olfactory, photonic, etc.) to that of the trained mice during the exercise sessions (60 min/day, five times/week). The running was mainly voluntary as no potentially harmful stimulations (such as electricity [95]) were applied to force the mice to run. To ensure that all the mice ran at the same speed and during the same period (similar exercise amounts), we used a small hand air pump to apply a light air stimulation a few times during the training sessions. We chose to conduct this part of our investigation on male mice because we aimed to explore the muscles in the context of exercise and ageing. Skeletal muscle development and response to exercise, as well as sarcopenic (age-related) muscle mass and strength loss, are more important in males compared to females [102,103]. In addition, exercise-induced SPARC levels correlate with skeletal muscle mass [104]. Therefore, a decline (*Sparc* KO/ageing) or enhancement (exercise) in functions and metabolic performances of the muscle would be more likely to be seen in males.

24.7.1.1. Mice Sacrifice and Tissue Weights

The mice were sacrificed at the age of 21 weeks for the young mice and at the age of 78 weeks for the old mice. They were sacrificed 48 h after the end of the 12 weeks of exercise training to avoid the acute impacts of the exercise on the measurements conducted afterwards. Immediately after the sacrifice of each mouse, its tissues were weighed. The tissues of concern were the Achilles tendon, brown adipose tissue (BAT), inguinal adipose tissue (IngAT), abdominal adipose tissue (AbdAT), retroperitoneal adipose tissue (RetAT), epididymal adipose tissue (EpiAT), and Mesenteric adipose tissue (MesAT) and the three muscles, gastrocnemius (GC), soleus (Sol), and extensor digitorum longus (EDL). As for the tissues that were used later for either Western blot or glycogen quantification, they were snap frozen in liquid nitrogen then moved to -80 ◦C and stored until use.

24.7.1.2. Oral Glucose Tolerance Test (OGTT)

In order to evaluate the glucose tolerance in mice [75], we performed an oral glucose tolerance test (OGTT). The mice were fasted for 6 h prior to the glucose gavage. After the glucose (prepared from 45% solution, Sigma-Aldrich Canada Co., Oakville, ON,

Canada) oral gavage (2 mg of glucose per 1 g of body weight), we measured the glycemia 5 times: 0, 15, 30, 60, and 120 min after glucose gavage. These 5 timepoints allowed us to obtain a curve and calculate the area under the curve (AUC) [105]. The OGTTs were administered three times (a total of three AUCs), at pre-training, at week 5, and at the end of week 12 of the training. The blood glucose levels were measured via tail pricking with a needle to collect blood samples on glucose test strips that were then inserted into a blood glucose meter (Accu Chek, Roche Diabetes Care, Inc., Mississauga, ON, Canada) to read the blood glucose levels.

24.7.1.3. Muscle Glucose Transporter Type 4 (GLUT4) Expression (Western Blot)

The glucose transporter type 4 (GLUT4) is a transporter that increases with exercise training, allowing glucose to enter the muscles [82]. We measured the expression of GLUT4 in the tibialis anterior (TA) muscle because it is the skeletal muscle for which we previously had an effect of age (decrease), genotype (decrease), and exercise (increase) on either its weight or its weight percentage [11]. To measure the expression of GLUT4, the total proteins were extracted from the TA muscle, using a radio-immunoprecipitation assay (RIPA) buffer and a protease inhibitor cocktail (Sigma-Aldrich Canada Co.), and followed by a protein quantification of each protein extract using a Bio-Rad protein assay (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). The protein extracts were kept at -80 ◦C until the Western blot was performed. Five micrograms of proteins was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the TGX Stain-Free FastCast acrylamide solutions (Bio-Rad Laboratories Ltd.), and the trihalo compound in the gels was activated under UV light. Then, the total proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad Laboratories Ltd.), and the gels (before and after the transfer) and membranes were visualized under UV light by using the AlphaImager TM 1220 (Alpha Innotech Co., San Leandro, CA, USA). The membranes were blocked using the Pierce™ Protein-Free (TBS) blocking buffer (Life Technologies Inc., Burlington, ON, Canada), incubated overnight with 1/1600 dilution (in the blocking buffer) of the primary antibody (sc-7938, Santa Cruz Biotechnology Inc., Dallas, TX, USA) and a 1 h incubation with a 1/10,000 dilution (in the blocking buffer) of the secondary antibody (sc-2004, Santa Cruz Biotechnology Inc.) and finally visualized with the Clarity™ Western ECL Blotting Substrate on a film (Bio-Rad Laboratories Ltd.). As the total number of mice (samples) was 95, we used many gels to load all the samples. Therefore, we needed an intermembranes and interfilms normalization method to both optimize and quantify these Western blot results. First, the visualized total proteins on the membranes and target proteins on the films were quantified using ImageJ software (ImageJ bundled with 64-bit Java 1.8.0_172, U. S. National Institutes of Health, Bethesda, MD, USA) [106]. The methodology of the lane and band quantifications, followed by the expression evaluations, was performed according to Taylor et al. [107,108], as we have detailed in one of our previous works [109].

24.7.1.4. Skeletal Muscle Glycogen Content

In order to further explore the metabolic phenotype of the muscle, we measured the glycogen content in the gastrocnemius (GC) muscle. The method was based on the protocol previously published [110]. We used the standard curve to quantify the glycogen in the solution. We divided the glycogen content by the GC weight to have the glycogen content in µg per mg of muscle.

24.7.1.5. Histological Analysis of the White Adipose Tissue

We analyzed both the inguinal and the epididymal adipocytes. These two locations are representative of the subcutaneous and visceral adipocytes, respectively. Immediately after the sacrifice, the adipose tissue depots (inguinal and epididymal) were harvested and fixed in 4% paraformaldehyde for 48 h and then paraffin-embedded for use in histology (hematoxylin and eosin staining). Slices of 5 µm were prepared for the paraffin-embedded tissue sections. The section images were taken using the microscope Nikon Eclipse E800 (magnification of ×20) combined with a digital camera Nikon D5500, Nikon corporation (magnification of ×2). The cross-sectional area analysis of the adipocyte diamaters was performed using the ImageJ [106]. For each mouse, we had 5 images, and we analysed the cross-sectional area of 7 adipocytes from each image. In total, we had 35 cross-sectional areas for each adipose tissue sample. We also, based on the cross-sectional area and the adipose tissue weight of each mouse, estimated the number of the adipocytes [111].

24.7.1.6. Statistical Analyses

The data were analyzed by three-way (age, genotype, and exercise) and four-way (plus time for the OGTT) ANOVA. When the ANOVA revealed a significant interaction between two or three variables, the Tukey Kramer post hoc test was performed to identify the significant difference between the groups ($p < 0.05$). A trend corresponds to $0.05 \leq p < 0.1$. In the results section, all the effects are significant ($p < 0.05$), unless mentioned as a trend. The number of mice (11–12 mice per experimental condition) was based on the results of a power analysis by setting the statistical power at 80% ($\alpha = 0.05$ and $\beta = 0.2$) with our previous study, which used the same strain of WT mice [112].

24.7.2. *Sparc* Overexpression (*Sparc* Tg) Experiment

We used transgenic mice overexpressing *Sparc* (*Sparc* Tg) and WT mice, both with the same genetic background as those used in the *Sparc* KO experiment. The PiggyBac transposase-mediated gene transfer was used to create transgenic lines expressing mouse *Sparc* under the control of a strong and ubiquitous CAG promoter (CMV early enhancer fused to modified chicken b-actin promoter) in a C57BL/6 mouse. In the PiggyBac vector, the "CAG promoter-Kozak-mouse *Sparc* CDS-polyA" cassette was flanked by two PiggyBac inverted terminal repeat sequences (ITRs) to facilitate the transpose-mediated transgene integration. The verified PiggyBac vector carrying the transgenic cassette was co-injected with transposases into the pronucleus of fertilized eggs, and the eggs were

implanted into surrogate mothers to obtain offspring. The pups were genotyped by PCR to identify the ones carrying the desired PiggyBac transgene. The positive founder mice were counter screened for transposes. Out of 25 pups screened, 6 were identified positive (Tg1 to Tg6, 4 males, and 2 females). The procedure above was performed by Cyagen Biosciences [113].

24.7.2.1. Confirming the SPARC/*Sparc* Gene Overexpression and Selecting Mice (Western blot and Q_RT-PCR)

After all 4 male and 2 female *Sparc* Tg mice were transfered to our animal facility, the F1 mice were produced with an average of 8.4 F1 pups per F0 mouse (the ratio of male to female pups was 0.84). As no known toxic component was included in this design, the only risk was that the transgenic cassette could have been randomly inserted into the genome and may have disrupted the endogenous genes. This is why we used multiple transgenic founders to minimize this artificial effect. The overall expression level in various tissues will be affected by the regulatory mechanism of the CAG promoters and the turnover time of the protein itself, which could be variable in every tissue. Thus, we also investigated the SPARC expression in several tissues using quantitative real-time PCR (Q_RT-PCR) and/or Western blot.

For the Western blot, we measured the expression of SPARC in the TA, heart, liver, BAT, IngAT, and gonadal (Gon) AT. On the day of the sacrifice, the tissues were removed and quickly put in liquid nitrogen (snap frozen), then moved to -80 ◦C and kept until the protein extraction procedure (See Section 24.7.1.3). The protein extracts were kept at -80 ◦C until the Western blot was performed. First, 20 and 40 micrograms of TA proteins (extract) of 7 mice (Tg1, Tg2, Tg4, Tg5, Tg6, and two WT) were separated by SDS-PAGE using the TGX Stain-Free FastCast acrylamide solutions, and the trihalo compound in the gels was activated under UV light. Then, the total proteins were transferred to PVDF membranes, and gels (before and after the transfer), and the membranes were visualized under UV light. The membranes were blocked for 2 h in 2% nonfat dry milk (Bio-Rad Laboratories Ltd.), incubated overnight with 1/250 dilution (in PierceTM Protein-Free (TBS) blocking buffer) of the primary antibody (AF942, R&D Systems, Inc., Minneapolis, MN, USA), and a 2 h incubation with 1/1000 dilution (in PierceTM Protein-Free (TBS) blocking buffer) of the secondary antibody (sc-2354, Santa Cruz Biotechnology Inc.), and finally visualized with the Clarity™ Western ECL Blotting Substrate on a film. Unlike the first part of this paper (*Sparc* KO experiment), the number of samples for each target protein allowed us to have all the samples loaded on the same gel. Therefore, there was no need for a normalization. We confirmed the similarity of the loaded proteins by visualizing the protein lanes on the membrane after the transfer. The visualized target proteins on the films were quantified using ImageJ software [106].

For the *Sparc* gene expression analyzed by Q_RT-PCR, the tissues (TA) were homogenized in Qiazol buffer (Qiagen Inc., Germantown, MD, USA), and the total RNA was extracted using the miRNeasy micro kit on-column DNase (Qiagen Inc.) treatment

following the manufacturer's instructions. The quantity of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and total RNA quality was assayed on an Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). First-strand cDNA synthesis was accomplished using 4 ug of isolated RNA in a reaction containing 200 U of Superscript IV Rnase H-RT (Life Technologies), 300 ng of oligo-dT18, 50 ng of random hexamers, 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl2, 500 uM deoxynucleotides triphosphate, 5 mM dithiothreitol, and 40 U of Protector RNase inhibitor (Roche Diagnostics, Indianapolis, IN, USA) in a final volume of 50 uL. The reaction was incubated at 25 ◦C for 10 min, then at 50 ◦C for 20 min and inactivated at 80 ◦C for 10 min. A PCR purification kit (Qiagen Inc.) was used to purify the cDNA. The oligo primer pair that allows the amplification of 95 bp was designed by GeneTools software (Biotools, Edmonton, Alberta, Canada), and their specificity was verified by blast in the GenBank database. The gene name, GenBank accession number, and the sequences of the primer pair were the following: *Mus musculus* secreted acidic cysteine rich glycoprotein (*Sparc*), NM_009242, and CCACACGTTTCTTTGGACC/GATGTCCTGCTCCTTGATGC. The oligo primer pair was performed by IDT (Integrated DNA Technology, Coralville, IA, USA). A quantity corresponding to 20 ng of total RNA was used to perform fluorescentbased real-time PCR quantification using the LightCycler 480 (Roche Diagnostics). Reagent LightCycler 480 SYBRGreen I Master (Roche Diagnostics) was used as described by the manufacturer with 2% DMSO. The conditions for the PCR reactions were: 45 cycles, denaturation at 98 ◦C for 10 s, annealing at 57 ◦C for 10 s, and elongation at 72 ◦C for 14 s and then 74 ◦C for 5 s (reading). A melting curve was performed to assess the non-specific signal. The calculation of the number of copies of each mRNA was performed according to Luu-The et al. [114] using a second derivative method and a standard curve of Cp versus logarithm of the quantity. The standard curve is established using known amounts of purified PCR products (10, 10^2, 10^3, 10^4, 10^5, and 10^6 copies) and a LightCycler 480 v1.5 program provided by the manufacturer (Roche Diagnostics). The PCR amplification efficiency was verified. The Q_RT-PCR measurements were performed by the CHU de Québec Research Center (CHUL) Gene Expression Platform, Quebec, Canada and were compliant with the MIQE guidelines [115,116].

The results confirming the expression of SPARC and *Sparc* in the Tg lines are shown in Figure 24.7. As shown in Figure 24.7, we generated Tg mice expressing the *Sparc* gene at the different levels (*Sparc* Tg lines 1, 2, 4, 5, and 6 expressed the *Sparc* gene 69-, 1-, 7-, 31- and 39-fold compared to the WT, respectively), whereas the germline transmission did not occur in the *Sparc* Tg line 3. Therefore, *Sparc* Tg lines 2 and 3 were excluded.

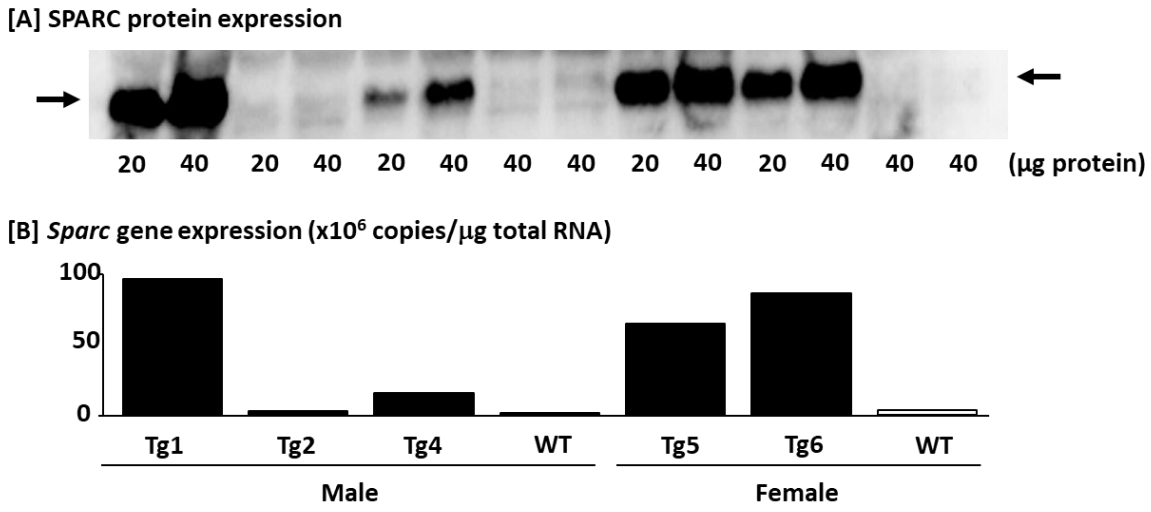


Figure 24.7. SPARC expression in the tibialis anterior muscle of *Sparc* transgenic (Tg) and wild-type (WT) F0 mice. (**A**) SPARC protein expression analyzed by Western blot. (**B**) *Sparc* gene expression analyzed by quantitative real-time PCR. Abbreviations: SPARC/*Sparc*, secreted protein acidic and rich in cysteine; Tg, transgenic (*Sparc* overexpression); Tg1, *Sparc* Tg line 1; Tg2, *Sparc* Tg line 2; Tg4, *Sparc* Tg line 4; Tg5, *Sparc* Tg line 5; Tg6, *Sparc* Tg line 6.

In addition, to have an overview/confirm the SPARC expression in different tissues, we measured the expression of SPARC in six tissues (TA, heart, liver, BAT, IngAT, and GonAT) in 4 male and 4 female mice, 2 WT mice, 1 mouse from the line Tg1, and 1 mouse from the line Tg6. For WT mice sample run in parallel with Tg mice sample, both the WT and the Tg mice had a parent from a similar Tg line (Tg1 or Tg6), as shown in Figure 24.8. The lines Tg1 and Tg6 have been selected because they represent the lines that express the most SPARC/*Sparc* (Figure 24.7). The Western blot protocol of Figure 24.8 was the same as above (Figure 24.7) but with 15 µg as the protein loading amount.

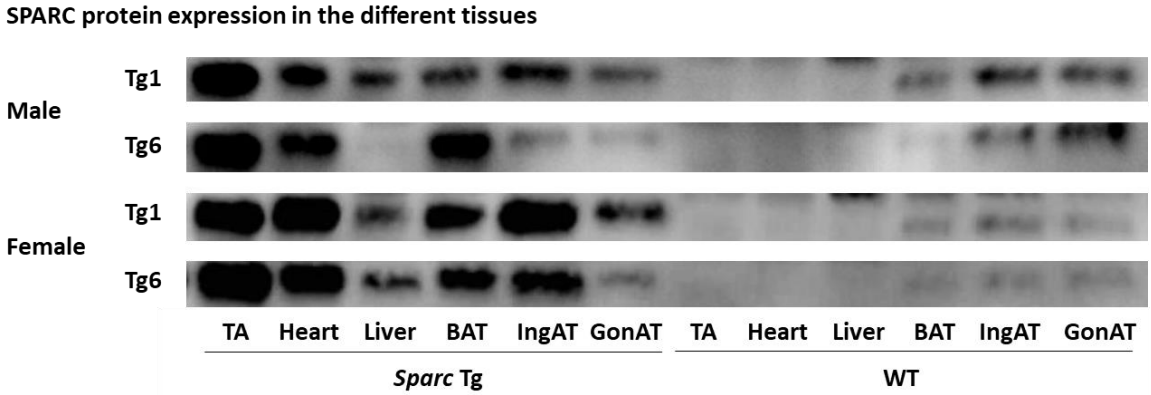


Figure 24.8. SPARC protein expression in the different tissues of transgenic (*Sparc* overexpression) and WT of F1 and F2 mice. Abbreviations: BAT, brown adipose tissue; GonAT, gonadal adipose tissue; IngAT, inguinal adipose tissue; TA, tibialis anterior; Tg, transgenic (*Sparc* overexpression); SPARC, secreted protein acidic and rich in cysteine; WT, wild-type.

At the end, the selected mice (for breeding and to generate both Tg and WT mice) all belong to the Tg lines 1, 5, or 6, which are the lines with the highest expression of SPARC/*Sparc* (Figure 24.7). For our study, the 32 mice constituted 4 groups, depending on genotype and sex, groups of males (M) or females (F), and Tg or WT (Figure 24.6). Therefore, we had four groups: Tg-M (n = 9), WT-M (n = 7), Tg-F (n = 11) and WT-F (n = 5). These were the numbers of mice for all our measurements except for those of Section 24.7.2.5. The average sacrifice age of each group was 6.0 ± 0.1 months. We ensured that there was no statistical age difference between the males and the females or between the WT and *Sparc* Tg mice (the two genotypes have similar ages). Therefore, a genotype effect would be explained only by the *Sparc* overexpression rather than age difference.

24.7.2.2. Body Weight and Tissue Weights

Living, fasted (12 h) mice were weighed just prior to their sacrifice. Immediately after the sacrifice of each mouse, its tissues were quickly removed and some of them weighed. The tissues of concern were skeletal muscles (GC, Sol, TA and EDL), IngAT, BAT, heart, liver, kidney, pancreas, spleen, and the femur. As for the tissues used later for Western blot, they were snap frozen in liquid nitrogen then moved to -80 ◦C and stored until use.

24.7.2.3. Grip Power Test

Prior to their sacrifice, the muscle strengths of all the mice were measured through performing a grip power test with a grip strength meter (Columbus Instruments International, Columbus, OH, USA). The grip strength was measured by allowing the mouse to grab (with two limbs) pull bar assemblies attached to the force transducer while the mouse was pulled horizontally by the tail away from the bars, similar to what has previously been described [117], especially to detect physiological changes in the skeletal muscle function of C57BL/6J mice [118]. The peak force applied by the mouse (g) was then shown on a digital display. This test was conducted five times (5 min apart) for each mouse, after which the mean forces in grams were calculated as being normalized to the body weight of the corresponding mouse.

24.7.2.4. Blood Glucose (Glycemia)

The mice were sacrificed following a 12 h fasting. At the end of the 12-h fasting and prior to the sacrifice, the blood glucose levels were measured via tail pricking with a needle to collect blood samples on glucose test strips that were then inserted into a blood glucose meter (Accu-Chek, Roche Diabetes Care, Inc. Mississauga, ON, Canada) to read the blood glucose value.

24.7.2.5. Muscular GLUT4 and Mitochondrially Encoded Cytochrome c Oxidase II (MT-CO2) Expression (Western Blot)

Whereas GLUT4 is a transporter allowing glucose to enter the muscle [82], mitochondrially encoded cytochrome c oxidase II (MT-CO2) is a mitochondrial protein

encoded by a mitochondrial gene that can be considered as an indicator of mitochondrial oxidative phosphorylation [119–123]. In addition to the glycemia, and in order to map the muscular metabolic patterns, we measured the TA expression of GLUT4 in 4 males and 4 females (2 WT mice, 1 mouse from the line Tg1, and 1 mouse from the line Tg6 for each sex) and the Soleus expression of MT-CO2 in 4 females (2 WT mice and 2 mice from the line Tg5). For a WT mice sample loaded in parallel with a Tg mice sample, they had a parent from a similar Tg line (Tg1, Tg5, or Tg6). We chose to measure the GLUT4 and MT-CO2 expression in the same muscles (TA and Soleus) where GLUT4 (above) and MT-CO1 [11] have been measured in a *Sparc* KO study to justify a later comparison of the results in our context. At the day of sacrifice, the two muscles were removed and quickly put in liquid nitrogen (snap frozen) then moved to -80 °C and kept until the protein extraction procedure. The Western blot steps were similar to the SPARC overexpression measures with different conditions. For GLUT4, 15 µg of proteins was loaded. The membranes were blocked using the Pierce™ Protein-Free (TBS) blocking buffer, incubated overnight with a 1/400 dilution of the primary antibody (sc-7938) before an incubation for 1 h with the secondary antibody (sc-2004) at a dilution of 1/10,000. For MT-CO2, 60 µg of proteins was loaded. The membranes were blocked using the Pierce™ Protein-Free (TBS) blocking buffer, incubated for 2 h with a 1/1600 dilution of primary antibody (sc-514489) before an incubation for 2 h with the secondary antibody (sc-516102) at a dilution of 1/10,000. All the antibodies were from Santa Cruz Biotechnology Inc. and diluted in Pierce™ Protein-Free (TBS) blocking buffer. Similarly to the SPARC protein expression quantification, the number of samples for each target protein allowed us to have all the samples loaded on the same gel. Therefore, there was no need for a normalization (similar to the one conducted in the *Sparc* KO Western blot experiment). We confirmed that there was no difference in protein loading by visualizing (under UV light) the protein lanes on the membrane after the transfer (rather than using beta actin, for instance, that might not be a reliable loading control [124]). The visualized bands of the films were quantified using ImageJ software [106]. To better see the correlation between the SPARC expression and both the GLUT4 and the MT-CO2, the three proteins were observed in parallel.

24.7.2.6. Statistical Analyses

The data were analyzed by two-way (genotype and sex) ANOVA. When the ANOVA revealed a significant interaction between two variables, the Tukey Kramer post hoc test was performed to identify the significant difference between the groups ($p < 0.05$). A trend corresponds to $0.05 \leq p \leq 0.1$. In the results section, all the effects are significant ($p < 0.05$), unless mentioned as a trend.

24.8 Conclusions and Perspectives

The importance of SPARC, the various roles it plays, and its wide distribution makes its deficiency affect more than one system, and therefore, it leads to a relatively

systemically distributed phenotype rather than limited organ-specific or a tissue-specific changes.

Our data highlight that SPARC is involved in both exercise-induced benefits, the ageing process, and the metabolic as well as the functional properties at various tissular and cellular levels. Indeed, SPARC declines with ageing and increases with exercise and its overexpression leads to an exercise-like effect. We noticed that *Sparc* KO and ageing lead to a similar phenotype in terms of reduced muscle mass, muscle power, and metabolic performance in addition to reduced glucose tolerance, glucose uptake, and collagen expression in the muscles. Furthermore, other in vivo and in vitro studies (reported above) on SPARC deficiency or inhibition highlight similar changes and have shown reduced muscle differentiation, decreased mitochondrial metabolism, osteopenia, and cataractogenesis, among other ageing-like patterns. On the other hand, SPARC overexpression in animals (this paper and our previous study [11]) or addition in cell culture (in vitro) [8,13] results in exercise-like effects, including enhanced mitochondrial oxidative phosphorylation, increased muscle mass and power, increased mitochondrial biogenesis, reduced glycemia and adipose tissue, increased glucose uptake by the muscle, and higher collagen and myogenin expression in muscle cells with increased myoblasts differentiation. All together, this evidence point out that *Sparc* KO leads to an ageing-like effect (or accelerated ageing) and that both the *Sparc* KO phenotype and ageing can be counteracted or reversed (at least in part) by either exercise or SPARC as SPARC-mediated effects mimic exercise. These can be exploited to both build/optimize animal models and potentially develop therapies (Figure 24.9). First, we could knockout or knock-down *Sparc* in order to build animal models (and even cellular models) to explore diseases and health conditions, as well as the pathways shown or suggested to interact with SPARC, such as its intracellular interactions and caspase-8, and in colon cancer cells [9], muscle AMPK signaling [81], integrin-linked kinase [8,26], RGS4 protein in pancreatic β cells [73], transforming growth factor-β1 in renal cell carcinoma [125], and beta-catenin in pulmonary fibroblasts [126]. This will deepen our understanding of the diseases and physiological conditions in which SPARC expression changes, such as cancer, obesity, muscle development, exercise, etc.

On the other hand, the exercise-like effects mediated by SPARC can open doors to use either SPARC or pharmacologically targeted SPARC-related pathways to achieve therapeutic goals that are usually obtained with physical exercise; in particular since SPARC has been suggested as a marker of exercise efficiency. Importantly, such benefits gave exercise the status of a therapeutic tool. The health problems and conditions for which exercise has been prescribed, and for which SPARC-related therapy can be explored to mimic exercise, include obesity, sarcopenia, diabetes, metabolic disorders, etc. This is of particular importance for those who need exercise but are unable to perform the prescribed amount because of physical disability, heart disease, or other health conditions. Indeed, such an “exercise pill” would overcome this challenge and still get the exercise benefits. Moreover, as SPARC would be required in the exercise-induced muscle changes,

combining exercise with SPARC overexpression/inoculation could also improve and optimise exercise benefits. Importantly, the hypothesis we gave earlier to explain why SPARC is overexpressed in situations such as obesity [127] and cancer [80] is precisely an attempt to correct the damages induced by these situations through the beneficial properties of SPARC (metabolic enhancement, anti-cancer, etc.), as suggested by the increased plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus [128]. However, the non identification of the SPARC receptor would represent a challenge that limits the exploration of SPARC, including using agonist or antagonist. Indeed, so far, putative receptors of SPARC have been reported [129,130], including alpha 5 beta 1 integrin complex (activates the Wnt/β-catenin), which has been identified as a candidate receptor [131]. Studies such as those overexpressing SPARC only in specific tissues and exploring the consequences of in vitro SPARC addition to cell/tissue cultures would also illuminate the path of understanding SPARC molecular patterns.

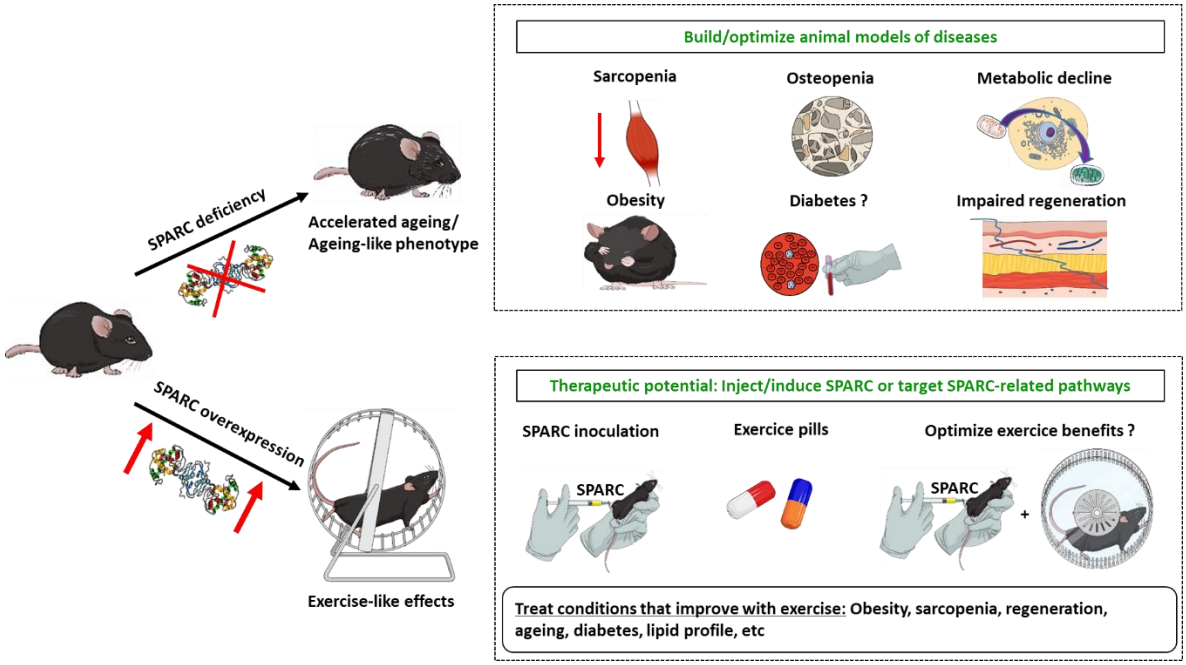


Figure 24.9. SPARC deficiency and ageing share similar phenotypes which are counteracted/improved by SPARC induction that mimics exercise effects. These can be exploited to both build/optimize animal models and potentially develop therapies to treat conditions that have common patterns with the SPARC-deficiency-related biological and functional changes. Abbreviations: SPARC, secreted protein acidic and rich in cysteine.

It is worth noting that sequences of the *SPARC* gene and protein are highly conserved among species [22] and are aimed towards extrapolating from animal results to human studies. Importantly, with exercise considered as a panacea [132], SPARC seems to mediate and is likely to mimic exercise benefits. Elucidating SPARC pathways in the context of human diseases can add strong, yet safe, tools to the available therapeutic options for as many diseases and health problems as those for which exercise has been

shown to have beneficial impacts. SPARC-based therapeutic pathways, with possible short comings, would be towards treating age-related health problems and metabolic disorders. Within this context, as SPARC expression was shown to have varying patterns based on targets and tissues, the rationale as to how to induce SPARC expression in selected tissues, or introduce it, can further optimize such a therapeutic approach. Indeed, based on SPARC-specific effects, including muscle growth, adipogenesis inhibition and anticancer, the development of new generations of pharmacological delivery systems would make a great contribution towards such novel molecular therapies by making them tissue-specific (muscles, tumors, etc.). That would increase the therapy precision, reduce possible side effects, and potentially lead to a personalised medicine.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/metabo12020125/s1, Figure 24.S1: reports the *Sparc* Tg experiment body weights of mice.

Author Contributions: Data curation, A.G., A.M., M.Y. and J.S.-A.; formal analysis, A.G., A.M. and M.Y.; funding acquisition, J.S.-A.; investigation, A.G., A.M., M.Y. and J.S.-A.; methodology, A.G., A.M., M.Y. and J.S.-A.; project administration, M.Y. and J.S. A.; supervision, M.Y. and J.S.-A.; validation, M.Y. and J.S.-A.; visualization, A.G., A.M., M.Y. and J.S.-A.; writing-original draft, A.G.; writing-review and editing, A.G., A.M., M.Y. and J.S.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Canadian Institutes of Health Research (CIHR), Number: 201309MOP-311306-BCA-CFBA-40425.

Institutional Review Board Statement: All animal experimentations were conducted in accord with the guidelines of the Canadian Council on Animal Care and approved by the Animal Protection Committee of Laval University (Identifications: 2014165 and 2014168 /Approval number for Tg mice: CHU-18-037). Mice were periodically checked by animal care technicians for health and wellness. Mice with any type of illness were immediately euthanized by cervical dislocation and excluded from the studies. Mice found with anatomical abnormalities (during the sacrifice) were also excluded from the studies. Moreover, the mice were always handled gently when taken from the cage and/or to the training device and vice versa.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in article or supplementary material.

Acknowledgments: We thank Amy D. Bradshaw for providing our research group with sperm of *Sparc* KO mice. We thank the research team of Jacques P. Tremblay (CHU de Québec-Université Laval Research Center, Quebec, Canada) for the microscope we have used during our histological analysis. Abdelaziz Ghanemi received a Merit scholarship for foreign students from the Ministry of Education and Higher Education of Quebec, Canada, The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship «Bourse Tremplin-Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec–Sante (FRQS), Quebec, Canada. Images from https://mindthegraph.com/ and https://smart.servier.com/ were used to create parts of the figures (Accessed date: 22 December 2021).

Conflicts of Interest: The authors declare no conflict of interest.

24.9 References

1. Chia, C.W.; Egan, J.M.; Ferrucci, L. Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk. *Circ. Res.* 2018, *123*, 886–904.
2. Cartee, G.D. Influence of age on skeletal muscle glucose transport and glycogen metabolism. *Med. Sci. Sports Exerc.* 1994, *26*, 577–585.
3. Fleg, J.L. Aerobic exercise in the elderly: A key to successful aging. *Discov. Med.* 2012, *13*, 223–228.
4. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. *Phys. Med. Rehabil. Clin. N. Am.* 2017, *28*, 659–669.
5. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. *Am. Fam. Physician* 2017, *95*, 425–432.
6. Consitt, L.A.; Dudley, C.; Saxena, G. Impact of Endurance and Resistance Training on Skeletal Muscle Glucose Metabolism in Older Adults. *Nutrients* 2019, *11*, 2636.
7. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
8. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.
9. Aoi, W.; Sakuma, K. Skeletal muscle: Novel and intriguing characteristics as a secretory organ. *BioDiscovery* 2013, *7*, e8942.
10. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
11. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
12. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. *Genes* 2021, *12*, 1832.
13. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the

modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
14. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. *Front. Physiol.* 2020, *11*, 18.
15. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. *Aging Cell* 2014, *13*, 175–184.
16. Delany, A.M.; Kalajzic, I.; Bradshaw, A.D.; Sage, E.H.; Canalis, E. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. *Endocrinology* 2003, *144*, 2588–2596.
17. Motamed, K. SPARC (osteonectin/BM-40). *Int. J. Biochem. Cell Biol.* 1999, *31*, 1363–1366.
18. Scavelli, K.; Chatterjee, A.; Rhee, D.J. Secreted Protein Acidic and Rich in Cysteine in Ocular Tissue. *J. Ocul. Pharmacol. Ther.* 2015, *31*, 396–405.
19. Sage, H.; Johnson, C.; Bornstein, P. Characterization of a novel serum albumin-binding glycoprotein secreted by endothelial cells in culture. *J. Biol. Chem.* 1984, *259*, 3993–4007.
20. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix. *Matrix Biol.* 2000, *19*, 569–580.
21. Norose, K.; Clark, J.I.; Syed, N.A.; Basu, A.; Heber-Katz, E.; Sage, E.H.; Howe, C.C. SPARC deficiency leads to early-onset cataractogenesis. *Investig. Ophthalmol. Vis. Sci.* 1998, *39*, 2674–2680.
22. Yan, Q.; Sage, E.H. SPARC, a matricellular glycoprotein with important biological functions. *J. Histochem. Cytochem.* 1999, *47*, 1495–1506.
23. Zhu, J.; Wang, L.Y.; Li, C.Y.; Wu, J.Y.; Zhang, Y.T.; Pang, K.P.; Wei, Y.; Du, L.Q.; Liu, M.; Wu, X.Y. SPARC promotes self-renewal of limbal epithelial stem cells and ocular surface restoration through JNK and p38-MAPK signaling pathways. *Stem Cells* 2020, *38*, 134–145.
24. Alachkar, H.; Santhanam, R.; Maharry, K.; Metzeler, K.H.; Huang, X.; Kohlschmidt, J.; Mendler, J.H.; Benito, J.M.; Hickey, C.; Neviani, P.; et al. SPARC promotes leukemic cell growth and predicts acute myeloid leukemia outcome. *J. Clin. Investig.* 2014, *124*, 1512–1524.
25. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectindeficient mice. *J. Clin. Investig.* 2000, *105*, 915–923.
26. Barker, T.H.; Baneyx, G.; Cardó-Vila, M.; Workman, G.A.; Weaver, M.; Menon, P.M.; Dedhar, S.; Rempel, S.A.; Arap, W.; Pasqualini, R.; et al. SPARC regulates extracellular matrix organization through its modulation of integrin-linked kinase activity. *J. Biol. Chem.* 2005, *280*, 36483–36493.
27. Bradshaw, A.D. The role of SPARC in extracellular matrix assembly. *J. Cell Commun. Signal.* 2009, *3*, 239–246.
28. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
29. Onorato, A.M.; Fiore, E.; Bayo, J.; Casali, C.; Fernandez-Tomé, M.; Rodríguez, M.; Domínguez, L.; Argemi, J.; Hidalgo, F.; Favre, C.; et al. SPARC inhibition accelerates NAFLD-associated hepatocellular carcinoma development by dysregulating hepatic lipid metabolism. *Liver Int.* 2021, *41*, 1677–1693.
30. Song, H.; Ding, L.; Zhang, S.; Wang, W. MiR-29 family members interact with SPARC to regulate glucose metabolism. *Biochem. Biophys. Res. Commun.* 2018, *497*, 667–674.
31. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
32. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). *J. Mol. Cell. Cardiol.* 2010, *48*, 544–549.
33. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. *Histol. Histopathol.* 2013, *28*, 1451–1460.

34. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J. Clin. Investig.* 2001, *107*, 1049–1054.
35. Kim, J.S.; Galvão, D.A.; Newton, R.U.; Gray, E.; Taaffe, D.R. Exercise-induced myokines and their effect on prostate cancer. *Nat. Rev. Urol.* 2021, *18*, 519–542.
36. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. *Biomolecules* 2021, *11*, 1689.
37. Frontera, W.R.; Ochala, J. Skeletal muscle: A brief review of structure and function. *Calcif. Tissue Int.* 2015, *96*, 183–195.
38. Barbalho, S.M.; Flato, U.A.P.; Tofano, R.J.; Goulart, R.A.; Guiguer, E.L.; Detregiachi, C.R.P.; Buchaim, D.V.; Araújo, A.C.; Buchaim, R.L.; Reina, F.T.R.; et al. Physical Exercise and Myokines: Relationships with Sarcopenia and Cardiovascular Complications. *Int. J. Mol. Sci.* 2020, *21*, 3607.
39. Lee, J.H.; Jun, H.S. Role of Myokines in Regulating Skeletal Muscle Mass and Function. *Front. Physiol.* 2019, *10*, 42.
40. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* 2013, *153*, 1194–1217.
41. Distefano, G.; Goodpaster, B.H. Effects of Exercise and Aging on Skeletal Muscle. *Cold Spring Harb. Perspect. Med.* 2018, *8*, a029785.
42. Stegeman, R.; Weake, V.M. Transcriptional Signatures of Aging. *J. Mol. Biol.* 2017, *429*, 2427–2437.
43. Dhingra, R.; Vasan, R.S. Age as a risk factor. *Med. Clin. N. Am.* 2012, *96*, 87–91.
44. Cheitlin, M.D. Cardiovascular physiology-changes with aging. *Am. J. Geriatr. Cardiol.* 2003, *12*, 9–13.
45. Kõks, S.; Dogan, S.; Tuna, B.G.; González-Navarro, H.; Potter, P.; Vandenbroucke, R.E. Mouse models of ageing and their relevance to disease. *Mech. Ageing Dev.* 2016, *160*, 41–53.
46. Vanhooren, V.; Libert, C. The mouse as a model organism in aging research: Usefulness, pitfalls and possibilities. *Ageing Res. Rev.* 2013, *12*, 8–21.
47. Azzu, V.; Valencak, T.G. Energy Metabolism and Ageing in the Mouse: A Mini-Review. *Gerontology* 2017, *63*, 327–336.
48. Azman, K.F.; Zakaria, R. D-Galactose-induced accelerated aging model: An overview. *Biogerontology* 2019, *20*, 763–782.
49. Santilli, V.; Bernetti, A.; Mangone, M.; Paoloni, M. Clinical definition of sarcopenia. *Clin. Cases Miner. Bone Metab.* 2014, *11*, 177–180.
50. Jørgensen, L.H.; Jepsen, P.L.; Boysen, A.; Dalgaard, L.B.; Hvid, L.G.; Ørtenblad, N.; Ravn, D.; Sellathurai, J.; Møller-Jensen, J.; Lochmüller, H.; et al. SPARC Interacts with Actin in Skeletal Muscle in Vitro and in Vivo. *Am. J. Pathol.* 2017, *187*, 457–474.
51. Kjær, M. Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. *Physiol. Rev.* 2004, *84*, 649–698.
52. Gelse, K.; Pöschl, E.; Aigner, T. Collagens—Structure, function, and biosynthesis. *Adv. Drug Deliv. Rev.* 2003, *55*, 1531–1546.
53. Wang, T.; Wagner, A.; Gehwolf, R.; Yan, W.; Passini, F.S.; Thien, C.; Weissenbacher, N.; Lin, Z.; Lehner, C.; Teng, H.; et al. Load-induced regulation of tendon homeostasis by SPARC, a genetic predisposition factor for tendon and ligament injuries. *Sci. Transl. Med.* 2021, *13*, eabe5738.
54. Gruber, J.; Schaffer, S.; Halliwell, B. The mitochondrial free radical theory of ageing–where do we stand? *Front. Biosci.* 2008, *13*, 6554–6579.
55. DeFronzo, R.A. Glucose Intolerance and Aging. *Diabetes Care* 1981, *4*, 493–501.
56. Kos, K.; Wong, S.; Tan, B.; Gummesson, A.; Jernas, M.; Franck, N.; Kerrigan, D.; Nystrom, F.H.; Carlsson, L.M.; Randeva, H.S.; et al. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. *Diabetes* 2009, *58*, 1780–1788.
57. Lee, S.H.; Lee, J.A.; Park, H.S.; Song, Y.S.; Jang, Y.J.; Kim, J.H.; Lee, Y.J.; Heo, Y. Associations among SPARC mRNA expression in adipose tissue, serum SPARC concentration and metabolic parameters in

Korean women. *Obesity* 2013, *21*, 2296–2302.
58. Mukherjee, S.; Choi, M.J.; Kim, S.W.; Yun, J.W. Secreted protein acidic and rich in cysteine (SPARC) regulates thermogenesis in white and brown adipocytes. *Mol. Cell. Endocrinol.* 2020, *506*, 110757.
59. Guo, S.S.; Zeller, C.; Chumlea, W.C.; Siervogel, R.M. Aging, body composition, and lifestyle: The Fels Longitudinal Study. *Am. J. Clin. Nutr.* 1999, *70*, 405–411.
60. Sackmann-Sala, L.; Berryman, D.E.; Munn, R.D.; Lubbers, E.R.; Kopchick, J.J. Heterogeneity among white adipose tissue depots in male C57BL/6J mice. *Obesity* 2012, *20*, 101–111.
61. Jo, J.; Gavrilova, O.; Pack, S.; Jou, W.; Mullen, S.; Sumner, A.E.; Cushman, S.W.; Periwal, V. Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. *PLoS Comput. Biol.* 2009, *5*, e1000324.
62. Nie, J.; Bradshaw, A.D.; Delany, A.M.; Sage, E.H. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. *Connect. Tissue Res.* 2011, *52*, 99–108.
63. Carosio, S.; Berardinelli, M.G.; Aucello, M.; Musarò, A. Impact of ageing on muscle cell regeneration. *Ageing Res. Rev.* 2011, *10*, 35–42.
64. Hwang, A.B.; Brack, A.S. Muscle Stem Cells and Aging. *Curr. Top. Dev. Biol.* 2018, *126*, 299–322.
65. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
66. Mansergh, F.C.; Wells, T.; Elford, C.; Evans, S.L.; Perry, M.J.; Evans, M.J.; Evans, B.A. Osteopenia in Sparc (osteonectin)-deficient mice: Characterization of phenotypic determinants of femoral strength and changes in gene expression. *Physiol. Genom.* 2007, *32*, 64–73.
67. Mansergh, F.C.; Wride, M.A.; Walker, V.E.; Adams, S.; Hunter, S.M.; Evans, M.J. Gene expression changes during cataract progression in Sparc null mice: Differential regulation of mouse globins in the lens. *Mol. Vis.* 2004, *10*, 490–511.
68. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. *Genes Immun.* 2007, *8*, 262–274.
69. Whittal, M.C.; Molladavoodi, S.; Zwambag, D.P.; Millecamps, M.; Stone, L.S.; Gregory, D.E. Mechanical Consequence of Induced Intervertebral Disc Degeneration in the SPARC-Null Mouse. *J. Biomech. Eng.* 2021, *143*, 024501.
70. Gruber, H.E.; Sage, E.H.; Norton, H.J.; Funk, S.; Ingram, J.; Hanley, E.N., Jr. Targeted deletion of the SPARC gene accelerates disc degeneration in the aging mouse. *J. Histochem. Cytochem.* 2005, *53*, 1131–1138.
71. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G.D. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. *Clin. Sci.* 2019, *133*, 351–365.
72. Harries, L.W.; McCulloch, L.J.; Holley, J.E.; Rawling, T.J.; Welters, H.J.; Kos, K. A role for SPARC in the moderation of human insulin secretion. *PLoS ONE* 2013, *8*, e68253.
73. Hu, L.; He, F.; Huang, M.; Zhao, Q.; Cheng, L.; Said, N.; Zhou, Z.; Liu, F.; Dai, Y.S. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. *Sci. Rep.* 2020, *10*, 17581.
74. Kahn, S.E.; Hull, R.L.; Utzschneider, K.M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006, *444*, 840–846.
75. Andrikopoulos, S.; Blair, A.R.; Deluca, N.; Fam, B.C.; Proietto, J. Evaluating the glucose tolerance test in mice. *Am. J. Physiol. Endocrinol. Metab.* 2008, *295*, E1323–E1332.
76. Songsorn, P.; Ruffino, J.; Vollaard, N.B. No effect of acute and chronic supramaximal exercise on circulating levels of the myokine SPARC. *Eur. J. Sport Sci.* 2017, *17*, 447–452.
77. Batsis, J.A.; Villareal, D.T. Sarcopenic obesity in older adults: Aetiology, epidemiology and treatment strategies. *Nat. Rev. Endocrinol.* 2018, *14*, 513–537.
78. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.

79. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. *Diseases* 2021, *9*, 87.
80. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
81. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. *FASEB J.* 2019, *33*, 10551–10562.
82. Richter, E.A.; Hargreaves, M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol. Rev.* 2013, *93*, 993–1017.
83. Pearson, A.M. Muscle growth and exercise. *Crit. Rev. Food Sci. Nutr.* 1990, *29*, 167–196.
84. Liberman, K.; Forti, L.N.; Beyer, I.; Bautmans, I. The effects of exercise on muscle strength, body composition, physical functioning and the inflammatory profile of older adults: A systematic review. *Curr. Opin. Clin. Nutr. Metab. Care* 2017, *20*, 30–53.
85. Willis, L.H.; Slentz, C.A.; Bateman, L.A.; Shields, A.T.; Piner, L.W.; Bales, C.W.; Houmard, J.A.; Kraus, W.E. Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults. *J. Appl. Physiol.* 2012, *113*, 1831–1837.
86. Francaux, M.; Deldicque, L. Exercise and the control of muscle mass in human. *Pflug. Arch.-Eur. J. Physiol.* 2019, *471*, 397–411.
87. Bishop, D.J.; Botella, J.; Genders, A.J.; Lee, M.J.; Saner, N.J.; Kuang, J.; Yan, X.; Granata, C. High-Intensity Exercise and Mitochondrial Biogenesis: Current Controversies and Future Research Directions. *Physiology* 2019, *34*, 56–70.
88. Kwon, J.H.; Moon, K.M.; Min, K.W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. *Healthcare* 2020, *8*, 378.
89. Huh, J.Y. The role of exercise-induced myokines in regulating metabolism. *Arch. Pharmacal Res.* 2018, *41*, 14–29.
90. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. *Cell Metab.* 2016, *23*, 554–562.
91. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
92. Rowlatt, C.; Chesterman, F.C.; Sheriff, M.U. Lifespan, age changes and tumour incidence in an ageing C57BL mouse colony. *Lab. Anim.* 1976, *10*, 419–442.
93. Kunstyr, I.; Leuenberger, H.G. Gerontological data of C57BL/6J mice. I. Sex differences in survival curves. *J. Gerontol.* 1975, *30*, 157–162.
94. Billat, V.L.; Mouisel, E.; Roblot, N.; Melki, J. Inter- and intrastrain variation in mouse critical running speed. *J. Appl. Physiol.* 2005, *98*, 1258–1263.
95. Schefer, V.; Talan, M.I. Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity. *Exp. Gerontol.* 1996, *31*, 387–392.
96. Teklad Global 18% Protein Rodent Diet (Sterilizable). Available online: https://insights.envigo.com/hubfs/resources/datasheets/2018s-datasheet-0915.pdf (accessed on 13 December 2020).
97. Norose, K.; Lo, W.K.; Clark, J.I.; Sage, E.H.; Howe, C.C. Lenses of SPARC-null mice exhibit an abnormal cell surface-basement membrane interface. *Exp. Eye Res.* 2000, *71*, 295–307.
98. Nishida, Y.; Tokuyama, K.; Nagasaka, S.; Higaki, Y.; Shirai, Y.; Kiyonaga, A.; Shindo, M.; Kusaka, I.; Nakamura, T.; Ishibashi, S.; et al. Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartment-labeled minimal model. *Diabetes* 2004, *53*, 315–320.
99. Tanaka, H.; Shindo, M. The benefits of the low intensity training. *Ann. Physiol. Anthr.* 1992, *11*, 365–368.
100. Motoyama, M.; Sunami, Y.; Kinoshita, F.; Kiyonaga, A.; Tanaka, H.; Shindo, M.; Irie, T.; Urata, H.;

Sasaki, J.; Arakawa, K. Blood pressure lowering effect of low intensity aerobic training in elderly hypertensive patients. *Med. Sci. Sports Exerc.* 1998, *30*, 818–823.
101. Sunami, Y.; Motoyama, M.; Kinoshita, F.; Mizooka, Y.; Sueta, K.; Matsunaga, A.; Sasaki, J.; Tanaka, H.; Shindo, M. Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. *Metabolism*
1999, *48*, 984–988.
102. Frontera, W.R.; Hughes, V.A.; Fielding, R.A.; Fiatarone, M.A.; Evans, W.J.; Roubenoff, R. Aging of skeletal muscle: A 12-yr longitudinal study. *J. Appl. Physiol.* 2000, *88*, 1321–1326.
103. Yamada, M.; Moriguch, Y.; Mitani, T.; Aoyama, T.; Arai, H. Age-dependent changes in skeletal muscle mass and visceral fat area in Japanese adults from 40 to 79 years-of-age. *Geriatr. Gerontol. Int.* 2014, *14* (Suppl. S1), 8–14.
104. Miyamoto, T.; Shimizu, Y.; Matsuo, Y.; Otaru, T.; Kanzawa, Y.; Miyamae, N.; Yamada, E.; Katsuno, T. Effects of exercise intensity and duration on a myokine, secreted protein acidic and rich in cysteine. *Eur. J. Sport Sci.* 2021, 1–10.
105. Potteiger, J.A.; Jacobsen, D.J.; Donnelly, J.E. A comparison of methods for analyzing glucose and insulin areas under the curve following nine months of exercise in overweight adults. *Int. J. Obes. Relat. Metab. Disord.* 2002, *26*, 87–89.
106. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 2012, *9*, 671–675.
107. Taylor, S.C.; Berkelman, T.; Yadav, G.; Hammond, M. A defined methodology for reliable quantification of Western blot data. *Mol. Biotechnol.* 2013, *55*, 217–226.
108. Taylor, S.C.; Posch, A. The design of a quantitative western blot experiment. *BioMed Res. Int.* 2014, *2014*, 361590.
109. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. *Life Sci.* 2018, *215*, 190–197.
110. Lo, S.; Russell, J.C.; Taylor, A.W. Determination of glycogen in small tissue samples. *J. Appl. Physiol.* 1970, *28*, 234–236.
111. Parlee, S.D.; Lentz, S.I.; Mori, H.; MacDougald, O.A. Quantifying size and number of adipocytes in adipose tissue. *Methods Enzymol.* 2014, *537*, 93–122.
112. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. *Obesity* 2013, *21*, 1389–1395.
113. Available online: https://www.cyagen.com/us/en/service/piggybac-transgenic-mouse.html (accessed on 15 December 2020).
114. Luu-The, V.; Paquet, N.; Calvo, E.; Cumps, J. Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction. *BioTechniques* 2005, *38*, 287–293.
115. Bustin, S.A.; Benes, V.; Garson, J.A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M.W.; Shipley, G.L.; et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 2009, *55*, 611–622.
116. Bustin, S.A.; Beaulieu, J.F.; Huggett, J.; Jaggi, R.; Kibenge, F.S.; Olsvik, P.A.; Penning, L.C.; Toegel, S. MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. *BMC Mol. Biol.* 2010, *11*, 74.
117. Smith, J.P.; Hicks, P.S.; Ortiz, L.R.; Martinez, M.J.; Mandler, R.N. Quantitative measurement of muscle strength in the mouse. *J. Neurosci. Methods* 1995, *62*, 15–19.
118. Takeshita, H.; Yamamoto, K.; Nozato, S.; Inagaki, T.; Tsuchimochi, H.; Shirai, M.; Yamamoto, R.; Imaizumi, Y.; Hongyo, K.; Yokoyama, S.; et al. Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice. *Sci. Rep.* 2017, *7*, 42323.
119. Capaldi, R.A.; Malatesta, F.; Darley-Usmar, V.M. Structure of cytochrome c oxidase. *Biochim. Biophys.*

Acta 1983, *726*, 135–148.
120. García-Horsman, J.A.; Barquera, B.; Rumbley, J.; Ma, J.; Gennis, R.B. The superfamily of heme-copper respiratory oxidases. *J. Bacteriol.* 1994, *176*, 5587–5600.
121. Saraste, M. Structure and evolution of cytochrome oxidase. *Antonie Leeuwenhoek* 1994, *65*, 285–287.
122. Azzi, A. Cytochrome c oxidase. Towards a clarification of its structure, interactions and mechanism. *Biochim. Biophys. Acta* 1980, *594*, 231–252.
123. Available online: https://www.ncbi.nlm.nih.gov/gene/17709 (accessed on 16 January 2022).
124. Dittmer, A.; Dittmer, J. Beta-actin is not a reliable loading control in Western blot analysis. *Electrophoresis* 2006, *27*, 2844–2845.
125. Bao, J.M.; Dang, Q.; Lin, C.J.; Lo, U.G.; Feldkoren, B.; Dang, A.; Hernandez, E.; Li, F.; Panwar, V.; Lee, C.F.; et al. SPARC is a key mediator of TGF-β-induced renal cancer metastasis. *J. Cell. Physiol.* 2021, *236*, 1926–1938.
126. Chang, W.; Wei, K.; Jacobs, S.S.; Upadhyay, D.; Weill, D.; Rosen, G.D. SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin. *J. Biol. Chem.* 2010, *285*, 8196–8206.
127. Shen, Y.; Zhao, Y.; Yuan, L.; Yi, W.; Zhao, R.; Yi, Q.; Yong, T. SPARC is over-expressed in adipose tissues of diet-induced obese rats and causes insulin resistance in 3T3-L1 adipocytes. *Acta Histochem.* 2014, *116*, 158–166.
128. Wu, D.; Li, L.; Yang, M.; Liu, H.; Yang, G. Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus. *Eur. J. Endocrinol.* 2011, *165*, 597–601.
129. Yiu, G.K.; Chan, W.Y.; Ng, S.W.; Chan, P.S.; Cheung, K.K.; Berkowitz, R.S.; Mok, S.C. SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells. *Am. J. Pathol.* 2001, *159*, 609–622.
130. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Matsuwaki, T.; Nishihara, M. Age-related resistance of skeletal muscle-derived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. *Aging* 2012, *4*, 40–48.
131. Nie, J.; Chang, B.; Traktuev, D.O.; Sun, J.; March, K.; Chan, L.; Sage, E.H.; Pasqualini, R.; Arap, W.; Kolonin, M.G. IFATS collection: Combinatorial peptides identify alpha5beta1 integrin as a receptor for the matricellular protein SPARC on adipose stromal cells. *Stem Cells* 2008, *26*, 2735–2745.
132. Jacunski, M.; Melville, P.; Currie, G.P. Exercise: The panacea in management of many ills. Now is the time to engage. *J. R. Coll. Physicians Edinb.* 2021, *51*, 120–122.

24.10 Appendix 24.1 (Figure 24.S1): *Sparc* Tg experiment body weights of mice

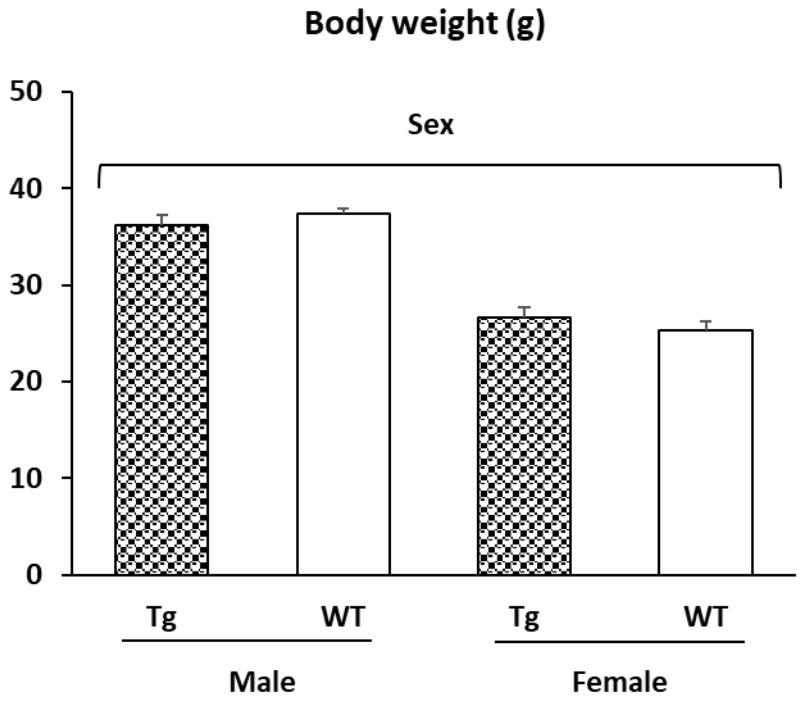


Figure 24.S 1: **Body weights of both WT and Tg mice (male and female)**.
Male mice had a higher body weight than female mice.
All data are mean ± SEM. The number of mice: 5-11 mice per experimental condition.
Abbreviations: g, gram; *Sparc*, secreted protein acidic and rich in cysteine; Tg, transgenic (*Sparc* overexpression); WT, wild-type.

Chapter 25. Opinion - Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, QC G1V 0A6, Canada

Received: 6 October 2021; Accepted: 17 November 2021; Published: 20 November 2021

25.1 Résumé (French abstract)

Les nombreux avantages de l'exercice pour la santé ainsi que les applications pour les maladies ont conduit à la prescription de l'exercice dans de nombreuses conditions pathologiques. L'expression génique de la secreted protein acidic and rich in cysteine (SPARC) est stimulée par l'exercice et SPARC a été suggéré comme médiateur moléculaire de l'exercice. Par conséquent, nous suggérons d'utiliser cette propriété pour la médecine personnalisée. Ceci peut être réalisé en prescrivant l'exercice avec un modèle (durée, intensité, etc.) qui correspond à l'expression optimale de *SPARC/Sparc*. Nous nous attendons à ce que cette approche optimise la thérapie par l'exercice dans les contextes préventifs et curatifs. Dans le domaine de la recherche, la mesure de l'expression de *Sparc* dépendante de l'exercice représenterait un outil moléculaire permettant d'optimiser davantage la sélection des modèles d'animaux de l'exercice.

25.2 Abstract

The numerous exercise benefits for health as well as applications for diseases has led to exercise being prescribed in many pathological conditions. Secreted protein acidic and rich in cysteine (SPARC) gene expression is stimulated by exercise and SPARC has been suggested as a molecular mediator of exercise. Therefore, we suggest using this property for personalized medicine. This can be achieved by prescribing the exercise with a pattern (duration, intensity, etc.) that corresponds to the optimum *SPARC/Sparc* expression. We expect this approach to optimize the exercise therapy in both the preventive and curative contexts. In the research field, measuring exercise -dependent expression of *Sparc* would represent a molecular tool to further optimize the selection of exercise animal models as well.

Keywords: exercise; secreted protein acidic and rich in cysteine; expression; medicine

25.3 Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine

With the development of non-pharmacological and non-surgical approaches in therapeutics, the medical applications of exercise are gaining increasing importance. Indeed, beyond being a habit for numerous individuals with positive impacts on mood [1,2], exercise represents a therapeutic option for a variety of diseases and health conditions. It has been used within medical protocols either as a therapy or as an adjuvant to treat, prevent or improve diseases and health problems in which effects including controlling energy balance or enhancing biological properties are therapeutic targets such as cardiovascular diseases [3,4], obesity [5–8], low back pain [9], metabolic disorders [10], chronic kidney disease [11], regeneration [12], cancer [13], diabetes [14], immunity and infections [15–17]. Such exercise applications find their origin in the very numerous benefits that exercise has on health. This includes lowering blood pressure [18], bone osteogenesis stimulation [19], reducing cachexia [11] and anti-inflammatory effects [20]. Mental health (anxiety, stress and depression), sports psychiatry [21–23], and improved sleep quality [24,25] are also in this list. These medical benefits were considered as "granted" for humans who lived before the current industrial area because they had a healthier lifestyle that included sufficient physical activity. Thus, it significantly contributed to positive public health. However, in the last decades, the development of technologies has made life easier and humans need less effort to achieve what required huge effort previously. This situation has led to a sedentary lifestyle and less active societies, which has contributed to the increase of various human diseases. As an attempt to correct this negative consequence of modernity, health professionals are recommending physical activity for diverse population categories.

Regarding the molecular mechanism linking exercise and the exercise-induced effects, exercise benefits have been suggested to be mediated through a variety of factors, mainly the muscle-secreted myokines [26] that are produced by skeletal muscles and increase in response to exercise [27]. Such an exercise-induced pattern of secretion suggests that these myokines would govern the molecular pathways underlying the phenotypic changes resulting from exercise in different organs and tissues leading to the known health benefits of the physical activity. May be the most interesting one is secreted protein acidic and rich in cysteine (SPARC), an exercise-responsive myokine [28] in both humans and mice [29]. Indeed, using powerful functional genomics that represent a strong strategy to study the dynamic expression of genes [30,31], *SPARC* has been characterized as an exercise-induced gene [32]. Initially, the serial analysis of gene expression revealed that the cycle ergometer training increased the *SPARC* expression in muscles following

endurance training [32]. Moreover, Aoi el al. showed that a single bout of exercise increased SPARC expression in the muscle and also in the plasma [29] and such a plasma exercise-induced increase becomes more important following training [29].

Following that, in vitro studies have been performed on C2C12 muscle cells to further explore SPARC-exercise mechanistic links. Electrical pulse stimulation (EPS), considered as the in vitro model of exercise [33–36], applied on C2C12 cells also induced *Sparc* expression [37]. The same in vitro studies showed that *Sparc* modulates mitochondrial functions [37] and that adding SPARC both increased myoblasts differentiation and mitochondrial proteins in C2C12 cells [38]. Importantly, a recent in vivo study on trained *Sparc* knock-out mice suggested that exercise-induced muscle phenotype changes, including metabolism, strength and development, are SPARC-dependent [39]. Together, these data highlight SPARC as a key mediator of the exercise-induced benefits. Furthermore, the roles and functions in which SPARC has been implicated or suggested to be involved correlate with exercise effects. For instance, beyond its known implications, mainly in tissue repair [40], SPARC has been suggested to be involved in metabolic changes [28,41–44], bone formation [45], regeneration [46–48], anticancer effects [29,49], anti-inflammatory paths [50], and regulating muscle mass and function [51], all of which have also been shown to improve with exercise; which further supports the existence of molecular links between SPARC functions and exercise effects [28,52]. These cellular and molecular properties may represent the rationale why *SPARC*/*Sparc* functions as an exercise-responsive gene and why SPARC is induced by exercise. Indeed, since exercise may promote health and enhance systemic health via various cellular responses (e.g., metabolic change, bone, regeneration, anti-cancer, anti-inflammatory and regulation of muscle mass and function) that have been shown to implicate SPARC, SPARC comes out as a molecular mediator secreted following exercise to enhance and stimulate biological properties and endogenous processes toward a healthy homeostatic phenotype.

Therefore, since exercise effects are mediated via SPARC, the optimum exercise would be the one that induces SPARC/*SPARC/Sparc* expression the most. Thus, we suggest for the first time to our knowledge-applying such a concept for personalized medicine. The process would be to challenge individuals with a variety of exercise patterns and programs that are different in terms of type of exercise type, the used device, the speed, duration, time (morning, night, etc.) and even the addition of other factors such as the temperature and incline setting (treadmill) for instance. Following the exercise, we proceed to a muscle biopsy, a common procedure [53,54], to measure the expression of *SPARC/Sparc*. Based on the results, the optimum exercise conditions (time, speed, environment, etc.) would be determined as those corresponding to the optimum *SPARC/Sparc* expression. Future studies would allow one to make further links not only between exercise and *SPARC/Sparc* gene expression but also between the exercise and the protein SPARC expression or its serum levels that increase following exercise [29,55], thus adding the protein expression and the serum concentrations of SPARC as novel exercise-efficacy evaluation tools. Such tools would allow one to estimate the benefits that an

exercise (depending on its patterns) would induce and open the door to a variety of potential applications (Figure 25.1). Measuring exercise induced SPARC/*SPARC*/*Sparc* can contribute to answering the questions discussed in diverse studies in terms of exercise "dose" [14,56–58]. Indeed, a possible correlation between the exercise intensity and SPARC serum level has been shown [59], which supports such SPARC-dependent evaluation of exercise effects.

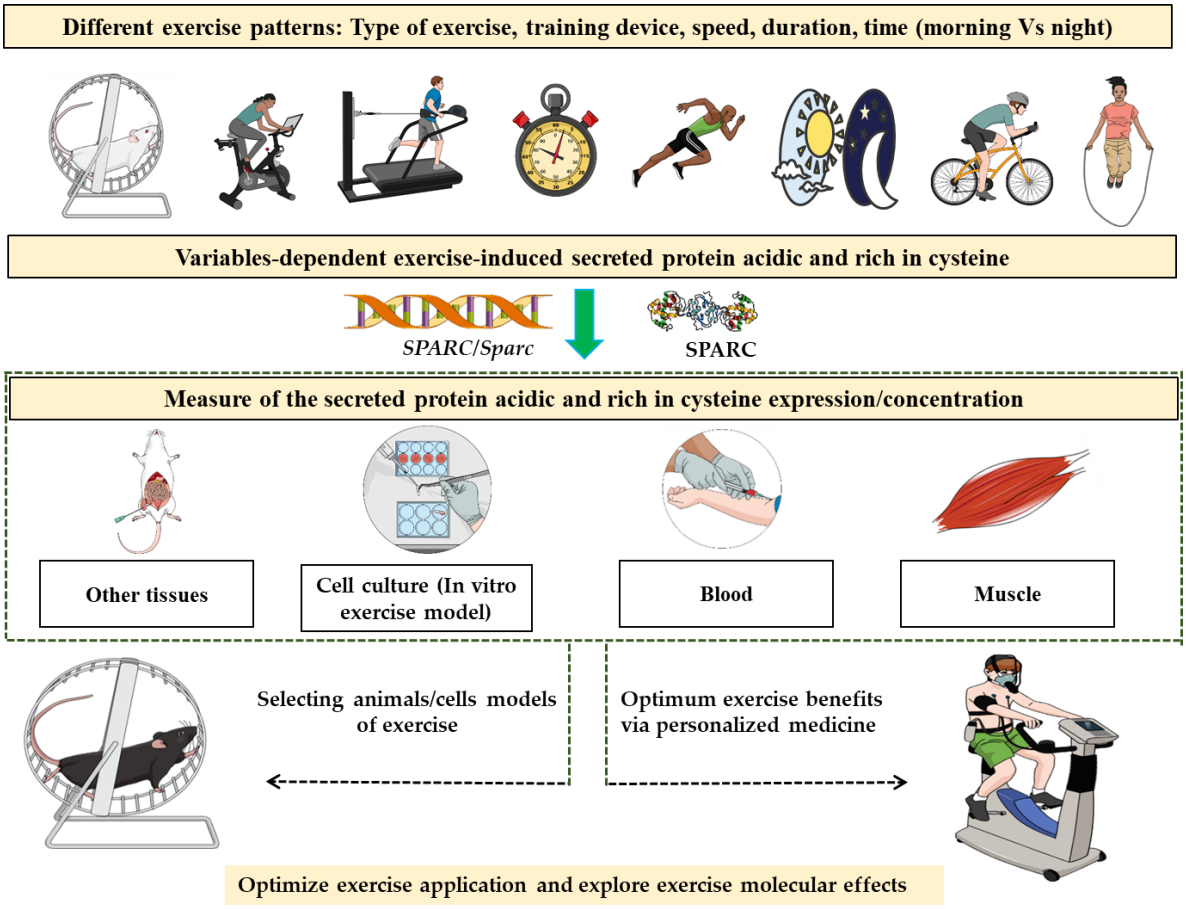


Figure 25.1. Measuring secreted protein acidic and rich in cysteine expression/concentration in biological samples following different patterns of exercise training would reflect the biological "responsiveness" to the physical activity and would predict the intensity of the benefits that exercise-induced changes will have. Such a property could be explored for instance to optimize the prescribed physical activity towards a personalized medicine approach and also select animal/cell models of exercise.

For clinical perspectives, which still require deeper investigations, the main application would be to determine the optimum parameters of the exercise to prescribe for patients suffering from diseases and health problems for which exercise represents a therapy. Of course, the tested exercise intensity, duration, strength, etc. would depend on each patient based on the physiological and biochemical parameters that limit the exercise ability such as oxygen saturation, lung capacity, heart status, glycemia and physical disabilities. Indeed, going beyond those physiological limits will not only be harmful but

could also have no exercise-induced benefits, as suggested by the fact that supramaximal exercise had no effect on SPARC levels [60]. Such a need to set a limit could be achieved by measuring SPARC via the evaluation of exercise-dependant SPARC expression as well.

Another application would be the optimization of animal models of exercise to better develop exercise science and exercise-related research towards an optimized application of exercise to treat patients, as a prevention for healthy individuals or to optimize training efficacy and outcome for athletes. Furthermore, the same *SPARC/Sparc* expression as a measure of exercise efficacy principle can be used not only to optimize the exercise pattern but also to compare different groups based on age, sex, diet, genetic polymorphism and species (animals). In this context, the in vitro models of exercise (electric pulse stimulation) would also provide additional data at the molecular and subcellular levels.

The importance of such new tools comes from the fact that exercise represents a "panacea" for limitless health problems. We believe that this suggested approach of measuring of SPARC/*SPARC/Sparc* expression/level in response to different exercise patterns could optimize exercise science and provide molecular evaluation tools to significantly improve public health via personalized medicine. One of the main applications would be to manage obesity and metabolic disorders. This concept would also be of a specific application for the older population that have many potential benefits from exercise but that need to be optimized in terms of intensity, type and duration for healthy ageing [61–63].

The evidence we have provided builds up a puzzle that suggests SPARC as a selective biological marker that reflects the physiological responsiveness to exercise, not only through muscle-related patterns [64–67] but also metabolism [68], adiposity [69], and other effects, and thus the quality and the level of the induced benefits we will see following that exercise. However, further studies are still required to confirm and quantify the exact correlation between SPARC/*SPARC* expression and the various factors that define the exercise amount, mainly the intensity and the duration. These studies should focus on numerical and quantitative correlation similar to what we have for biomarkers used in clinical practice or biomedical research [70–87] including SPARC itself, which was also suggested as a physiological and pathological biomarker [88]. Personalized medicine [89–93] and precision medicine [94–101] are growing areas in respect to exercise [102–107], which further highlights the potential of measuring SPARC/*SPARC*/*Sparc* expression/level in optimizing and developing medical practice.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, the figure, references selection, etc.) throughout the writing process, edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published.
All authors have read and agreed to the published version of the manuscript.

Funding: This work received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship « Bourse Tremplin -Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec–Sante (FRQS), Quebec, Canada. Figure 25.1 was created using images from https:// mindthegraph.com/ (Accessed on 28 September 2021) and http://smart.servier.com (Accessed on 28 September 2021). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

Conflicts of Interest: The authors declare that there is no conflict of interest.

25.4 References

1. Hosker, D.K.; Elkins, R.M.; Potter, M.P. Promoting Mental Health and Wellness in Youth Through Physical Activity, Nutrition, and Sleep. *Child. Adolesc. Psychiatr. Clin. N. Am.* 2019, *28*, 171–193.
2. Peluso, M.A.; de Andrade, L.H.G. Physical activity and mental health: The association between exercise and mood. *Clinics* 2005, *60*, 61–70.
3. Myers, J.; McAuley, P.; Lavie, C.J.; Despres, J.P.; Arena, R.; Kokkinos, P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: Their independent and interwoven importance to health status. *Prog. Cardiovasc. Dis.* 2015, *57*, 306–314.
4. Katzmarzyk, P.T.; Ross, R.; Blair, S.N.; Després, J.P. Should we target increased physical activity or less sedentary behavior in the battle against cardiovascular disease risk development? *Atherosclerosis* 2020, *311*, 107–115.
5. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. *Medicina* 2021, *57*, 399.
6. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. *Eur. J. Intern. Med.* 2018, *55*, 20–22.
7. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. *J. Clin. Med.* 2018, *7*, 453.
8. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. *Medicina* 2021, *57*, 66.
9. Owen, P.J.; Miller, C.T.; Mundell, N.L.; Verswijveren, S.; Tagliaferri, S.D.; Brisby, H.; Bowe, S.J.; Belavy, D.L. Which specific modes of exercise training are most effective for treating low back pain? Network meta-analysis. *Br. J. Sports Med.* 2020, *54*, 1279–1287.
10. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.

11. Wilkinson, T.J.; Shur, N.F.; Smith, A.C. “Exercise as medicine” in chronic kidney disease. *Scand. J. Med. Sci. Sports* 2016, *26*, 985–988.
12. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. *Animals* 2020, *10*, 2344.
13. Idorn, M.; Straten, P.T. Exercise and cancer: From “healthy” to “therapeutic”? *Cancer Immunol. Immunother.* 2017, *66*, 667–671.
14. Balducci, S.; Sacchetti, M.; Haxhi, J.; Orlando, G.; D’Errico, V.; Fallucca, S.; Menini, S.; Pugliese, G. Physical exercise as therapy for type 2 diabetes mellitus. *Diabetes Metab. Res. Rev.* 2014, *30*, 13–23.
15. Simpson, R.J.; Kunz, H.; Agha, N.; Graff, R. Exercise and the Regulation of Immune Functions. *Prog. Mol. Biol. Transl. Sci.* 2015, *135*, 355–380.
16. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. *Biology* 2021, *10*, 545.
17. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? *Med. Hypotheses* 2020, *144*, 110042.
18. Sharman, J.E.; La Gerche, A.; Coombes, J.S. Exercise and cardiovascular risk in patients with hypertension. *Am. J. Hypertens.* 2015, *28*, 147–158.
19. Benedetti, M.G.; Furlini, G.; Zati, A.; Mauro, G.L. The Effectiveness of Physical Exercise on Bone Density in Osteoporotic Patients. *Biomed. Res. Int.* 2018, *2018*, 4840531.
20. Metsios, G.S.; Moe, R.H.; Kitas, G.D. Exercise and inflammation. *Best Pract. Res. Clin. Rheumatol.* 2020, *34*, 101504.
21. Ströhle, A. Sports psychiatry: Mental health and mental disorders in athletes and exercise treatment of mental disorders. *Eur. Arch. Psychiatry Clin. Neurosci.* 2019, *269*, 485–498.
22. Zhang, Y.; Zhang, H.; Ma, X.; Di, Q. Mental Health Problems during the COVID-19 Pandemics and the Mitigation Effects of Exercise: A Longitudinal Study of College Students in China. *Int. J. Environ. Res. Public Health* 2020, *17*, 3722.
23. Mikkelsen, K.; Stojanovska, L.; Polenakovic, M.; Bosevski, M.; Apostolopoulos, V. Exercise and mental health. *Maturitas* 2017, *106*, 48–56.
24. Jurado-Fasoli, L.; De-la, O.A.; Molina-Hidalgo, C.; Migueles, J.H.; Castillo, M.J.; Amaro-Gahete, F.J. Exercise training improves sleep quality: A randomized controlled trial. *Eur. J. Clin. Investig.* 2020, *50*, e13202.
25. Yang, P.Y.; Ho, K.H.; Chen, H.C.; Chien, M.Y. Exercise training improves sleep quality in middle-aged and older adults with sleep problems: A systematic review. *J. Physiother.* 2012, *58*, 157–163.
26. Kwon, J.H.; Moon, K.M.; Min, K.-W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. *Healthcare* 2020, *8*, 378.
27. Aoi, W.; Sakuma, K. Skeletal muscle: Novel and intriguing characteristics as a secretory organ. *BioDiscovery* 2013, *7*.
28. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. *Faseb J.* 2019, *33*, 10551–10562.
29. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
30. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
31. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res. Rev. Mutat. Res.* 2019, *781*, 175–185.
32. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.

33. Scheler, M.; Irmler, M.; Lehr, S.; Hartwig, S.; Staiger, H.; Al-Hasani, H.; Beckers, J.; de Angelis, M.H.; Häring, H.U.; Weigert, C. Cytokine response of primary human myotubes in an in vitro exercise model. *Am. J. Physiol. Cell Physiol.* 2013, *305*, C877–C886.
34. Nikoli´c, N.; Bakke, S.S.; Kase, E.T.; Rudberg, I.; Halle, I.F.; Rustan, A.C.; Thoresen, G.H.; Aas, V. Correction: Electrical Pulse Stimulation of Cultured Human Skeletal Muscle Cells as an In Vitro Model of Exercise. *PLoS ONE* 2013, *8*, e33203.
35. Tarum, J.; Folkesson, M.; Atherton, P.J.; Kadi, F. Electrical pulse stimulation: An in vitro exercise model for the induction of human skeletal muscle cell hypertrophy. A proof-of-concept study. *Exp. Physiol.* 2017, *102*, 1405–1413.
36. Feng, Y.Z.; Nikoli´c, N.; Bakke, S.S.; Kase, E.T.; Guderud, K.; Hjelmesæth, J.; Aas, V.; Rustan, A.C.; Thoresen, G.H. Myotubes from lean and severely obese subjects with and without type 2 diabetes respond differently to an in vitro model of exercise. *Am. J. Physiol. Cell. Physiol.* 2015, *308*, C548–C556.
37. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.
38. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
39. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
40. Phan, E.; Ahluwalia, A.; Tarnawski, A.S. Role of SPARC—Matricellular protein in pathophysiology and tissue injury healing. Implications for gastritis and gastric ulcers. *Med. Sci. Monit.* 2007, *13*, RA25–RA30.
41. Harries, L.W.; McCulloch, L.J.; Holley, J.E.; Rawling, T.J.; Welters, H.J.; Kos, K. A role for SPARC in the moderation of human insulin secretion. *PLoS ONE* 2013, *8*, e68253.
42. Hu, L.; He, F.; Huang, M.; Zhao, Q.; Cheng, L.; Said, N.; Zhou, Z.; Liu, F.; Dai, Y.S. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. *Sci. Rep.* 2020, *10*, 17581.
43. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
44. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
45. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. *Matrix Biol.* 2016, *52–54*, 78–87.
46. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
47. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. *Histol. Histopathol.* 2013, *28*, 1451–1460.
48. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schrøder, H.D. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. *J. Histochem. Cytochem.* 2009, *57*, 29–39.
49. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
50. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
51. Lee, J.H.; Jun, H.S. Role of Myokines in Regulating Skeletal Muscle Mass and Function. *Front. Physiol.* 2019, *10*, 42.
52. Kim, J.S.; Galvão, D.A.; Newton, R.U.; Gray, E.; Taaffe, D.R. Exercise-induced myokines and their

effect on prostate cancer. *Nat. Rev. Urol.* 2021, *18*, 519–542.
53. Joyce, N.C.; Oskarsson, B.; Jin, L.W. Muscle biopsy evaluation in neuromuscular disorders. *Phys. Med. Rehabil. Clin. N. Am.* 2012, *23*, 609–631.
54. Pearl, G.S.; Ghatak, N.R. Muscle biopsy. *Arch. Pathol. Lab. Med.* 1995, *119*, 303–306.
55. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. *Front. Physiol.* 2020, *11*, 18.
56. Brown, J.C.; Damjanov, N.; Courneya, K.S.; Troxel, A.B.; Zemel, B.S.; Rickels, M.R.; Ky, B.; Rhim, A.D.; Rustgi, A.K.; Schmitz, K.H. A randomized dose-response trial of aerobic exercise and health-related quality of life in colon cancer survivors. *Psychooncology* 2018, *27*, 1221–1228.
57. Dunn, A.L.; Trivedi, M.H.; Kampert, J.B.; Clark, C.G. Chambliss HO: Exercise treatment for depression: Efficacy and dose response. *Am. J. Prev. Med.* 2005, *28*, 1–8.
58. Malliaras, P.; Johnston, R.; Street, G.; Littlewood, C.; Bennell, K.; Haines, T.; Buchbinder, R. The Efficacy of Higher Versus Lower Dose Exercise in Rotator Cuff Tendinopathy: A Systematic Review of Randomized Controlled Trials. *Arch. Phys. Med. Rehabil.* 2020, *101*, 1822–1834.
59. Miyamoto, T.; Shimizu, Y.; Matsuo, Y.; Otaru, T.; Kanzawa, Y.; Miyamae, N.; Yamada, E.; Katsuno, T. Effects of exercise intensity and duration on a myokine, secreted protein acidic and rich in cysteine. *Eur. J. Sport Sci.* 2021, *8*, 1–10.
60. Songsorn, P.; Ruffino, J.; Vollaard, N.B. No effect of acute and chronic supramaximal exercise on circulating levels of the myokine SPARC. *Eur. J. Sport Sci.* 2017, *17*, 447–452.
61. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. *Phys. Med. Rehabil. Clin. N. Am.* 2017, *28*, 659–669.
62. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. *Am. Fam. Physician.* 2017, *95*, 425–432.
63. Baker, M.K.; Atlantis, E.; Singh, M.A.F. Multi-modal exercise programs for older adults. *Age Ageing* 2007, *36*, 375–381.
64. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Transdisciplinary Approach for Sarcopenia. Molecular mechanism of sarcopenia: The role of skeletal muscle niche component SPARC in the regulation of myogenesis and adipogenesis and its alteration with age. *Clin Calcium* 2014, *24*, 1471–1478.
65. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. *J. Vet. Med. Sci.* 2019, *81*, 1649–1654.
66. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. *Aging Cell* 2014, *13*, 175–184.
67. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Matsuwaki, T.; Nishihara, M. Age-related resistance of skeletal muscle-derived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. *Aging (Albany NY)* 2012, *4*, 40–48.
68. Song, H.; Ding, L.; Zhang, S.; Wang, W. MiR-29 family members interact with SPARC to regulate glucose metabolism. *Biochem. Biophys. Res. Commun.* 2018, *497*, 667–674.
69. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. *Nat. Rev. Endocrinol.* 2010, *6*, 225–235.
70. Pierrakos, C.; Vincent, J.L. Sepsis biomarkers: A review. *Crit Care* 2010, *14*, R15.
71. Capecchi, R.; Puxeddu, I.; Pratesi, F.; Migliorini, P. New biomarkers in SLE: From bench to bedside. *Rheumatology (Oxford)* 2020, *59*, v12–v18.
72. Kunc, P.; Fabry, J.; Lucanska, M.; Pecova, R. Biomarkers of Bronchial Asthma. *Physiol. Res.* 2020, *69*, S29–S34.
73. Aronson, J.K.; Ferner, R.E. Biomarkers-A General Review. *Curr. Protoc. Pharmacol.* 2017, *76*, 9–23.
74. Sandokji, I.; Greenberg, J.H. Novel biomarkers of acute kidney injury in children: An update on recent findings. *Curr. Opin. Pediatr.* 2020, *32*, 354–359.

75. Kawata, K.; Tierney, R.; Langford, D. Blood and cerebrospinal fluid biomarkers. *Handb. Clin. Neurol.* 2018, *158*, 217–233.
76. Avramouli, A.; Vlamos, P. Buccal Mucosa Biomarkers in Alzheimer's Disease. *Adv. Exp. Med. Biol.* 2020, *1195*, 49–56.
77. Zhou, L.T.; Lv, L.L.; Liu, B.C. Urinary Biomarkers of Renal Fibrosis. *Adv. Exp. Med. Biol.* 2019, *1165*, 607–623.
78. Shen, L.; Liu, X.; Zhang, H.; Lin, J.; Feng, C.; Iqbal, J. Biomarkers in autism spectrum disorders: Current progress. *Clin. Chim. Acta.* 2020, *502*, 41–54.
79. Steckl, A.J.; Ray, P. Stress Biomarkers in Biological Fluids and Their Point-of-Use Detection. *ACS Sens.* 2018, *3*, 2025–2044.
80. Gururajan, A.; Clarke, G.; Dinan, T.G.; Cryan, J.F. Molecular biomarkers of depression. *Neurosci. Biobehav. Rev.* 2016, *64*, 101–133.
81. Watt, F.E. Osteoarthritis biomarkers: Year in review. *Osteoarthritis Cartilage* 2018, *26*, 312–318.
82. Chen, X.H.; Huang, S.; Kerr, D. Biomarkers in clinical medicine. *IARC Sci. Publ.* 2011, 303–322.
83. Jing, J.; Gao, Y. Urine biomarkers in the early stages of diseases: Current status and perspective. *Discov. Med.* 2018, *25*, 57–65.
84. Agah, E.; Saleh, F.; Sanjari, M.H.; Saghazadeh, A.; Tafakhori, A.; Rezaei, N. CSF and blood biomarkers in amyotrophic lateral sclerosis: Protocol for a systematic review and meta-analysis. *Syst. Rev.* 2018, *7*, 237.
85. Bhide, A.A.; Cartwright, R.; Khullar, V.; Digesu, G.A. Biomarkers in overactive bladder. *Int. Urogynecol. J.* 2013, *24*, 1065–1072.
86. Ahn, M.H.; Kim, H.A. Biomarkers for adult-onset still's disease: Up-to-date. *Expert. Rev. Mol. Diagn.* 2019, *19*, 655–657.
87. Zhao, X.; Modur, V.; Carayannopoulos, L.N.; Laterza, O.F. Biomarkers in Pharmaceutical Research. *Clin. Chem.* 2015, *61*, 1343–1353.
88. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. *Biomolecules* 2021, *11*, 1689.
89. Curigliano, G. Personalizing medicine through personalized communication: Individuality of the patient across borders and cultures. *J. Natl. Compr. Canc. Netw.* 2010, *8*, 277–278.
90. Ziegelstein, R.C. Perspectives in Primary Care: Knowing the Patient as a Person in the Precision Medicine Era. *Ann. Fam. Med.* 2018, *16*, 4–5.
91. Erden, A. Personalized Medicine: How Studying Individual Differences Can Shed Light on Treating Thousands of Patients: An Interview with Richard Lifton, PhD. *Yale. J. Biol. Med.* 2015, *88*, 407–411.
92. Abettan, C.; Welie, J.V.M. The impact of twenty-first century personalized medicine versus twenty-first century medicine's impact on personalization. *Philos. Ethics Humanit Med.* 2020, *15*, 11.
93. Boersma, G.J.; Benthem, L.; van Beek, A.P.; van Dijk, G.; Scheurink, A.J. Personality, a key factor in personalized medicine? *Eur. J. Pharmacol.* 2011, *667*, 23–25.
94. Khatri, V.P.; Petrelli, N.J. Precision Medicine. *Surg. Oncol. Clin. N. Am.* 2020, *29*, xv–xvi.
95. Ramaswami, R.; Bayer, R.; Galea, S. Precision Medicine from a Public Health Perspective. *Annu. Rev. Public Health* 2018, *39*, 153–168.
96. Beckmann, J.S.; Lew, D. Reconciling evidence-based medicine and precision medicine in the era of big data: Challenges and opportunities. *Genome Med.* 2016, *8*, 134.
97. Antman, E.M.; Loscalzo, J. Precision medicine in cardiology. *Nat. Rev. Cardiol.* 2016, *13*, 591–602.
98. Weil, A.R. Precision Medicine. *Health Aff. (Millwood)* 2018, *37*, 687.
99. Xie, F.; Chan, J.C.; Ma, R.C. Precision medicine in diabetes prevention, classification and management. *J. Diabetes Investig.* 2018, *9*, 998–1015.
100. Forrest, S.J.; Geoerger, B.; Janeway, K.A. Precision medicine in pediatric oncology. *Curr. Opin. Pediatr.* 2018, *30*, 17–24.
101. König, I.R.; Fuchs, O.; Hansen, G.; von Mutius, E.; Kopp, M.V. What is precision medicine? *Eur. Respir. J.* 2017, *50*.

102. Yardley, J.E.; Campbell, M.D. Moving Toward Precision Medicine with Diabetes, Exercise and Physical Activity. *Can. J. Diabetes* 2020, *44*, 679.
103. Bonafiglia, J.T.; Ramic, L.; Islam, H. Challenges to personalized exercise medicine: Lack of repeatability and influence of genetics. *J. Physiol.* 2020, *598*, 5021–5023.
104. Buford TW, Roberts MD, Church TS: Toward exercise as personalized medicine. *Sports Med.* 2013, *43*, 157–165.
105. Garner, S.; Fenton, T.; Martin, L.; Creaser, C.; Johns, C.; Barnabe, C. Personalized diet and exercise recommendations in early rheumatoid arthritis: A feasibility trial. *Musculoskeletal Care* 2018, *16*, 167–172.
106. Kostek, M. Precision Medicine and Exercise Therapy in Duchenne Muscular Dystrophy. *Sports (Basel)* 2019, *7*, 64.
107. Ross, R.; Goodpaster, B.H.; Koch, L.G.; Sarzynski, M.A.; Kohrt, W.M.; Johannsen, N.M.; Skinner, J.S.; Castro, A.; Irving, B.A.; Noland, R.C.; et al. Precision exercise medicine: Understanding exercise response variability. *Br. J. Sports Med.* 2019, *53*, 1141–1153.

Chapter 26. Opinion - Secreted Protein Acidic and Rich in Cysteine as an Exercise-Induced Gene: Towards Novel Molecular Therapies for Immobilization-Related Muscle Atrophy in Elderly Patients

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [2], Jonny St-Amand [1,2]

[1] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

[2] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec-Université Laval Research Center, Québec, QC G1V 4G2, Canada

Received: 22 February 2022; Accepted: 1 June 2022; Published: 4 June 2022

26.1 Résumé (French abstract)

De longues périodes d'immobilisation, entre autres étiologies, entraîneraient une atrophie musculaire. L'exercice est la meilleure approche pour inverser cette atrophie. Cependant, la capacité limitée ou l'incapacité d'effectuer l'activité physique requise pour ces patients et les options pharmacologiques limitées rendent nécessaire le développement de nouvelles approches thérapeutiques. Dans ce contexte, la secreted protein acidic and rich in cysteine (*SPARC*) est un gène induit par l'exercice. Alors que la déficience en ce gène conduit à un phénotype qui imite un certain nombre de changements induits par le vieillissement et liés à la sarcopénie, la surexpression de SPARC chez les souris ou son ajout à la culture de cellules musculaire produit des effets similaires à ceux de l'exercice. Par conséquent, cet article vise à fournir des preuves à l'appui de l'utilisation potentielle de *SPARC* / SPARC comme thérapie moléculaire pour l'atrophie musculaire dans le contexte de l'immobilisation, en particulier chez les patients âgés.

26.2 Abstract

Long periods of immobilization, among other etiologies, would result is muscle atrophy. Exercise is the best approach to reverse this atrophy. However, the limited or the non-ability to perform the required physical activity for such patients and the limited pharmacological options make developing novel therapeutic approaches a necessity. Within this context, secreted protein acidic and rich in cysteine (*SPARC*) has been characterized as an exercise-induced gene. Whereas the knock-out of this gene leads to a phenotype that mimics number of the ageing-induced and sarcopenia-related changes including muscle atrophy, overexpressing SPARC in mice or adding it to muscular cell culture produces

similar effects as exercise including enhanced muscle mass, strength and metabolism. Therefore, this piece of writing aims to provide evidence supporting the potential use of *SPARC*/SPARC as a molecular therapy for muscle atrophy in the context of immobilization especially for elderly patients.

Keywords: SPARC; muscle atrophy; immobilization; ageing

26.3 Secreted Protein Acidic and Rich in Cysteine as an Exercise-Induced Gene: Towards Novel Molecular Therapies for Immobilization-Related Muscle Atrophy in Elderly Patients

The increased number of hospitalized individuals lead to the development of various fields aiming to improve and optimize the healthcare within hospitals [1–5]. Patients admitted to hospitals have, beside treating the reasons of their admission, also to face other challenges such as possible nosocomial infections [6], bedsores [7,8] and musculoskeletal atrophy. Furthermore, post-hospitalization recovery of the mobility remains a challenge due to the immobilization (bed rest)-induced muscle atrophy. Such bed resting (immobilization) does not only lead to muscle atrophy, but also reduces both muscle strength as well as key regulators of mitochondrial biogenesis/remodeling and activity; it also alters genes expression and leads to metabolic decline including insulin resistance [9–12]. Bed resting also impacts bones and reduces their mineral density [13]. Cardiovascular complications and cardiac atrophy have also been reported following bed rest [14,15]. The consequences on the locomotor system impact the mass, the strength and the metabolism. Thus, patients, especially elderly people, have a difficulty to return to normal life after a certain period of bed rest caused by hospitalization or immobilization mainly because of muscle atrophy. In addition, ageing reduces both myogenesis [16] and skeletal muscle stem cells regenerative capacity [17]. Ageing also has specific genes expression signature [18,19] and shares numerous patterns with obesity such as epigenetic changes, inflammation and metabolic impairments [20]. These elements show the seriousness of the clinical outcomes of combining immobilization and ageing. The increased hospitalization rate represents one of the features of the current ongoing COVID-19 pandemic especially among the elderly patients who are already vulnerable. Intensive care unit patients (also increased with COVID-19) have more muscle loss especially with long hospitalization periods [21]. Furthermore, the elderly population has a limited physical activity within their lifestyle. Indeed, many of them spend long periods of immobilization due to some diseases or accidents requiring bed rest or hospitalizations. Ageing is another factor which, either independently or combined to immobilization, significantly contributes to the muscle and bone loss. Sarcopenia is an age-related decline in muscles mass and strength [22]. Age related comorbidities such as chronic heart failure [23] and chronic obstructive pulmonary diseases [24] accelerate sarcopenia [25]. Clinically, sarcopenia epidemiological profile is

increasing and enhances mortality [26] especially with the increasing number of elderly people who develop a poor lifestyle (reduced activity, unhealthy diet, etc.).

Muscle atrophy includes protein degradation, mitochondrial dysregulation and inflammation among its key biological features [27–29]. Biological markers suggested for sarcopenia [25,30] would represent significant diagnosis tools for muscle atrophy as well. Both muscle atrophy and bone loss (key tissues of the locomotor system) can be reversed by physical activity [31,32]. Exercise is known for its benefits in respect to muscle function and metabolism including as sarcopenia treatment [33–36]. The effects of exercise, including pre-training, on muscle atrophy and recovery has also been highlighted [37–39]. Indeed, muscle atrophy could be prevented by exercise [40], including a pretraining as suggested by electrical stimulation studies [41,42]. Exercise represents the main treatment approach and electrical stimulation and "cytoprotective" dietary interventions are also used against muscle atrophy [43,44]. Other therapeutic options represent potential approaches such as gene therapy and epigenetic drugs [45–47]. Pharmacological therapies, however, remain limited to some growth factors among which we cite insulin, ghrelin/IGF-1 analogues, testosterone and growth hormone [45,47]. The limitation in therapeutic options is in part due to the limited knowledge on the underlying molecular pathways and physiopathological processes.

To reveal such mechanism and deepen our understating of these immobilizationinduced atrophy, animal models of immobilization-induced muscle atrophy (rats, mice, rabbit) [26,48–50] have been developed. Mice remain the best choice due to their affordable cost, genetic manipulation possibilities and short lifespan; in addition to the ageing process similarities, they share with humans [51–55]. Cast immobilization is the most used because it mimics prolonged immobilization in terms of muscle atrophy [56,57]. The immobilization also induces bone loss in both growing and adult mice [58]. Thus, such immobilization alters the two main parts of the locomotor system, muscles and bones. Bone and muscle mass are reduced with immobilization in which various biological changes such as inflammation, increased muscle RING finger 1 and mRNA contents of polyubiquitin and the ubiquitin ligases muscle atrophy F-box along with reduced rapamycin complex 1 signaling and reducing the myofiber size were reported [49,57,59–61]. Immobilization-induced muscle loss depends on factors such as age and sex. For instance, unilateral hindlimb immobilization in rats of different ages leads to a muscle mass loss inversely proportional to age [61]. The difference between male and female in muscle atrophy depends on whether it is aging-induced or inflammation-based [21]. In addition, hindlimb unloading induced more muscle loss in female rats than in males [62]. This could indicate that females would be more impacted by bed resting. Such age and sex differences suggest the need to adapt the treatment (nature and intensity) based on these two factors as well.

Functional genomics and genes expression patterns can lead to the identification of potential novel therapies for the atrophy resulting from the immobilization including during bed rest. Herein, we focus on the gene secreted protein acidic and rich in cysteine (*SPARC/Sparc*). SPARC is a non-collagenous protein that is abundant in mineralized

tissues [63]. It is expressed in various situations in which tissues renewal and cell remodeling occur (exercise, regeneration, obesity, cancer, inflammation, etc.) [64]. It is also associated with cell turnover, remodeling and tissue repair [65]. Based on this expression pattern, we and others previously suggested using SPARC as a molecular physiological and pathological biomarker [64,66]. SPARC, also known as osteonectin or basement membrane-40 (BM-40) [67], has a calcium and collagen binding property [68]. It is a secreted protein that comprises three distinct structural domains [69] and its biosynthesis is regulated by various growth factors and cytokines [70–72]. As exemplified below, SPARC plays important roles in muscles biology. This gene was initially characterized as induced by exercise [73,74], potentially mediating exercise-induced muscle phenotype changes [75] and as up-regulated during skeletal muscle regeneration [76]. *Sparc* overexpression mimics exercise, including enhancing muscle mass, strength, metabolism as well as ameliorating glycemia [77]. SPARC is expressed both in fetal and neonatal muscle and following muscle damage as well [78]. Adding SPARC to muscle C2C12 (myoblast cell) culture increased myoblasts differentiation in addition to myogenic and mitochondrial proteins expression [79]. Moreover, SPARC plays roles in muscle stiffness maintenance [80], muscle morphological change [81] and promotes muscle progenitor cells myogenic differentiation in vitro [80]. On the other hand, *Sparc* expression [82] and muscle mass [83] decline with ageing. Such age-related decline in SPARC expression would explain why SPARC downregulation using siRNA reduced myogenesis in young rats skeletal muscle progenitor cells (SMPCs) but had little effect in SMPCs from old rats [84] since old rats would already have low SPARC levels. A resistance to SPARC with age is suggested by the fact that exogenous SPARC improved differentiation in young SMPCs, but exogenous SPARC did not affect old SMPCs [84]. This indicate that SPARC would be combined to other therapies which require further investigation especially with the other effects SPARC has on muscles as we detail below.

Furthermore, *Sparc* KO leads to a phenotype that mimics number of the ageinginduced and sarcopenia-related changes including muscle atrophy with a decrease in muscle mass, strength and metabolism [77]. Small interfering RNA (siRNA)-mediated transient suppression of SPARC leads to muscle atrophy [59] and myofibers atrophic changes [80]. Anti-SPARC antibodies reduced C2C12 differentiation and decreased myogenin expression [79,81]. These suggest that the muscle atrophy could have the decline of SPARC expression as one of its key underlying pathways. Thus, SPARC decline would be implicated within both sarcopenia as well as ageing process that impacts muscles as well.

Such similarities between SPARC impacts on muscles (enhanced functional, structural and metabolic properties) and the exercise-induced muscle changes hypothesize that exercise effects are mediated, at least in part, by SPARC. Therefore, increasing *SPARC* expression (gene therapy) or administering SPARC protein would possibly lead to exercise-like effects similarly to those seen in mice overexpressing *Sparc* [77]. This would result in increasing muscles mass, strength and metabolism and counteract the atrophy resulting

from hospitalization (immobilization), ageing, or more importantly hospitalization of elderly patients (combines ageing and immobilization). Indeed, hospitalized patients have long periods of immobilization during which they are not able to perform physical activity. Similarly, elderly individuals usually have a limited ability to perform high amounts of exercise. Therefore, administering SPARC or inducing its expression could be an option to overcome these struggles by generating some of the exercise-induced effects without in fact performing exercise. As muscle atrophy is among the most important health problems for these patients (immobilized and/or aged), SPARC comes as a potential therapy as its specific impacts on muscles are well documents. Importantly, the literature also shows the divers beneficial properties and implications of SPARC including metabolic properties [85,86], anticancer [87], anti-inflammatory [88], collagen regulation in the heart [89], tissue repair and regeneration [90,91]. These SPARC properties allowed us to classify it as a regeneration factor [90] that would create a biological environment with optimum conditions for regeneration, muscle differentiation and growth properties.

The importance of SPARC in bones increases the potential of SPARC in managing the bed rest-induced atrophy since immobilization also leads to bone loss. Indeed, SPARC is important for bone formation, remodeling and regeneration [90]. *Sparc* KO mice develop osteopenia [92], decreased bone formation [93]. SPARC deficiency also affects bone marrow stromal function [94]. In addition, SPARC also plays roles in bone remodeling [95] and osteoblast maturation [67]. It also regulates hydroxyapatite crystals formation and growth [96] and influence osteogenic differentiation [97]. Furthermore, the implication of SPARC in other locomotor system constituents (such as ligaments [98,99] and tendons [100,101]) would make that treating with SPARC would not only improve muscle phenotype but could also have positive effects on the whole locomotion system. Therefore, SPARC administration might contribute to the maintenance of the musculoskeletal system responsible for the individual mobility during hospitalization and recovery periods. It is worth highlighting that increased SPARC expression has been reported in negative biological status such as metabolic disorders [102], rheumatoid arthritis [70], cancer [103], coronary artery disease [104] and intracranial aneurysms [105]. We have hypothesized that such expression would not indicate the involvement of SPARC in the pathogenesis or prognosis but rather represents an attempt to counteract the effects generated by such pathologies or disorders via the beneficial SPARC-mediated effects. Examples of SPARC counteracting inflammation [88] and cancer [87,106] would be two illustrations of such “regulatory feedback”.

Such approach can also be extended to those chronically bedridden, with physical disability or even space missions (microgravity environment) [107] as summarized in Figure 1. Evidence suggests that *Sparc* decline contributes to the muscle atrophy, ageing and the resulting phenotypes, whereas its overexpression induced by exercise would be a mechanism via which exercise corrects and improves muscle atrophy and ageing. Therefore, we suggested measuring exercise-induced SPARC/*SPARC*/*Sparc* expression as a molecular tool to optimize exercise therapy towards a personalized medicine [108] and

also using SPARC as a potential "exercise substitute" [109]. Such measure could be applied to immobilized patients during a potential pre-training session aiming to counteract muscle atrophy. We believe that further animal and clinical studies could lead to a new generation of molecular therapies for muscle atrophy based on *SPARC* and permit the overcoming of this challenging atrophy resulting from hospitalization, immobility and ageing. The best option, when available, is to rather focus on exercise-induced SPARC as a possible treatment and we emphasize that further studies are needed to further map the mechanistic links between exercise, the exercise-induced myokines (including SPARC) and the exercise- induced effects.

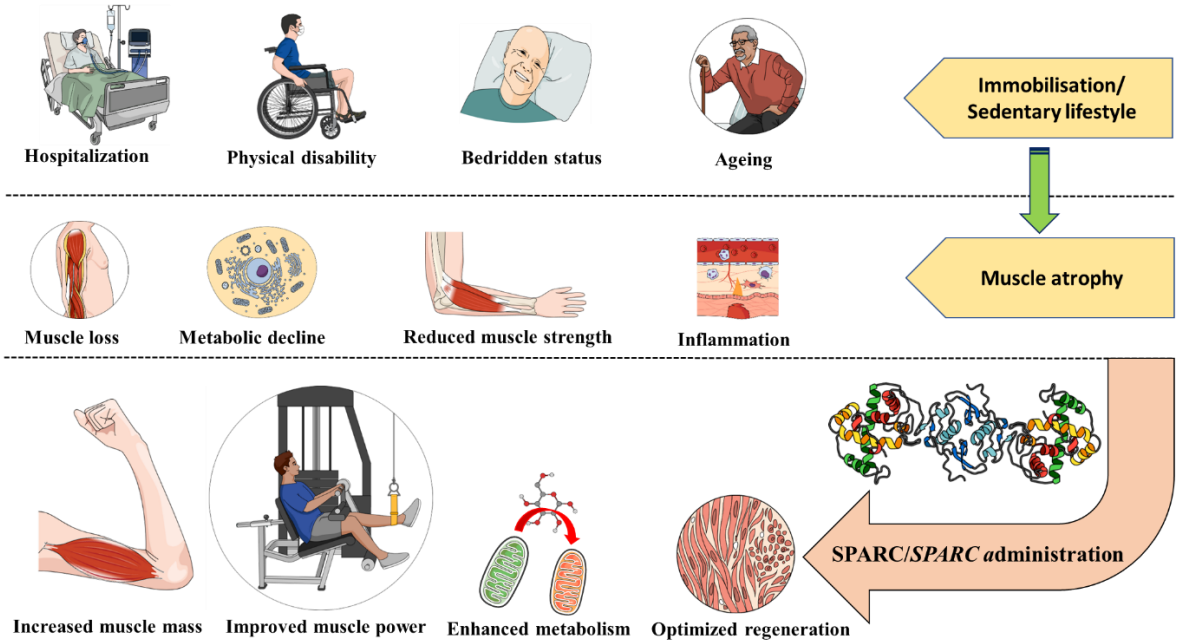


Figure 26.1. Secreted Protein Acidic and Rich in Cysteine (SPARC/*SPARC*) as a muscle atrophy therapy. Situations such as hospitalization, physical disability or being bedridden represent an immobilization that might lead to muscle atrophy. Ageing (usually accompanied with a sedentary lifestyle) is another risk factor for the muscle atrophy. SPARC properties of enhancing muscles mass, strength and metabolism are towards counteracting muscle atrophy and highlight SPARC/*SPARC* (protein administration or gene therapy) as a molecular therapy for muscle atrophy.

Author Contributions: A.G. designed the manuscript structure and wrote it. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, the figures, references selection, etc.) throughout the writing process, edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship « Bourse Tremplin -Stage en milieu de pratique » (Internship scholarship) from the Fonds de recherche du Québec-Sante (FRQS), Quebec, Canada. Figure 1 was created using images from https://mindthegraph.com/ (accessed on 17 February 2022).

Conflicts of Interest: The authors declare no conflict of interest.

26.4 References

1. Wong, H.J.; Morra, D. Excellent hospital care for all: Open and operating 24/7. *J. Gen. Intern. Med.* 2011, 26, 1050–1052.
2. Lopez, M.A.; Hall, M.; Auger, K.A.; Bettenhausen, J.L.; Colvin, J.D.; Cutler, G.J.; Fieldston, E.; Macy, M.L.; Morse, R.; Raphael, J.L.; et al. Care of Pediatric High-Cost Hospitalizations Across Hospital Types. *Hosp. Pediatr.* 2020, *10*, 206–213.
3. Copnell, B.; Hagger, V.; Wilson, S.G.; Evans, S.M.; Sprivulis, P.C.; Cameron, P.A. Measuring the quality of hospital care: An inventory of indicators. *Intern. Med. J.* 2009, *39*, 352–360.
4. Buttigieg, S.C.; Abela, L.; Pace, A. Variables affecting hospital length of stay: A scoping review. *J. Health Organ. Manag.* 2018, *32*, 463–493.
5. Hua, M.; Lu, Y.; Ma, X.; Morrison, R.S.; Li, G.; Wunsch, H. Association Between the Implementation of Hospital-Based Palliative Care and Use of Intensive Care During Terminal Hospitalizations. *JAMA Netw. Open* 2020, *3*, e1918675.
6. Liu, J.Y.; Dickter, J.K. Nosocomial Infections: A History of Hospital-Acquired Infections. *Gastrointest. Endosc. N. Am.* 2020, *30*, 637–652.
7. Alwasel, A.; Alossimi, B.; Alsadun, M.; Alhussaini, K. Bedsores Management: Efficiency Simulation of a New Mattress Design. *Healthcare* 2021, *9*, 1701.
8. Bluestein, D.; Javaheri, A. Pressure ulcers: Prevention, evaluation, and management. *Am. Fam. Physician* 2008, *78*, 1186–1194.
9. Buso, A.; Comelli, M.; Picco, R.; Isola, M.; Magnesa, B.; Pišot, R.; Rittweger, J.; Salvadego, D.; Šimuniˇc, B.; Grassi, B.; et al. Mitochondrial Adaptations in Elderly and Young Men Skeletal Muscle Following 2 Weeks of Bed Rest and Rehabilitation. *Front. Physiol.* 2019, *10*, 474.
10. LeBlanc, A.; Gogia, P.; Schneider, V.; Krebs, J.; Schonfeld, E.; Evans, H. Calf muscle area and strength changes after five weeks of horizontal bed rest. *Am. J. Sports Med.* 1988, *16*, 624–629.
11. Mahmassani, Z.S.; Reidy, P.T.; McKenzie, A.I.; Stubben, C.; Howard, M.T.; Drummond, M.J. Age-dependent skeletal muscle transcriptome response to bed rest-induced atrophy. *J. Appl. Physiol.* 2019, *126*, 894–902.

12. Dirks, M.L.; Smeets, J.S.J.; Holwerda, A.M.; Kouw, I.W.K.; Marzuca-Nassr, G.N.; Gijsen, A.P.; Holloway, G.P.; Verdijk, L.B.; van Loon, L.J.C. Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest. *Am. J. Physiol. Endocrinol. Metab.* 2019, *316*, E536–E545.
13. Bloomfield, S.A. Changes in musculoskeletal structure and function with prolonged bed rest. *Med. Sci. Sports Exerc.* 1997, *29*, 197–206.
14. Perhonen, M.A.; Franco, F.; Lane, L.D.; Buckey, J.C.; Blomqvist, C.G.; Zerwekh, J.E.; Peshock, R.M.; Weatherall, P.T.; Levine, B.D. Cardiac atrophy after bed rest and spaceflight. *J. Appl. Physiol.* 2001, *91*, 645–653.
15. Dittmer, D.K.; Teasell, R. Complications of immobilization and bed rest. Part 1: Musculoskeletal and cardiovascular complications. *Can. Fam. Physician* 1993, *39*, 1428–1432, 1435–1437.
16. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. *Aging Cell* 2014, *13*, 175–184.
17. Sousa-Victor, P.; Muñoz-Cánoves, P. Regenerative decline of stem cells in sarcopenia. *Mol. Asp. Med.* 2016, *50*, 109–117.
18. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
19. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res. Rev. Mutat. Res.* 2019, *781*, 175–185.
20. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. *Diseases* 2021, *9*, 87.
21. Rosa-Caldwell, M.E.; Greene, N.P. Muscle metabolism and atrophy: Let's talk about sex. *Biol. Sex Differ.* 2019, *10*, 43.
22. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Transdisciplinary Approach for Sarcopenia. Molecular mechanism of sarcopenia: The role of skeletal muscle niche component SPARC in the regulation of myogenesis and adipogenesis and its alteration with age. *Clin. Calcium.* 2014, *24*, 1471–1478.
23. Li, H.; Hastings, M.H.; Rhee, J.; Trager, L.E.; Roh, J.D.; Rosenzweig, A. Targeting Age-Related Pathways in Heart Failure. *Circ. Res.* 2020, *126*, 533–551.
24. Easter, M.; Bollenbecker, S.; Barnes, J.W.; Krick, S. Targeting Aging Pathways in Chronic Obstructive Pulmonary Disease. *Int. J. Mol. Sci.* 2020, *21*, 6924.
25. Qaisar, R.; Karim, A.; Muhammad, T.; Shah, I.; Khan, J. Prediction of sarcopenia using a battery of circulating biomarkers. *Sci. Rep.* 2021, *11*, 8632.
26. Baek, K.W.; Jung, Y.K.; Kim, J.S.; Park, J.S.; Hah, Y.S.; Kim, S.J.; Yoo, J.I. Rodent Model of Muscular Atrophy for Sarcopenia Study. *J. Bone Metab.* 2020, *27*, 97–110.
27. Bonaldo, P.; Sandri, M. Cellular and molecular mechanisms of muscle atrophy. *Dis. Mech.* 2013, *6*, 25–39.
28. Ji, L.L.; Yeo, D. Mitochondrial dysregulation and muscle disuse atrophy. *F1000Res* 2019, *8*, F1000.
29. Tuttle, C.S.L.; Thang, L.A.N.; Maier, A.B. Markers of inflammation and their association with muscle strength and mass: A systematic review and meta-analysis. *Ageing Res. Rev.* 2020, *64*, 101185.
30. Kwak, J.Y.; Hwang, H.; Kim, S.K.; Choi, J.Y.; Lee, S.M.; Bang, H.; Kwon, E.S.; Lee, K.P.; Chung, S.G.; Kwon, K.S. Prediction of sarcopenia using a combination of multiple serum biomarkers. *Sci. Rep.* 2018, *8*, 8574.
31. Miokovic, T.; Armbrecht, G.; Gast, U.; Rawer, R.; Roth, H.J.; Runge, M.; Felsenberg, D.; Belavý, D.L. Muscle atrophy, pain, and damage in bed rest reduced by resistive (vibration) exercise. *Med. Sci. Sports Exerc.* 2014, *46*, 1506–1516.
32. Leblanc, A.D.; Schneider, V.S.; Evans, H.J.; Engelbretson, D.A.; Krebs, J.M. Bone mineral loss and recovery after 17 weeks of bed rest. *J. Bone Min. Res.* 1990, *5*, 843–850.
33. Landi, F.; Marzetti, E.; Martone, A.M.; Bernabei, R.; Onder, G. Exercise as a remedy for sarcopenia. *Curr. Opin. Clin. Nutr. Metab. Care* 2014, *17*, 25–31.

34. Spaulding, H.R.; Selsby, J.T. Is Exercise the Right Medicine for Dystrophic Muscle? *Med. Sci. Sports Exerc.* 2018, *50*, 1723–1732.
35. Phu, S.; Boersma, D.; Duque, G. Exercise and Sarcopenia. *J. Clin. Densitom.* 2015, *18*, 488–492.
36. Marzetti, E.; Calvani, R.; Tosato, M.; Cesari, M.; Di Bari, M.; Cherubini, A.; Broccatelli, M.; Savera, G.; D'Elia, M.; Pahor, M.; et al. Physical activity and exercise as countermeasures to physical frailty and sarcopenia. *Aging Clin. Exp. Res.* 2017, *29*, 35–42.
37. He, N.; Ye, H. Exercise and Muscle Atrophy. *Adv. Exp. Med. Biol.* 2020, *1228*, 255–267.
38. Theilen, N.T.; Kunkel, G.H.; Tyagi, S.C. The Role of Exercise and TFAM in Preventing Skeletal Muscle Atrophy. *J. Cell Physiol.* 2017, *232*, 2348–2358.
39. Salles, J.I.; Guimarães, J.M.; Filho, G.M.; Morrissey, D. Effect of a specific exercise strategy on strength and proprioception in volleyball players with infraspinatus muscle atrophy. *Scand. J. Med. Sci. Sports* 2018, *28*, 2093–2099.
40. Czerwinski, S.M.; Kurowski, T.G.; O'Neill, T.M.; Hickson, R.C. Initiating regular exercise protects against muscle atrophy from glucocorticoids. *J. Appl. Physiol.* 1987, *63*, 1504–1510.
41. Baldi, J.C.; Jackson, R.D.; Moraille, R.; Mysiw, W.J. Muscle atrophy is prevented in patients with acute spinal cord injury using functional electrical stimulation. *Spinal Cord* 1998, *36*, 463–469.
42. Lake, D.A. Neuromuscular electrical stimulation. An overview and its application in the treatment of sports injuries. *Sports Med.* 1992, *13*, 320–336.
43. Granic, A.; Sayer, A.A.; Robinson, S.M. Dietary Patterns, Skeletal Muscle Health, and Sarcopenia in Older Adults. *Nutrients* 2019, *11*, 745.
44. Anton, S.D.; Hida, A.; Mankowski, R.; Layne, A.; Solberg, L.M.; Mainous, A.G.; Buford, T. Nutrition and Exercise in Sarcopenia. *Curr. Protein. Pept. Sci.* 2018, *19*, 649–667.
45. Urso, M.L. Disuse atrophy of human skeletal muscle: Cell signaling and potential interventions. *Med. Sci. Sports Exerc.* 2009, *41*, 1860–1868.
46. Guasconi, V.; Puri, P.L. Epigenetic drugs in the treatment of skeletal muscle atrophy. *Curr. Opin. Clin. Nutr. Metab. Care* 2008, *11*, 233–241.
47. Ding, S.; Dai, Q.; Huang, H.; Xu, Y.; Zhong, C. An Overview of Muscle Atrophy. *Adv. Exp. Med. Biol.* 2018, *1088*, 3–19.
48. Son, J.S.; Kim, J.H.; Kim, H.J.; Yoon, D.H.; Kim, J.S.; Song, H.S.; Song, W. Effect of resistance ladder training on sparc expression in skeletal muscle of hindlimb immobilized rats. *Muscle Nerve* 2016, *53*, 951–957.
49. Caron, A.Z.; Drouin, G.; Desrosiers, J.; Trensz, F.; Grenier, G. A novel hindlimb immobilization procedure for studying skeletal muscle atrophy and recovery in mouse. *J. Appl. Physiol.* 2009, *106*, 2049–2059.
50. Herbert, R.D.; Balnave, R.J. The effect of position of immobilisation on resting length, resting stiffness, and weight of the soleus muscle of the rabbit. *J. Orthop. Res.* 1993, *11*, 358–366.
51. Yuan, R.; Peters, L.L.; Paigen, B. Mice as a mammalian model for research on the genetics of aging. *Ilar. J.* 2011, *52*, 4–15.
52. Yuan, R.; Tsaih, S.W.; Petkova, S.B.; Marin de Evsikova, C.; Xing, S.; Marion, M.A.; Bogue, M.A.; Mills, K.D.; Peters, L.L.; Bult, C.J.; et al. Aging in inbred strains of mice: Study design and interim report on median lifespans and circulating IGF1 levels. *Aging Cell* 2009, *8*, 277–287.
53. Barreto, G.; Huang, T.T.; Giffard, R.G. Age-related defects in sensorimotor activity, spatial learning, and memory in C57BL/6 mice. *J. Neurosurg. Anesth.* 2010, *22*, 214–219.
54. Graber, T.G.; Ferguson-Stegall, L.; Kim, J.H.; Thompson, L.V. C57BL/6 neuromuscular healthspan scoring system. *J. Gerontol. A Biol. Sci. Med. Sci.* 2013, *68*, 1326–1336.
55. Parks, R.J.; Fares, E.; Macdonald, J.K.; Ernst, M.C.; Sinal, C.J.; Rockwood, K.; Howlett, S.E. A procedure for creating a frailty index based on deficit accumulation in aging mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 2012, *67*, 217–227.
56. Palus, S.; Springer, J.I.; Doehner, W.; von Haehling, S.; Anker, M.; Anker, S.D.; Springer, J. Models of

sarcopenia: Short review. *Int. J. Cardiol.* 2017, *238*, 19–21.
57. St-Amand, J.; Okamura, K.; Matsumoto, K.; Shimizu, S.; Sogawa, Y. Characterization of control and immobilized skeletal muscle: An overview from genetic engineering. *Faseb J.* 2001, *15*, 684–692.
58. Friedman, M.A.; Zhang, Y.; Wayne, J.S.; Farber, C.R.; Donahue, H.J. Single limb immobilization model for bone loss from unloading. *J. Biomech.* 2019, *83*, 181–189.
59. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Nishihara, M. Loss of SPARC in mouse skeletal muscle causes myofiber atrophy. *Muscle Nerve* 2013, *48*, 791–799.
60. Krawiec, B.J.; Frost, R.A.; Vary, T.C.; Jefferson, L.S.; Lang, C.H. Hindlimb casting decreases muscle mass in part by proteasomedependent proteolysis but independent of protein synthesis. *Am. J. Physiol. Endocrinol. Metab.* 2005, *289*, E969–E980.
61. Kelleher, A.R.; Pereira, S.L.; Jefferson, L.S.; Kimball, S.R. REDD2 expression in rat skeletal muscle correlates with nutrient-induced activation of mTORC1: Responses to aging, immobilization, and remobilization. *Am. J. Physiol. Endocrinol. Metab.* 2015, *308*, E122–E129.
62. Yoshihara, T.; Natsume, T.; Tsuzuki, T.; Chang, S.W.; Kakigi, R.; Sugiura, T.; Naito, H. Sex differences in forkhead box O3a signaling response to hindlimb unloading in rat soleus muscle. *J. Physiol. Sci.* 2019, *69*, 235–244.
63. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. *Matrix Biol.* 2016, *52–54*, 78–87.
64. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. *Biomolecules* 2021, *11*, 1689.
65. Yan, Q.; Sage, E.H. SPARC, a matricellular glycoprotein with important biological functions. *J. Histochem. Cytochem.* 1999, *47*, 1495–1506.
66. Kao, S.C.; Kirschner, M.B.; Cooper, W.A.; Tran, T.; Burgers, S.; Wright, C.; Korse, T.; van den Broek, D.; Edelman, J.; Vallely, M.; et al. A proteomics-based approach identifies secreted protein acidic and rich in cysteine as a prognostic biomarker in malignant pleural mesothelioma. *Br. J. Cancer* 2016, *114*, 524–531.
67. Delany, A.M.; Kalajzic, I.; Bradshaw, A.D.; Sage, E.H.; Canalis, E. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. *Endocrinology* 2003, *144*, 2588–2596.
68. Sage, H.; Johnson, C.; Bornstein, P. Characterization of a novel serum albumin-binding glycoprotein secreted by endothelial cells in culture. *J. Biol. Chem.* 1984, *259*, 3993–4007.
69. Scavelli, K.; Chatterjee, A.; Rhee, D.J. Secreted Protein Acidic and Rich in Cysteine in Ocular Tissue. *J. Ocul. Pharm.* 2015, *31*, 396–405.
70. Nakamura, S.; Kamihagi, K.; Satakeda, H.; Katayama, M.; Pan, H.; Okamoto, H.; Noshiro, M.; Takahashi, K.; Yoshihara, Y.; Shimmei, M.; et al. Enhancement of SPARC (osteonectin) synthesis in arthritic cartilage. Increased levels in synovial fluids from patients with rheumatoid arthritis and regulation by growth factors and cytokines in chondrocyte cultures. *Arthritis Rheum.* 1996, *39*, 539–551.
71. Chandrasekhar, S.; Harvey, A.K.; Johnson, M.G.; Becker, G.W. Osteonectin/SPARC is a product of articular chondrocytes/cartilage and is regulated by cytokines and growth factors. *Biochim. Biophys. Acta* 1994, *1221*, 7–14.
72. Fujita, T.; Shiba, H.; Van Dyke, T.E.; Kurihara, H. Differential effects of growth factors and cytokines on the synthesis of SPARC DNA fibronectin and alkaline phosphatase activity in human periodontal ligament cells. *Cell Biol. Int.* 2004, *28*, 281–286.
73. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
74. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.
75. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.

76. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. *Histol. Histopathol.* 2013, *28*, 1451–1460.
77. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Leads to an Accelerated Ageing Phenotype Which Is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice. *Metabolites* 2022, *12*, 125.
78. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schrøder, H.D. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. *J. Histochem. Cytochem.* 2009, *57*, 29–39.
79. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
80. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. *J. Vet. Med. Sci.* 2019, *81*, 1649–1654.
81. Cho, W.J.; Kim, E.J.; Lee, S.J.; Kim, H.D.; Shin, H.J.; Lim, W.K. Involvement of SPARC in in vitro differentiation of skeletal myoblasts. *Biochem. Biophys. Res. Commun.* 2000, *271*, 630–634.
82. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
83. Balachandran, A.; Krawczyk, S.N.; Potiaumpai, M.; Signorile, J.F. High-speed circuit training vs hypertrophy training to improve physical function in sarcopenic obese adults: A randomized controlled trial. *Exp. Gerontol.* 2014, *60*, 64–71.
84. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Matsuwaki, T.; Nishihara, M. Age-related resistance of skeletal musclederived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. *Aging* 2012, *4*, 40–48.
85. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
86. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
87. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
88. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
89. Harris, B.S.; Zhang, Y.; Card, L.; Rivera, L.B.; Brekken, R.A.; Bradshaw, A.D. SPARC regulates collagen interaction with cardiac fibroblast cell surfaces. *Am. J. Physiol. Heart Circ. Physiol.* 2011, *301*, H841–H847.
90. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
91. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Exercise, Diet and Sleeping as Regenerative Medicine Adjuvants: Obesity and Ageing as Illustrations. *Medicines* 2022, *9*, 7.
92. Mansergh, F.C.; Wells, T.; Elford, C.; Evans, S.L.; Perry, M.J.; Evans, M.J.; Evans, B.A. Osteopenia in Sparc (osteonectin)-deficient mice: Characterization of phenotypic determinants of femoral strength and changes in gene expression. *Physiol. Genom.* 2007, *32*, 64–73.
93. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectindeficient mice. *J. Clin. Investig.* 2000, *105*, 915–923.
94. Luo, Z.; Zhou, Y.; Luo, P.; Zhao, Q.; Xiao, N.; Yu, Y.; Yan, Q.; Lu, G.; Cheng, L. SPARC deficiency affects bone marrow stromal function, resulting in impaired B lymphopoiesis. *J. Leukoc. Biol.* 2014, *96*, 73–

82.
95. Ribeiro, N.; Sousa, S.R.; Brekken, R.A.; Monteiro, F.J. Role of SPARC in bone remodeling and cancer-related bone metastasis. *J. Cell Biochem.* 2014, *115*, 17–26.
96. Sodek, J.; Zhu, B.; Huynh, M.H.; Brown, T.J.; Ringuette, M. Novel functions of the matricellular proteins osteopontin and osteonectin/SPARC. *Connect. Tissue Res.* 2002, *43*, 308–319.
97. Rowe, D.W. Chapter 61—Osteogenesis imperfecta. In *Principles of Bone Biology*, 4th ed.; Bilezikian, J.P., Martin, T.J., Clemens, T.L., Rosen, C.J., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 1489–1505.
98. Trombetta, J.M.; Bradshaw, A.D. SPARC/osteonectin functions to maintain homeostasis of the collagenous extracellular matrix in the periodontal ligament. *J. Histochem. Cytochem.* 2010, *58*, 871–879.
99. Rosset, E.M.; Trombetta-eSilva, J.; Hepfer, G.; Yao, H.; Bradshaw, A.D. SPARC and the N-propeptide of collagen I influence fibroblast proliferation and collagen assembly in the periodontal ligament. *PLoS ONE* 2017, *12*, e0173209.
100. Gehwolf, R.; Wagner, A.; Lehner, C.; Bradshaw, A.D.; Scharler, C.; Niestrawska, J.A.; Holzapfel, G.A.; Bauer, H.C.; Tempfer, H.; Traweger, A. Pleiotropic roles of the matricellular protein Sparc in tendon maturation and ageing. *Sci. Rep.* 2016, *6*, 32635.
101. Wang, T.; Wagner, A.; Gehwolf, R.; Yan, W.; Passini, F.S.; Thien, C.; Weissenbacher, N.; Lin, Z.; Lehner, C.; Teng, H.; et al. Load-induced regulation of tendon homeostasis by SPARC, a genetic predisposition factor for tendon and ligament injuries. *Sci. Transl. Med.* 2021, *13*, eabe5738.
102. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. *Nat. Rev. Endocrinol.* 2010, *6*, 225–235.
103. Chang, C.-H.; Yen, M.-C.; Liao, S.-H.; Hsu, Y.-L.; Lai, C.-S.; Chang, K.-P.; Hsu, Y.-L. Secreted Protein Acidic and Rich in Cysteine (SPARC) Enhances Cell Proliferation, Migration, and Epithelial Mesenchymal Transition, and SPARC Expression is Associated with Tumor Grade in Head and Neck Cancer. *Int. J. Mol. Sci.* 2017, *18*, 1556.
104. Takahashi, M.; Nagaretani, H.; Funahashi, T.; Nishizawa, H.; Maeda, N.; Kishida, K.; Kuriyama, H.; Shimomura, I.; Maeda, K.; Hotta, K.; et al. The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease. *Obes. Res.* 2001, *9*, 388–393.
105. Tan, X.; Li, T.; Zhu, S.; Zhong, W.; Li, F.; Wang, Y. Induction of SPARC on Oxidative Stress, Inflammatory Phenotype Transformation, and Apoptosis of Human Brain Smooth Muscle Cells Via TGF-β1-NOX4 Pathway. *J. Mol. Neurosci.* 2020, *70*, 1728–1741.
106. Ma, J.; Gao, S.; Xie, X.; Sun, E.; Zhang, M.; Zhou, Q.; Lu, C. SPARC inhibits breast cancer bone metastasis and may be a clinical therapeutic target. *Oncol. Lett.* 2017, *14*, 5876–5882.
107. Droppert, P.M. A review of muscle atrophy in microgravity and during prolonged bed rest. *J. Br. Interplanet. Soc.* 1993, *46*, 83–86.
108. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. *Genes* 2021, *12*, 1832.
109. Ghanemi A, Yoshioka M, St-Amand J: Genetic Expression between Ageing and Exercise: Secreted Protein Acidic and Rich in Cysteine as a Potential "Exercise Substitute" Antiageing Therapy. *Genes* 2022, *13*, 950.

Chapter 27. Opinion - Genetic Expression between Ageing and Exercise: Secreted Protein Acidic and Rich in Cysteine as a Potential "Exercise Substitute" Antiageing Therapy

Abdelaziz Ghanemi [1,2], Mayumi Yoshioka [1], Jonny St-Amand [1,2]

[1] Functional Genomics Laboratory, Endocrinology and Nephrology Axis, CHU de Québec Université Laval Research Center, Québec, QC G1V 4G2, Canada

[2] Department of Molecular Medicine, Faculty of Medicine, Laval University, Québec, Québec G1V 0A6, Canada

Received: 22 April 2022; Accepted: 24 May 2022; Published: 26 May 2022

27.1 Résumé (French abstract)

Le vieillissement représente un facteur de risque pour plusieurs maladies. La gériatrie moderne prescrit l'exercice pour contrer les effets du vieillissement. Cet article présente la secreted protein acidic and rich in cysteine (SPARC) comme un potentiel traitement anti-âge. Nous passons du fait que SPARC diminue avec le vieillissement et que l'exercice induit l'expression du SPARC à l'hypothèse que SPARC est un facteur induit par l'exercice qui induit - au moins une partie - de ses effets anti-âge induits par l'exercice car surexprimer SPARC imite l'exercice. Ceci est d'une importance particulière car le vieillissement et les maladies liées à l'âge pourraient réduire la capacité d'effectuer l'activité physique requise. Les possibilités d'imiter les avantages de l'exercice via SPARC ne se limitent pas au vieillissement et peuvent être appliquées dans divers contextes dans lesquels l'exercice ne peut pas être effectué en raison d'handicaps physiques, de troubles de santé ou d'une mobilité limitée.

27.2 Abstract

Ageing is the effect of time on biological entities. It represents a risk factor for a variety of diseases and health disorders; thus, therapeutic options are required to tackle ageing issues. Modern geriatric medicine prescribes exercise to counteract ageing effects. This work presents secreted protein acidic and rich in cysteine (SPARC) as a potential antiageing therapy. Indeed, SPARC declines with ageing, exercise induces SPARC, and SPARC overexpression in mice mimics exercise. Thus, we hypothesize that SPARC is an exercise-induced factor that is beyond—at least part of—the antiageing effects induced by exercise. This could become a potential antiageing therapy for the elderly that counteracts

ageing by mimicking the effects of exercise without needing to perform exercise. This is of particular importance because ageing usually reduces mobility and age-related diseases can reduce the ability to perform the required physical activity. On the other hand, the possibilities of mimicking exercise benefits via SPARC are not limited to ageing, and can be applied in various contexts in which exercise cannot be performed because of physical disabilities, health disorders, or limited mobility.

Keywords: Secreted Protein Acidic and Rich in Cysteine; ageing; exercise, antiageing

27.3 Genetic Expression between Ageing and Exercise: Secreted Protein Acidic and Rich in Cysteine as a Potential "Exercise Substitute" Antiageing Therapy

Ageing is defined as the biological decline of diverse functions and processes within cells, tissues, and organisms over time [1,2]. Biological ageing can also be defined as the cellular and tissue changes that develop through one's lifespan. These changes include metabolic decline [3], skeletal muscle mass loss [4], adipose tissue dysfunction [5,6], cognitive decline [7], and immunosenescence [8]. Ageing involves molecular and cellular changes such as epigenetic modifications, inflammation, and impaired regeneration [2]. Delaying ageing has been the focus of humans for a long time, with ancient philosophers/civilizations describing the fountain of youth [9]. Millennia later, the development of healthcare systems has led to ageing societies [10]. Ageing is an important risk factor for various diseases and health problems. Thus, biomedical research is focused on how to tackle ageing and diverse studies have pointed out factors that could contribute to either slowing down ageing or accelerating it. Both exercise [11,12] and calorie restriction [13] are among the most well-known approaches to counteracting the effects of ageing. More specifically, the diverse benefits of exercise [14–19] are the reason it is prescribed to the elderly in order to counteract/limit the metabolic and functional decline associated with ageing. Therefore, we suggest the existence of molecular patterns shared between ageing and exercise, as two physiological changes, that can explain the antiageing effect of exercise. Understanding the mechanistic links between exercise and antiageing effects at molecular and cellular levels will allow us to deepen our knowledge towards developing and optimizing antiageing therapies.

Thus, there are potentially molecular pathways beyond the antiageing effect of exercise. Within this context, here, we specifically focus on secreted protein acidic and rich in cysteine (SPARC). *SPARC/Sparc* has been identified as a gene with an expression level that changes with both exercise and ageing. Interestingly, these changes take place in opposite directions. Indeed, while exercise (as well as the in vivo model of exercise) increases the *SPARC*/*Sparc* expression [20–22], this gene expression decreases with ageing [21]. Such expression patterns indicate that SPARC represents a key molecular pathway in both exercise and ageing, and explain, at least in part, both the ageing process and the

antiageing effects of exercise. In addition, the effects of exercise on skeletal muscle counteract those of ageing [23]. Not only has *SPARC* been characterized as an exercise induced gene, but *Sparc* KO in mice or SPARC inhibition in the cell culture leads to an ageing-like phenotype [24,25]. Moreover, SPARC overexpression mimics exercise-induced changes [24]. Therefore, it seems that a decrease in *SPARC* expression might contribute to the ageing process, while an increase in *SPARC* expression could be involved in the exercise-induced changes. Although more evidence is still required, we focused on SPARC because we have shown that it is extremely upregulated by exercise (aerobic exercise rather than resistance training [26,27]) compared with other exercise-induced genes [28], in addition to being downregulated with ageing [21]. The measure of SPARC/*SPARC*/*Sparc* expression has also been suggested as a molecular physiological and pathological biomarker [29], as well as a molecular tool to optimize personalized medicine based on exercise prescription [30].

We previously suggested that the antiageing effect of exercise might be mediated by the exercise-induced increase in SPARC expression, which reverses/counteracts the ageing-associated decline in SPARC/*Sparc* expression [24]. This is supported by a study suggesting that exercise-induced muscle phenotype changes are SPARC-dependent [31]. The association between the ageing phenotype and SPARC decline is further supported by the fact that animal models of *Sparc* KO exhibit ageing-like phenotypes, including accelerated degeneration [32,33], osteopenia [34], early onset of cataractogenesis [35,36], lack of immune response to lipopolysaccharides [37], and decreased bone formation [38]. Furthermore, the involvement of SPARC in exercise-induced antiageing effects is confirmed by SPARC overexpression in mice [24] or the addition of SPARC to the muscle cell cultures [25], which also mimics exercise in terms of metabolism and muscle properties. Therefore, SPARC expression levels could be an indicator of whether the phenotype would be for ageing (low SPARC expression) or rather an exercise-induced (antiageing) phenotype (high SPARC expression).

On the one hand, the similarities between *SPARC* properties and exercise-induced effects and the *SPARC*-induced effects indicate that *SPARC* acts towards counteracting ageing; on the other hand, they represent elements that present *SPARC* as a molecule that can both mimic exercise and counteract ageing. Indeed, SPARC has been shown to have diverse properties, such as anti-inflammatory [39], anticancer [40], and regenerative properties [41]. SPARC is also involved in metabolism [42,43] and obesity [44], among others, all of which are properties that would be beneficial against ageing. Thus, SPARC would be a selective target towards a potential antiageing therapy. This could be achieved either by injecting SPARC; inducing *SPARC* expression (gene therapy); or, as a more specific therapy, stimulating selected SPARC-induced pathways. Such an approach would generate antiageing effects, including those induced by exercise (Figure 1). The result would be an antiageing therapy for the elderly that counteracts ageing by mimicking the effects of exercise without the need to do exercise. This is of particular importance, because

ageing usually reduces mobility and age-related diseases could also reduce the ability to perform the required physical activity.

These *SPARC*-related properties illustrate how genetics might contribute to developing and optimizing antiageing therapies. Functional genomics studies the changes in gene expression under various conditions, including diet [45,46], ageing [47,48], and exercise [49]. The aim of our hypothesis, presented herein, is to target gene(s) that are both overexpressed during exercise and at the same time downregulated with ageing. This expression pattern suggests that such gene(s) are involved in both ageing and exercise (antiageing).

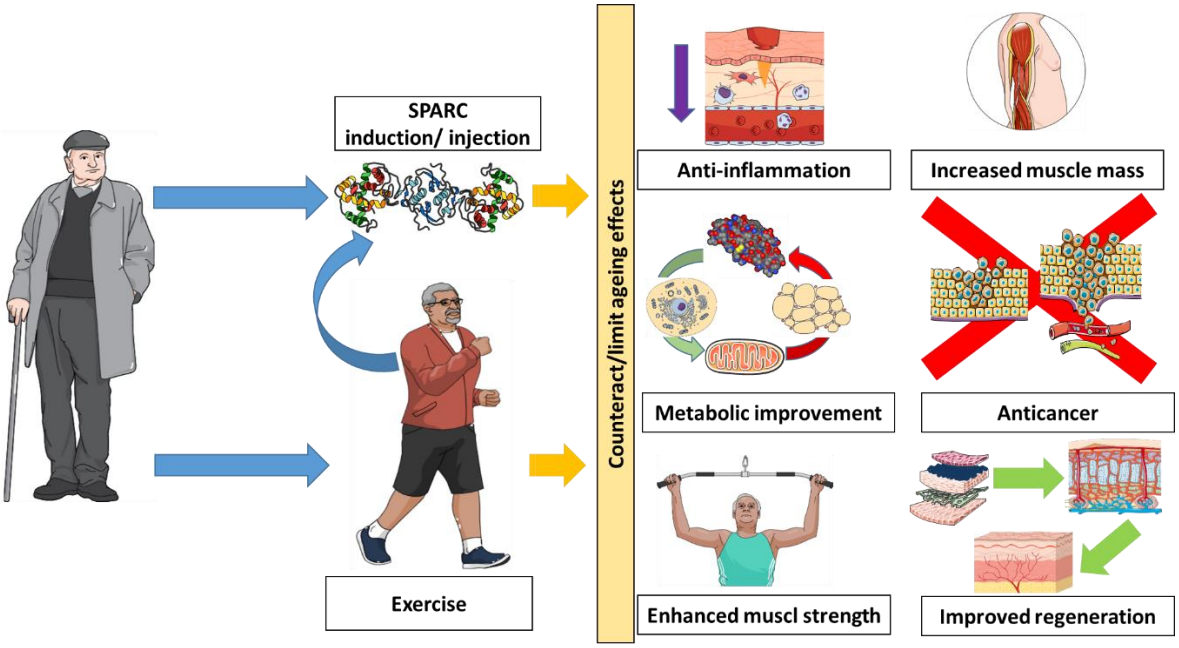


Figure 27.1. Secreted protein acidic and rich in cysteine (SPARC) as a potential antiageing "exercise substitute". SPARC (which is induced by exercise) represents a potential therapy that can mimic exercise and produce antiageing effects. This is of particular importance because ageing usually reduces mobility and age-related diseases could also reduce the ability to perform the required physical activity.

Based on the fact that SPARC declines with ageing and that exercise induces SPARC, we hypothesized that SPARC is an exercise-induced antiageing factor, after we showed that SPARC overexpression mimics the effects of exercise. The same logic could be carefully applied to diet. Indeed, diets such as calorie restriction diets are prescribed to counteract the effects of ageing. Thus, studying the variations in gene expression induced by such diets and how the expressions of such genes change with ageing could identify novel targets. Pharmacological intervention on such targets would mimic the therapeutic outcome of calorie-restriction diets (antiageing). On the other hand, and although more evidence is required, the possibilities of mimicking exercise benefits via SPARC are not limited to ageing and could be applied in various contexts in which exercise cannot be performed because of physical disabilities, health disorders, or limited mobility.

Author Contributions: A.G. designed the manuscript structure and wrote the study. A.G., M.Y. and J.S.-A. discussed the content and exchanged ideas and suggestions (concepts to add, the figures, references selection, etc.) throughout the writing process, as well as edited and critically revised the paper. J.S.-A. gave the final approval for the version to be published. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: Abdelaziz Ghanemi received a scholarship under the Merit Scholarship Program for foreign students from the Ministry of Education and Higher Education of Quebec, Canada. The Fonds de recherche du Québec-Nature et technologies (FRQNT) is responsible for managing the program (Bourses d'excellence pour étudiants étrangers du Ministère de l'Éducation et de l'Enseignement supérieur du Québec, Le Fonds de recherche du Québec-Nature et technologies (FRQNT) est responsable de la gestion du programme). Abdelaziz Ghanemi received the scholarship «Bourse Tremplin -Stage en milieu de pratique» (Internship scholarship) from the Fonds de recherche du Québec-Sante (FRQS), Québec, Canada. Abdelaziz Ghanemi received the scholarship "Inspirational journey" («Un parcours inspirant») from la Caisse Desjardins de l'Université Laval, Quebec, Canada. Figure 1 was created using images from available online: https://mindthegraph.com/ (acceded on 4 April 2022) and http://smart.servier.com (accessed on 4 April 2022). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License. There are no copyright issues with the figure.

Conflicts of Interest: The authors declare no conflict of interest.

27.4 References

1. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. *Cell* 2013, *153*, 1194–1217.

2. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. *Diseases* 2021, *9*, 87.

3. Covarrubias, A.J.; Kale, A.; Perrone, R.; Lopez-Dominguez, J.A.; Pisco, A.O.; Kasler, H.G.; Schmidt, M.S.; Heckenbach, I.; Kwok, R.; Wiley, C.D.; et al. Senescent cells promote tissue NAD (+) decline during ageing via the activation of CD38(+) macrophages. *Nat. Metab.* 2020, *2*, 1265–1283.

4. Wilkinson, D.J.; Piasecki, M.; Atherton, P.J. The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. *Ageing Res. Rev.* 2018, *47*, 123–132.

5. Liu, Z.; Wu, K.K.L.; Jiang, X.; Xu, A.; Cheng, K.K.Y. The role of adipose tissue senescence in obesity-

and ageing-related metabolic disorders. *Clin. Sci.* 2020, *1343*, 315–330.
6. Spinelli, R.; Parrillo, L.; Longo, M.; Florese, P.; Desiderio, A.; Zatterale, F.; Miele, C.; Raciti, G.A.; Beguinot, F. Molecular basis of ageing in chronic metabolic diseases. *J. Endocrinol. Investig.* 2020, *43*, 1373–1389.
7. Konar, A.; Singh, P.; Thakur, M.K. Age-associated Cognitive Decline: Insights into Molecular Switches and Recovery Avenues. *Aging Dis.* 2016, *7*, 121–129.
8. Pawelec, G. Immunity and ageing in man. *Exp. Gerontol.* 2006, *41*, 1239–1242.
9. Hopkins, E.W. The Fountain of Youth. *J. Am. Orient. Soc.* 1905, *26*, 1–67.
10. Sims, J. Our ageing society: A view from the streets. *Australas. J. Ageing* 2018, *37*, 168.
11. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. *Phys. Med. Rehabil. Clin. N. Am.* 2017, *28*, 659–669.
12. Mora, J.C.; Valencia, W.M. Exercise and Older Adults. *Clin. Geriatr. Med.* 2018, *34*, 145–162.
13. López-Lluch, G.; Navas, P. Calorie restriction as an intervention in ageing. *J. Physiol.* 2016, *594*, 2043–2060.
14. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. *Medicines* 2021, *8*, 57.
15. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Exercise, Diet and Sleeping as Regenerative Medicine Adjuvants: Obesity and Ageing as Illustrations. *Medicines* 2022, *9*, 7.
16. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. *Am. Fam. Physician* 2017, *95*, 425–432.
17. Han, P.; Zhang, W.; Kang, L.; Ma, Y.; Fu, L.; Jia, L.; Yu, H.; Chen, X.; Hou, L.; Wang, L.; et al. Clinical Evidence of Exercise Benefits for Stroke. *Adv. Exp. Med. Biol.* 2017, *1000*, 131–151.
18. Halabchi, F.; Alizadeh, Z.; Sahraian, M.A.; Abolhasani, M. Exercise prescription for patients with multiple sclerosis; potential benefits and practical recommendations. *BMC Neurol.* 2017, *17*, 185.
19. Rosenfeldt, A.B.; Linder, S.M.; Davidson, S.; Clark, C.; Zimmerman, N.M.; Lee, J.J.; Alberts, J.L. Combined Aerobic Exercise and Task Practice Improve Health-Related Quality of Life Poststroke: A Preliminary Analysis. *Arch. Phys. Med. Rehabil.* 2019, *100*, 923–930.
20. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. *Front. Physiol.* 2020, *11*, 18.
21. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. *Gut* 2013, *62*, 882–889.
22. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. *Life Sci.* 2019, *229*, 277–287.
23. Distefano, G.; Goodpaster, B.H. Effects of Exercise and Aging on Skeletal Muscle. *Cold Spring Harb. Perspect. Med.* 2018, *8*, a029785.

24. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Leads to an Accelerated Ageing Phenotype Which Is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice. *Metabolites* 2022, *12*, 125.
25. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. *PLoS ONE* 2018, *13*, e0192714.
26. Deane, C.S.; Ames, R.M.; Phillips, B.E.; Weedon, M.N.; Willis, C.R.G.; Boereboom, C.; Abdulla, H.; Bukhari, S.S.I.; Lund, J.N.; Williams, J.P.; et al. The acute transcriptional response to resistance exercise: Impact of age and contraction mode. *Aging* 2019, *11*, 2111–2126.
27. Pillon, N.J.; Gabriel, B.M.; Dollet, L.; Smith, J.A.B.; Sardón Puig, L.; Botella, J.; Bishop, D.J.; Krook, A.; Zierath, J.R. Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. *Nat. Commun.* 2020, *11*, 470.
28. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. *Exp. Gerontol.* 2010, *45*, 896–903.
29. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. *Biomolecules* 2021, *11*, 1689.
30. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. *Genes* 2021, *12*, 1832.
31. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. *Appl. Sci.* 2020, *10*, 9108.
32. Gruber, H.E.; Sage, E.H.; Norton, H.J.; Funk, S.; Ingram, J.; Hanley, E.N., Jr. Targeted deletion of the SPARC gene accelerates disc degeneration in the aging mouse. *J. Histochem. Cytochem.* 2005, *53*, 1131–1138.
33. Whittal, M.C.; Molladavoodi, S.; Zwambag, D.P.; Millecamps, M.; Stone, L.S.; Gregory, D.E. Mechanical Consequence of Induced Intervertebral Disc Degeneration in the SPARC-Null Mouse. *J. Biomech. Eng.* 2021, *143*, 024501.
34. Mansergh, F.C.; Wells, T.; Elford, C.; Evans, S.L.; Perry, M.J.; Evans, M.J.; Evans, B.A. Osteopenia in Sparc (osteonectin)-deficient mice: Characterization of phenotypic determinants of femoral strength and changes in gene expression. *Physiol. Genom.* 2007, *32*, 64–73.
35. Norose, K.; Clark, J.I.; Syed, N.A.; Basu, A.; Heber-Katz, E.; Sage, E.H.; Howe, C.C. SPARC deficiency leads to early-onset cataractogenesis. *Investig. Ophthalmol. Vis. Sci.* 1998, *39*, 2674–2680.
36. Mansergh, F.C.; Wride, M.A.; Walker, V.E.; Adams, S.; Hunter, S.M.; Evans, M.J. Gene expression changes during cataract progression in Sparc null mice: Differential regulation of mouse globins in the lens. *Mol. Vis.* 2004, *10*, 490–511.
37. Luo, Z.; Zhou, Y.; Luo, P.; Zhao, Q.; Xiao, N.; Yu, Y.; Yan, Q.; Lu, G.; Cheng, L. SPARC deficiency affects bone marrow stromal function, resulting in impaired B lymphopoiesis. *J. Leukoc. Biol.* 2014, *96*, 73–

82.
38. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectindeficient mice. *J. Clin. Investig.* 2000, *105*, 915–923.
39. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? *Cytokine* 2020, *133*, 155179.
40. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? *Cytokine* 2020, *127*, 154996.
41. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. *Life* 2021, *11*, 38.
42. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. *Appl. Sci.* 2020, *10*, 2388.
43. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. *Int. J. Biochem. Cell Biol.* 2019, *117*, 105627.
44. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. *Nat. Rev. Endocrinol.* 2010, *6*, 225–235.
45. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. *Nutr. Metab.* 2017, *14*, 66.
46. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 Expression as an Indicator of the Severity of the High-Fat Diet-Induced Obesity. *Genes* 2021, *12*, 1505.
47. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. *Ageing Res. Rev.* 2018, *41*, 53–63.
48. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. *Mutat. Res./Rev. Mutat. Res.* 2019, *781*, 175–185.
49. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. *Genes* 2020, *11*, 875.

Conclusion

Both diet, especially HDF, and exercise are at the center of obesity development and management. Therefore, I have explored two selected genes related to both HFD and exercise. The data and literature reported within this thesis focus on these two genes and the properties they have, mainly but not only, in the context of energy balance, a key parameter in obesity and metabolism. Indeed, obesity and other related disorders result from metabolic imbalances leading to disorders. The results reported in this thesis characterize the metabolic variations related to *Tff2* and *Sparc*, two genes identified via functional genomics-based approaches and expressed following HFD and exercise respectively.

As *Tff2* is specifically induced by HDF, I explored selected metabolic patterns in *Tff2* KO mice challenged with both HFD and LFD. Overall, the data indicated a metabolic phenotype towards increased energy usage and lower energy storage. The mapped metabolic and biochemical pathways of lipids and glucose involving the adipose tissue, liver, skeletal muscle and sympathetic nervous system that protect *Tff2* KO mice from the HF diet-induced obesity highlight *Tff2*-related pathways as potential targets for obesity therapies. In addition, other properties we report from the literature, put such TFF2 metabolic implications in the context of homeostasis and in intercorrelation with other processes such as inflammation and tissue protection. For *Sparc*, and as it is induced by exercise, we explored its implications via various studies in both *Sparc* KO mice as well as mice overexpressing *Sparc* in the contexts of metabolics, exercise-induced changes and ageing. Overall, the data suggested that SPARC mediates, at least in part, the exercise-induced benefits especially at the metabolic level. We also highlight the potential implications such as using *Sparc*/SPARC as a biomarker in various conditions and pathologies. Importantly, our data, support the potential use or targeting of SPARC or its related pathways as therapeutic options against obesity and metabolic disorders. Such options, would also be of benefits for conditions in which exercise has therapeutic effects and/or for which SPARC induce beneficial effects such as sarcopenia, ageing, cancer, etc.

Following the same line of thought, studying other factors (genes, proteins, hormones) related to either *Tff2* and *Sparc* remains of a significant importance to deepen

our knowledge on energy metabolism and probably learn more about obesity-related mechanisms. For instance, proceeding to similar investigations of the other trefoil factors (TFF1 and TFF3) could be a starting point to identify other pharmacological targets for obesity especially that they are also related to food intake and share similar biological functions with TFF2 [2391]. The identified *Sparc*-related pathways might be targeted to treat obesity and other metabolic diseases by gene therapy or injection of the recombinant protein (for which different strategies have been developed to produce and purify it [2392]). This thesis also further highlights the great input functional genomics provides for the identification of genes worth exploring within the contexts of obesity and energy metabolism. It can lead to a new generation of therapies with specific applications in personalized medicine. Such exploratory approaches will be strengthened with the developments of functional genomics methods and those of metabolics and proteomics. Therefore, identify novel potential functional genomics-based molecular targets to develop novel therapies for obesity and the related diseases, including diabetes, metabolic syndromes and hyperlipidemia, and hopefully overcome the undesirable side effects known for the long-term obesity treatments either currently used [2393] or withdrawn [2394].

Within this context, further therapeutic-oriented research and explorations are required for a deeper investigation of the potential pharmacological application. Indeed, beyond the implications in obesity, exercise, diet-induced pathways or energy balance homeostais, *Tff2* and *Sparc* are involved in other biological processes. Such implications make exploring their properties a strong addition towards understating various pathways and develop novel therapies. For instance, with the ongoing COVID-19 pandemic, SPARC could be an important option as it mimics exercise, reduces/limits both ageing and obesity and controls inflammation. All these properties are towards reducing the vulnerability to COVID-19 and improving the prognosis of COVID-19 patients. The post-COVID-19 era could also benefit from the properties of SPARC to treat or provide a therapeutic addition for health problems related to ageing, obesity, inflammation, cancer, regeneration, etc.

We hope that the content of this thesis can stimulate other research toward tackling the health problems our generations are facing and improve the human well-being.

Bibliography

1. Jéquier E: Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci 2002, 967:379-388.
2. Health topics. Obesity. World Health Organization. 2018 [http://www.who.int/topics/obesity/en/ (Accessed date: March, 2nd 2018)]
3. Janssen I: The public health burden of obesity in Canada. Can J Diabetes 2013, 37:90-96.
4. Nardocci M, Leclerc BS, Louzada ML, Monteiro CA, Batal M, Moubarac JC: Consumption of ultra-processed foods and obesity in Canada. Can J Public Health 2019, 110:4-14.
5. Campbell-Scherer D, Sharma AM: Improving Obesity Prevention and Management in Primary Care in Canada. Curr Obes Rep 2016, 5:327-332.
6. Sharma AM, Ramos Salas X: Obesity Prevention and Management Strategies in Canada: Shifting Paradigms and Putting People First. Curr Obes Rep 2018, 7:89-96.
7. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000, 894:i-xii, 1-253.
8. Lissner L, Levitsky DA, Strupp BJ, Kalkwarf HJ, Roe DA: Dietary fat and the regulation of energy intake in human subjects. Am J Clin Nutr 1987, 46:886-892.
9. Flatt JP, Ravussin E, Acheson KJ, Jéquier E: Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 1985, 76:1019-1024.
10. Schutz Y, Flatt JP, Jéquier E: Failure of dietary fat intake to promote fat oxidation: a factor favoring the development of obesity. Am J Clin Nutr 1989, 50:307-314.
11. Yoshioka M, Bolduc C, Raymond V, St-Amand J: High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity (Silver Spring) 2008, 16:2302-2307.
12. Mucunguzi O, Melouane A, Ghanemi A, Yoshioka M, Boivin A, Calvo EL, St-Amand J: Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr Metab (Lond) 2017, 14:66.
13. De Giorgio MR, Yoshioka M, Riedl I, Moreault O, Cherizol RG, Shah AA, Blin N, Richard D, St-Amand J: Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity (Silver Spring) 2013, 21:1389-1395.
14. Riedl I, Yoshioka M, Nishida Y, Tobina T, Paradis R, Shono N, Tanaka H, St-Amand J: Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp Gerontol 2010, 45:896-903.
15. Melouane A, Yoshioka M, Kanzaki M, St-Amand J: Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci 2019, 229:277-287.
16. Obesity. Available online: http://www.who.int/topics/obesity/en/ (accessed on 20 November 2018). 2. Obesity: Preventing and managing the global epidemic: Report of a WHO consultation. WHO Tech. Rep.Ser. 2000, 894, 1–253.
17. Arsenault, B.J.; Lemieux, I.; Despres, J.P.; Wareham, N.J.; Kastelein, J.J.; Khaw, K.T.; Boekholdt, S.M. The hypertriglyceridemic-waist phenotype and the risk of coronary artery disease, results from the EPICNorfolk prospective population study. CMAJ 2010, 182, 1427–1432.
18. Misigoj-Durakovic, M.; Soric, M.; Durakovic, Z. Anthropometry in cardio-metabolic risk assessment. Arh.Hig. Rada. Toksikol. 2014, 65, 19–27.
19. Al-Domi, H.; Al-Shorman, A. Increased waist circumference is associated with subclinical atherosclerosis in schoolchildren. Diabetes Metab. Syndr. Clin. Res. Rev. 2019, 13, 264–269.
20. Sharma, A.M.; Kushner, R.F. A proposed clinical staging system for obesity. Int. J. Obes. (Lond) 2009, 33, 289–295.
21. American Diabetes Association. Consensus Development Conference on Insulin Resistance: 5–6 November 1997. Diabetes Care 1998, 21, 310–314.
22. Saltiel, A.R.; Olefsky, J.M. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 1996, 45, 1661–1669.
23. Kuhlmann, J.; Neumann-Haefelin, C.; Belz, U.; Kalisch, J.; Juretschke, H.P.; Stein, M.; Kleinschmidt, E.; Kramer, W.; Herling, A.W. Intramyocellular lipid and insulin resistance: A longitudinal in vivo 1Hspectroscopic study in Zucker diabetic fatty rats. Diabetes 2003, 52, 138–144.

24. Li, Z.; Lai, Z.W.; Christiano, R.; Gazos-Lopes, F.; Walther, T.C.; Farese, R.V. Global Analyses of Selective Insulin Resistance in Hepatocytes due to Palmitate Lipotoxicity. Mol. Cell. Proteomics 2018, 17, 836–849.
25. Siri-Tarino, P.W.; Sun, Q.; Hu, F.B.; Krauss, R.M. Saturated fatty acids and risk of coronary heart disease: Modulation by replacement nutrients. Curr. Atheroscler. Rep. 2010, 12, 384–390.
26. Hur, S.J.; Min, B.; Nam, K.C.; Lee, E.J.; Ahn, D.U. Effect of dietary cholesterol and cholesterol oxides on blood cholesterol, lipids, and the development of atherosclerosis in rabbits. Int. J. Mol. Sci. 2013, 14, 12593– 12606.
27. Wood, P.D.; Stefanick, M.L.; Dreon, D.M.; Frey-Hewitt, B.; Garay, S.C.; Williams, P.T.; Superko, H.R.; Fortmann, S.P.; Albers, J.J.; Vranizan, K.M.; et al. Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. N. Engl. J. Med. 1988, 319, 1173–1179.
28. Anderson, J.W.; Brinkman-Kaplan, V.L.; Lee, H.; Wood, C.L. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. J. Am. Coll. Nutri. 1994, 13, 256–261.
29. Gaesser, G.A.; Angadi, S.S.; Sawyer, B.J. Exercise and diet, independent of weight loss, improve cardiometabolic risk profile in overweight and obese individuals. Phys. Sportsmed. 2011, 39, 87–97.
30. Despres, J.P. Body fat distribution and risk of cardiovascular disease, an update. Circulation 2012, 126, 1301–1313.
31. Gastaldelli, A.; Gaggini, M. Ectopic fat: A target for cardiometabolic risk management. Expert Rev. Cardiovasc. Ther. 2016, 14, 1301–1303.
32. Buhmann, H.; Le Roux, C.W.; Bueter, M. The gut-brain axis in obesity. Best Pract. Res. Clin. Gastroenterol. 2014, 28, 559–571.
33. Maher, T.; Clegg, M.E. Dietary lipids with potential to affect satiety: Mechanisms and evidence. Crit. Rev. Food Sci. Nutr. 2018, 23, 1–26.
34. Liu, M.; Shen, L.; Liu, Y.; Woods, S.C.; Seeley, R.J.; D'Alessio, D.; Tso, P. Obesity induced by a high-fat diet downregulates apolipoprotein A-IV gene expression in rat hypothalamus. Am. J. Physiol. Endocrinol.Metab. 2004, 287, E366–E370.
35. Ziotopoulou, M.; Mantzoros, C.S.; Hileman, S.M.; Flier, J.S. Differential expression of hypothalamic
36. neuropeptides in the early phase of diet-induced obesity in mice. Am. J. Physiol. Endocrinol. Metab. 2000, 279, E838–E845.
37. Fothergill, E.; Guo, J.; Howard, L.; Kerns, J.C.; Knuth, N.D.; Brychta, R.; Chen, K.Y.; Skarulis, M.C.;
38. Walter, M.; Walter, P.J.; et al. Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. Obesity 2016, 24, 1612–1619.
39. Richard, D. Cognitive and autonomic determinants of energy homeostasis in obesity. Nat. Rev. Endocrinol. 2015, 11, 489–501.
40. Blüher, M.; Mantzoros, C.S. From leptin to other adipokines in health and disease: Facts and expectations at the beginning of the 21st century. Metabolism 2015, 64, 131–145.
41. Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L.; Friedman, J.M. Positional cloning of the mouse obese gene and its human homologue. Nature 1994, 372, 425–432.
42. Halaas, J.L.; Gajiwala, K.S.; Maffei, M.; Cohen, S.L.; Chait, B.T.; Rabinowitz, D.; Lallone, R.L.; Burley,
43. S.K.; Friedman, J.M. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995, 269, 543–546.
44. Agusti, A.; Garcia-Pardo, M.P.; Lopez-Almela, I.; Campillo, I.; Maes, M.; Romani-Perez, M.; Sanz, Y.Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function. Front. Neurosci. 2018, 12, 155.
45. Popkin, B.M.; Adair, L.S.; Ng, S.W. Global nutrition transition and the pandemic of obesity in developing countries. Nutr. Rev. 2012, 70, 3–21.
46. Candi, E.; Tesauro, M.; Cardillo, C.; Lena, A.M.; Schinzari, F.; Rodia, G.; Sica, G.; Gentileschi, P.;
47. Rovella, V.; Annicchiarico-Petruzzelli, M.; et al. Metabolic profiling of visceral adipose tissue from obese subjects with or without metabolic syndrome. Biochem. J. 2018, 475, 1019–1035.
48. Kruk, J. Overweight, obesity, oxidative stress and the risk of breast cancer. Asian Pac. J. Cancer Prev. 2014, 15, 9579–9586.
49. Marseglia, L.; Manti, S.; D'Angelo, G.; Nicotera, A.; Parisi, E.; Di Rosa, G.; Gitto, E.; Arrigo, T. Oxidative stress in obesity, a critical component in human diseases. Int. J. Mol. Sci. 2014, 16, 378–400.
50. Reuter, S.; Gupta, S.C.; Chaturvedi, M.M.; Aggarwal, B.B. Oxidative stress, inflammation, and cancer, how are they linked? Free Radic. Biol. Med. 2010, 49, 1603–1616.

51. Anjani, K.; Lhomme, M.; Sokolovska, N.; Poitou, C.; Aron-Wisnewsky, J.; Bouillot, J.L.; Lesnik, P.; Bedossa, P.; Kontush, A.; Clement, K.; et al. Circulating phospholipid profiling identifies portal contribution to NASH signature in obesity. J. Hepatol. 2015, 62, 905–912.
52. Lovren, F.; Teoh, H.; Verma, S. Obesity and atherosclerosis, mechanistic insights. Can. J. Cardiol. 2015, 31, 177–183.
53. Polsky, S.; Ellis, S.L. Obesity, insulin resistance, and type 1 diabetes mellitus. Curr. Opin. Endocrinol. Diabetes Obes. 2015, 22, 277–282.
54. Dimitriadis, G.; Mitrou, P.; Lambadiari, V.; Maratou, E.; Raptis, S.A. Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. 2011, 93, S52–S59.
55. Paz-Filho, G.; Mastronardi, C.; Wong, M.L.; Licinio, J. Leptin therapy, insulin sensitivity, and glucose homeostasis. Indian J. Endocrinol. Metab. 2012, 16, S549–S555.
56. Wada, N.; Hirako, S.; Takenoya, F.; Kageyama, H.; Okabe, M.; Shioda, S. Leptin and its receptors. J. Chem. Neuroanat. 2014, 61–62, 191–199.
57. Li, S.; Li, X. Leptin in normal physiology and leptin resistance. Sci. Bull. 2016, 61, 1480–1488.
58. Díaz, B.B.; González, D.A.; Gannar, F.; Pérez, M.C.R.; De León, A.C. Myokines, physical activity, insulin resistance and autoimmune diseases. Immunol. Letters 2018, 203, 1–5.
59. Despres, J.P.; Lemieux, I. Abdominal obesity and metabolic syndrome. Nature 2006, 444, 881–887.
60. Andoh, T. Subchapter 19A—Insulin. In Handbook of Hormones; Takei, Y., Ando, H., Tsutsui, K., Eds.; Academic Press: San Diego, CA, USA, 2016.
61. Villarroya, F.; Vidal-Puig, A. Beyond the sympathetic tone: The new brown fat activators. Cell. Metab. 2013, 17, 638–643.
62. Bayon, V.; Leger, D.; Gomez-Merino, D.; Vecchierini, M.F.; Chennaoui, M. Sleep debt and obesity. Ann.
Med. 2014, 46, 264–272.
63. Ogilvie, R.P.; Patel, S.R. The epidemiology of sleep and obesity. Sleep Health 2017, 3, 383–388.
64. Koren, D.; Dumin, M.; Gozal, D. Role of sleep quality in the metabolic syndrome. Diabetes Metab. Syndr.Obe. Targets Ther. 2016, 9, 281–310.
65. Poggiogalle, E.; Jamshed, H.; Peterson, C.M. Circadian regulation of glucose, lipid, and energy metabolism in humans. Metabolism 2018, 84, 11–27.
66. Spiegel, K.; Leproult, R.; Van Cauter, E. Impact of sleep debt on metabolic and endocrine function. Lancet 1999, 354, 1435–1439.
67. Spiegel, K.; Tasali, E.; Penev, P.; Van Cauter, E. Brief communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. Ann. Intern. Med. 2004, 141, 846–850.
68. Reutrakul, S.; Van Cauter, E. Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. Metabolism 2018, 84, 56–66.
69. Baron, K.G.; Reid, K.J. Chapter 26—Relationship between Circadian Rhythms, Feeding, and Obesity. In Modulation of Sleep by Obesity, Diabetes, Age, and Diet; Watson, R.R., Ed.; Academic Press: San Diego, CA, USA, 2015; pp. 243–253.
70. Xanthopoulos, M.S.; Berkowitz, R.I.; Tapia, I.E. Effects of obesity therapies on sleep disorders. Metabolism 2018, 84, 109–117.
71. Lasikiewicz, N.; Myrissa, K.; Hoyland, A.; Lawton, C.L. Psychological benefits of weight loss following behavioural and/or dietary weight loss interventions: A systematic research review. Appetite 2014, 72, 123– 137.
72. Atlantis, E.; Goldney, R.D.; Wittert, G.A. Obesity and depression or anxiety. BMJ 2009, 339, 871.
73. Sagar, R.; Gupta, T. Psychological Aspects of Obesity in Children and Adolescents. Indian J. Pediatr. 2018, 85, 554–559.
74. Chu, D.-T.; Minh Nguyet, N.T.; Nga, V.T.; Thai Lien, N.V.; Vo, D.D.; Lien, N.; Nhu Ngoc, V.T.; Son, L.H.; Le, D.-H.; Nga, V.B.; et al. An update on obesity: Mental consequences and psychological interventions. Diabetes Metab. Syndr. Clin. Res. Rev. 2019, 13, 155–160.
75. Bose, M.; Olivan, B.; Laferrere, B. Stress and obesity: The role of the hypothalamic-pituitary-adrenal axis in metabolic disease. Curr. Opin. Endocrinol. Diabetes Obes. 2009, 16, 340–346.
76. Singh, M. Mood, food, and obesity. Front. Psychol. 2014, 5, 925.
77. Soto, L.; Armendariz-Anguiano, A.L.; Bacardi-Gascon, M.; Jimenez Cruz, A. Beliefs, attitudes and phobias among Mexican medical and psychology students towards people with obesity. Nutr. Hosp. 2014, 30, 37–41.

78. Pan, L.; Li, X.; Feng, Y.; Hong, L. Psychological assessment of children and adolescents with obesity. J. Int. Med. Res. 2018, 46, 89–97.
79. Burgess, E.; Hassmen, P.; Welvaert, M.; Pumpa, K.L. Behavioural treatment strategies improve adherence to lifestyle intervention programmes in adults with obesity: A systematic review and meta-analysis. Clin. Obes. 2017, 7, 105–114.
80. Morelli, V.; Davis, C. The potential role of sports psychology in the obesity epidemic. Prim. Care 2013, 40, 507–523.
81. Chisholm, A.; Hart, J.; Mann, K.; Perry, M.; Duthie, H.; Rezvani, L.; Peters, S. Investigating the feasibility and acceptability of health psychology-informed obesity training for medical students. Psychol. Health Med. 2016, 21, 368–376.
82. Ghanemi, A. Psychiatric neural networks and neuropharmacology: Selected advances and novel implications. Saudi Pharm. J. 2014, 22, 95–100.
83. Kusminski, C.M.; Bickel, P.E.; Scherer, P.E. Targeting adipose tissue in the treatment of obesityassociated diabetes. Nat. Rev. Drug Discov. 2016, 15, 639–660.
84. Uusitupa, M. Remission of type 2 diabetes: Mission not impossible. Lancet 2018, 391, 515–516.
85. Catoi, A.F.; Parvu, A.; Muresan, A.; Busetto, L. Metabolic Mechanisms in Obesity and Type 2 Diabetes, Insights from Bariatric/Metabolic Surgery. Obes. Facts 2015, 8, 350–363.
86. Tremblay, A.; Picard-Deland, E.; Panahi, S.; Marette, A. Adipose tissue and sustainable development: A connection that needs protection. Front. Pharmacol. 2015, 6, 110.
87. Fernandes, M.R.; Lima, N.V.; Rezende, K.S.; Santos, I.C.; Silva, I.S.; Guimaraes, R.C. Animal models of obesity in rodents: An integrative review. Acta. Cir. Bras. 2016, 31, 840–844.
88. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
89. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894(i-xii):1–253.
90. Heymsfield SB, Wadden TA. Mechanisms, pathophysiology, and management of obesity. N Engl J Med 2017; 376:254–66.
91. Chandler M, Cunningham S, Lund EM, Khanna C, Naramore R, Patel A, et al. Obesity and associated comorbidities in people and companion animals: a one health perspective. J Comp Pathol 2017; 156:296 309.
92. Busutil R, Espallardo O, Torres A, Martinez-Galdeano L, Zozaya N, Hidalgo-Vega A. The impact of obesity on health-related quality of life in Spain. Health Qual Life Outcomes 2017; 15:197.
93. Noor MI. Obesity epidemic in Asia: issues and challenges. J Nutr Sci Vitaminol (Tokyo) 2015;61(Suppl): S212.
94. Katzmarzyk PT. The Canadian obesity epidemic: an historical perspective. Obes Res 2002; 10:666–74.
95. Statistics Canada. Overweight and obese adults (self-reported). Government of Canada, in. 2016.
96. Rabkin SW, Mathewson FA, Hsu PH. Relation of body weight to development of ischemic heart disease in a cohort of young north American men after a 26 year observation period: the Manitoba study. Am J Cardiol 1977; 39:452–8.
97. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham heart study. Circulation 1983; 67:968–77.
98. Bierman EL, Bagdade JD, Porte Jr. D. Obesity and diabetes: the odd couple. Am J Clin Nutr 1968; 21:1434–7.
99. Despres JP. Abdominal obesity and the risk of coronary artery disease. Can J Cardiol 1992; 8:561–2.
100. Mobley D, Baum N. The obesity epidemic and its impact on urologic care. Rev Urol 2015; 17:165–70.
101. Corey KE, Kaplan LM. Obesity and liver disease: the epidemic of the twenty-first century. Clin Liver Dis 2014; 18:1–18.
102. McElroy SL. The epidemic of depression with obesity. J Clin Psychiatry 2015;76: e1340–2.
103. Raveendran R, Wong J, Chung F. Morbid obesity, sleep apnea, obesity hypoventilation syndrome: are we sleepwalking into disaster? Perioperative Care and Operating Room Management 2017; 9:24–32.
104. Camacho DR, LeMasters PE. Gastroesophageal reflux disease in the obese. Techniques in Gastrointestinal Endoscopy 2015; 17:83–8.
105. Bonfrate L, Wang DQH, Garruti G, Portincasa P. Obesity and the risk and prognosis of gallstone disease and pancreatitis. Best Pract Res Clin Gastroenterol 2014;2 8:623–35.

106. Kulkarni K, Karssiens T, Kumar V, Pandit H. Obesity and osteoarthritis. Maturitas 2016; 89:22–8.
107. Banerjee A, Heiden E. Chapter 11 - Obesity and the Effects on the Respiratory System A2 - Weaver, Jolanta Urszula, in: Practical Guide to Obesity Medicine. Elsevier; 2018. p. 109–21.
108. Hoffman EL, VonWald T, Hansen K. The metabolic syndrome. S D Med, Spec No 2015:24–8.
109. Samson SL, Garber AJ. Metabolic syndrome. Endocrinol Metab Clin North Am 2014; 43:1–23.
110. Silva AO, Silva MV, Pereira LKN, Feitosa WMN, Ritti-Dias RM, Diniz PRB, et al. Association between general and abdominal obesity with high blood pressure: difference between genders. J Pediatr (Rio J) 2016; 92:174–80.
111. Wood PD, Stefanick ML, Dreon DM, Frey-Hewitt B, Garay SC, Williams PT, et al. Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. N Engl J Med 1988; 319:1173–9.
112. Anderson JW, Brinkman-Kaplan VL, Lee H, Wood CL. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. J Am Coll Nutr 1994; 13:256–61.
113. Ades PA, Savage PD. Obesity in coronary heart disease: an unaddressed behavioral risk factor. Prev Med 2017; 104:117–9.
114. Druso JE, Fischbach C. Biophysical properties of extracellular matrix: linking obesity and Cancer. Trends in Cancer 2018; 4:271–3.
115. D'Apuzzo MR, Novicoff WM, Browne JA. The John Insall award: morbid obesity independently impacts complications, mortality, and resource use after TKA. Clin Orthop Relat Res 2015; 473:57–63.
116. Nasraway Jr. SA, Albert M, Donnelly AM, Ruthazer R, Shikora SA, Saltzman E. Morbid obesity is an independent determinant of death among surgical critically ill patients. Crit Care Med 2006; 34:964–70. [quiz 971].
117. Upadhyay J, Farr O, Perakakis N, Ghaly W, Mantzoros C. Obesity as a disease. Med Clin North Am 2018; 102:13–33.
118. Whitaker BN, Fisher PL, Jambhekar S, Com G, Razzaq S, Thompson JE, et al. Impact of degree of obesity on sleep, quality of life, and depression in youth. J Pediatr Health Care 2018;32: e37–44.
119. Mucunguzi O, Melouane A, Ghanemi A, Yoshioka M, Boivin A, Calvo E-L, et al. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr Metab 2017; 14:66.
120. Fothergill E, Guo J, Howard L, Kerns JC, Knuth ND, Brychta R, et al. Persistent metabolic adaptation 6 years after "the biggest loser" competition. Obesity (Silver Spring) 2016; 24:1612–9.
121. Saunders KH, Umashanker D, Igel LI, Kumar RB, Aronne LJ. Obesity pharmacotherapy. Med Clin North Am 2018; 102:135–48.
122. Tremblay A, Picard-Deland E, Panahi S, Marette A. Adipose tissue and sustainable development: a connection that needs protection. Front Pharmacol 2015; 6:110.
123. Stefan N, Haring HU, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. Lancet Diabetes Endocrinol 2013; 1:152–62.
124. Wang B, Zhang M, Wang S, Wang C, Wang J, Li L, et al. Dynamic status of metabolically healthy overweight/obesity and metabolically unhealthy and normal weight and the risk of type 2 diabetes mellitus: a cohort study of a rural adult Chinese population. Obes Res Clin Pract 2018; 12:61–71.
125. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically healthy obese and incident cardiovascular disease events among 3.5 million men and women. J Am Coll Cardiol 2017; 70:1429–37.
126. Lemieux I. Energy partitioning in gluteal-femoral fat: does the metabolic fate of triglycerides affect coronary heart disease risk? Arterioscler Thromb Vasc Biol 2004; 24:795–7.
127. Arsenault BJ, Lemieux I, Despres JP, Wareham NJ, Kastelein JJ, Khaw KT, et al. The hypertriglyceridemic-waist phenotype and the risk of coronary artery disease: results from the EPIC-Norfolk prospective population study. CMAJ 2010; 182:1427–32.
128. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
129. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
130. Green, S.R.; Storey, K.B. Regulation of the α-ketoglutarate dehydrogenasecomplex during hibernation in a small mammal, the Richardson's ground squirrel (Urocitellus richardsonii). Biochim. Biophys. Acta Proteins Proteom. 2020, 1868, 140448.

131. Ruberto, A.A.; Logan, S.M.; Storey, K.B. Temperature and serine phosphorylation regulate glycerol-3-phosphate dehydrogenase in skeletal muscle of hibernating Richardson's ground squirrels. Biochem. Cell Biol. 2019, 97, 148–157.
132. Bell, R.A.V.; Storey, K.B. Purification and characterization of skeletal muscle pyruvate kinase from the hibernating ground squirrel, Urocitellus richardsonii: Potential regulation by posttranslational modification during torpor. Mol. Cell. Biochem. 2018, 442, 47–58.
133. Nelson, O.L.; Robbins, C.T. Cardiovascular function in large to small hibernators: Bears to ground squirrels. J. Comp. Physiol. B 2015, 185, 265–279.
134. Neeland, I.J.; Ross, R.; Després, J.P.; Matsuzawa, Y.; Yamashita, S.; Shai, I.; Seidell, J.; Magni, P.; Santos, R.D.; Arsenault, B.; et al.Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: A position statement. Lancet Diabetes Endocrinol. 2019, 7, 715–725.
135. Hyland, L.; Park, S.B.; Abdelaziz, Y.; Abizaid, A. Metabolic effects of ghrelin delivery into the hypothalamic ventral premammilary nucleus of male mice. Physiol. Behav. 2020, 228, 113208.
136. Drucker, D.J. Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. Cell Metab. 2018, 27, 740–756.
137. Alhadeff, A.L.; Golub, D.; Hayes, M.R.; Grill HJ: Peptide, Y.Y. signaling in the lateral parabrachial nucleus increases food intake through the Y1 receptor. Am. J. Physiol. Endocrinol. Metab. 2015, 309, E759– E766.
138. Poher, A.L.; Tschöp, M.H.; Müller, T.D. Ghrelin regulation of glucose metabolism. Peptides 2018, 100, 236–242.
139. Yu, Z.W.; Liu, R.; Li, X.; Wang, Y.; Fu, Y.H.; Li, H.Y.; Yuan, Y.; Gao, X.Y. Potential roles of Glucagonlike peptide-1 and its analogues in cognitive impairment associated with type 2 diabetes mellitus. Mech. Ageing Dev. 2020, 190, 111294.
140. Salcedo, I.; Tweedie, D.; Li, Y.; Greig, N.H. Neuroprotective and neurotrophic actions of glucagon-like peptide-1: An emerging opportunity to treat neurodegenerative and cerebrovascular disorders. Br. J. Pharmacol. 2012, 166, 1586–1599.
141. Fletcher, P.C.; Kenny, P.J. Food addiction: A valid concept? Neuropsychopharmacology 2018, 43, 2506–2513.
142. Gordon, E.L.; Ariel-Donges, A.H.; Bauman, V.; Merlo, L.J. What Is the Evidence for "Food Addiction"? A Systematic Review. Nutrients 2018, 10, 477.
143. Lennerz, B.; Lennerz, J.K. Food Addiction, High-Glycemic-Index Carbohydrates, and Obesity. Clin. Chem. 2018, 64, 64–71.
144. Lindgren, E.; Gray, K.; Miller, G.; Tyler, R.; Wiers, C.E.; Volkow, N.D.; Wang, G.J. Food addiction: A common neurobiological mechanism with drug abuse. Front. Biosci. 2018, 23, 811–836.
145. Solinas, M.; Belujon, P.; Fernagut, P.O.; Jaber, M.; Thiriet, N. Dopamine and addiction: What have we learned from 40 years of research. J. Neural Transm. 2019, 126, 481–516.
146. Gulyaeva, N.V. Molecular Mechanisms of Neuroplasticity: An Expanding Universe. Biochemistry 2017, 82, 237–242.
147. Lau, J.; Bloch, P.; Schäffer, L.; Pettersson, I.; Spetzler, J.; Kofoed, J.; Madsen, K.; Knudsen, L.B.; McGuire, J.; Steensgaard, D.B.; et al. Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide. J. Med. Chem. 2015, 58, 7370–7380.
148. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
149. Małodobra-Mazur, M.; Alama, A.; Bednarska-Chabowska, D.; Pawelka, D.; Myszczyszyn, A.; Dobosz, T. Obesity-induced insulin resistance via changes in the DNA methylation profile of insulin pathway genes. Adv. Clin. Exp. Med. 2019, 28, 1599–1607.
150. Izquierdo, A.G.; Crujeiras, A.B.; Casanueva, F.F.; Carreira, M.C. Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later? Nutrients 2019, 11, 2704.
151. Shimazu, T. Innervation of the liver and glucoregulation: Roles of the hypothalamus and autonomic nerves. Nutrition 1996, 12, 65–66.
152. Zeng, X.; Ye, M.; Resch, J.M.; Jedrychowski, M.P.; Hu, B.; Lowell, B.B.; Ginty, D.D.; Spiegelman, B.M. Innervation of thermogenic adipose tissue via a calsyntenin 3β-S100b axis. Nature 2019, 569, 229–235.
153. Johannsen, D.L.; Knuth, N.D.; Huizenga, R.; Rood, J.C.; Ravussin, E.; Hall, K.D. Metabolic slowing with massive weight loss despite preservation of fat-free mass. J. Clin. Endocrinol. Metab. 2012, 97, 2489–2496.

154. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
155. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity 2013, 21, 1389–1395.
156. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet induced obesity. Life Sci. 2018, 215, 190–197.
157. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
158. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
159. Hargreaves, M.; Spriet, L.L. Skeletal muscle energy metabolism during exercise. Nat. Metab. 2020, 2, 817–828.
160. Beer, N.J.; Dimmock, J.A.; Jackson, B.; Guelfi, K.J. Exercise-related factors that influence post-exercise energy intake: A psychological perspective. J. Sci. Med. Sport 2020, 23, 1068–1073.
161. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
162. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
163. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine (SPARC) as a regeneration factor: Beyond the tissue repair. Life 2021, 11, 38.
164. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
165. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
166. Ambrosi, T.H.; Scialdone, A.; Graja, A.; Gohlke, S.; Jank, A.-M.; Bocian, C.; Woelk, L.; Fan, H.; Logan, D.W.; Schürmann, A.; et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. Cell Stem Cell 2017, 20, 771–784.e6.
167. Tencerova, M.; Figeac, F.; Ditzel, N.; Taipaleenmäki, H.; Nielsen, T.K.; Kassem, M. High-Fat DietInduced Obesity Promotes Expansion of Bone Marrow Adipose Tissue and Impairs Skeletal Stem Cell Functions in Mice. J. Bone Miner. Res. 2018, 33, 1154–1165.
168. Da Silva, S.V.; Renovato-Martins, M.; Ribeiro-Pereira, C.; Citelli, M.; Barja-Fidalgo, C. Obesity modifies bone marrow microenvironment and directs bone marrow mesenchymal cells to adipogenesis. Obeity 2016, 24, 2522–2532.
169. Ulum, B.; Teker, H.T.; Sarikaya, A.; Balta, G.; Ku¸skonmaz, B.; Uckan, D.; Aerts-Kaya, F. Bone marrow mesenchymal stem cell donors with a high body mass index display elevated endoplasmic reticulum stress and are functionally impaired. J. Cell. Physiol. 2018, 233, 8429–8436.
170. Pérez, L.M.; Bernal, A.; Martín, N.S.; Gálvez, B.G. Obese-derived ASCs show impaired migration and angiogenesis properties. Arch. Physiol. Biochem. 2013, 119, 195–201.
171. Wu, C.-L.; Diekman, B.O.; Jain, D.; Guilak, F. Diet-induced obesity alters the differentiation potential of stem cells isolated from bone marrow, adipose tissue and infrapatellar fat pad: The effects of free fatty acids. Int. J. Obes. 2012, 37, 1079–1087.
172. Ma, S.; Wong, W.T.; Wang, D.H. Obesity reshapes stem cell extracellular vesicles. Cytom. Part A 2018, 93, 177–179.
173. Fuster, J.J.; Ouchi, N.; Gokce, N.; Walsh, K. Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease. Circ. Res. 2016, 118, 1786–1807.
174. Iyengar, N.M.; Gucalp, A.; Dannenberg, A.J.; Hudis, C.A. Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. J. Clin. Oncol. 2016, 34, 4270–4276.
175. Kraakman, M.J.; Murphy, A.J.; Jandeleit-Dahm, K.; Kammoun, H.L. Macrophage Polarization in Obesity and Type 2 Diabetes: Weighing Down Our Understanding of Macrophage Function? Front. Immunol. 2014, 5, 470.

176. Esser, N.; Legrand-Poels, S.; Piette, J.; Scheen, A.; Paquot, N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res. Clin. Pract. 2014, 105, 141–150.
177. Varga, T.; Mounier, R.; Patsalos, A.; Gogolák, P.; Peloquin, M.; Horvath, A.; Pap, A.; Daniel, B.; Nagy, G.; Pintye, E.; et al. Macrophage PPARγ, a Lipid Activated Transcription Factor Controls the Growth Factor GDF3 and Skeletal Muscle Regeneration. Immunity 2016, 45, 1038–1051.
178. Varga, T.; Mounier, R.; Gogolak, P.; Poliska, S.; Chazaud, B.; Nagy, L. Tissue LyC6- Macrophages Are Generated in the Absence of Circulating LyC6- Monocytes and Nur77 in a Model of Muscle Regeneration. J. Immunol. 2013, 191, 5695–5701.
179. Varga, T.; Mounier, R.; Horvath, A.; Cuvellier, S.; Dumont, F.; Póliska, S.; Ardjoune, H.; Juban, G.; Nagy, L.; Chazaud, B. Highly Dynamic Transcriptional Signature of Distinct Macrophage Subsets during Sterile Inflammation, Resolution, and Tissue Repair. J. Immunol. 2016, 196, 4771–4782.
180. Fu, X.; Zhu, M.; Zhang, S.; Foretz, M.; Viollet, B.; Du, M. Obesity impairs skeletal muscle regeneration via inhibition of AMP-activated protein kinase. Diabetes 2015, 65, 188–200.
181. Xu, P.; Werner, J.-U.; Milerski, S.; Hamp, C.M.; Kuzenko, T.; Jähnert, M.; Gottmann, P.; De Roy, L.; Warnecke, D.; Abaei, A.; et al. Diet-Induced Obesity Affects Muscle Regeneration After Murine Blunt Muscle Trauma—A Broad Spectrum Analysis. Front. Physiol. 2018, 9, 674.
182. Geiger, A.E.; Daughtry, M.R.; Yen, C.; Kirkpatrick, L.T.; Shi, H.; Gerrard, D.E. Dual effects of obesity on satellite cells and muscle regeneration. Physiol. Rep. 2020, 8, e14511.
183. Dungan, C.M.; Peck, B.D.; Walton, R.G.; Huang, Z.; Bamman, M.M.; Kern, P.A.; Peterson, C.A. In vivo analysis of γH2AX+ cells in skeletal muscle from aged and obese humans. FASEB J. 2020, 34, 7018–7035.
184. Liu, X.; Qu, H.; Zheng, Y.; Liao, Q.; Zhang, L.; Liao, X.; Xiong, X.; Wang, Y.; Zhang, R.; Wang, H.; et al. Mitochondrial glycerol 3-phosphate dehydrogenase promotes skeletal muscle regeneration. EMBO Mol. Med. 2018, 10, e9390.
185. Duguez, S.; Féasson, L.; Denis, C.; Freyssenet,D.Mitochondrial biogenesis during skeletal muscle regeneration. Am. J. Physiol. Metab. 2002, 282, E802–E809.
186. Wagatsuma, A.; Kotake, N.; Yamada, S. Muscle regeneration occurs to coincide with mitochondrial biogenesis. Mol. Cell. Biochem. 2010, 349, 139–147.
187. Wagatsuma, A.; Kotake, N.; Kawachi, T.; Shiozuka, M.; Yamada, S.; Matsuda, R. Mitochondrial adaptations in skeletal muscle to hindlimb unloading. Mol. Cell. Biochem. 2010, 350, 1–11.
188. Hood, D.A. Invited Review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. J. Appl. Physiol. 2001, 90, 1137–1157.
189. Hood, D.A.; Irrcher, I.; Ljubicic, V.; Joseph, A.-M. Coordination of metabolic plasticity in skeletal muscle. J. Exp. Biol. 2006, 209, 2265–2275.
190. Owens, D.J.; Sharples, A.P.; Polydorou, I.; Alwan, N.; Donovan, T.F.; Tang, J.; Fraser, W.D.; Cooper, R.G.; Morton, J.P.; E Stewart, C.; et al. A systems-based investigation into vitamin D and skeletal muscle repair, regeneration, and hypertrophy. Am. J. Physiol. Metab. 2015, 309, E1019–E1031.
191. Pereira-Santos, M.; Costa, P.R.F.; Assis, A.M.O.; Santos, C.A.S.T.; Santos, D.B. Obesity and vitamin D deficiency: A systematic review and meta-analysis. Obes. Rev. 2015, 16, 341–349.
192. Farrell, N.J.; Norris, G.H.; Ryan, J.; Porter, C.M.; Jiang, C.; Blesso, C.N. Black elderberry extract attenuates inflammation and metabolic dysfunction in diet-induced obese mice. Br. J. Nutr. 2015, 114, 1123– 1131.
193. Van Der Heijden, R.A.; Sheedfar, F.; Morrison, M.C.; Hommelberg, P.P.H.; Kor, D.; Kloosterhuis, N.J.; Gruben, N.; A Youssef, S.; De Bruin, A.; Hofker, M.H.; et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. Aging 2015, 7, 256–268.
194. Vieira-Potter, V.J.; Valentine, R.J.; Wilund, K.R.; Antao, N.; Baynard, T.; Woods, J.A. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. Am. J. Physiol. Metab. 2009, 296, E1164–E1171.
195. Perandini, L.A.; Chimin, P.; Lutkemeyer, D.D.S.; Câmara, N.O.S. Chronic inflammation in skeletal muscle impairs satellite cells function during regeneration: Can physical exercise restore the satellite cell niche? FEBS J. 2018, 285, 1973–1984.
196. Polyzos, S.A.; Margioris, A.N. Sarcopenic obesity. Hormones 2018, 17, 321–331.
197. Hong, S.-H.; Choi, K.M. Sarcopenic Obesity, Insulin Resistance, and Their Implications in Cardiovascular and Metabolic Consequences. Int. J. Mol. Sci. 2020, 21, 494.
198. Hurr, C.; Simonyan, H.; Morgan, D.A.; Rahmouni, K.; Young, C.N. Liver sympathetic denervation reverses obesity-induced hepatic steatosis. J. Physiol. 2019, 597, 4565–4580.

199. Khoo, N.K.; Fazzari, M.; Chartoumpekis, D.V.; Li, L.; Guimaraes, D.A.; Arteel, G.E.; Shiva, S.; Freeman, B.A. Electrophilic nitro-oleic acid reverses obesity-induced hepatic steatosis. Redox Biol. 2019, 22, 101132.
200. Allaire, M.; Gilgenkrantz, H. The impact of steatosis on liver regeneration. Horm. Mol. Biol. Clin. Investig. 2018, 41.
201. Lee, Y.; Berglund, E.D.; Yu, X.; Wang, M.-Y.; Evans, M.R.; Scherer, P.E.; Holland, W.L.; Charron, M.J.; Roth, M.G.; Unger, R.H. Hyperglycemia in rodent models of type 2 diabetes requires insulin-resistant alpha cells. Proc. Natl. Acad. Sci. USA 2014, 111, 13217–13222.
202. Sáinz, N.; Barrenetxe, J.; Moreno-Aliaga, M.J.; Martínez, J.A. Leptin resistance and diet-induced obesity: Central and peripheral actions of leptin. Metabolism 2015, 64, 35–46.
203. Raut, P.K.; Kim, S.-H.; Choi, D.Y.; Jeong, G.-S.; Park, P.-H. Growth of breast cancer cells by leptin is mediated via activation of the inflammasome: Critical roles of estrogen receptor signaling and reactive oxygen species production. Biochem. Pharmacol. 2019, 161, 73–88.
204. Barrios-Correa, A.A.; Estrada, J.A.; Contreras, I. Leptin Signaling in the Control of Metabolism and Appetite: Lessons from Animal Models. J. Mol. Neurosci. 2018, 66, 390–402.
205. Pérez-Pérez, A.; Vilariño-García, T.; Fernández-Riejos, P.; Martín-González, J.; Segura-Egea, J.J.; Sánchez-Margalet, V. Role of leptin as a link between metabolism and the immune system. Cytokine Growth Factor Rev. 2017, 35, 71–84.
206. Titchenell, P.M.; Lazar, M.A.; Birnbaum, M.J. Unraveling the Regulation of Hepatic Metabolism by Insulin. Trends Endocrinol. Metab. 2017, 28, 497–505.
207. Laron, Z.; Werner, H. Insulin: A Growth Hormone and Potential Oncogene. Pediatr. Endocrinol. Rev. 2020, 17, 191–197.
208. Tencerova, M.; Okla, M.; Kassem, M. Insulin Signaling in Bone Marrow Adipocytes. Curr. Osteoporos. Rep. 2019, 17, 446–454.
209. Proietto, J.; Tencerova, M.; Frost, M.; Figeac, F.; Nielsen, T.K.; Ali, D.; Lauterlein, J.L.; Andersen, T.L.; Haakonsson, A.K.; Rauch, A.; et al. Faculty Opinions recommendation of Obesity-Associated Hypermetabolism and Accelerated Senescence of Bone Marrow Stromal Stem Cells Suggest a Potential Mechanism for Bone Fragility. Fac. Opin. Post-Publ. Peer Rev. Biomed. Lit. 2020, 27, 2050–2062.
210. Cornish, J.; Wang, T.; Lin, J.-M. Role of Marrow Adipocytes in Regulation of Energy Metabolism and Bone Homeostasis. Curr. Osteoporos. Rep. 2018, 16, 116–122.
211. Muruganandan, S.; Govindarajan, R.; Sinal, C.J.; Shanmugam, M.; Rajgopal, G. Bone Marrow Adipose Tissue and Skeletal Health. Curr. Osteoporos. Rep. 2018, 16, 434–442.
212. Röszer, T.; Józsa, T.; Kiss-Tóth, E.D.; De Clerck, N.; Balogh, L. Leptin receptor deficient diabetic (db/db) mice are compromised in postnatal bone regeneration. Cell Tissue Res. 2013, 356, 195–206.
213. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. Metabolism 2019, 92, 121–135.
214. Li, H.-M.; Ye, Z.-H. Microenvironment of liver regeneration in liver cancer. Chin. J. Integr. Med. 2017, 23, 555–560.
215. Boilly, B.; Faulkner, S.; Jobling, P.; Hondermarck, H. Nerve Dependence: From Regeneration to Cancer. Cancer Cell 2017, 31, 342–354.
216. Gao, F.; Lv, T.-R.; Zhou, J.-C.; Qin, X.-D. Effects of obesity on the healing of bone fracture in mice. J. Orthop. Surg. Res. 2018, 13, 145.
217. Yu, H.; Harrison, F.E.; Xia, F. Altered DNA repair; an early pathogenic pathway in Alzheimer's disease and obesity. Sci. Rep. 2018, 8, 1–6.
218. Włodarczyk, M.; Nowicka, G. Obesity, DNA Damage, and Development of Obesity-Related Diseases. Int. J. Mol. Sci. 2019, 20, 1146.
219. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. Cell Metab. 2019, 29, 1028–1044.
220. Mazon, J.N.; De Mello, A.H.; Ferreira, G.K.; Rezin, G.T. The impact of obesity on neurodegenerative diseases. Life Sci. 2017, 182, 22–28.
221. Payab, M.; Goodarzi, P.; Heravani, N.F.; Hadavandkhani, M.; Zarei, Z.; Falahzadeh, K.; Larijani, B.; Rahim, F.; Arjmand, B. Stem Cell and Obesity: Current State and Future Perspective. Atherosclerosis 2018, 1089, 1–22.
222. Matsushita, K.; Dzau, V.J. Mesenchymal stem cells in obesity: Insights for translational applications. Lab. Investig. 2017, 97, 1158–1166.

223. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet induced obesity. Life Sci. 2018, 215, 190–197.
224. Ahmad, A.; Ali, T.; Kim, M.W.; Khan, A.; Jo, M.H.; Rehman, S.U.; Khan, M.S.; Bin Abid, N.; Khan, M.; Ullah, R.; et al. Adiponectin homolog novel osmotin protects obesity/diabetes-induced NAFLD by upregulating AdipoRs/PPARα signaling in ob/ob and db/db transgenic mouse models. Metabolism 2019, 90, 31–43.
225. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). Int J Surgery (London, England). 2020; 76:71–6.
226. Hagerty SL, Williams LM. The impact of COVID-19 on mental health: the interactive roles of brain biotypes and human connection. Brain Behav Immun – Health 2020; 5:100078.
227. Torales J, O'Higgins M, Castaldelli-Maia JM, Ventriglio A. The outbreak of COVID- 19 coronavirus and its impact on global mental health. Int J Soc Psychiatry 2020.
228. Grover S, Dua D, Sahoo S, Mehra A, Nehra R, Chakrabarti S. Why all COVID-19 Hospitals should have Mental Health Professionals: the importance of mental health in a worldwide crisis!. Asian J Psychiatry 2020;102147.
229. Altena E, Baglioni C, Espie CA, Ellis J, Gavriloff D, Holzinger B, et al. Dealing with sleep problems during home confinement due to the COVID-19 outbreak: practical recommendations from a task force of the European CBT-I Academy. J Sleep Res 2020: e13052.
230. João K, Jesus SN, Carmo C, Pinto P. The impact of sleep quality on the mental health of a non-clinical population. Sleep Med 2018; 46:69–73.
231. Stone KL, Xiao Q. Impact of poor sleep on physical and mental health in older women. Sleep Med Clinics 2018;13(3):457–65.
232. Thorsteinsson EB, Brown RF, Owens MT. Modeling the effects of stress, anxiety, and depression on rumination, sleep, and fatigue in a nonclinical sample. J Nerv Ment Dis 2019; 207(5):355–9.
233. Kalmbach DA, Anderson JR, Drake CL. The impact of stress on sleep: Pathogenic sleep reactivity as a vulnerability to insomnia and circadian disorders. J Sleep Res 2018; 27(6): e12710.
234. St-Onge MP. Sleep-obesity relation: underlying mechanisms and consequences for treatment. Obesity Rev 2017; 18(Suppl 1):34–9.
235. Bayon V, Leger D, Gomez-Merino D, Vecchierini MF, Chennaoui M. Sleep debt and obesity. Ann Med 2014;46(5): 264–72.
236. Chan WS, Levsen MP, McCrae CS. A meta-analysis of associations between obesity and insomnia diagnosis and symptoms. Sleep Med Rev 2018; 40:170–82.
237. Ghanemi A, Yoshioka M, St-Amand J. Broken energy homeostasis and obesity pathogenesis: the surrounding concepts. J Clin Med. 2018;7(11).
238. Atlantis E, Goldney RD, Wittert GA. Obesity and depression or anxiety. BMJ (Clinical research ed). 2009;339: b3868.
239. Gafoor R, Booth HP, Gulliford MC. Antidepressant utilisation and incidence of weight gain during 10 years' follow-up: population based cohort study. BMJ (Clinical research ed). 2018; 361:k1951.
240. Traversy G, Chaput JP. Alcohol consumption and obesity: an update. Current Obesity Rep 2015; 4(1):122–30.
241. Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: a systematic review. Nutr Rev 2011;69(8): 419–31.
242. Sharif A, Aloui C, Yarovaya L. COVID-19 pandemic, oil prices, stock market, geopolitical risk andpolicy uncertainty nexus in the US economy: fresh evidence from the wavelet-based approach. Int Rev Financ Anal 2020; 70:101496.
243. Shammi M, Bodrud-Doza M, Towfiqul Islam ARM, Rahman MM. COVID-19 pandemic, socioeconomic crisis and human stress in resource-limited settings: a case from Bangladesh. Heliyon 2020;6(5): e04063.
244. Payab M, Kelishadi R, Qorbani M, Motlagh ME, Ranjbar SH, Ardalan G, et al. Association of junk food consumption with high blood pressure and obesity in Iranian children and adolescents: the Caspian-IV Study. Jornal de Pediatria (Versão em Português) 2015;91(2): 196–205.
245. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ (Clinical research ed). 2020; 368:m1091.

246. Hartman ME, Hernandez RA, Patel K, Wagner TE, Trinh T, Lipke AB, et al. COVID- 19 respiratory failure: targeting inflammation on VV-ECMO support. ASAIO J (American Society for Artificial Internal Organs: 1992) 2020.
247. Ghanemi A, St-Amand J. Redefining obesity toward classifying as a disease. Eur J Intern Med. 2018;55: 20–2.
248. Sanchis-Gomar F, Lavie CJ, Mehra MR, Henry BM, Lippi G. Obesity and outcomes in COVID-19: when an epidemic and pandemic collide. Mayo Clin Proc 2020.
249. Bray GA, Macdiarmid J. The epidemic of obesity. Western J Med 2000;172(2):78–9.
250. Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. In WHO Technical Report Series 894; World Health Organization: Geneva, Switzerland, 2000; pp. i-xii, 1-253.
251. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
252. Inoue, Y.; Qin, B.; Poti, J.; Sokol, R.; Gordon-Larsen, P. Epidemiology of Obesity in Adults: Latest Trends. Curr. Obes. Rep. 2018, 7, 276–288.
253. Ng, M.; Fleming, T.; Robinson, M.; Thomson, B.; Graetz, N.; Margono, C.; Mullany, E.C.; Biryukov, S.; Abbafati, C.; Abera, S.F.; et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014, 384, 766–781.
254. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
255. Andolfi, C.; Fisichella, P.M. Epidemiology of Obesity and Associated Comorbidities. J. Laparoendosc. Adv. Surg. Tech. A 2018, 28, 919–924.
256. Bhupathiraju, S.N.; Hu, F.B. Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. Circ. Res. 2016, 118, 1723–1735.
257. Wang, J.; Wu, Y.; Xiong, G.; Chao, T.; Jin, Q.; Liu, R.; Hao, L.; Wei, S.; Yang, N.; Yang, X. Introduction of complementary feeding before 4 months of age increases the risk of childhood overweight or obesity: A meta-analysis of prospective cohort studies. Nutr. Res. 2016, 36, 759–770.
258. Segal, M.; Eliasziw, M.; Phillips, S.; Bandini, L.; Curtin, C.; Kral, T.V.E.; Sherwood, N.E.; Sikich, L.; Stanish, H.; Must, A. Intellectual disability is associated with increased risk for obesity in a nationally representative sample of U.S. children. Disabil. Health J. 2016, 9, 392–398.
259. Michopoulos, V. Stress-Induced alterations in estradiol sensitivity increase risk for obesity in women. Physiol. Behav. 2016.
260. Heymsfield, S.B.; Wadden, T.A. Mechanisms, Pathophysiology, and Management of Obesity. N. Engl. J. Med. 2017, 376, 254–266.
261. Kim, J.Y.; Park, D.I.; Yu, J.; Jung, Y.S.; Park, J.H.; Kim, H.J.; Cho, Y.K.; Sohn, C.I.; Jeon, W.K.; Kim, B.I.; et al. Increased Risk of Advanced Colorectal Neoplasia Among Korean Men With Metabolic Abnormality and Obesity. Clin. Gastroenterol. Hepatol. 2016, 14.
262. Fu, M.C.; D0Ambrosia, C.; McLawhorn, A.S.; Schairer, W.W.; Padgett, D.E.; Cross, M.B. Malnutrition Increases With Obesity and Is a Stronger Independent Risk Factor for Postoperative Complications: A Propensity-Adjusted Analysis of Total Hip Arthroplasty Patients. J. Arthroplast. 2016.
263. Ricketts, T.A.; Sui, X.; Lavie, C.J.; Blair, S.N.; Ross, R. Addition of Cardiorespiratory Fitness Within an Obesity Risk Classification Model Identifies Men at Increased Risk of All-Cause Mortality. Am. J. Med. 2016, 129, 536.e13–536.e20.
264. Fontaine, K.R.; Redden, D.T.; Wang, C.; Westfall, A.O.; Allison, D.B. Years of life lost due to obesity. JAMA 2003, 289, 187–193.
265. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
266. le Roux, C.W.; Heneghan, H.M. Bariatric Surgery for Obesity. Med. Clin. N. Am. 2018, 102, 165–182.
267. Nuffer, W. Chapter 5-Pharmacologic Agents Chapter for Abdominal Obesity. In Nutrition in the Prevention and Treatment of Abdominal Obesity, 2nd ed.; Watson, R.R., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 51–66.
268. Kim, G.W.; Lin, J.E.; Blomain, E.S.; Waldman, S.A. Antiobesity pharmacotherapy: New drugs and emerging targets. Clin. Pharmacol. Ther. 2014, 95, 53–66.
269. Gadde, K.M.; Pritham Raj, Y. Pharmacotherapy of Obesity: Clinical Trials to Clinical Practice. Curr. Diab. Rep. 2017, 17, 34.

270. Gadde, K.M.; Apolzan, J.W.; Berthoud, H.R. Pharmacotherapy for Patients with Obesity. Clin. Chem. 2017.
271. Canella, D.S.; Novaes, H.M.; Levy, R.B. Medicine expenses and obesity in Brazil: An analysis based on the household budget survey. BMC Public Health 2016, 16, 54.
272. Verhaeghe, N.; De Greve, O.; Annemans, L. The potential health and economic effect of a Body Mass Index decrease in the overweight and obese population in Belgium. Public Health 2016.
273. Westerterp, K.R. Control of Energy Expenditure in Humans. In Endotext; Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., Dungan, K., Grossman, A., Hershman, J.M., Kaltsas, G., Koch, C., et al., Eds.; MDText.com, Inc.: South Dartmouth, MA, USA, 2000.
274. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. Phys. Med. Rehabil. Clin. N Am. 2017, 28, 659–669.
275. Myers, J. Cardiology patient pages. Exercise and cardiovascular health. Circulation 2003, 107, e2–e5.
276. Tran, Z.V.; Weltman, A. Differential effects of exercise on serum lipid and lipoprotein levels seen with changes in body weight. A meta-Analysis. Jama 1985, 254, 919–924.
277. Blundell, J.E.; Gibbons, C.; Caudwell, P.; Finlayson, G.; Hopkins, M. Appetite control and energy balance: Impact of exercise. Obes. Rev. 2015, 16 (Suppl. 1), 67–76.
278. Chin, S.H.; Kahathuduwa, C.N.; Binks, M. Physical activity and obesity: What we know and what we need to know. Obes. Rev. 2016, 17, 1226–1244.
279. Marcin, T.; Eser, P.; Prescott, E.; Mikkelsen, N.; Prins, L.F.; Kolkman, E.K.; Lado-Baleato, Ó.; CardasoSuaréz, C.; Bruins, W.; van der Velde, A.E.; et al. Predictors of pre-Rehabilitation exercise capacity in elderly European cardiac patients-The EU-CaRE study. Eur. J. Prev. Cardiol. 2019.
280. McLeod, M.; Breen, L.; Hamilton, D.L.; Philp, A. Live strong and prosper: The importance of skeletal muscle strength for healthy ageing. Biogerontology 2016, 17, 497–510.
281. Brook, M.S.; Wilkinson, D.J.; Phillips, B.E.; Perez-Schindler, J.; Philp, A.; Smith, K.; Atherton, P.J. Skeletal muscle homeostasis and plasticity in youth and ageing: Impact of nutrition and exercise. Acta Physiol. (Oxf.) 2016, 216, 15–41.
282. Shad, B.J.; Wallis, G.; van Loon, L.J.; Thompson, J.L. Exercise prescription for the older population: The interactions between physical activity, sedentary time, and adequate nutrition in maintaining musculoskeletal health. Maturitas 2016, 93, 78–82.
283. Figueira, A.C.C.; Figueira, M.C.; Silva, C.; Padrao, A.; Oliveira, P.A.; Ferreira, R.P.; Duarte, J.A. Exercise Training-Induced Modulation in Microenvironment of Rat Mammary Neoplasms. Int. J. Sports Med. 2018.
284. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. Cell Metab. 2016, 23, 554–562.
285. Antunes, J.M.M.; Ferreira, R.M.P.; Moreira-Goncalves, D. Exercise Training as Therapy for CancerInduced Cardiac Cachexia. Trends Mol. Med. 2018, 24, 709–727.
286. Wesnes, K.; Myhr, K.M.; Riise, T.; Cortese, M.; Pugliatti, M.; Bostrom, I.; Landtblom, A.M.; Wolfson, C.; Bjornevik, K. Physical activity is associated with a decreased multiple sclerosis risk: The EnvIMS study. Mult. Scler. 2018, 24, 150–157.
287. Han, P.; Zhang, W.; Kang, L.; Ma, Y.; Fu, L.; Jia, L.; Yu, H.; Chen, X.; Hou, L.; Wang, L.; et al. Clinical Evidence of Exercise Benefits for Stroke. Adv. Exp. Med. Biol. 2017, 1000, 131–151.
288. Panchik, D.; Masco, S.; Zinnikas, P.; Hillriegel, B.; Lauder, T.; Suttmann, E.; Chinchilli, V.; McBeth, M.; Hermann, W. The Effect of Exercise on Breast Cancer-Related Lymphedema: What the Lymphatic Surgeon Needs to Know. J. Reconstr. Microsurg. 2018.
289. Keilani, M.; Hasenoehrl, T.; Baumann, L.; Ristl, R.; Schwarz, M.; Marhold, M.; Sedghi Komandj, T.; Crevenna, R. Effects of resistance exercise in prostate cancer patients: A meta-analysis. Support Care Cancer 2017, 25, 2953–2968.
290. Hart, N.H.; Galvao, D.A.; Newton, R.U. Exercise medicine for advanced prostate cancer. Curr. Opin. Support Palliat. Care 2017, 11, 247–257.
291. Erlich, A.T.; Brownlee, D.M.; Beyfuss, K.; Hood, D.A. Exercise induces TFEB expression and activity in skeletal muscle in a PGC-1alpha-Dependent manner. Am. J. Physiol. Cell Physiol. 2017.
292. Vainshtein, A.; Tryon, L.D.; Pauly, M.; Hood, D.A. Role of PGC-1alpha during acute exercise-Induced autophagy and mitophagy in skeletal muscle. Am. J. Physiol. Cell Physiol. 2015, 308, C710–C719.

293. Wang, P.; Li, C.G.; Qi, Z.; Cui, D.; Ding, S. Acute exercise stress promotes Ref1/Nrf2 signalling and increases mitochondrial antioxidant activity in skeletal muscle. Exp. Physiol. 2016, 101, 410–420.
294. Choi, S.; Liu, X.; Li, P.; Akimoto, T.; Lee, S.Y.; Zhang, M.; Yan, Z. Transcriptional profiling in mouse skeletal muscle following a single bout of voluntary running: Evidence of increased cell proliferation. J. Appl. Physiol. (Bethesda, Md. 1985) 2005, 99, 2406–2415.
295. Hu, Z.; Zhou, L.; He, T. Potential effect of exercise in ameliorating insulin resistance at transcriptome level. J. Sports Med. Phys. Fit. 2017.
296. Larose, M.; St-Amand, J.; Yoshioka, M.; Belleau, P.; Morissette, J.; Labrie, C.; Raymond, V.; Labrie, F. Transcriptome of mouse uterus by serial analysis of gene expression (SAGE): Comparison with skeletal muscle. Mol. Reprod. Dev. 2004, 68, 142–148.
297. Vissing, K.; Schjerling, P. Simplified data access on human skeletal muscle transcriptome responses to differentiated exercise. Sci. Data 2014, 1, 140041.
298. Lindholm, M.E.; Huss, M.; Solnestam, B.W.; Kjellqvist, S.; Lundeberg, J.; Sundberg, C.J. The human skeletal muscle transcriptome: Sex differences, alternative splicing, and tissue homogeneity assessed with RNA sequencing. FASEB J. 2014, 28, 4571–4581.
299. Seaborne, R.A.; Strauss, J.; Cocks,M.; Shepherd, S.; O0Brien, T.D.; van Someren, K.A.; Bell, P.G.; Murgatroyd, C.; Morton, J.P.; Stewart, C.E.; et al. Human Skeletal Muscle Possesses an Epigenetic Memory of Hypertrophy. Sci. Rep. 2018, 8, 1898.
300. Lindholm, M.E.; Marabita, F.; Gomez-Cabrero, D.; Rundqvist, H.; Ekstrom, T.J.; Tegner, J.; Sundberg, C.J. An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. Epigenetics 2014, 9, 1557–1569.
301. Sharples, A.P.; Stewart, C.E.; Seaborne, R.A. Does skeletal muscle have an 'epi'-memory? The role of epigenetics in nutritional programming, metabolic disease, aging and exercise. Aging Cell 2016, 15, 603–616.
302. Gensous, N.; Bacalini, M.G.; Pirazzini, C.; Marasco, E.; Giuliani, C.; Ravaioli, F.; Mengozzi, G.; Bertarelli, C.; Palmas, M.G.; Franceschi, C.; et al. The epigenetic landscape of age-related diseases: The geroscience perspective. Biogerontology 2017, 18, 549–559.
303. Li, S.; Chen, Y.; Zhang, Y.; Zhang, H.; Wu, Y.; He, H.; Gong, L.; Zeng, F.; Shi, L. Exercise during pregnancy enhances vascular function via epigenetic repression of Ca(V)1.2 channel in offspring of hypertensive rats. Life Sci. 2019, 231, 116576.
304. Hopkins, S.A.; Baldi, J.C.; Cutfield, W.S.; McCowan, L.; Hofman, P.L. Exercise training in pregnancy reduces offspring size without changes in maternal insulin sensitivity. J. Clin. Endocrinol. Metab. 2010, 95, 2080–2088.
305. Yoshioka, M.; Tanaka, H.; Shono, N.; Snyder, E.E.; Shindo, M.; St-Amand, J. Serial analysis of gene expression in the skeletal muscle of endurance athletes compared to sedentary men. FASEB J. 2003, 17, 1812–1819.
306. Yoshioka, M.; Tanaka, H.; Shono, N.; Shindo, M.; St-Amand, J. Gene expression profile of sprinter's muscle. Int. J. Sports Med. 2007, 28, 1053–1058.
307. Cochran, A.J.; Percival, M.E.; Tricarico, S.; Little, J.P.; Cermak, N.; Gillen, J.B.; Tarnopolsky, M.A.; Gibala, M.J. Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations. Exp. Physiol. 2014, 99, 782–791.
308. Nishida, Y.; Tanaka, H.; Tobina, T.; Murakami, K.; Shono, N.; Shindo, M.; Ogawa, W.; Yoshioka, M.; St-Amand, J. Regulation of muscle genes by moderate exercise. Int. J. Sports Med. 2010, 31, 656–670.
309. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
310. Timmons, J.A.; Larsson, O.; Jansson, E.; Fischer, H.; Gustafsson, T.; Greenhaff, P.L.; Ridden, J.; Rachman, J.; Peyrard-Janvid, M.; Wahlestedt, C.; et al. Human muscle gene expression responses to endurance training provide a novel perspective on Duchenne muscular dystrophy. Faseb J. 2005, 19, 750– 760.
311. Huang, J.H.; Hood, D.A. Age-Associated mitochondrial dysfunction in skeletal muscle: Contributing factors and suggestions for long-Term interventions. IUBMB Life 2009, 61, 201–214.
312. Marzetti, E.; Lawler, J.M.; Hiona, A.; Manini, T.; Seo, A.Y.; Leeuwenburgh, C. Modulation of age induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle. Free Radic Biol. Med. 2008, 44, 160–168.

313. Lourenço Dos Santos, S.; Baraibar, M.A.; Lundberg, S.; Eeg-Olofsson, O.; Larsson, L.; Friguet, B. Oxidative proteome alterations during skeletal muscle ageing. Redox Biol. 2015, 5, 267–274.
314. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
315. Norheim, F.; Raastad, T.; Thiede, B.; Rustan, A.C.; Drevon, C.A.; Haugen, F. Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. Am. J. Physiol. Endocrinol. Metab. 2011, 301, E1013–E1021.
316. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
317. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
318. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
319. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
320. Sage, H.; Vernon, R.B.; Funk, S.E.; Everitt, E.A.; Angello, J. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. J. Cell Biol. 1989, 109, 341–356.
321. Francki, A.; Motamed, K.; McClure, T.D.; Kaya, M.; Murri, C.; Blake, D.J.; Carbon, J.G.; Sage, E.H. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-Dependent signaling. J. Cell Biochem. 2003, 88, 802–811.
322. Mason, I.J.; Taylor, A.; Williams, J.G.; Sage, H.; Hogan, B.L. Evidence from molecular cloning that SPARC, a major product of mouse embryo parietal endoderm, is related to an endothelial cell 'culture shock' glycoprotein of Mr 43,000. Embo J. 1986, 5, 1465–1472.
323. Cho, W.J.; Kim, E.J.; Lee, S.J.; Kim, H.D.; Shin, H.J.; Lim, W.K. Involvement of SPARC in in vitro differentiation of skeletal myoblasts. Biochem. Biophys. Res. Commun. 2000, 271, 630–634.
324. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
325. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
326. Hood, D.A. Mechanisms of exercise-induced mitochondrial biogenesis in skeletal muscle. Appl. Physiol. Nutr. Metab. 2009, 34, 465–472.
327. Hoffman, N.J.; Parker, B.L.; Chaudhuri, R.; Fisher-Wellman, K.H.; Kleinert, M.; Humphrey, S.J.; Yang, P.; Holliday, M.; Trefely, S.; Fazakerley, D.J.; et al. Global Phosphoproteomic Analysis of Human Skeletal Muscle Reveals a Network of Exercise-Regulated Kinases and AMPK Substrates. Cell Metab. 2015, 22, 922– 935.
328. Hamilton, M.T.; Booth, F.W. Skeletal muscle adaptation to exercise: A century of progress. J. Appl. Physiol. (1985) 2000, 88, 327–331.
329. Melov, S.; Tarnopolsky, M.A.; Beckman, K.; Felkey, K.; Hubbard, A. Resistance exercise reverses aging in human skeletal muscle. PLoS ONE 2007, 2, e465.
330. Petriz, B.A.; Gomes, C.P.; Almeida, J.A.; de Oliveira, G.P., Jr.; Ribeiro, F.M.; Pereira, R.W.; Franco, O.L. The Effects of Acute and Chronic Exercise on Skeletal Muscle Proteome. J. Cell Physiol. 2017, 232, 257–269.
331. Holloway, K.V.; O0Gorman, M.; Woods, P.; Morton, J.P.; Evans, L.; Cable, N.T.; Goldspink, D.F.; Burniston, J.G. Proteomic investigation of changes in human vastus lateralis muscle in response to intervalExercise training. Proteomics 2009, 9, 5155–5174.
332. Burniston, J.G. Changes in the rat skeletal muscle proteome induced by moderate-intensity endurance exercise. Biochim. Biophys. Acta 2008, 1784, 1077–1086.
333. Ruas, J.L.; White, J.P.; Rao, R.R.; Kleiner, S.; Brannan, K.T.; Harrison, B.C.; Greene, N.P.; Wu, J.; Estall, J.L.; Irving, B.A.; et al. A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell 2012, 151, 1319–1331.

334. Coffey, V.G.; Hawley, J.A. Concurrent exercise training: Do opposites distract? J. Physiol. 2017, 595, 2883–2896.
335. Raue, U.; Trappe, T.A.; Estrem, S.T.; Qian, H.R.; Helvering, L.M.; Smith, R.C.; Trappe, S. Transcriptome signature of resistance exercise adaptations: Mixed muscle and fiber type specific profiles in young and old adults. J. Appl. Physiol. (1985) 2012, 112, 1625–1636.
336. St-Amand, J.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Shono, N.; Tanaka, H. Effects of mild-exercise training cessation in human skeletal muscle. Eur. J. Appl. Physiol. 2012, 112, 853–869.
337. Jacobs, K.A.; Krauss, R.M.; Fattor, J.A.; Horning, M.A.; Friedlander, A.L.; Bauer, T.A.; Hagobian, T.A.; Wolfel, E.E.; Brooks, G.A. Endurance training has little effect on active muscle free fatty acid, lipoprotein cholesterol, or triglyceride net balances. Am. J. Physiol. Endocrinol. Metab. 2006, 291, E656–E665.
338. Shono, N.; Urata, H.; Saltin, B.; Mizuno, M.; Harada, T.; Shindo, M.; Tanaka, H. Effects of low intensity aerobic training on skeletal muscle capillary and blood lipoprotein profiles. J. Atheroscler. Thromb. 2002, 9, 78–85.
339. Laguna-Camacho, A. Influence on Adiposity and Atherogenic Lipaemia of Fatty Meals and Snacks in Daily Life. J. Lipids 2017, 2017, 1375342.
340. Ribaroff, G.A.; Wastnedge, E.; Drake, A.J.; Sharpe, R.M.; Chambers, T.J.G. Animal models of maternal high fat diet exposure and effects on metabolism in offspring: A meta-regression analysis. Obes. Rev. 2017, 18, 673–686.
341. Flatt, J.P. The difference in the storage capacities for carbohydrate and for fat, and its implications in the regulation of body weight. Ann. N. Y. Acad. Sci. 1987, 499, 104–123.
342. Maher, T.; Clegg, M.E. Dietary lipids with potential to affect satiety: Mechanisms and evidence. Crit. Rev. Food Sci. Nutr. 2018, 1–26.
343. Lissner, L.; Levitsky, D.A.; Strupp, B.J.; Kalkwarf, H.J.; Roe, D.A. Dietary fat and the regulation of energy intake in human subjects. Am. J. Clin. Nutr. 1987, 46, 886–892.
344. Flatt, J.P.; Ravussin, E.; Acheson, K.J.; Jequier, E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J. Clin. Investig. 1985, 76, 1019–1024.
345. Schutz, Y.; Flatt, J.P.; Jequier, E. Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. Am. J. Clin. Nutr. 1989, 50, 307–314.
346. Keleher, M.R.; Zaidi, R.; Shah, S.; Oakley, M.E.; Pavlatos, C.; El Idrissi, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. PLoS ONE 2018, 13, e0192606.
347. Keleher, M.R.; Zaidi, R.; Hicks, L.; Shah, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. A high-Fat diet alters genome-Wide DNA methylation and gene expression in SM/J mice. BMC Genom. 2018, 19, 888.
348. Suter, M.A.; Ma, J.; Vuguin, P.M.; Hartil, K.; Fiallo, A.; Harris, R.A.; Charron, M.J.; Aagaard, K.M. In utero exposure to a maternal high-Fat diet alters the epigenetic histone code in a murine model. Am. J. Obstet. Gynecol. 2014, 210, 463.E1–463.E11.
349. Wankhade, U.D.; Zhong, Y.; Kang, P.; Alfaro, M.; Chintapalli, S.V.; Thakali, K.M.; Shankar, K. Enhanced offspring predisposition to steatohepatitis with maternal high-Fat diet is associated with epigenetic and microbiome alterations. PLoS ONE 2017, 12, e0175675.
350. Glendining, K.A.; Fisher, L.C.; Jasoni, C.L. Maternal high fat diet alters offspring epigenetic regulators, amygdala glutamatergic profile and anxiety. Psychoneuroendocrinology 2018, 96, 132–141.
351. Smilowitz, J.T.; German, J.B.; Zivkovic, A.M. Food Intake and Obesity: The Case of Fat. In Fat Detection: Taste, Texture, and Post Ingestive Effects; Montmayeur, J.P., le Coutre, J., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2010.
352. Astrup, A. The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of lowFat diets. Int. J. Obes. Relat. Metab. Disord. J. Int. Assoc. Study Obes. 2001, 25 (Suppl. 1), S46–S50.
353. Liu, L.; Wang, Q.; Liu, A.; Lan, X.; Huang, Y.; Zhao, Z.; Jie, H.; Chen, J.; Zhao, Y. Physiological Implications of Orexins/Hypocretins on Energy Metabolism and Adipose Tissue Development. ACS Omega 2020, 5, 547–555.
354. Milbank, E.; López, M. Orexins/Hypocretins: Key Regulators of Energy Homeostasis. Front. Endocrinol. 2019, 10, 830.
355. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-Fat meal-Induced changes in the duodenum mucosa transcriptome. Obes. (Silver Spring Md.) 2008, 16, 2302–2307.

356. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
357. Douglass, J.D.; Malik, N.; Chon, S.H.; Wells, K.; Zhou, Y.X.; Choi, A.S.; Joseph, L.B.; Storch, J. Intestinal mucosal triacylglycerol accumulation secondary to decreased lipid secretion in obese and high fat fed mice. Front. Physiol. 2012, 3, 25.
358. Smolders, L.; Mensink, R.P.; Boekschoten, M.V.; de Ridder, R.J.J.; Plat, J. The acute effects on duodenal gene expression in healthy men following consumption of a low-Fat meal enriched with theobromine or fat. Sci. Rep. 2018, 8, 1700.
359. Martin, C.R.; Osadchiy, V.; Kalani, A.; Mayer, E.A. The Brain-Gut-Microbiome Axis. Cell Mol. Gastroenterol. Hepatol. 2018, 6, 133–148.
360. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-Fat diet-Induced obesity. Obes. (Silver Spring) 2013, 21, 1389–1395.
361. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-Fat diet-Induced obesity. Life Sci. 2018, 215, 190–197.
362. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding regulates the expression of pancreatic genes in gastric mucosa. J. Obes. 2010, 2010, 371950.
363. Bolduc, C.; Yoshioka, M.; St-Amand, J. Acute molecular mechanisms responsive to feeding and meal constitution in mesenteric adipose tissue. Obes. (Silver Spring) 2010, 18, 410–413.
364. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding induced changes in the hypothalamic transcriptome. Clin. Chim. Acta 2009, 406, 103–107.
365. Gondret, F.; Vincent, A.; Houee-Bigot, M.; Siegel, A.; Lagarrigue, S.; Louveau, I.; Causeur, D. Molecular alterations induced by a high-Fat high-Fiber diet in porcine adipose tissues: Variations according to the anatomical fat location. BMC Genom. 2016, 17, 120.
366. Moraes, R.C.; Blondet, A.; Birkenkamp-Demtroeder, K.; Tirard, J.; Orntoft, T.F.; Gertler, A.; Durand, P.; Naville, D.; Bégeot, M. Study of the Alteration of Gene Expression in Adipose Tissue of Diet-Induced Obese Mice by Microarray and Reverse Transcription-Polymerase Chain Reaction Analyses. Endocrinology 2003, 144, 4773–4782.
367. Perez-Schindler, J.; Kanhere, A.; Edwards, L.; Allwood, J.W.; Dunn, W.B.; Schenk, S.; Philp, A. Exercise and high-fat feeding remodel transcript-metabolite interactive networks in mouse skeletal muscle. Sci. Rep. 2017, 7, 13485.
368. Hui, X.; Zhu, W.; Wang, Y.; Lam, K.S.; Zhang, J.; Wu, D.; Kraegen, E.W.; Li, Y.; Xu, A. Major urinary protein-1 increases energy expenditure and improves glucose intolerance through enhancing mitochondrial function in skeletal muscle of diabetic mice. J. Biol. Chem. 2009, 284, 14050–14057.
369. Zhou, Y.; Rui, L. Major urinary protein regulation of chemical communication and nutrient metabolism. Vitam. Horm. 2010, 83, 151–163.
370. Zhou, Y.; Jiang, L.; Rui, L. Identification of MUP1 as a regulator for glucose and lipid metabolism in mice. J. Biol. Chem. 2009, 284, 11152–11159.
371. van Schothorst, E.M.; Keijer, J.; Pennings, J.L.; Opperhuizen, A.; van den Brom, C.E.; Kohl, T.; Franssen-van Hal, N.L.; Hoebee, B. Adipose gene expression response of lean and obese mice to short-Term dietary restriction. Obes. (Silver Spring) 2006, 14, 974–979.
372. Harrell, C.S.; Burgado, J.; Kelly, S.D.; Johnson, Z.P.; Neigh, G.N. High-Fructose diet during periadolescent development increases depressive-Like behavior and remodels the hypothalamic transcriptome in male rats. Psychoneuroendocrinology 2015, 62, 252–264.
373. Ziotopoulou, M.; Mantzoros, C.S.; Hileman, S.M.; Flier, J.S. Differential expression of hypothalamic neuropeptides in the early phase of diet-Induced obesity in mice. Am. J. Physiol. Endocrinol. Metab. 2000, 279, E838–E845.
374. St-Amand, J.; Yoshioka, M.; Tanaka, K.; Nishida, Y. Transcriptome-Wide identification of preferentially expressed genes in the hypothalamus and pituitary gland. Front. Endocrinol. 2011, 2, 111.
375. Bedard, K.; Bedard, J.; Rocheleau, G.; Ferland, G.; Gaudreau, P. Aging and diets regulate the rat anterior pituitary and hypothalamic transcriptome. Neuroendocrinology 2013, 97, 146–159.
376. Takahashi, J.; Waki, S.; Matsumoto, R.; Odake, J.; Miyaji, T.; Tottori, J.; Iwanaga, T.; Iwahashi, H. Oligonucleotide microarray analysis of dietary-induced hyperlipidemia gene expression profiles in miniature pigs. PLoS ONE 2012, 7, e37581.

377. Maruthur, N.M.; Tseng, E.; Hutfless, S.; Wilson, L.M.; Suarez-Cuervo, C.; Berger, Z.; Chu, Y.; Iyoha, E.; Segal, J.B.; Bolen, S. Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. Ann. Intern. Med. 2016, 164, 740–751.
378. Salomaki, H.; Heinaniemi, M.; Vahatalo, L.H.; Ailanen, L.; Eerola, K.; Ruohonen, S.T.; Pesonen, U.; Koulu, M. Prenatal metformin exposure in a maternal high fat diet mouse model alters the transcriptome and modifies the metabolic responses of the offspring. PLoS ONE 2014, 9, e115778.
379. Yoon, G.; Cho, K.A.; Song, J.; Kim, Y.-K. Transcriptomic Analysis of High Fat Diet Fed Mouse Brain Cortex. Front. Genet. 2019, 10.
380. Hansen, D.; Dendale, P.; Berger, J.; van Loon, L.J.; Meeusen, R. The effects of exercise training on fat mass loss in obese patients during energy intake restriction. Sports Med. 2007, 37, 31–46.
381. Miller, C.T.; Fraser, S.F.; Levinger, I.; Straznicky, N.E.; Dixon, J.B.; Reynolds, J.; Selig, S.E. The effects of exercise training in addition to energy restriction on functional capacities and body composition in obese adults during weight losss: A systematic review. PLoS ONE 2013, 8, e81692.
382. Blüher, M. Obesity: Global epidemiology and pathogenesis. Nat. Rev. Endocrinol. 2019, 15, 288–298.
383. Spieker, E.A.; Pyzocha, N. Economic impact of obesity. Prim. Care 2016, 43, 83–95.
384. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
385. Weir, C.B.; Jan, A. BMI Classification Percentile and Cut Off Points; StatPearls Publishing: Treasure Island, FL, USA, 2020.
386. Gutin, I. In BMI we trust: Reframing the body mass index as a measure of health. Soc. Theory Health 2018, 16, 256–271.
387. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken energy homeostasis and obesity pathogenesis: The surrounding concepts. J. Clin. Med. 2018, 7, 453.
388. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a neuroendocrine reprogramming. Medicina 2021, 57, 66.
389. Donini, L.M.; Rosano, A.; di Lazzaro, L.; Lubrano, C.; Carbonelli, M.; Pinto, A.; Giusti, A.M.; Lenzi, A.; Siervo, M. Impact of disability, psychological status, and comorbidity on health-related quality of life perceived by subjects with obesity. Obes. Facts 2020, 13, 191–200.
390. Jokinen, E. Obesity and cardiovascular disease. Minerva Pediatr. 2015, 67, 25–32.
391. Saliba, L.J.; Maffett, S. Hypertensive heart disease and obesity: A review. Heart Fail. Clin. 2019, 15, 509–517.
392. Karczewski, J.; Sledzi´nska, E.; Baturo, A.; Jo´nczyk, I.; Maleszko, A.; Samborski, P.; Begier-Krasi´nska, B.; Dobrowolska, A. Obesity and inflammation. Eur. Cytokine Netw. 2018, 29, 83–94.
393. Cox, A.J.; West, N.P.; Cripps, A.W. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol. 2015, 3, 207–215.
394. Dixon, A.E.; Peters, U. The effect of obesity on lung function. Expert Rev. Respir. Med. 2018, 12, 755–767.
395. Meurling, I.J.; Shea, D.O.; Garvey, J.F. Obesity and sleep: A growing concern. Curr. Opin. Pulm. Med. 2019, 25, 602–608.
396. Tang, X.N.; Liebeskind, D.S.; Towfighi, A. The Role of Diabetes, Obesity, and Metabolic Syndrome in Stroke. Semin. Neurol. 2017, 37, 267–273.
397. Yawoot, N.; Govitrapong, P.; Tocharus, C.; Tocharus, J. Ischemic stroke, obesity, and the antiinflammatory role of melatonin. BioFactors 2021, 47, 41–58.
398. Chobot, A.; Górowska-Kowolik, K.; Sokołowska, M.; Jarosz-Chobot, P. Obesity and diabetes—Not only a simple link between two epidemics. Diabetes Metab. Res. Rev. 2018, 34, e3042.
399. Barnes, A.S. The epidemic of obesity and diabetes: Trends and treatments. Texas Heart Inst. J. 2011, 38, 142–144.
400. Milaneschi, Y.; Simmons, W.K.; van Rossum, E.F.C.; Penninx, B.W. Depression and obesity: Evidence of shared biological mechanisms. Mol. Psychiatry 2019, 24, 18–33.
401. Romain, A.J.; Marleau, J.; Baillot, A. Impact of obesity and mood disorders on physical comorbidities, psychological well-being, health behaviours and use of health services. J. Affect. Disord. 2018, 225, 381–388.
402. Aigner, E.; Feldman, A.; Datz, C. Obesity as an emerging risk factor for iron deficiency. Nutrients 2014, 6, 3587–3600.

403. Cohen, A.; Dempster, D.W.; Recker, R.R.; Lappe, J.M.; Zhou, H.; Zwahlen, A.; Müller, R.; Zhao, B.; Guo, X.E.; Lang, T.; et al. Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: A transiliac bone biopsy study. J. Clin. Endocrinol. Metab. 2013, 98, 2562– 2572.
404. Lloret, A.; Monllor, P.; Esteve, D.; Cervera-Ferri, A.; Lloret, M.-A. Obesity as a risk factor for Alzheimer's disease: Implication of leptin and glutamate. Front. Neurosci. 2019, 13, 508.
405. Shinohara, M.; Tashiro, Y.; Shinohara, M.; Hirokawa, J.; Suzuki, K.; Onishi-Takeya, M.; Mukouzono, M.; Takeda, S.; Saito, T.; Fukumori, A.; et al. Increased levels of Aβ42 decrease the lifespan of ob/ob mice with dysregulation of microglia and astrocytes. FASEB J. 2020, 34, 2425–2435.
406. Mazon, J.N.; de Mello, A.H.; Ferreira, G.K.; Rezin, G.T. The impact of obesity on neurodegenerative diseases. Life Sci. 2017, 182, 22–28.
407. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. Metabolism 2019, 92, 121–135.
408. Pischon, T.; Nimptsch, K. Obesity and risk of cancer: An introductory overview. Methods Mol. Biol. 2016, 208, 1–15.
409. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during obesity: An impaired homeostasis. Animals 2020, 10, 2344.
410. Andersen, T.; Gluud, C. Liver morphology in morbid obesity: A literature study. Int. J. Obes. 1984, 8, 97– 106.
411. Di Meo, S.; Iossa, S.; Venditti, P. Improvement of obesity-linked skeletal muscle insulin resistance by strength and endurance training. J. Endocrinol. 2017, 234, R159–R181.
412. Barazzoni, R.; Bischoff, S.; Boirie, Y.; Busetto, L.; Cederholm, T.; Dicker, D.; Toplak, H.; Van Gossum, A.; Yumuk, V.; Vettor, R. Sarcopenic Obesity: Time to Meet the Challenge. Obes. Facts 2018, 11, 294–305.
413. Dashty, M. A quick look at biochemistry: Carbohydrate metabolism. Clin. Biochem. 2013, 46, 1339–1352.
414. Mili´c, S.; Luli´c, D.; Štimac, D. Non-alcoholic fatty liver disease and obesity: Biochemical, metabolic and clinical presentations. World J. Gastroenterol. 2014, 20, 9330–9337.
415. Engin, A. The Definition and Prevalence of Obesity and Metabolic Syndrome. Adv. Exp. Med. Biol. 2017, 960, 1–17.
416. Unamuno, X.; Gómez-Ambrosi, J.; Rodríguez, A.; Becerril, S.; Frühbeck, G.; Catalán, V. Adipokine dysregulation and adipose tissue inflammation in human obesity. Eur. J. Clin. Investig. 2018, 48, e12997.
417. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
418. Wilding, J.P.H.; Mooney, V.; Pile, R. Should obesity be recognised as a disease? BMJ 2019, 366, l4258.
419. Arrieta, F.; Pedro-Botet, J. Recognizing obesity as a disease: A true challenge. Rev. Clín. Esp. 2020.
420. De Lorenzo, A.; Romano, L.; di Renzo, L.; di Lorenzo, N.; Cenname, G.; Gualtieri, P. Obesity: A preventable, treatable, but relapsing disease. Nutrition 2020, 71, 110615.
421. Christensen, S. Recognizing obesity as a disease. J. Am. Assoc. Nurse Pract. 2020, 32, 497–503.
422. Fuijkschot, W.W.; Morrison, M.C.; Zethof, I.P.; Krijnen, P.A.; Kleemann, R.; Niessen, H.W.; Smulders, Y.M. LPS-induced systemic inflammation does not alter atherosclerotic plaque area or inflammation in APOE3*LEIDEN mice in the early phase up to 15 days. Shock 2018, 50, 360–365.
423. Furman, B.L. Streptozotocin-induced diabetic models in mice and rats. Curr. Protoc. Pharmacol. 2015, 70, 5.47.1–5.47.20.
424. Lutz, T.A.; Woods, S.C. Overview of animal models of obesity. Curr. Protoc. Pharmacol. 2012, 58, 5.61.1–5.61.18.
425. Kleinert, M.; Clemmensen, C.; Hofmann, S.M.; Moore, M.C.; Renner, S.; Woods, S.C.; Huypens, P.; Beckers, J.; de Angelis, M.H.; Schürmann, A.; et al. Animal models of obesity and diabetes mellitus. Nat. Rev. Endocrinol. 2018, 14, 140–162.
426. Soto, M.; Orliaguet, L.; Reyzer, M.L.; Manier, M.L.; Caprioli, R.M.; Kahn, C.R. Pyruvate induces torpor in obese mice. Proc. Natl. Acad. Sci. USA 2018, 115, 810–815.
427. Wang, C.Y.; Liao, J.K. A mouse model of diet-induced obesity and insulin resistance. Methods Mol. Biol. 2012, 821, 421–433.
428. Shin, J.-H.; Nam, M.H.; Lee, H.; Lee, J.-S.; Kim, H.; Chung, M.-J.; Seo, J.-G. Amelioration of obesityrelated characteristics by a probiotic formulation in a high-fat diet-induced obese rat model. Eur. J. Nutr. 2017, 57, 2081–2090.

429. Val-Laillet, D. Review: Impact of food, gut–brain signals and metabolic status on brain activity in the pig model: 10 years of nutrition research using in vivo brain imaging. Animal 2019, 13, 2699–2713.
430. Jerez, S.; Scacchi, F.; Sierra, L.; Karbiner, S.; de Bruno, M.P. Vascular hyporeactivity to angiotensin II and noradrenaline in a rabbit model of obesity. J. Cardiovasc. Pharmacol. 2012, 59, 49–57.
431. Siino, V.; Amato, A.; di Salvo, F.; Caldara, G.F.; Filogamo, M.; James, P.; Vasto, S. Impact of dietinduced obesity on the mouse brain phosphoproteome. J. Nutr. Biochem. 2018, 58, 102–109.
432. Lopomo, A.; Burgio, E.; Migliore, L. Epigenetics of obesity. Prog. Mol. Biol. Transl. Sci. 2016, 140, 151– 184.
433. Catalano, P.M.; Shankar, K. Obesity and pregnancy: Mechanisms of short term and long term adverse consequences for mother and child. BMJ 2017, 356, j1.
434. Goossens, G.H. The metabolic phenotype in obesity: Fat mass, body fat distribution, and adipose tissue function. Obes. Facts 2017, 10, 207–215.
435. Yin, D.P.; Gao, Q.; Ma, L.L.; Yan, W.; Williams, P.E.; McGuinness, O.P.; Wasserman, D.H.; Abumrad, N.N. Assessment of different bariatric surgeries in the treatment of obesity and insulin resistance in mice. Ann. Surg. 2011, 254, 73–82.
436. Houpt, K.A.; Houpt, T.R.; Pond, W.G. The pig as a model for the study of obesity and of control of food intake: A review. Yale J. Boil. Med. 1979, 52, 307–329.
437. Tang, L.-L.; Tang, X.-H.; Li, X.; Yu, H.-B.; Xie, Z.-G.; Liu, X.-Y.; Zhou, Z.-G. Effect of high-fat or highglucose diet on obesity and visceral adipose tissue in mice. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2014, 36, 614–619.
438. Chen, H.; Guan, K.; Qi, X.; Wang, R.; Ma, Y. α-lactalbumin ameliorates hepatic lipid metabolism in highfat-diet induced obese C57BL/6J mice. J. Funct. Foods 2020, 75, 104253.
439. Chu, D.-T.; Malinowska, E.; Jura, M.; Kozak, L.P. C57BL/6J mice as a polygenic developmental model of diet-induced obesity. Physiol. Rep. 2017, 5, e13093.
440. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
441. Zhou, A.; Ward, R. Milk polar lipids modulate lipid metabolism, gut permeability, and systemic inflammation in high-fat-fed C57BL/6J ob/ob mice, a model of severe obesity. J. Dairy Sci. 2019, 102, 4816– 4831.
442. Ahmad, A.; Ali, T.; Kim, M.W.; Khan, A.; Jo, M.H.; Rehman, S.U.; Khan, M.S.; Abid, N.B.; Khan, M.; Ullah, R.; et al. Adiponectin homolog novel osmotin protects obesity/diabetes-induced NAFLD by upregulating AdipoRs/PPARα signaling in ob/ob and db/db transgenic mouse models. Metabolism 2019, 90, 31–43.
443. Yanagita, T.; Tsuge, K.; Koga, M.; Inoue, N.; Nagao, K. Eicosapentaenoic acid-containing polar lipids from seaweed Susabinori (Pyropia yezoensis) alleviate hepatic steatosis in obese db/db mice. Arch. Biochem. Biophys. 2020, 691, 108486.
444. Noratto, G.D.; Murphy, K.; Chew, B.P. Quinoa intake reduces plasma and liver cholesterol, lessens obesity-associated inflammation, and helps to prevent hepatic steatosis in obese db/db mouse. Food Chem. 2019, 287, 107–114.
445. Grigorova, N.; Ivanova, Z.; Bjorndal, B.; Vachkova, E.; Penchev, G.; Berge, R.; Ribarski, S.; Georgieva, T.M.; Yonkova, P.; Georgiev, I.P. Effect of fish oil supplementation and restricted feeding on body fat distribution and blood lipid profile in a rabbit model of castration-induced obesity. Res. Vet. Sci. 2019, 124, 99–105.
446. Georgiev, I.P.; Georgieva, T.M.; Ivanov, V.; Dimitrova, S.; Kanelov, I.; Vlaykova, T.; Tanev, S.; Zaprianova, D.; Dichlianova, E.; Penchev, G.; et al. Effects of castration-induced visceral obesity and antioxidant treatment on lipid profile and insulin sensitivity in New Zealand white rabbits. Res. Vet. Sci. 2011, 90, 196–204.
447. Keleher, M.R.; Zaidi, R.; Shah, S.; Oakley, M.E.; Pavlatos, C.; El Idrissi, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. PLoS ONE 2018, 13, e0192606.
448. Liu, J.; He, Z.; Ma, N.; Chen, Z.Y. Beneficial Effects of Dietary Polyphenols on High-Fat Diet-Induced Obesity Linking with Modulation of Gut Microbiota. J. Agric. Food Chem. 2020, 68, 33–47.
449. Herbert, D.; Franz, S.; Popkova, Y.; Anderegg, U.; Schiller, J.; Schwede, K.; Lorz, A.; Simon, J.C.; Saalbach, A. High-fat diet exacerbates early psoriatic skin inflammation independent of obesity: Saturated fatty acids as key players. J. Investig. Dermatol. 2018, 138, 1999–2009.

450. Liu, Y.; Lu, X.; Li, X.; Du, P.; Qin, G. High-fat diet triggers obesity-related early infiltration of macrophages into adipose tissue and transient reduction of blood monocyte count. Mol. Immunol. 2020, 117, 139–146.
451. Tung, Y.-T.; Chen, H.-L.; Wu, H.-S.; Ho, M.-H.; Chong, K.-Y.; Chen, C.-M. Kefir peptides prevent hyperlipidemia and obesity in high-fat-diet-induced obese rats via lipid metabolism modulation. Mol. Nutr. Food Res. 2018, 62, 62.
452. Heydemann, A. An overview of murine high fat diet as a model for type 2 diabetes mellitus. J. Diabetes Res. 2016, 2016, 2902351.
453. Varlamov, O. Western-style diet, sex steroids and metabolism. Biochim. Biophys. Acta Mol. Basis Dis. 2017, 1863, 1147–1155.
454. Komorowski, J.M. Role of the hypothalamus in the pathogenesis of obesity in the light of modern studies. Pol. Tyg. Lek. 1978, 33, 657–659.
455. Mercer, J.G.; Speakman, J.R. Hypothalamic neuropeptide mechanisms for regulating energy balance: From rodent models to human obesity. Neurosci. Biobehav. Rev. 2001, 25, 101–116.
456. Nishida, Y.; Yoshioka, M.; St-Amand, J. Regulation of hypothalamic gene expression by glucocorticoid: Implications for energy homeostasis. Physiol. Genomics 2006, 25, 96–104.
457. Nishida, Y.; Yoshioka, M.; St-Amand, J. The top 10 most abundant transcripts are sufficient to characterize the organs functional specificity: Evidences from the cortex, hypothalamus and pituitary gland. Gene 2005, 344, 133–141.
458. Thaler, J.P.; Yi, C.X.; Schur, E.A.; Guyenet, S.J.; Hwang, B.H.; Dietrich, M.O.; Zhao, X.; Sarruf, D.A.; Izgur, V.; Maravilla, K.R.; et al. Obesity is associated with hypothalamic injury in rodents and humans. J. Clin. Investig. 2012, 122, 153–162.
459. Velloso, L.A.; Schwartz, M.W. Altered hypothalamic function in diet-induced obesity. Int. J. Obes. (Lond.) 2011, 35, 1455–1465.
460. Mercer, J.G.; Archer, Z.A. Putting the diet back into diet-induced obesity: Diet-induced hypothalamic gene expression. Eur. J. Pharmacol. 2008, 585, 31–37.
461. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding induced changes in the hypothalamic transcriptome. Clin. Chim. Acta 2009, 406, 103–107.
462. Stahl, S.M. Neuropharmacology of obesity: My receptors made me eat it. J. Clin. Psychiatry 1998, 59, 447–448.
463. Moore, C.F.; Panciera, J.I.; Sabino, V.; Cottone, P. Neuropharmacology of compulsive eating. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2018, 373, 20170024.
464. Leibowitz, S.F.; Alexander, J.T. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biol. Psychiatry 1998, 44, 851–864.
465. Weiss, G.F.; Rogacki, N.; Fueg, A.; Buchen, D.; Leibowitz, S.F. Impact of hypothalamic d norfenfluramine and peripheral d-fenfluramine injection on macronutrient intake in the rat. Brain Res. Bull. 1990, 25, 849–859.
466. Weiss, G.F.; Rogacki, N.; Fueg, A.; Buchen, D.; Suh, J.S.; Wong, D.T.; Leibowitz, S.F. Effect of hypothalamic and peripheral fluoxetine injection on natural patterns of macronutrient intake in the rat. Psychopharmacology 1991, 105, 467–476.
467. van Lingen, M.; Sidorova, M.; Alenina, N.; Klempin, F. Lack of Brain Serotonin Affects Feeding and Differentiation of Newborn Cells in the Adult Hypothalamus. Front. Cell Dev. Biol. 2019, 7, 65.
468. Rao, D.P.; Patel, P.; Roberts, K.C.; Thompson, W. Obesity and healthy aging: Social, functional and mental well-being among older Canadians. Health Promot. Chronic Dis. Prev. Can. 2018, 38, 437–444.
469. Anonymous. Body mass index (BMI) charts. Pediatr. Dent. 2017, 39, 461–462.
470. Chu, D.-T.; Nguyet, N.T.M.; Nga, V.T.; Lien, N.V.T.; Vo, D.D.; Ngoc, V.T.N.; Son, L.H.; Le, D.-H.; Van Tu, P.; Van To, T.; et al. An update on obesity: Mental consequences and psychological interventions. Diabetes Metab. Syndr. Clin. Res. Rev. 2019, 13, 155–160.
471. Oliosa, P.R.; Zaniqueli, D.; Alvim, R.O.; Barbosa, M.C.R.; Mill, J.G. Body fat percentage is better than indicators of weight status to identify children and adolescents with unfavorable lipid profile. J. Pediatr. 2019, 95, 112–118.
472. Rogers, P.; Webb, G.P. Estimation of body fat in normal and obese mice. Br. J. Nutr. 1980, 43, 83–86.
473. Air, E.L.; Strowski, M.Z.; Benoit, S.C.; Conarello, S.L.; Salituro, G.M.; Guan, X.-M.; Liu, K.; Woods, S.C.; Zhang, B.B. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nat. Med. 2002, 8, 179–183.

474. Melega, W.P.; Lacan, G.; Gorgulho, A.A.; Behnke, E.J.; de Salles, A.A.F. Hypothalamic deep brain stimulation reduces weight gain in an obesity-animal model. PLoS ONE 2012, 7, e30672.
475. Shoelson, S.E.; Herrero, L.; Naaz, A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007, 132, 2169–2180.
476. Fock, K.M.; Khoo, J. Diet and exercise in management of obesity and overweight. J. Gastroenterol. Hepatol. 2013, 28, 59–63.
477. Rebello, C.J.; Greenway, F.L. Obesity medications in development. Expert Opin. Investig. Drugs 2020, 29, 63–71.
478. Wolfe, B.M.; Kvach, E.; Eckel, R.H. Treatment of obesity: Weight loss and bariatric surgery. Circ. Res. 2016, 118, 1844–1855.
479. Monteiro, R.; Azevedo, I. Chronic inflammation in obesity and the metabolic syndrome. Mediat. Inflamm. 2010, 2010, 1–10.
480. Sonne, S.B.; Yadav, R.; Yin, G.; Dalgaard, M.D.; Myrmel, L.S.; Gupta, R.; Wang, J.; Madsen, L.; Kajimura, S.; Kristiansen, K. Obesity is associated with depot-specific alterations in adipocyte DNA methylation and gene expression. Adipocyte 2017, 6, 124–133.
481. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and high-fat diet in obesity: Functional genomics perspectives of two energy homeostasis pillars. Genes 2020, 11, 875.
482. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
483. Sardon Puig, L.; Pillon, N.J.; Näslund, E.; Krook, A.; Zierath, J.R. Influence of obesity, weight loss, and free fatty acids on skeletal muscle clock gene expression. Am. J. Physiol. Endocrinol. Metab. 2020, 318, e1– e10.
484. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-fat diet-induced trefoil factor family member 2 (TFF2) to counteract the immunemediated damage in mice. Animals 2021, 11, 258.
485. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine as a regeneration factor: Beyond the tissue repair. Life 2021, 11, 38.
486. Drel, V.R.; Mashtalir, N.; Ilnytska, O.; Shin, J.; Li, F.; Lyzogubov, V.V.; Obrosova, I.G. The leptindeficient (ob/ob) mouse: A new animal model of peripheral neuropathy of type 2 diabetes and obesity. Diabetes 2006, 55, 3335–3343.
487. Malloy, V.L.; Perrone, C.E.; Mattocks, D.A.; Ables, G.P.; Caliendo, N.S.; Orentreich, D.S.; Orentreich, N. Methionine restriction prevents the progression of hepatic steatosis in leptin-deficient obese mice. Metabolism 2013, 62, 1651–1661.
488. Neelankal John, A.; Ram, R.; Jiang, F.X. RNA-seq analysis of islets to characterise the dedifferentiation in type 2 diabetes model mice db/db. Endocr. Pathol. 2018, 29, 207–221.
489. Bogdanov, P.; Corraliza, L.; Villena, J.A.; Carvalho, A.R.; García-Arumí, J.; Ramos, D.; Ruberte, J.; Simó, R.; Hernández, C. The db/db mouse: A useful model for the study of diabetic retinal neurodegeneration. PLoS ONE 2014, 9, e97302.
490. Vareniuk, I.; Pavlov, I.A.; Drel, V.R.; Lyzogubov, V.V.; Ilnytska, O.; Bell, S.R.; Tibrewala, J.; Groves, J.T.; Obrosova, I.G. Nitrosative stress and peripheral diabetic neuropathy in leptin-deficient (ob/ob) mice. Exp. Neurol. 2007, 205, 425–436.
491. Yu, F.; Han, W.; Zhan, G.; Li, S.; Jiang, X.; Wang, L.; Xiang, S.; Zhu, B.; Yang, L.; Luo, A.; et al. Abnormal gut microbiota composition contributes to the development of type 2 diabetes mellitus in db/db mice. Aging 2019, 11, 10454–10467.
492. Larter, C.Z.; Yeh, M.M.; van Rooyen, D.M.; Teoh, N.C.; Brooling, J.; Hou, J.Y.; Williams, J.; Clyne, M.; Nolan, C.J.; Farrell, G.C. Roles of adipose restriction and metabolic factors in progression of steatosis to steatohepatitis in obese, diabetic mice. J. Gastroenterol. Hepatol. 2009, 24, 1658–1668.
493. Lauterbach, M.A.R.; Wunderlich, F.T. Macrophage function in obesity-induced inflammation and insulin resistance. Pflügers Archiv 2017, 469, 385–396.
494. Van der Heijden, R.A.; Sheedfar, F.; Morrison, M.C.; Hommelberg, P.P.; Kor, D.; Kloosterhuis, N.J.; Gruben, N.; Youssef, S.A.; de Bruin, A.; Hofker, M.H.; et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. Aging 2015, 7, 256–268.
495. Engin, A. Obesity-associated breast cancer: Analysis of risk factors. Adv. Exp. Med. Biol. 2017, 960, 571–606.
496. Kompaniyets, L.; Goodman, A.B.; Belay, B.; Freedman, D.S.; Sucosky, M.S.; Lange, S.J.; Gundlapalli, A.V.; Boehmer, T.K.; Blanck, H.M. Body mass index and risk for COVID-19—Related hospitalization,

intensive care unit admission, invasive mechanical ventilation, and death—United States, March–December 2020. MMWR Morb. Mortal Wkly. Rep. 2021, 70, 355–361.
497. Torre, L.A.; Siegel, R.L.; Ward, E.M.; Jemal, A. Global cancer incidence and mortality rates and trends—An update. Cancer Epidemiol. Biomark. Prev. 2015, 25, 16–27.
498. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer 2014, 136, E359–E386.
499. Duranti, S.; Ferrario, C.; van Sinderen, D.; Ventura, M.; Turroni, F. Obesity and microbiota: An example of an intricate relationship. Genes Nutr. 2017, 12, 18.
500. Madsen, L.; Myrmel, L.S.; Fjære, E.; Liaset, B.; Kristiansen, K. Links between Dietary Protein Sources, the Gut Microbiota, and Obesity. Front Physiol. 2017, 8, 1047.
501. Cuevas-Sierra, A.; Ramos-Lopez, O.; Riezu-Boj, J.I.; Milagro, F.I.; Martinez, J.A. Diet, Gut Microbiota, and Obesity: Links with Host Genetics and Epigenetics and Potential Applications. Adv. Nutr. 2019, 10, s17– s30.
502. Lee, P.; Yacyshyn, B.R.; Yacyshyn, M.B. Gut microbiota and obesity: An opportunity to alter obesity through faecal microbiota transplant (FMT). Diabetes Obes. Metab. 2019, 21, 479–490.
503. Marotz, C.A.; Zarrinpar, A. Treating Obesity and Metabolic Syndrome with Fecal Microbiota Transplantation. Yale J. Biol. Med. 2016, 89, 383–388.
504. Aron-Wisnewsky, J.; Clément, K.; Nieuwdorp, M. Fecal Microbiota Transplantation: A Future Therapeutic Option for Obesity/Diabetes? Curr. Diab. Rep. 2019, 19, 51.
505. Kang, Y.; Cai, Y. Gut microbiota and obesity: Implications for fecal microbiota transplantation therapy. Hormones (Athens) 2017, 16, 223–234.
506. Mazloom, K.; Siddiqi, I.; Covasa, M. Probiotics: How Effective Are They in the Fight against Obesity? Nutrients 2019, 11, 258.
507. Fontané, L.; Benaiges, D.; Goday, A.; Llauradó, G.; Pedro-Botet, J. Influence of the microbiota and probiotics in obesity. Clin. Investig. Arterioscler. 2018, 30, 271–279.
508. Nova, E.; Pérez de Heredia, F.; Gómez-Martínez, S.; Marcos, A. The Role of Probiotics on the Microbiota: Effect on Obesity. Nutr Clin. Pract. 2016, 31, 387–400.
509. Katsiki, N.; Tousoulis, D. Diabetes mellitus and comorbidities: A bad romance. Hellenic J. Cardiol. 2020, 61, 23–25.
510. Lehrke, M.; Marx, N. Diabetes mellitus and heart failure. Am. J. Cardiol. 2017, 120, S37–S47.
511. Wojciechowska, J.; Krajewski, W.; Bolanowski, M.; Kr˛ecicki, T.; Zato´nski, T. Diabetes and cancer: A review of current knowledge. Exp. Clin. Endocrinol. Diabetes 2016, 124, 263–275.
512. Cryer, M.J.; Horani, T.; DiPette, D.J. Diabetes and hypertension: A comparative review of current guidelines. J. Clin. Hypertens. 2016, 18, 95–100.
513. Shifflett, K.; Marzi, A. Marburg virus pathogenesis—Differences and similarities in humans and animal models. Virol. J. 2019, 16, 165.
514. Chooi, Y.C.; Ding, C.; Magkos, F. The epidemiology of obesity. Metabolism 2019, 92, 6–10.
515. Habek, M. Immune and autonomic nervous system interactions in multiple sclerosis: Clinical implications. Clin. Auton. Res. 2019, 29, 267–275.
516. Renner, U.; Sapochnik, M.; Lucia, K.; Stalla, G.K.; Arzt, E. Intrahypophyseal Immune-Endocrine Interactions: Endocrine Integration of the Inflammatory Inputs. Front. Horm. Res. 2017, 48, 37–47.
517. Brizi´c, I.; Hiršl, L.; Britt, W.J.; Krmpoti´c, A.; Jonji´c, S. Immune responses to congenital cytomegalovirus infection. Microbes. Infect. 2018, 20, 543–551.
518. Brown, K.; DeCoffe, D.; Molcan, E.; Gibson, D.L. Diet-Induced Dysbiosis of the Intestinal Microbiota and the Effects on Immunity and Disease. Nutrients 2012, 4, 1095–1119.
519. Sato Mito, N.; Suzui, M.; Yoshino, H.; Kaburagi, T.; Sato, K. Long term effects of high fat and sucrose diets on obesity and lymphocyte proliferation in mice. J. Nutr. Health Aging 2009, 13, 602–606.
520. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
521. Keleher, M.R.; Zaidi, R.; Shah, S.; Oakley, M.E.; Pavlatos, C.; El Idrissi, S.; Xing, X.; Li, D.; Wang, T.; Cheverud, J.M. Maternal high-fat diet associated with altered gene expression, DNA methylation, and obesity risk in mouse offspring. PLoS ONE 2018, 13, e0192606.
522. Heydemann, A. An Overview of Murine High Fat Diet as a Model for Type 2 Diabetes Mellitus. J. Diabetes Res. 2016, 2016, 2902351.

523. Udomkasemsab, A.; Prangthip, P. High fat diet for induced dyslipidemia and cardiac pathological alterations in Wistar rats compared to Sprague Dawley rats. Clin. Investig. Arterioscler. 2019, 31, 56–62.
524. Recena Aydos, L.; Aparecida do Amaral, L.; Serafim de Souza, R.; Jacobowski, A.C.; Freitas Dos Santos, E.; Rodrigues Macedo, M.L. Nonalcoholic Fatty Liver Disease Induced by High-Fat Diet in C57bl/6 Models. Nutrients 2019, 11, 3067.
525. Kurt-Jones, E.A.; Cao, L.; Sandor, F.; Rogers, A.B.; Whary, M.T.; Nambiar, P.R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; et al. Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. Infect. Immun. 2007, 75, 471– 480.
526. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an InflammatoryInduced and AntiInflammatory Tissue Repair Factor. Animals 2020, 10, 1646.
527. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 1–10.
528. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity (Silver Spring) 2008, 16, 2302–2307.
529. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity (Silver Spring) 2013, 21, 1389–1395.
530. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
531. Nakandakari, S.; Muñoz, V.R.; Kuga, G.K.; Gaspar, R.C.; Sant'Ana, M.R.; Pavan, I.C.B.; da Silva, L.G.S.; Morelli, A.P.; Simabuco, F.M.; da Silva, A.S.R.; et al. Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice. Brain Behav. Immun.
2019, 79, 284–293.
532. Schachter, J.; Martel, J.; Lin, C.S.; Chang, C.J.; Wu, T.R.; Lu, C.C.; Ko, Y.F.; Lai, H.C.; Ojcius, D.M.; Young, J.D. Effects of obesity on depression: A role for inflammation and the gut microbiota. Brain Behav. Immun. 2018, 69, 1–8.
533. Lissner, L.; Levitsky, D.A.; Strupp, B.J.; Kalkwarf, H.J.; Roe, D.A. Dietary fat and the regulation of energy intake in human subjects. Am. J. Clin. Nutr. 1987, 46, 886–892.
534. Schutz, Y.; Flatt, J.P.; Jéquier, E. Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. Am. J. Clin. Nutr. 1989, 50, 307–314.
535. Andersen, C.J.; Murphy, K.E.; Fernandez, M.L. Impact of Obesity and Metabolic Syndrome on Immunity. Adv. Nutr. 2016, 7, 66–75.
536. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
537. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. Nat. Rev. Mol. Cell Biol. 2003, 4, 721–732.
538. Tran, C.P.; Cook, G.A.; Yeomans, N.D.; Thim, L.; Giraud, A.S. Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. Gut 1999, 44, 636–642.
539. Baus-Loncar, M.; Kayademir, T.; Takaishi, S.; Wang, T. Trefoil factor family 2 deficiency and immune response. Cell Mol. Life Sci. 2005, 62, 2947–2955.
540. Baus-Loncar, M.; Schmid, J.; Lalani el, N.; Rosewell, I.; Goodlad, R.A.; Stamp, G.W.; Blin, N.; Kayademir, T. Trefoil factor 2 (TFF2) deficiency in murine digestive tract influences the immune system. Cell Physiol. Biochem. 2005, 16, 31–42.
541. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. Am. J. Physiol. Gastrointest.Liver. Physiol. 2015, 308, G12–G24.
542. Kaise, M.; Miwa, J.; Fujimoto, A.; Tashiro, J.; Tagami, D.; Sano, H.; Ohmoto, Y. Influence of Helicobacter pylori status and eradication on the serum levels of trefoil factors and pepsinogen test: Serum trefoil factor 3 is a stable biomarker. Gastric Cancer 2013, 16, 329–337.

543. Fox, J.G.; Rogers, A.B.; Whary, M.T.; Ge, Z.; Ohtani, M.; Jones, E.K.; Wang, T.C. Accelerated progression of gastritis to dysplasia in the pyloric antrum of TFF2 -/- C57BL6 x Sv129 Helicobacter pyloriinfected mice. Am. J. Pathol. 2007, 171, 1520–1528.
544. Mobley, H.L. The role of Helicobacter pylori urease in the pathogenesis of gastritis and peptic ulceration. Aliment. Pharmacol. Ther. 1996, 10 (Suppl. 1), 57–64.
545. Kalwat, M.A.; Cobb, M.H. Mechanisms of the amplifying pathway of insulin secretion in the β cell. Pharmacol. Ther. 2017, 179, 17–30.
546. Madsen, J.; Nielsen, O.; Tornøe, I.; Thim, L.; Holmskov, U. Tissue localization of human trefoil factors 1, 2, and 3. J. Histochem. Cytochem. 2007, 55, 505–513.
547. Hoffmann, W.; Jagla, W.; Wiede, A. Molecular medicine of TFF-peptides: From gut to brain. Histol. Histopathol. 2001, 16, 319–334.
548. Hasebe, K.; Rivera, L.R.; Smith, C.M.; Allnutt, T.; Crowley, T.; Nelson, T.M.; Dean, O.M.; McGee, S.L.; Walder, K.; Gray, L. Modulation of high fat diet-induced microbiome changes, but not behaviour, by minocycline. Brain Behav. Immun. 2019, 82, 309–318.
549. Hassan, A.M.; Mancano, G.; Kashofer, K.; Fröhlich, E.E.; Matak, A.; Mayerhofer, R.; Reichmann, F.; Olivares, M.; Neyrinck, A.M.; Delzenne, N.M.; et al. High-fat diet induces depression-like behaviour in mice associated with changes in microbiome, neuropeptide Y, and brain metabolome. Nutr. Neurosci. 2019, 22, 877–893.
550. Lambring, C.B.; Siraj, S.; Patel, K.; Sankpal, U.T.; Mathew, S.; Basha, R. Impact of the Microbiome on the Immune System. Crit. Rev. Immunol. 2019, 39, 313–328.
551. Shi, N.; Li, N.; Duan, X.; Niu, H. Interaction between the gut microbiome and mucosal immune system. Mil. Med. Res. 2017, 4, 14.
552. Ticinesi, A.; Lauretani, F.; Tana, C.; Nouvenne, A.; Ridolo, E.; Meschi, T. Exercise and immune system as modulators of intestinal microbiome: Implications for the gut-muscle axis hypothesis. Exerc. Immunol.
Rev. 2019, 25, 84–95.
553. Cândido, F.G.; Valente, F.X.; Grze´skowiak, Ł.M.; Moreira, A.P.B.; Rocha, D.; Alfenas, R.C.G. Impact of dietary fat on gut microbiota and low-grade systemic inflammation: Mechanisms and clinical implications on obesity. Int. J. Food Sci. Nutr. 2018, 69, 125–143.
554. Netto Candido, T.L.; Bressan, J.; Alfenas, R.C.G. Dysbiosis and metabolic endotoxemia induced by highfat diet. Nutr. Hosp. 2018, 35, 1432–1440.
555. Khoder, G.; Al-Yassir, F.; Al Menhali, A.; Saseedharan, P.; Sugathan, S.; Tomasetto, C.; Karam, S.M. Probiotics Upregulate Trefoil Factors and Downregulate Pepsinogen in the Mouse Stomach. Int. J. Mol. Sci. 2019, 20, 3901.
556. Chen, X.; Hu, Y.; Xie, Y.; Wang, Y. High salt diet can down-regulate TFF2 expression level in gastric mucosa of MGs after H. pylori infection. Microb. Pathog. 2018, 118, 316–321.
557. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
558. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
559. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Méd. Hypotheses 2020, 144, 110042.
560. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
561. Jørgensen, K.H.; Thim, L.; Jacobsen, H.E. Pancreatic spasmolytic polypeptide (PSP): I. Preparation and initial chemical characterization of a new polypeptide from porcine pancreas. Regul. Pept. 1982, 3, 207–219.
562. Royce, S.G.; Lim, C.; Muljadi, R.C.; Samuel, C.S.; Ververis, K.; Karagiannis, T.C.; Giraud, A.S.; Tang, M.L. Trefoil factor-2 reverses airway remodeling changes in allergic airways disease. Am. J. Respir. Cell Mol. Biol. 2013, 48, 135–144.
563. Farrell, J.J.; Taupin, D.; Koh, T.J.; Chen, D.; Zhao, C.-M.; Podolsky, D.K.; Wang, T.C. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. J. Clin. Investig. 2002, 109, 193–204.
564. Aamann, L.; Vestergaard, E.M.; Grønbæk, H. Trefoil factors in inflammatory bowel disease. World J. Gastroenterol. 2014, 20, 3223–3230.

565. Playford, R.J.; Marchbank, T.; Chinery, R.; Evison, R.; Pignatelli, M.; Boulton, R.A.; Thim, L.; Hanby, A.M. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroenterology 1995, 108, 108–116.
566. McKenzie, C.; Marchbank, T.; Playford, R.J.; Otto, W.; Thim, L.; Parsons, M.E. Pancreatic spasmolytic polypeptide protects the gastric mucosa but does not inhibit acid secretion or motility. Am. J. Physiol. 1997, 273 Pt 1, G112–G117.
567. Aihara, E.; Engevik, K.A.; Montrose, M.H. Trefoil Factor Peptides and Gastrointestinal Function. Annu. Rev. Physiol. 2017, 79, 357–380.
568. Greeley, M.A.; Van Winkle, L.S.; Edwards, P.C.; Plopper, C.G. Airway trefoil factor expression during naphthalene injury and repair. Toxicol. Sci. 2010, 113, 453–467.
569. Heuer, F.; Stürmer, R.; Heuer, J.; Kalinski, T.; Lemke, A.; Meyer, F.; Hoffmann, W. Different Forms of TFF2, A Lectin of the Human Gastric Mucus Barrier: In Vitro Binding Studies. Int. J. Mol. Sci. 2019, 20, 5871.
570. Royce, S.G.; Lim, C.; Muljadi, R.C.; Tang, M.L. Trefoil factor 2 regulates airway remodeling in animal models of asthma. J. Asthma 2011, 48, 653–659.
571. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
572. Katoh, M. Trefoil factors and human gastric cancer (review). Int. J. Mol. Med. 2003, 12, 3–9.
573. Konturek, P.C.; Brzozowski, T.; Pierzchalski, P.; Kwiecien, S.; Pajdo, R.; Hahn, E.G.; Konturek, S.J. Activation of genes for spasmolytic peptide, transforming growth factor alpha and for cyclooxygenase (COX)-1 and COX-2 during gastric adaptation to aspirin damage in rats. Aliment. Pharm. 1998, 12, 767–777.
574. Hanisch, F.G.; Bonar, D.; Schloerer, N.; Schroten, H. Human trefoil factor 2 is a lectin that binds α-GlcNAc-capped mucin glycans with antibiotic activity against Helicobacter pylori. J. Biol. Chem. 2014, 289, 27363–27375.
575. Sébert, M.; Sola-Tapias, N.; Mas, E.; Barreau, F.; Ferrand, A. Protease-Activated Receptors in the Intestine: Focus on Inflammation and Cancer. Front. Endocrinol. (Lausanne) 2019, 10, 717.
576. Ma, K.; Lu, N.; Zou, F.; Meng, F.Z. Sirtuins as novel targets in the pathogenesis of airway inflammation in bronchial asthma. Eur. J. Pharm. 2019, 865, 172670.
577. Schuliga, M. NF-kappaB Signaling in Chronic Inflammatory Airway Disease. Biomolecules 2015, 5, 1266–1283.
578. Hoffmann, W. TFF2, a MUC6-binding lectin stabilizing the gastric mucus barrier and more (Review). Int. J. Oncol. 2015, 47, 806–816.
579. Ortiz-Masiá, D.; Hernández, C.; Quintana, E.; Velázquez, M.; Cebrián, S.; Riaño, A.; Calatayud, S.; Esplugues, J.V.; Barrachina, M.D. iNOS-derived nitric oxide mediates the increase in TFF2 expression associated with gastric damage: Role of HIF-1. FASEB J. 2010, 24, 136–145.
580. Nikolaidis, N.M.; Zimmermann, N.; King, N.E.; Mishra, A.; Pope, S.M.; Finkelman, F.D.; Rothenberg, M.E. Trefoil factor-2 is an allergen-induced gene regulated by Th2 cytokines and STAT6 in the lung. Am. J. Respir. Cell Mol. Biol. 2003, 29, 458–464.
581. Knipper, J.A.; Willenborg, S.; Brinckmann, J.; Bloch, W.; Maaß, T.; Wagener, R.; Krieg, T.; Sutherland, T.; Munitz, A.; Rothenberg, M.E.; et al. Interleukin-4 Receptor α Signaling in Myeloid Cells Controls Collagen Fibril Assembly in Skin Repair. Immunity 2015, 43, 803–816.
582. Balaji, S.; Wang, X.; King, A.; Le, L.D.; Bhattacharya, S.S.; Moles, C.M.; Butte, M.J.; de Jesus Perez, V.A.; Liechty, K.W.; Wight, T.N.; et al. Interleukin-10-mediated regenerative postnatal tissue repair is dependent on regulation of hyaluronan metabolism via fibroblast-specific STAT3 signaling. FASEB J. 2017, 31, 868–881.
583. Hadian, Y.; Bagood, M.D.; Dahle, S.E.; Sood, A.; Isseroff, R.R. Interleukin-17: Potential Target for Chronic Wounds. Mediat. Inflamm. 2019, 2019, 1297675.
584. Hernández, C.; Santamatilde, E.; McCreath, K.J.; Cervera, A.M.; Díez, I.; Ortiz-Masiá, D.; Martínez, N.; Calatayud, S.; Esplugues, J.V.; Barrachina, M.D. Induction of trefoil factor (TFF)1, TFF2 and TFF3 by hypoxia is mediated by hypoxia inducible factor-1: Implications for gastric mucosal healing. Br. J. Pharmacol. 2009, 156, 262–272.
585. Azarschab, P.; Al-Azzeh, E.; Kornberger, W.; Götte, P. Aspirin promotes TFF2 gene activation in human gastric cancer cell lines. FEBS Lett. 2001, 488, 206–210.

586. Hu, G.Y.; Yu, B.P.; Dong, W.G.; Li, M.Q.; Yu, J.P.; Luo, H.S.; Rang, Z.X. Expression of TFF2 and Helicobacter pylori infection in carcinogenesis of gastric mucosa. World J. Gastroenterol. 2003, 9, 910–914.
587. Tran, C.P.; Cook, G.A.; Yeomans, N.D.; Thim, L.; Giraud, A.S. Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. Gut 1999, 44, 636–642.
588. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. Am. J. Physiol. Gastrointest. Liver Physiol. 2015, 308, G12–G24.
589. Ellermann, M.; Gharaibeh, R.Z.; Fulbright, L.; Dogan, B.; Moore, L.N.; Broberg, C.A.; Lopez, L.R.; Rothemich, A.M.; Herzog, J.W.; Rogala, A.; et al. Yersiniabactin-Producing Adherent/Invasive Escherichia coli Promotes Inflammation-Associated Fibrosis in Gnotobiotic Il10(-/-) Mice. Infect. Immun. 2019, 87, e00587-19.
590. Gordon, I.O.; Agrawal, N.; Willis, E.; Goldblum, J.R.; Lopez, R.; Allende, D.; Liu, X.; Patil, D.Y.; Yerian, L.; El-Khider, F.; et al. Fibrosis in ulcerative colitis is directly linked to severity and chronicity of mucosal inflammation. Aliment. Pharmacol. Ther. 2018, 47, 922–939.
591. Soriano-Izquierdo, A.; Gironella, M.; Massaguer, A.; May, F.E.; Salas, A.; Sans, M.; Poulsom, R.; Thim, L.; Piqué, J.M.; Panés, J. Trefoil peptide TFF2 treatment reduces VCAM-1 expression and leukocyte recruitment in experimental intestinal inflammation. J. Leukoc. Biol. 2004, 75, 214–223.
592. Giraud, A.S.; Pereira, P.M.; Thim, L.; Parker, L.M.; Judd, L.M. TFF-2 inhibits iNOS/NO in monocytes, and nitrated protein in healing colon after colitis. Peptides 2004, 25, 803–809.
593. Dhar, D.K.; Wang, T.C.; Maruyama, R.; Udagawa, J.; Kubota, H.; Fuji, T.; Tachibana, M.; Ono, T.; Otani, H.; Nagasue, N. Expression of Cytoplasmic TFF2 Is a Marker of Tumor Metastasis and Negative Prognostic Factor in Gastric Cancer. Lab. Investig. 2003, 83, 1343–1352.
594. Schmidt, P.H.; Lee, J.R.; Joshi, V.; Playford, R.J.; Poulsom, R.; Wright, N.A.; Goldenring, J.R. Identification of a metaplastic cell lineage associated with human gastric adenocarcinoma. Lab. Investig. 1999, 79, 639–646.
595. Cai, Y.; Yi, M.; Chen, D.; Liu, J.; Guleng, B.; Ren, J.; Shi, H. Trefoil factor family 2 expression inhibits gastric cancer cell growth and invasion in vitro via interactions with the transcription factor Sp3. Int. J. Mol. Med. 2016, 38, 1474–1480.
596. Longman, R.J.; Douthwaite, J.; Sylvester, P.A.; Poulsom, R.; Corfield, A.P.; Thomas, M.G.; Wright, N.A. Coordinated localisation of mucins and trefoil peptides in the ulcer associated cell lineage and the gastrointestinal mucosa. Gut 2000, 47, 792–800.
597. Khoder, G.; Al-Yassir, F.; Al Menhali, A.; Saseedharan, P.; Sugathan, S.; Tomasetto, C.; Karam, S.M. Probiotics Upregulate Trefoil Factors and Downregulate Pepsinogen in the Mouse Stomach. Int. J. Mol. Sci. 2019, 20, 3901.
598. Lukic, J.; Chen, V.; Strahinic, I.; Begovic, J.; Lev-Tov, H.; Davis, S.C.; Tomic-Canic, M.; Pastar, I. Probiotics or pro-healers: The role of beneficial bacteria in tissue repair. Wound Repair Regen. 2017, 25, 912– 922.
599. Plaza-Díaz, J.; Ruiz-Ojeda, F.J.; Vilchez-Padial, L.M.; Gil, A. Evidence of the Anti-Inflammatory Effects of Probiotics and Synbiotics in Intestinal Chronic Diseases. Nutrients 2017, 9, 555.
600. Liu, Y.; Alookaran, J.J.; Rhoads, J.M. Probiotics in Autoimmune and Inflammatory Disorders. Nutrients 2018, 10, 1537.
601. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity (Silver Spring) 2008, 16, 2302–2307.
602. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity (Silver Spring) 2013, 21, 1389–1395.
603. Ghanemi, A.; St-Amand, J. Interleukin-6 as a “metabolic hormone”. Cytokine 2018, 112, 132–136.
604. Hong, J.T.; Son, D.J.; Lee, C.K.; Yoon, D.Y.; Lee, D.H.; Park, M.H. Interleukin 32, inflammation and cancer. Pharmacol. Ther. 2017, 174, 127–137.
605. Petruzzelli, M.; Wagner, E.F. Mechanisms of metabolic dysfunction in cancer-associated cachexia. Genes Dev. 2016, 30, 489–501.
606. Tanaka, T.; Narazaki, M.; Kishimoto, T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb. Perspect. Biol. 2014, 6, a016295.

607. Judd, L.M.; Alderman, B.M.; Howlett, M.; Shulkes, A.; Dow, C.; Moverley, J.; Grail, D.; Jenkins, B.J.; Ernst, M.; Giraud, A.S. Gastric cancer development in mice lacking the SHP2 binding site on the IL-6 family co-receptor gp130. Gastroenterology 2004, 126, 196–207.
608. Peritore, A.F.; Siracusa, R.; Crupi, R.; Cuzzocrea, S. Therapeutic Efficacy of Palmitoylethanolamide and Its New Formulations in Synergy with Different Antioxidant Molecules Present in Diets. Nutrients 2019, 11, 2175.
609. Lasker, S.; Rahman, M.M.; Parvez, F.; Zamila, M.; Miah, P.; Nahar, K.; Kabir, F.; Sharmin, S.B.; Subhan, N.; Ahsan, G.U.; et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. Sci. Rep. 2019, 9, 20026.
610. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
611. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes (Basel) 2020, 11, 875.
612. Sindhu, S.; Akhter, N.; Kochumon, S.; Thomas, R.; Wilson, A.; Shenouda, S.; Tuomilehto, J.; Ahmad, R. Increased Expression of the Innate Immune Receptor TLR10 in Obesity and Type-2 Diabetes: Association with ROS-Mediated Oxidative Stress. Cell. Physiol. Biochem. 2018, 45, 572–590.
613. Karczewski, J.; Sledzi´nska, E.; Baturo, A.; Jo´nczyk, I.; Maleszko, A.; Samborski, P.; Begier-Krasi´nska, B.; Dobrowolska, A. Obesity and inflammation. Eur. Cytokine Netw. 2018, 29, 83–94.
614. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
615. Taupin, D.; Wu, D.-C.; Jeon, W.-K.; Devaney, K.; Wang, T.C.; Podolsky, D.K. The trefoil gene family are coordinately expressed immediate-early genes: EGF receptor– and MAP kinase–dependent interregulation. J. Clin. Investig. 1999, 103, R31–R38.
616. Lefebvre, O.; Chenard, M.-P.; Masson, R.; Linares, J.; Dierich, A.; LeMeur, M.; Wendling, C.; Tomasetto, C.; Chambon, P.; Rio, M.-C. Gastric Mucosa Abnormalities and Tumorigenesis in Mice Lacking the pS2 Trefoil Protein. Science 1996, 274, 259–262.
617. Hoffmann, W. Trefoil Factor Family (TFF) Peptides and Their Diverse Molecular Functions in Mucus Barrier Protection and More: Changing the Paradigm. Int. J. Mol. Sci. 2020, 21, 4535.
618. Kirikoshi, H.; Katoh, M. Expression of TFF1, TFF2 and TFF3 in gastric cancer. Int. J. Oncol. 2002, 21, 655–659.
619. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
620. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
621. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
622. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
623. M.R. De Giorgio, et al., Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat dietinduced obesity, Obesity 21 (7) (2013) 1389–1395.
624. E. Jequier, Leptin signaling, adiposity, and energy balance, Ann. N. Y. Acad. Sci. 967 (2002) 379–388.
625. L. Thim, Trefoil peptides: from structure to function, Cell. Mol. Life Sci. 53 (11-12) (1997) 888–903.
626. J. Madsen, et al., Tissue localization of human trefoil factors 1, 2, and 3, J. Histochem. Cytochem. 55 (5) (2007) 505–513.
627. W. Hoffmann, W. Jagla, A. Wiede, Molecular medicine of TFF-peptides: from gut to brain, Histol. Histopathol. 16 (1) (2001) 319–334.
628. D. Taupin, D.K. Podolsky, Trefoil factors: initiators of mucosal healing, Nat Rev Mol Cell Biol 4 (9) (2003) 721–732.
629. M. Hinz, et al., Trefoil factor family (TFF) expression in the mouse brain and pituitary: changes in the developing cerebellum, Peptides 25 (5) (2004) 827–832.
630. C. Bolduc, M. Yoshioka, J. St-Amand, Acute molecular mechanisms responsive to feeding and meal constitution in mesenteric adipose tissue, Obesity 18 (2) (2010) 410–413.
631. M. Yoshioka, et al., High-fat meal-induced changes in the duodenum mucosa transcriptome, Obesity 16 (10) (2008) 2302–2307.

632. M.R. De Giorgio, M. Yoshioka, J. St-Amand, Feeding induced changes in the hypothalamic transcriptome, Clin. Chim. Acta 406 (1–2) (2009) 103–107.
633. V.L. Veum, et al., The nuclear receptors NUR77, NURR1 and NOR1 in obesity and during fat loss, Int. J. Obes. 36 (9) (2012) 1195–1202.
634. V. Luu-The, et al., Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction, BioTechniques 38 (2) (2005) 287–293.
635. C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH image to ImageJ: 25 years of image analysis, Nat. Methods 9 (7) (2012) 671–675.
636. S.C. Taylor, et al., A defined methodology for reliable quantification of Western blot data, Mol. Biotechnol. 55 (3) (2013) 217–226.
637. S.C. Taylor, A. Posch, The Design of a Quantitative Western Blot Experiment, Biomed. Res. Int. 2014 (2014) 8.
638. J.L. Halaas, et al., Weight-reducing effects of the plasma protein encoded by the obese gene, Science 269 (5223) (1995) 543–546.
639. A. Matthias, et al., Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scidinduced thermogenesis, J. Biol. Chem. 275 (33) (2000) 25073–25081.
640. J.A. Harrold, G. Williams, P.S. Widdowson, Changes in hypothalamic agouti-related protein (AGRP), but not alpha-MSH or pro-opiomelanocortin concentrations in dietary-obese and food-restricted rats, Biochem. Biophys. Res. Commun. 258 (3) (1999) 574–577.
641. M.M. Ollmann, et al., Antagonism of central melanocortin receptors in vitro and in vivo by agoutirelated protein, Science 278 (5335) (1997) 135–138.
642. Y. Zhang, et al., Positional cloning of the mouse obese gene and its human homologue, Nature 372 (6505) (1994) 425–432.
643. F. Depocas, The calorigenic response of cold-acclimated white rats to infused noradrenaline, Can. J. Biochem. Physiol. 38 (2) (1960) 107–114.
644. G.M. Heaton, et al., Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation, Eur. J. Biochem. 82 (2) (1978) 515–521.
645. J. Orava, et al., Different metabolic responses of human brown adipose tissue to activation by cold and insulin, Cell Metab. 14 (2) (2011) 272–279.
646. A. Bartelt, et al., Brown adipose tissue activity controls triglyceride clearance, Nat. Med. 17 (2) (2011) 200–205.
647. A. Fedorenko, P.V. Lishko, Y. Kirichok, Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria, Cell 151 (2) (2012) 400–413.
648. N.J. Rothwell, M.J. Stock, Influence of noradrenaline on blood flow to brown adipose tissue in rats exhibiting diet-induced thermogenesis, Pflugers Arch. 389 (3) (1981) 237–242.
649. M.A. Paschoalini, R.H. Migliorini, Participation of the CNS in the control of FFA mobilization during fasting in rabbits, Physiol. Behav. 47 (3) (1990) 461–465.
650. S. Klein, et al., Effect of short- and long-term beta-adrenergic blockade on lipolysis during fasting in humans, Am. J. Phys. 257 (1 Pt 1) (1989) E65–E73.
651. R.A. Defronzo, et al., The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization, Diabetes 30 (12) (1981) 1000–1007.
652. P. Nuutila, et al., Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans, Am. J. Phys. 267 (6 Pt 1) (1994) E941–E946.
653. M.A. Maxwell, et al., Nur77 regulates lipolysis in skeletal muscle cells. Evidence for cross-talk between the beta-adrenergic and an orphan nuclear hormone receptor pathway, J. Biol. Chem. 280 (13) (2005) 12573– 12584.
654. L.C. Chao, et al., Skeletal muscle Nur77 expression enhances oxidative metabolism and substrate utilization, J. Lipid Res. 53 (12) (2012) 2610–2619.
655. L.C. Chao, et al., Nur77 coordinately regulates expression of genes linked to glucose metabolism in skeletal muscle, Mol. Endocrinol. 21 (9) (2007) 2152–2163.
656. D.J. Mahoney, et al., Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise, FASEB J. 19 (11) (2005) 1498–1500.
657. K. Orime, et al., Trefoil factor 2 promotes cell proliferation in pancreatic beta-cells through CXCR-4-mediated ERK1/2 phosphorylation, Endocrinology 154 (1) (2013) 54–64.

658. L.C. Chao, et al., Inhibition of adipocyte differentiation by Nur77, Nurr1, and Nor1, Mol. Endocrinol. 22 (12) (2008) 2596–2608.
659. W.S. Au, et al., The NR4A family of orphan nuclear receptors are not required for adipogenesis, Int. J. Obes. 32 (2) (2008) 388–392.
660. Y. Fu, et al., NR4A orphan nuclear receptors modulate insulin action and the glucose transport system: potential role in insulin resistance, J. Biol. Chem. 282 (43) (2007) 31525–31533.
661. D.J. Klemm, et al., Insulin-induced adipocyte differentiation. Activation of CREB rescues adipogenesis from the arrest caused by inhibition of prenylation, J. Biol. Chem. 276 (30) (2001) 28430–28435.
662. K.N. Frayn, et al., Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to postprandial transition, Am. J. Phys. 266 (3 Pt 1) (1994) E308–E317.
663. J.J. Geerling, et al., Sympathetic nervous system control of triglyceride metabolism: novel concepts derived from recent studies, J. Lipid Res. 55 (2) (2014) 180–189.
664. T.J. Bartness, C.K. Song, Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue, J. Lipid Res. 48 (8) (2007) 1655–1672.
665. S. Rosell, Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation, Acta Physiol. Scand. 67 (3) (1966) 343–351.
666. T.J. Bartness, C.H. Vaughan, C.K. Song, Sympathetic and sensory innervation of brown adipose tissue, Int. J. Obes. 34 (2010) S36.
667. P.R. Shepherd, B.B. Kahn, Glucose transporters and insulin action–implications for insulin resistance and diabetes mellitus, N. Engl. J. Med. 341 (4) (1999) 248–257.
668. W. Wahli, O. Braissant, B. Desvergne, Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more, Chem. Biol. 2 (5) (1995) 261–266.
669. P. Angulo, Nonalcoholic fatty liver disease, N. Engl. J. Med. 346 (16) (2002) 1221–1231.
670. P.H. Schmelck, J. Hanoune, The hepatic adrenergic receptors, Mol. Cell. Biochem. 33 (1–2) (1980) 35–48.
671. J.L. Benovic, et al., Regulation of adenylyl cyclase-coupled beta-adrenergic receptors, Annu. Rev. Cell Biol. 4 (1988) 405–428.
672. S.A. Myers, et al., Beta-adrenergic signaling regulates NR4A nuclear receptor and metabolic gene expression in multiple tissues, Mol. Cell. Endocrinol. 309 (1–2) (2009) 101–108.
673. L. Pei, et al., NR4A orphan nuclear receptors are transcriptional regulators of hepatic glucose metabolism, Nat. Med. 12 (9) (2006) 1048–1055.
674. G.A. Leveille, K. Chakrabarty, Diurnal variations in tissue glycogen and liver weight of meal-fed rats, J. Nutr. 93 (4) (1967) 546–554.
675. S.N. Kreitzman, A.Y. Coxon, K.F. Szaz, Glycogen storage: illusions of easy weight loss, excessive weight regain, and distortions in estimates of body composition, Am. J. Clin. Nutr. 56 (1 Suppl) (1992) 292S– 293S.
676. M.R. De Giorgio, et al., Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat dietinduced obesity, Obesity (Silver Spring) 21 (7) (2013) 1389–1395.
677. M. Yoshioka, et al., High-fat meal-induced changes in the duodenum mucosa transcriptome, Obesity (Silver Spring) 16 (10) (2008) 2302–2307.
678. O. Mucunguzi, et al., Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine, Nutr. Metab. (Lond.) 14 (2017) 66.
679. H. Ge, et al., Trefoil factor 3 (TFF3) is regulated by food intake, improves glucose tolerance and induces mucinous metaplasia, PLoS One 10 (6) (2015) e0126924.
680. Y. Xue, et al., Tff3, as a novel peptide, regulates hepatic glucose metabolism, PLoS One 8 (9) (2013) e75240.
681. D. Taupin, et al., The trefoil gene family are coordinately expressed immediateearly genes: EGF receptor- and MAP kinase-dependent interregulation, J. Clin. Invest. 103 (9) (1999) R31–R38.
682. O. Lefebvre, et al., Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein, Science 274 (5285) (1996) 259–262.
683. I.H. Rosenberg, Summary comments, Am. J. Clin. Nutr. 50 (5) (1989) 1231–1233.
684. J.E. Morley, Protein-energy malnutrition in older subjects, Proc. Nutr. Soc. 57 (4) (1998) 587–592.
685. Yoshioka, M.; St-Pierre, S.; Drapeau, V.; Dionne, I.; Doucet, E.; Suzuki, M.; Tremblay, A. Effect of red pepper on feeding behavior and energy intake. Br. J. Nutr. 1999, 82, 115–123.
686. Lusis, A.J. Genetic factors affecting blood lipoproteins: The candidate gene approach. J. Lipid Res. 1988, 29, 397–429.

687. Da Silva, M.S.; Julien, P.; Perusse, L.; Vohl, M.-C.; Rudkowska, I. Natural Rumen-Derived trans Fatty Acids Are Associated with Metabolic Markers of Cardiac Health. Lipids 2015, 50, 873–882.
688. Perusse, L.; Tremblay, A.; Leblanc, C.; Cloninger, C.R.; Reich, T.; Rice, J.; Bouchard, C. Familial resemblance in energy intake: Contribution of genetic and environmental factors. Am. J. Clin. Nutr. 1988, 47, 629–635.
689. Locke, A.; Kahali, B.; Berndt, S.I.; Justice, A.E.; Pers, T.; Day, F.; Powell, C.; Vedantam, S.; Buchkovich, M.L.; Yang, J.; et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015, 518, 197–206.
690. Rudkowska, I.; Pérusse, L.; Bellis, C.; Blangero, J.; Després, J.-P.; Bouchard, C.; Vohl, M.-C. Interaction between Common Genetic Variants and Total Fat Intake on Low-Density Lipoprotein Peak Particle Diameter: A Genome-Wide Association Study. J. Nutr. Nutr. 2015, 8, 44–53.
691. Perusse, L.; Rankinen, T.; Zuberi, A.; Chagnon, Y.C.; Weisnagel, S.J.; Argyropoulos, G.; Walts, B.; Snyder, E.E.; Bouchard, C. The Human Obesity Gene Map: The 2004 Update. Obes. Res. 2005, 13, 381–490.
692. Bierman, E.L.; Bagdade, J.D.; Porte, D. Obesity and Diabetes: The Odd Couple. Am. J. Clin. Nutr. 1968, 21, 1434–1437.
693. Government of Canada. Statistics Canada. Overweight and Obese Adults (Self-Reported). 2016. Available online: http://www.statcan.gc.ca/eng/help/bb/info/obesity (accessed on 11 October 2016).
694. Shungin, D.; Winkler, T.W.; Croteau-Chonka, D.C.; Ferreira, T.; Locke, A.; Mägi, R.; Strawbridge, R.; Pers, T.; Fischer, K.; Justice, A.E.; et al. New genetic loci link adipose and insulin biology to body fat distribution. Nature 2015, 518, 187–196.
695. Paffenbarger, R.S.J.; Lee, I.-M. Age-specific physical activities and life style patterns as related to allcauses mortality and to longevity. In Exercise for Preventing Common Diseases; Tanaka, H., Shindo, M., Eds.; Springer: Tokyo, Japan, 1999; pp. 121–130.
696. Paffenbarger, R.S., Jr. Factors predisposing to fatal stroke in longshoremen. Prev. Med. 1972, 1, 522–528.
697. Després, J.P.; Pouliot, M.C.; Moorjani, S.; Nadeau, A.; Tremblay, A.; Lupien, P.J.; Thériault, G.; Bouchard, C. Loss of abdominal fat and metabolic response to exercise training in obese women. Am. J. Physiol. Metab. 1991, 261 (Pt 1), E159–E167.
698. Pérusse, L.; Bouchard, C. Genotype-environment interaction in human obesity. Nutr. Rev. 1999, 57, S31–
S37.
699. Pérusse, L.; Bouchard, C. Gene-diet interactions in obesity. Am. J. Clin. Nutr. 2000, 72, 1285s–1290s.
700. St-Amand, J.; Prud'Homme, D.; Moorjani, S.; Nadeau, A.; Tremblay, A.; Bouchard, C.; Lupien, P.J.; Després, J.P. Apolipoprotein E polymorphism and the relationships of physical fitness to plasma lipoproteinlipid levels in men and women. Med. Sci. Sports Exerc. 1999, 31, 692–697.
701. St-Amand, J.; Moorjani, S.; Lupien, P.J.; Prud'Homme, D.; Després, J.P. The relation of plasma triglyceride, apolipoprotein B and high-density lipoprotein-cholesterol to postheparin lipoprotein lipase activity is dependent upon apolipoprotein E polymorphism. Metabolism 1996, 45, 261–268.
702. St-Amand, J.; Okamura, K.; Matsumoto, K.; Shimizu, S.; Sogawa, Y. Characterization of control and immobilized skeletal muscle: An overview from genetic engineering. FASEB J. 2001, 15, 684–692.
703. Lissner, L.; Levitsky, D.A.; Strupp, B.J.; Kalkwarf, H.J.; Roe, D.A. Dietary fat and the regulation of energy intake in human subjects. Am. J. Clin. Nutr. 1987, 46, 886–892.
704. Flatt, J.P.; Ravussin, E.; Acheson, K.J.; Jéquier, E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J. Clin. Investig. 1985, 76, 1019–1024.
705. Schutz, Y.; Flatt, J.P.; Jéquier, E. Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. Am. J. Clin. Nutr. 1989, 50.
706. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
707. World Health Organization. Obesity: Preventing and Managing the Global Epidemic; Report of a WHO Consultation; World Health Organization Technical Report Series; WHO: Geneva, Switzerland, 2000; Volume 894, pp. 1–253.
708. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
709. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.

710. Afshin, A. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N. Engl. J. Med. 2017, 377, 13–27.
711. Després, J.P. Abdominal obesity and the risk of coronary artery disease. Can. J. Cardiol. 1992, 8, 561–562.
712. Hubert, H.B.; Feinleib, M.; McNamara, P.M.; Castelli, W.P. Obesity as an independent risk factor for cardiovascular disease: A 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983, 67, 968–977.
713. Rabkin, S.W.; Mathewson, F.A.; Hsu, P.-H. Relation of body weight to development of ischemic heart disease in a cohort of young north American men after a 26 year observation period: The manitoba study. Am. J. Cardiol. 1977, 39, 452–458.
714. Mobley, D.; Baum, N. The Obesity Epidemic and Its Impact on Urologic Care. Rev. Urol. 2015, 17, 165–170.
715. Corey, K.E.; Kaplan, L.M. Obesity and liver disease: The epidemic of the twenty-first century. Clin. Liver Dis. 2014, 18, 1–18.
716. McElroy, S.L. The epidemic of depression with obesity. J. Clin. Psychiatry 2015, 76.
717. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
718. D'Apuzzo, M.R.; Novicoff, W.M.; Browne, J.A. The John Insall Award: Morbid Obesity Independently Impacts Complications, Mortality, and Resource Use After TKA. Clin. Orthop. Relat. Res. 2015, 473, 57–63.
719. Nasraway, S.A.; Albert, M.; Donnelly, A.M.; Ruthazer, R.; Shikora, S.A.; Saltzman, E. Morbid obesity is an independent determinant of death among surgical critically ill patients. Crit. Care Med. 2006, 34, 964–970, quiz 971.
720. Wood, P.D.; Stefanick, M.L.; Dreon, D.M.; Frey-Hewitt, B.; Garay, S.C.; Williams, P.T.; Superko, H.R.; Fortmann, S.P.; Albers, J.J.; Vranizan, K.M.; et al. Changes in Plasma Lipids and Lipoproteins in Overweight Men during Weight Loss through Dieting as Compared with Exercise. N. Engl. J. Med. 1988, 319, 1173– 1179.
721. Anderson, J.W.; Brinkman-Kaplan, V.L.; Lee, H.; Wood, C.L. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. J. Am. Coll. Nutr. 1994, 13, 256–261.
722. Dumont, M.; Mauriège, P.; Bergeron, J.; Despres, J.P.; Prud'homme, D. Effect of a six month gemfibrozil treatment and dietary recommendations on the metabolic risk profile of visceral obese men. Int. J. Obes. Relat. Metab. Disord. 2001, 25, 1136–1143.
723. Bray, G.A.; Greenway, F.L. Current and potential drugs for treatment of obesity. Endocr. Rev. 1999, 20, 805–875.
724. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
725. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
726. Jequier, E. Pathways to obesity. Int. J. Obes. Relat. Metab. Disord. 2002, 26 (Suppl. S2), S12–S17.
727. Kalra, S.P.; Dube, M.G.; Pu, S.; Xu, B.; Horvath, T.L.; Kalra, P.S. Interacting Appetite-Regulating Pathways in the Hypothalamic Regulation of Body Weight. Endocr. Rev. 1999, 20, 68–100.
728. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat Meal-induced Changes in the Duodenum Mucosa Transcriptome. Obesity 2008, 16, 2302–2307.
729. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding Regulates the Expression of Pancreatic Genes in Gastric Mucosa. J. Obes. 2010, 2010, 1–10.
730. Bolduc, C.; Yoshioka, M.; St-Amand, J. Acute Molecular Mechanisms Responsive to Feeding and Meal Constitution in Mesenteric Adipose Tissue. Obesity 2010, 18, 410–413.
731. De Giorgio, M.R.; Yoshioka, M.; St-Amand, J. Feeding induced changes in the hypothalamic transcriptome. Clin. Chim. Acta 2009, 406, 103–107.
732. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
733. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
734. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res. 2019, 781, 175–185.

735. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.-L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
736. Thim, L.; May, F.E. Structure of mammalian trefoil factors and functional insights. Cell Mol. Life Sci. 2005, 62, 2956–2973.
737. Hoffmann, W.; Jagla, W.; Wiede, A. Molecular medicine of TFF-peptides: From gut to brain. Histol. Histopathol. 2001, 16, 319–334.
738. Gajhede, M.; Petersen, T.; Henriksen, A.; Petersen, J.; Dauter, Z.; Wilson, K.; Thim, L. Pancreatic spasmolytic polypeptide: First three-dimensional structure of a member of the mammalian trefoil family of peptides. Structure 1993, 1, 253–262.
739. Dignass, A.U.; Sturm, A. Peptide growth factors in the intestine. Eur. J. Gastroenterol. Hepatol. 2001, 13, 763–770.
740. Beld, J.; Woycechowsky, K.J.; Hilvert, D. Diselenides as universal oxidative folding catalysts of diverse proteins. J. Biotechnol. 2010, 150, 481–489.
741. Hua, Q.X.; Mayer, P.J.; Jia, W.; Zhang, J.; Weiss, A.M. The folding nucleus of the insulin superfamily: A flexible peptide model foreshadows the native state. J. Biol. Chem. 2006, 281, 28131–28142.
742. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. Nat. Rev. Mol. Cell Biol. 2003, 4, 721–732.
743. Thim, L. Trefoil peptides: From structure to function. Cell. Mol. Life Sci. 1997, 53, 888–903.
744. Playford, R.J.; Marchbank, T.; Chinery, R.; Evison, R.; Pignatelli, M.; Boulton, R.A.; Thim, L.; Hanby, A.M. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. Gastroenterology 1995, 108, 108–116.
745. Tomasetto, C.; Masson, R.; Linares, J.; Wendling, C.; Lefebvre, O.; Chenard, M.; Rio, M. pS2/TFF1 interacts directly with the VWFC cysteine-rich domains of mucins. Gastroenterology 2000, 118, 70–80.
746. Westley, B.R.; Griffin, S.M.; May, F.E.B. Interaction between TFF1, a Gastric Tumor Suppressor Trefoil Protein, and TFIZ1, a Brichos Domain-Containing Protein with Homology to SP-C. Biochemistry 2005, 44, 7967–7975.
747. Madsen, J.; Nielsen, O.; Tornøe, I.; Thim, L.; Holmskov, U. Tissue Localization of Human Trefoil Factors 1, 2, and 3. J. Histochem. Cytochem. 2007, 55, 505–513.
748. Poulsen, S.S.; Thulesen, J.; Christensen, L.; Nexo, E.; Thim, L. Metabolism of oral trefoil factor 2 (TFF2) and the effect of oral and parenteral TFF2 on gastric and duodenal ulcer healing in the rat. Gut 1999, 45, 516– 522.
749. Huang, Z.; Zhang, X.; Lu, H.; Wu, L.; Wang, D.; Zhang, Q.; Ding, H. Serum trefoil factor 3 is a promising non-invasive biomarker for gastric cancer screening: A monocentric cohort study in China. BMC Gastroenterol. 2014, 14, 74.
750. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.-G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity 2013, 21, 1389–1395.
751. Farrell, J.J.; Taupin, D.; Koh, T.J.; Chen, D.; Zhao, C.M.; Podolsky, D.K.; Wang, T.C. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. J. Clin. Investig. 2002, 109, 193–204.
752. Baus-Loncar, M.; Schmid, J.; Lalani, E.-N.; Rosewell, I.; Goodlad, R.; Stamp, G.W.H.; Blin, N.; Kayademir, T. Trefoil Factor 2 (Tff2) Deficiency in Murine Digestive Tract Influences the Immune System. Cell. Physiol. Biochem. 2005, 16, 31–42.
753. Baus-Loncar, M.; Schmid, J.; Lalani, E.N.; Rosewell, I.; Goodlad, R.; Stamp, G.; Blin, N.; Kayademir, T. Trefoil factor family 2 deficiency and immune response. Cell Mol. Life Sci. 2005, 62, 2947–2955.
754. Salzman, N.H.; Hung, K.; Haribhai, D.; Chu, H.; Karlsson-Sjöberg, J.; Amir, E.; Teggatz, P.; Barman, M.; Hayward, M.; Eastwood, D.; et al. Enteric defensins are essential regulators of intestinal microbial ecology. Nat. Immunol. 2010, 11, 76–83.
755. Sell, H.; Habich, C.; Eckel, J. Adaptive immunity in obesity and insulin resistance. Nat. Rev. Endocrinol. 2012, 8, 709–716.
756. Zhao, L. The gut microbiota and obesity: From correlation to causality. Nat. Rev. Microbiol. 2013, 11, 639–647.
757. Kurt-Jones, E.A.; Cao, L.; Sandor, F.; Rogers, A.B.; Whary, M.T.; Nambiar, P.R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; et al. Trefoil Family Factor 2 Is Expressed in Murine Gastric and Immune Cells and

Controls both Gastrointestinal Inflammation and Systemic Immune Responses. Infect. Immun. 2007, 75, 471– 480.
758. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an InflammatoryInduced and AntiInflammatory Tissue Repair Factor. Animals 2020, 10, 1646.
759. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice. Animals 2021, 11, 258.
760. Vowinkel, T.; Mori, M.; Krieglstein, C.F.; Russell, J.; Saijo, F.; Bharwani, S.; Turnage, R.H.; Davidson, W.; Tso, P.; Granger, D.N.; et al. Apolipoprotein A-IV inhibits experimental colitis. J. Clin. Investig. 2004, 114, 260–269.
761. Okumura, T.; Fukagawa, K.; Tso, P.; Taylor, I.L.; Pappas, T. Intracisternal injection of apolipoprotein AIV inhibits gastric secretion in pylorus-ligated conscious rats. Gastroenterology 1994, 107, 1861–1864.
762. Tso, P.; Sun, W.; Liu, M. Gastrointestinal satiety signals IV. Apolipoprotein A-IV. Am. J. Physiol. Gastrointest. Liver Physiol. 2004, 286, G885–G890.
763. Johns, C.E.; Newton, J.L.; Westley, B.R.; May, F.E. Human pancreatic polypeptide has a marked diurnal rhythm that is affected by ageing and is associated with the gastric TFF2 circadian rhythm. Peptides 2006, 27, 1341–1348.
764. Shimada, T.; Fujii, Y.; Koike, T.; Tabei, K.; Namatame, T.; Yamagata, M.; Tajima, A.; Yoneda, M.; Trano, A.; Hiraishi, H. Peroxisome proliferator-activated receptor gamma (PPARgamma) regulates trefoil factor family 2 (TFF2) expression in gastric epithelial cells. Int. J. Biochem. Cell Biol. 2007, 39, 626–637.
765. Vázquez, M.; Silvestre, J.S.; Prous, J.R. Experimental approaches to study PPAR gamma agonists as antidiabetic drugs. Methods Find. Exp. Clin. Pharmacol. 2002, 24, 515–523.
766. Dubeykovskaya, Z.; Dubeykovskiy, A.; Solal-Cohen, J.; Wang, T.C. Secreted Trefoil Factor 2 Activates the CXCR4 Receptor in Epithelial and Lymphocytic Cancer Cell Lines. J. Biol. Chem. 2009, 284, 3650–3662.
767. Chinery, R.; Cox, H.M. Immunoprecipitation and characterization of a binding protein specific for the peptide, intestinal trefoil factor. Peptides 1995, 16, 749–755.
768. Tan, X.-D.; Hsueh, W.; Chang, H.; Wei, K.R.; Crussi, F.G. Characterization of a Putative Receptor for Intestinal Trefoil Factor in Rat Small Intestine: Identification byin SituBinding and Ligand Blotting. Biochem. Biophys. Res. Commun. 1997, 237, 673–677.
769. Thim, L.; Mørtz, E. Isolation and characterization of putative trefoil peptide receptors. Regul. Pept. 2000, 90, 61–68.
770. Otto, W.R.; Patel, K.; McKinnell, I.; Evans, M.D.; Lee, C.-Y.; Frith, D.; Hanrahan, S.; Blight, K.; Blin, N.; Kayademir, T.; et al. Identification of blottin: A novel gastric trefoil factor family-2 binding protein. Proteomics 2006, 6, 4235–4245.
771. Rimland, J.; Xin, W.; Sweetnam, P.; Saijoh, K.; Nestler, E.J.; Duman, R.S. Sequence and expression of a neuropeptide Y receptor cDNA. Mol. Pharmacol. 1991, 40, 869–875.
772. Banisadr, G.; Fontanges, P.; Haour, F.; Kitabgi, P.; Rostene, W.; Parsadaniantz, S.M. Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. Eur. J. Neurosci. 2002, 16, 1661–1671.
773. Vucetic, Z.; Reyes, T.M. Central dopaminergic circuitry controlling food intake and reward: Implications for the regulation of obesity. Wiley Interdiscip. Rev. Syst. Biol. Med. 2010, 2, 577–593.
774. Richard, D. Energy expenditure: A critical determinant of energy balance with key hypothalamic controls. Minerva Endocrinol. 2007, 32, 173–183.
775. Trecki, J.; Unterwald, E. Modulation of cocaine-induced activity by intracerebral administration of CXCL12. Neuroscience 2009, 161, 13–22.
776. Pervin, B.; Aydın, G.; Visser, T.; Uçkan-Çetinkaya, D.; Aerts-Kaya, F.S.F. CXCR4 expression by mesenchymal stromal cells is lost after use of enzymatic dissociation agents, but preserved by use of non enzymatic methods. Int. J. Hematol. 2021, 113, 5–9.
777. Mousessian, A.S.; da Silva, C.P.N.; Oba-Shinjo, S.M.; Kolias, A.G.; Paiva, W.S.; Marie, S.K.N. CXCR7, CXCR4, and Their Ligand Expression Profile in Traumatic Brain Injury. World Neurosurg. 2021, 147, e16– e24.
778. Singh, A.K.; Arya, R.K.; Trivedi, A.; Sanyal, S.; Baral, R.; Dormond, O.; Briscoe, D.; Datta, D. Chemokine receptor trio: CXCR3, CXCR4 and CXCR7 crosstalk via CXCL11 and CXCL12. Cytokine Growth Factor Rev. 2013, 24, 41–49.

779. Hsiao, J.J.; Ng, B.H.; Smits, M.M.; Wang, J.; Jasavala, R.J.; Martinez, H.D.; Lee, J.; Alston, J.J.; Misonou, H.; Trimmer, J.S.; et al. Androgen receptor and chemokine receptors 4 and 7 form a signaling axis to regulate CXCL12-dependent cellular motility. BMC Cancer 2015, 15, 204.
780. Jiang, Q.; Sun, Y.; Liu, X. CXCR4 as a prognostic biomarker in gastrointestinal cancer: A meta-analysis. Biomarkers 2019, 24, 510–516.
781. Li, L.-N.; Jiang, K.-T.; Tan, P.; Wang, A.-H.; Kong, Q.-Y.; Wang, C.-Y.; Lu, H.-R.; Wang, J. Prognosis and Clinicopathology of CXCR4 in Colorectal Cancer Patients: A Meta-analysis. Asian Pac. J. Cancer Prev. 2015, 16, 4077–4080.
782. Mouhieddine, T.H.; Ahmad, Y.; Barlogie, B.; Jagannath, S.; Teruya-Feldstein, J.; Richter, J. Increased Muscle CXCR4 Expression in the Setting of Rare Muscle-invasive Multiple Myeloma. Clin. Lymphoma Myeloma Leuk. 2020, 20, e341–e344.
783. Guo, Z.; Chen, W.; Dai, G.; Huang, Y. Cordycepin suppresses the migration and invasion of human liver cancer cells by downregulating the expression of CXCR4. Int. J. Mol. Med. 2020, 45, 141–150.
784. Yang, J.; Zhang, L.; Jiang, Z.; Ge, C.; Zhao, F.; Jiang, J.; Tian, H.; Chen, T.; Xie, H.; Cui, Y.; et al. TCF12 promotes the tumorigenesis and metastasis of hepatocellular carcinoma via upregulation of CXCR4 expression. Theranostics 2019, 9, 5810–5827.
785. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
786. Iqbal, J.; Hussain, M.M. Intestinal lipid absorption. Am. J. Physiol. Endocrinol. Metab. 2009, 296, E1183–E1194.
787. Timofeeva, E.; Baraboi, E.D.; Richard, D. Contribution of the vagus nerve and lamina terminalis to brain activation induced by refeeding. Eur. J. Neurosci. 2005, 22, 1489–1501.
788. Guesdon, B.; Samson, P.; Richard, D. Effects of intracerebroventricular and intra-accumbens melaninconcentrating hormone agonism on food intake and energy expenditure. Am. J. Physiol. Integr. Comp. Physiol. 2009, 296, R469–R475.
789. Arvaniti, K.; Huang, Q.; Richard, D. Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. Neuroendocrinology 2001, 73, 227–236.
790. Huang, Q.; Rivest, R.; Richard, D. Effects of leptin on corticotropin-releasing factor (CRF) synthesis and CRF neuron activation in the paraventricular hypothalamic nucleus of obese (ob/ob) mice. Endocrinology 1998, 139, 1524–1532.
791. Timofeeva, E.; Picard, F.; Duclos, M.; Deshaies, Y.; Richard, D. Neuronal activation and corticotropin releasing hormone expression in the brain of obese (fa/fa) and lean (fa/?) Zucker rats in response to refeeding. Eur. J. Neurosci. 2002, 15, 1013–1029.
792. Coppola, A.; Liu, Z.-W.; Andrews, Z.B.; Paradis, E.; Roy, M.-C.; Friedman, J.M.; Ricquier, D.; Richard, D.; Horvath, T.L.; Gao, X.-B.; et al. A Central Thermogenic-like Mechanism in Feeding Regulation: An Interplay between Arcuate Nucleus T3 and UCP2. Cell Metab. 2007, 5, 21–33.
793. Nadreau, E.; Baraboi, E.-D.; Samson, P.; Blouin, A.; Hould, F.-S.; Marceau, P.; Biron, S.; Richard, D. Effects of the biliopancreatic diversion on energy balance in the rat. Int. J. Obes. 2006, 30, 419–429.
794. Cabanac, M.; Richard, D. Acute intraventricular CRF lowers the hoarding threshold in male rats. Physiol. Behav. 1995, 57, 705–710.
795. Dagnault, A.; Ouerghi, D.; Richard, D. Treatment with alpha-helical-CRF (9-41) prevents the anorectic effect of 17-beta-estradiol. Brain Res. Bull. 1993, 32, 689–692.
796. Richard, D.; Rivest, R.; Naimi, N.; Timofeeva, E.; Rivest, S. Expression of corticotropin-releasing factor and its receptors in the brain of lean and obese Zucker rats. Endocrinology 1996, 137, 4786–4795.
797. Rivest, S.; Richard, D. Hypothalamic paraventricular nucleus lesions do not prevent anorectic effect of exercise in male rats. Am. J. Physiol. Integr. Comp. Physiol. 1990, 259 (Pt 2), R579–R584.
798. Mailhot, G.; Ravid, Z.; Barchi, S.; Moreau, A.; Rabasa-Lhoret, R.; Levy, E. CFTR knockdown stimulates lipid synthesis and transport in intestinal Caco-2/15 cells. Am. J. Physiol. Gastrointest. Liver Physiol. 2009, 297, G1239–G1249.
799. Baraboi, E.-D.; Michel, C.; Smith, P.; Thibaudeau, K.; Ferguson, A.V.; Richard, D. Effects of albuminconjugated PYY on food intake: The respective roles of the circumventricular organs and vagus nerve. Eur. J. Neurosci. 2010, 32, 826–839.
800. Levy, E.; Harmel, E.; Laville, M.; Sanchez, R.; Emonnot, L.; Sinnett, D.; Ziv, E.; Delvin, E.; Couture, P.; Marcil, V.; et al. Expression of Sar1b Enhances Chylomicron Assembly and Key Components of the

Coat Protein Complex II System Driving Vesicle Budding. Arterioscler. Thromb. Vasc. Biol. 2011, 31, 2692–2699.
801. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
802. Wang, F.; Kohan, A.B.; Lo, C.-M.; Liu, M.; Howles, P.; Tso, P. Apolipoprotein A-IV: A protein intimately involved in metabolism. J. Lipid Res. 2015, 56, 1403–1418.
803. Filippatos, T.D.; Derdemezis, C.S.; Gazi, I.F.; Nakou, E.S.; Mikhailidis, D.P.; Elisaf, M.S. Orlistat associated adverse effects and drug interactions: A critical review. Drug Saf. 2008, 31, 53–65.
804. Khalil, H.; Ellwood, L.; Lord, H.; Fernandez, R. Pharmacological Treatment for Obesity in Adults: An Umbrella Review. Ann. Pharmacother. 2020, 54, 691–705.
805. Al-Azzeh, E.; Dittrich-Breiholz, O.; Vervoorts, J.; Blin, N.; Gött, P.; Lüscher, B. Gastroprotective peptide trefoil factor family 2 gene is activated by upstream stimulating factor but not by c-Myc in gastrointestinal cancer cells. Gut 2002, 51, 685–690.
806. Shah, A.A.; Leidinger, P.; Keller, A.; Wendschlag, A.; Meese, E.; Blin, N. Altered miRNA expression patterns in Tff2 knock-out mice correlate with cellular pathways of neoplastic development and caloric metabolism. Int. J. Mol. Med. 2012, 29, 637–643.
807. Shah, A.A.; Mihalj, M.; Ratkaj, I.; Lubka-Pathak, M.; Balogh, P.; Klingel, K.; Bohn, E.; Blin, N.; Baus-Loncar, M. Increased Susceptibility to Yersinia enterocolitica Infection of Tff2 Deficient Mice. Cell. Physiol.Biochem. 2012, 30, 853–862.
808. Chao, L.C.; Zhang, Z.; Pei, L.; Saito, T.; Tontonoz, P.; Pilch, P. Nur77 Coordinately Regulates Expression of Genes Linked to Glucose Metabolism in Skeletal Muscle. Mol. Endocrinol. 2007, 21, 2152– 2163.
809. Maxwell, M.A.; Cleasby, M.E.; Harding, A.; Stark, A.; Cooney, G.J.; Muscat, G.E. Nur77 regulates lipolysis in skeletal muscle cells. Evidence for cross-talk between the beta-adrenergic and an orphan nuclear hormone receptor pathway. J. Biol. Chem. 2005, 280, 12573–12584.
810. Pearen, M.A.; Muscat, G.E.O. Minireview: Nuclear Hormone Receptor 4A Signaling: Implications for Metabolic Disease. Mol. Endocrinol. 2010, 24, 1891–1903.
811. Guerremillo, M. Adiponectin: An update. Diabetes Metab. 2008, 34, 12–18.
812. Siersbaek, R.; Nielsen, R.; Mandrup, S. PPARgamma in adipocyte differentiation and metabolism Novel insights from genomewide studies. FEBS Lett. 2010, 584, 3242–3249.
813. Albashir, A.A.D. The potential impacts of obesity on COVID-19. Clin. Med. 2020, 20, e109–e113.
814. Petrakis, D.; Margină, D.; Tsarouhas, K.; Tekos, F.; Stan, M.; Nikitovic, D.; Kouretas, D.; Spandidos, D.A.; Tsatsakis, A. Obesity—A risk factor for increased COVID-19 prevalence, severity and lethality (Review). Mol. Med. Rep. 2020, 22, 9–19.
815. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
816. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.
817. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
818. Zachariae, C.; Skov, L. Obesity as a risk factor for psoriasis. J. Eur. Acad. Dermatol. Venereol. 2020, 34, 915–916.
819. Mandviwala, T.; Khalid, U.; Deswal, A. Obesity and Cardiovascular Disease: A Risk Factor or a Risk Marker? Curr. Atheroscler. Rep. 2016, 18, 21.
820. De Pergola, G.; Silvestris, F. Obesity as a major risk factor for cancer. J. Obes. 2013, 2013, 291546.
821. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
822. Withrow, D.; Alter, D.A. The economic burden of obesity worldwide: A systematic review of the direct costs of obesity. Obes Rev. 2011, 12, 131–141.
823. Tremmel, M.; Gerdtham, U.G.; Nilsson, P.M.; Saha, S. Economic Burden of Obesity: A Systematic Literature Review. Int. J. Environ. Res. Public Health 2017, 14, 435.
824. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
825. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.

826. Hoffmann, W. Trefoil Factor Family (TFF) Peptides and Their Diverse Molecular Functions in Mucus Barrier Protection and More: Changing the Paradigm. Int. J. Mol. Sci. 2020, 21, 4535.
827. Braga Emidio, N.; Hoffmann, W.; Brierley, S.M.; Muttenthaler, M. Trefoil Factor Family: Unresolved Questions and Clinical Perspectives. Trends Biochem. Sci. 2019, 44, 387–390.
828. Hoffmann, W.; Jagla, W.; Wiede, A. Molecular medicine of TFF-peptides: From gut to brain. Histol. Histopathol. 2001, 16, 319–334.
829. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. Nat. Rev. Mol. Cell Biol. 2003, 4, 721–732.
830. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an InflammatoryInduced and AntiInflammatory Tissue Repair Factor. Animals 2020, 10, 1646.
831. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice. Animals 2021, 11, 258.
832. Ge, Y.; Ma, G.; Liu, H.; Lin, Y.; Zhang, G.; Du, M.; Wang, M.; Chu, H.; Zhang, H.; Zhang, Z. MUC1 is associated with TFF2 methylation in gastric cancer. Clin. Epigenet. 2020, 12, 37.
833. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
834. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity 2008, 16, 2302–2307.
835. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
836. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity 2013, 21, 1389–1395.
837. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. Am. J. Physiol Gastrointest. Liver Physiol. 2015, 308, G12–G24.
838. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet induced obesity. Life Sci. 2018, 215, 190–197.
839. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2: From a High-Fat-Induced Gene to a Potential Obesity Therapy Target. Metabolites 2021, 11, 536.
840. Fan, Y.; Pedersen, O. Gut microbiota in human metabolic health and disease. Nat. Rev. Microbiol. 2021, 19, 55–71.
841. Karlsson, F.H.; Tremaroli, V.; Nookaew, I.; Bergström, G.; Behre, C.J.; Fagerberg, B.; Nielsen, J.; Bäckhed, F. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013, 498, 99–103.
842. Wu, H.; Tremaroli, V.; Schmidt, C.; Lundqvist, A.; Olsson, L.M.; Krämer, M.; Gummesson, A.; Perkins, R.; Bergström, G.; Bäckhed, F. The Gut Microbiota in Prediabetes and Diabetes: A Population-Based CrossSectional Study. Cell Metab. 2020, 32, 379–390.e373.
843. Yu, Y.; Raka, F.; Adeli, K. The Role of the Gut Microbiota in Lipid and Lipoprotein Metabolism. J. Clin. Med. 2019, 8, 2227.
844. Festi, D.; Schiumerini, R.; Eusebi, L.H.; Marasco, G.; Taddia, M.; Colecchia, A. Gut microbiota and metabolic syndrome. World J. Gastroenterol. 2014, 20, 16079–16094.
845. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. Eur. Rev. Med. Pharmacol. Sci. 2016, 20, 4742–4749.
846. Yin, J.; Li, Y.; Han, H.; Chen, S.; Gao, J.; Liu, G.; Wu, X.; Deng, J.; Yu, Q.; Huang, X.; et al. Melatonin reprogramming of gut microbiota improves lipid dysmetabolism in high-fat diet-fed mice. J. Pineal Res. 2018, 65, e12524.
847. Bisanz, J.E.; Upadhyay, V.; Turnbaugh, J.A.; Ly, K.; Turnbaugh, P.J. Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host Microbe 2019, 26, 265–272.e264.
848. Khoder, G.; Al-Yassir, F.; Al Menhali, A.; Saseedharan, P.; Sugathan, S.; Tomasetto, C.; Karam, S.M. Probiotics Upregulate Trefoil Factors and Downregulate Pepsinogen in the Mouse Stomach. Int. J. Mol. Sci. 2019, 20, 3901.

849. Ishibashi, Y.; Ohtsu, H.; Ikemura, M.; Kikuchi, Y.; Niwa, T.; Nishioka, K.; Uchida, Y.; Miura, H.; Aikou, S.; Gunji, T.; et al. Serum TFF1 and TFF3 but not TFF2 are higher in women with breast cancer than in women without breast cancer. Sci. Rep. 2017, 7, 4846.
850. Hoffmann, W. TFF2, a MUC6-binding lectin stabilizing the gastric mucus barrier and more (Review). Int. J. Oncol. 2015, 47, 806–816.
851. May, F.E.; Semple, J.I.; Newton, J.L.; Westley, B.R. The human two domain trefoil protein, TFF2, is glycosylated in vivo in the stomach. Gut 2000, 46, 454–459.
852. Cook, G.A.; Familari, M.; Thim, L.; Giraud, A.S. The trefoil peptides TFF2 and TFF3 are expressed in rat lymphoid tissues and participate in the immune response. FEBS Lett. 1999, 456, 155–159.
853. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
854. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat Res. Rev. Mutat Res. 2019, 781, 175–185.
855. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
856. Caballero, B. Humans against Obesity: Who Will Win? Adv. Nutr. 2019, 10, S4–S9.
857. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
858. Apovian, C.M. Obesity: Definition, comorbidities, causes, and burden. Am. J. Manag. Care 2016, 22, s176–s185.
859. Hulbert, A.J.; Else, P.L. Basal metabolic rate: History, composition, regulation, and usefulness. Physiol. Biochem. Zool. 2004, 77, 869–876.
860. van Baak, M.A. Physical activity and energy balance. Public Health Nutr. 1999, 2, 335–339.
861. Westerterp, K.R. Exercise, energy balance and body composition. Eur. J. Clin. Nutr. 2018, 72, 1246–1250.
862. Saito, M.; Matsushita, M.; Yoneshiro, T.; Okamatsu-Ogura, Y. Brown Adipose Tissue, Diet-Induced Thermogenesis, and Thermogenic Food Ingredients: From Mice to Men. Front. Endocrinol. 2020, 11, 222.
863. Roesler, A.; Kazak, L. UCP1-independent thermogenesis. Biochem. J. 2020, 477, 709–725.
864. Fenzl, A.; Kiefer, F.W. Brown adipose tissue and thermogenesis. Horm. Mol. Biol. Clin. Investig. 2014, 19, 25–37.
865. Wang, F.; Deeney, J.T.; Denis, G.V. Brd2 gene disruption causes “metabolically healthy” obesity: Epigenetic and chromatin-based mechanisms that uncouple obesity from type 2 diabetes. Vitam. Horm. 2013, 91, 49–75.
866. Wang, F.; Liu, H.; Blanton, W.P.; Belkina, A.; Lebrasseur, N.K.; Denis, G.V. Brd2 disruption in mice causes severe obesity without Type 2 diabetes. Biochem. J. 2009, 425, 71–83.
867. Rossi, H.L.; Raj, N.R.; Marquez de Prado, B.; Kuburas, A.; Luu, A.K.S.; Barr, G.A.; Recober, A. Trigeminal Pain Responses in Obese ob/ob Mice Are Modality-Specific. Neuroscience 2019, 415, 121–134.
868. Lindström, P. beta-cell function in obese-hyperglycemic mice [ob/ob Mice]. Adv. Exp. Med. Biol. 2010, 654, 463–477.
869. Lopomo, A.; Burgio, E.; Migliore, L. Epigenetics of Obesity. Prog. Mol. Biol. Transl. Sci. 2016, 140, 151–184.
870. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. Cell Metab. 2019, 29, 1028–1044.
871. Rohde, K.; Keller, M.; la Cour Poulsen, L.; Blüher, M.; Kovacs, P.; Böttcher, Y. Genetics and epigenetics in obesity. Metab. Clin. Exp. 2019, 92, 37–50.
872. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. Diseases 2021, 9, 87.
873. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
874. Lim, Y.Y.; Lee, Y.S.; Ooi, D.S.Q. Engineering the Gut Microbiome for Treatment of Obesity: A Review of Current Understanding and Progress. Biotechnol. J. 2020, 15, e2000013.
875. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
876. Conway, B.; Rene, A. Obesity as a disease: No lightweight matter. Obes. Rev. 2004, 5, 145–151.

877. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
878. Després, J.P.; Lemieux, I. Abdominal obesity and metabolic syndrome. Nature 2006, 444, 881–887.
879. Piché, M.E.; Tchernof, A.; Després, J.P. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. Circ. Res. 2020, 126, 1477–1500.
880. Piché, M.E.; Poirier, P.; Lemieux, I.; Després, J.P. Overview of Epidemiology and Contribution of Obesity and Body Fat Distribution to Cardiovascular Disease: An Update. Prog. Cardiovasc. Dis. 2018, 61, 103–113.
881. Vekic, J.; Zeljkovic, A.; Stefanovic, A.; Jelic-Ivanovic, Z.; Spasojevic-Kalimanovska, V. Obesity and dyslipidemia. Metabolism 2019, 92, 71–81.
882. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
883. Stolarczyk, E. Adipose tissue inflammation in obesity: A metabolic or immune response? Curr. Opin. Pharm. 2017, 37, 35–40.
884. Silva Junior, G.B.; Bentes, A.C.; Daher, E.F.; Matos, S.M. Obesity and kidney disease. J. Bras. Nephrol. 2017, 39, 65–69.
885. Kolb, R.; Sutterwala, F.S.; Zhang, W. Obesity and cancer: Inflammation bridges the two. Curr. Opin. Pharm. 2016, 29, 77–89.
886. Fang, X.; Henao-Mejia, J.; Henrickson, S.E. Obesity and immune status in children. Curr. Opin. Pediatr. 2020, 32, 805–815.
887. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. Medicines 2021, 8, 57.
888. Andersen, C.J.; Murphy, K.E.; Fernandez, M.L. Impact of Obesity and Metabolic Syndrome on Immunity. Adv. Nutr. 2016, 7, 66–75.
889. Rojas-Osornio, S.A.; Cruz-Hernández, T.R.; Drago-Serrano, M.E.; Campos-Rodríguez, R. Immunity to influenza: Impact of obesity. Obes. Res. Clin. Pract. 2019, 13, 419–429.
890. Bischof, G.N.; Park, D.C. Obesity and Aging: Consequences for Cognition, Brain Structure, and Brain Function. Psychosom. Med. 2015, 77, 697–709.
891. Xanthopoulos, M.; Tapia, I.E. Obesity and common respiratory diseases in children. Paediatr. Respir. Rev. 2017, 23, 68–71.
892. Jubber, A.S. Respiratory complications of obesity. Int. J. Clin. Pract. 2004, 58, 573–580.
893. Best, D.; Bhattacharya, S. Obesity and fertility. Horm. Mol. Biol. Clin. Investig. 2015, 24, 5–10.
894. Silvestris, E.; de Pergola, G.; Rosania, R.; Loverro, G. Obesity as disruptor of the female fertility. Reprod. Biol. Endocrinol. 2018, 16, 22.
895. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis Measures: Health Protective Properties? Medicines 2021, 8, 49.
896. Ammar, A.; Brach, M.; Trabelsi, K.; Chtourou, H.; Boukhris, O.; Masmoudi, L.; Bouaziz, B.; Bentlage, E.; How, D.; Ahmed, M.; et al. Effects of COVID-19 Home Confinement on Eating Behaviour and Physical Activity: Results of the ECLB-COVID19 International Online Survey. Nutrients 2020, 12, 1583.
897. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
898. Kwok, S.; Adam, S.; Ho, J.H.; Iqbal, Z.; Turkington, P.; Razvi, S.; Le Roux, C.W.; Soran, H.; Syed, A.A. Obesity: A critical risk factor in the COVID-19 pandemic. Clin. Obes. 2020, 10, e12403.
899. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.
900. Albashir, A.A.D. The potential impacts of obesity on COVID-19. Clin. Med. 2020, 20, e109–e113.
901. Castañeda-Babarro, A.; Arbillaga-Etxarri, A.; Gutiérrez-Santamaría, B.; Coca, A. Physical Activity Change during COVID-19 Confinement. Int. J. Environ. Res. Public Health 2020, 17, 6878.
902. Pellegrini, C.A.; Webster, J.; Hahn, K.R.; Leblond, T.L.; Unick, J.L. Relationship between stress and weight management behaviors during the COVID-19 pandemic among those enrolled in an internet program. Obes. Sci. Pract. 2021, 7, 129–134.
903. Popkin, B.M.; Du, S.; Green, W.D.; Beck, M.A.; Algaith, T.; Herbst, C.H.; Alsukait, R.F.; Alluhidan, M.; Alazemi, N.; Shekar, M. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. Obes. Rev. 2020, 21, e13128.

904. Petrakis, D.; Margină, D.; Tsarouhas, K.; Tekos, F.; Stan, M.; Nikitovic, D.; Kouretas, D.; Spandidos, D.A.; Tsatsakis, A. Obesity—A risk factor for increased COVID-19 prevalence, severity and lethality (Review). Mol. Med. Rep. 2020, 22, 9–19.
905. Korakas, E.; Ikonomidis, I.; Kousathana, F.; Balampanis, K.; Kountouri, A.; Raptis, A.; Palaiodimou, L.; Kokkinos, A.; Lambadiari, V. Obesity and COVID-19: Immune and metabolic derangement as a possible link to adverse clinical outcomes. Am. J. Physiol. Endocrinol. Metab. 2020, 319, E105–E109.
906. Caci, G.; Albini, A.; Malerba, M.; Noonan, D.M.; Pochetti, P.; Polosa, R. COVID-19 and Obesity: Dangerous Liaisons. J. Clin. Med. 2020, 9, 2511.
907. Ritter, A.; Kreis, N.N.; Louwen, F.; Yuan, J. Obesity and COVID-19: Molecular Mechanisms Linking Both Pandemics. Int. J. Mol. Sci. 2020, 21, 5793.
908. Verma, S.; Hussain, M.E. Obesity and diabetes: An update. Diabetes Metab. Syndr. 2017, 11, 73–79.
909. Malone, J.I.; Hansen, B.C. Does obesity cause type 2 diabetes mellitus (T2DM)? Or is it the opposite? Pediatr. Diabetes 2019, 20, 5–9.
910. Zhou, Y.; Chi, J.; Lv, W.; Wang, Y. Obesity and diabetes as high-risk factors for severe coronavirus disease 2019 (COVID-19). Diabetes Metab. Res. Rev. 2021, 37, e3377.
911. Landstra, C.P.; de Koning, E.J.P. COVID-19 and Diabetes: Understanding the Interrelationship and Risks for a Severe Course. Front. Endocrinol. 2021, 12, 649525.
912. Rajpal, A.; Rahimi, L.; Ismail-Beigi, F. Factors leading to high morbidity and mortality of COVID-19 in patients with type 2 diabetes. J. Diabetes 2020, 12, 895–908.
913. Neeland, I.J.; Poirier, P.; Després, J.P. Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. Circulation 2018, 137, 1391–1406.
914. Ferrara, D.; Montecucco, F.; Dallegri, F.; Carbone, F. Impact of different ectopic fat depots on cardiovascular and metabolic diseases. J. Cell Physiol. 2019, 234, 21630–21641.
915. Del Turco, S.; Vianello, A.; Ragusa, R.; Caselli, C.; Basta, G. COVID-19 and cardiovascular consequences: Is the endothelial dysfunction the hardest challenge? Thromb. Res. 2020, 196, 143–151.
916. Lima-Martínez, M.M.; Carrera Boada, C.; Madera-Silva, M.D.; Marín, W.; Contreras, M. COVID-19 and diabetes: A bidirectional relationship. Clin. Investig. Arterioscler. 2021, 33, 151–157.
917. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Post-Coronavirus Disease-2019 (COVID-19): Toward a Severe Multi-Level Health Crisis? Med. Sci. 2021, 9, 68.
918. Soleimanpour, S.; Yaghoubi, A. COVID-19 vaccine: Where are we now and where should we go? Expert Rev. Vaccines 2021, 20, 23–44.
919. Tremmel, M.; Gerdtham, U.G.; Nilsson, P.M.; Saha, S. Economic Burden of Obesity: A Systematic Literature Review. Int. J. Environ. Res. Public Health 2017, 14, 435.
920. Cole, A.G.; Laxer, R.E.; Leatherdale, S. Social Consequences of Overweight and Obesity among Ontario High School Students. Can. J. Diabetes 2015, 39, S67.
921. Bacchini, D.; Licenziati, M.R.; Garrasi, A.; Corciulo, N.; Driul, D.; Tanas, R.; Fiumani, P.M.; Di Pietro, E.; Pesce, S.; Crinò, A.; et al. Bullying and Victimization in Overweight and Obese Outpatient Children and Adolescents: An Italian Multicentric Study. PLoS ONE 2015, 10, e0142715.
922. Jackson, V.M.; Breen, D.M.; Fortin, J.P.; Liou, A.; Kuzmiski, J.B.; Loomis, A.K.; Rives, M.L.; Shah, B.; Carpino, P.A. Latest approaches for the treatment of obesity. Expert Opin. Drug Discov. 2015, 10, 825–839.
923. Rebello, C.J.; Greenway, F.L. Obesity medications in development. Expert Opin. Investig. Drugs 2020, 29, 63–71.
924. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. Metabolism 2019, 92, 163–169.
925. Fonseca-Junior, S.J.; Sá, C.G.; Rodrigues, P.A.; Oliveira, A.J.; Fernandes-Filho, J. Physical exercise and morbid obesity: A systematic review. Arq. Bras. Cir. Dig. 2013, 26 (Suppl. 1), 67–73.
926. Fock, K.M.; Khoo, J. Diet and exercise in management of obesity and overweight. J. Gastroenterol. Hepatol. 2013, 28 (Suppl. 4), 59–63.
927. Ikeda, K.; Yamada, T. UCP1 Dependent and Independent Thermogenesis in Brown and Beige Adipocytes. Front. Endocrinol. 2020, 11, 498.
928. Harvey-Berino, J. Calorie restriction is more effective for obesity treatment than dietary fat restriction. Ann. Behav. Med. 1999, 21, 35–39.
929. Avena, N.M.; Rada, P.; Hoebel, B.G. Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. Neurosci. Biobehav. Rev. 2008, 32, 20–39.

930. Lennerz, B.; Lennerz, J.K. Food Addiction, High-Glycemic-Index Carbohydrates, and Obesity. Clin. Chem. 2018, 64, 64–71.
931. Ghosh, S.; O'Connell, J.F.; Carlson, O.D.; González-Mariscal, I.; Kim, Y.; Moaddel, R.; Ghosh, P.; Egan, J.M. Linoleic acid in diets of mice increases total endocannabinoid levels in bowel and liver: Modification by dietary glucose. Obes. Sci. Pract. 2019, 5, 383–394.
932. Lecheminant, J.D.; Gibson, C.A.; Sullivan, D.K.; Hall, S.; Washburn, R.; Vernon, M.C.; Curry, C.; Stewart, E.; Westman, E.C.; Donnelly, J.E. Comparison of a low carbohydrate and low fat diet for weight maintenance in overweight or obese adults enrolled in a clinical weight management program. Nutr. J. 2007, 6, 36.
933. Brinkworth, G.D.; Wycherley, T.P.; Noakes, M.; Buckley, J.D.; Clifton, P.M. Long-term effects of a verylow-carbohydrate weight-loss diet and an isocaloric low-fat diet on bone health in obese adults. Nutrition 2016, 32, 1033–1036.
934. LeCheminant, J.D.; Smith, B.K.; Westman, E.C.; Vernon, M.C.; Donnelly, J.E. Comparison of a reduced carbohydrate and reduced fat diet for LDL, HDL, and VLDL subclasses during 9-months of weight maintenance subsequent to weight loss. Lipids Health Dis. 2010, 9, 54.
935. Meckling, K.A.; O'Sullivan, C.; Saari, D. Comparison of a low-fat diet to a low-carbohydrate diet on weight loss, body composition, and risk factors for diabetes and cardiovascular disease in free-living, overweight men and women. J. Clin. Endocrinol. Metab. 2004, 89, 2717–2723.
936. Schlundt, D.G.; Hill, J.O.; Pope-Cordle, J.; Arnold, D.; Virts, K.L.; Katahn, M. Randomized evaluation of a low fat ad libitum carbohydrate diet for weight reduction. Int. J. Obes. Relat. Metab. Disord. 1993, 17, 623– 629.
937. Astrup, A.; Astrup, A.; Buemann, B.; Flint, A.; Raben, A. Low-fat diets and energy balance: How does the evidence stand in 2002? Proc. Nutr. Soc. 2002, 61, 299–309.
938. Astrup, A. The role of dietary fat in the prevention and treatment of obesity. Efficacy and safety of low-fat diets. Int. J. Obes. Relat. Metab. Disord. 2001, 25 (Suppl. 1), S46–S50.
939. Hunter, P.M.; Hegele, R.A. Functional foods and dietary supplements for the management of dyslipidaemia. Nat. Rev. Endocrinol. 2017, 13, 278–288.
940. Asgary, S.; Rastqar, A.; Keshvari, M. Functional Food and Cardiovascular Disease Prevention and Treatment: A Review. J. Am. Coll. Nutr. 2018, 37, 429–455.
941. Alkhatib, A.; Tsang, C.; Tiss, A.; Bahorun, T.; Arefanian, H.; Barake, R.; Khadir, A.; Tuomilehto, J. Functional Foods and Lifestyle Approaches for Diabetes Prevention and Management. Nutrients 2017, 9, 1310.
942. Aronson, J.K. Defining Nutraceuticals: Neither nutritious nor pharmaceutical. Br. J. Clin. Pharm. 2017, 83, 8–19.
943. Sachdeva, V.; Roy, A.; Bharadvaja, N. Current Prospects of Nutraceuticals: A Review. Curr. Pharm. Biotechnol. 2020, 21, 884–896.
944. Tramontin, N.D.S.; Luciano, T.F.; Marques, S.O.; de Souza, C.T.; Muller, A.P. Ginger and avocado as nutraceuticals for obesity and its comorbidities. Phytother. Res. 2020, 34, 1282–1290.
945. Manganaris, G.A.; Goulas, V.; Vicente, A.R.; Terry, L.A. Berry antioxidants: Small fruits providing large benefits. J. Sci. Food Agric. 2014, 94, 825–833.
946. Skrovankova, S.; Sumczynski, D.; Mlcek, J.; Jurikova, T.; Sochor, J. Bioactive Compounds and Antioxidant Activity in Different Types of Berries. Int. J. Mol. Sci. 2015, 16, 24673–24706.
947. Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Crit. Rev. Food Sci. Nutr. 2018, 58, 1260–1270.
948. Maleki, S.J.; Crespo, J.F.; Cabanillas, B. Anti-inflammatory effects of flavonoids. Food Chem 2019, 299, 125124.
949. Sanlier, N.; Gökcen, B.B.; Sezgin, A.C. Health benefits of fermented foods. Crit. Rev. Food Sci. Nutr. 2019, 59, 506–527.
950. Baby, B.; Antony, P.; Vijayan, R. Antioxidant and anticancer properties of berries. Crit. Rev. Food Sci. Nutr. 2018, 58, 2491–2507.
951. Boivin, D.; Blanchette, M.; Barrette, S.; Moghrabi, A.; Béliveau, R. Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFkappaB by edible berry juice. Anticancer Res. 2007, 27, 937–948.
952. Sekizawa, H.; Ikuta, K.; Mizuta, K.; Takechi, S.; Suzutani, T. Relationship between polyphenol content and anti-influenza viral effects of berries. J. Sci. Food Agric. 2013, 93, 2239–2241.
953. Slavin, J. Fiber and prebiotics: Mechanisms and health benefits. Nutrients 2013, 5, 1417–1435.

954. Choi, B.S.; Varin, T.V.; St-Pierre, P.; Pilon, G.; Tremblay, A.; Marette, A. A polyphenol-rich cranberry extract protects against endogenous exposure to persistent organic pollutants during weight loss in mice. Food Chem. Toxicol. 2020, 146, 111832.
955. Pannu, P.K.; Calton, E.K.; Soares, M.J. Calcium and Vitamin D in Obesity and Related Chronic Disease. Adv. Food Nutr. Res. 2016, 77, 57–100.
956. Kuroda, M.; Sakaue, H. Role of vitamin D and calcium in obesity and type 2 diabetes. Clin. Calcium. 2016, 26, 349–354.
957. Sathyamoorthy, N.; Wang, T.T.; Phang, J.M. Stimulation of pS2 expression by diet-derived compounds. Cancer Res. 1994, 54, 957–961.
958. Van Horn, L.; Huffman, M.D. Eat to Treat Heart Failure. Circ. Heart Fail. 2018, 11, e005367.
959. Angelidi, A.M.; Kokkinos, A.; Katechaki, E.; Ros, E.; Mantzoros, C.S. Mediterranean diet as a nutritional approach for COVID-19. Metabolism 2021, 114, 154407.
960. Yoshioka, M.; St-Pierre, S.; Drapeau, V.; Dionne, I.; Doucet, E.; Suzuki, M.; Tremblay, A. Effects of red pepper on appetite and energy intake. Br. J. Nutr. 1999, 82, 115–123.
961. Yoshioka, M.; Imanaga, M.; Ueyama, H.; Yamane, M.; Kubo, Y.; Boivin, A.; St-Amand, J.; Tanaka, H.; Kiyonaga, A. Maximum tolerable dose of red pepper decreases fat intake independently of spicy sensation in the mouth. Br. J. Nutr. 2004, 91, 991–995.
962. Westerterp-Plantenga, M.S.; Smeets, A.; Lejeune, M.P. Sensory and gastrointestinal satiety effects of capsaicin on food intake. Int. J. Obes. 2005, 29, 682–688.
963. Cani, P.D.; Joly, E.; Horsmans, Y.; Delzenne, N.M. Oligofructose promotes satiety in healthy human: A pilot study. Eur. J. Clin. Nutr. 2006, 60, 567–572.
964. Howarth, N.C.; Saltzman, E.; Roberts, S.B. Dietary fiber and weight regulation. Nutr. Rev. 2001, 59, 129–139.
965. Mathern, J.R.; Raatz, S.K.; Thomas, W.; Slavin, J.L. Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. Phytother. Res. 2009, 23, 1543–1548.
966. Choi, B.S.; Daniel, N.; Houde, V.P.; Ouellette, A.; Marcotte, B.; Varin, T.V.; Vors, C.; Feutry, P.; Ilkayeva, O.; Ståhlman, M.; et al. Feeding diversified protein sources exacerbates hepatic insulin resistance via increased gut microbial branched-chain fatty acids and mTORC1 signaling in obese mice. Nat. Commun. 2021, 12, 3377.
967. Chevrier, G.; Mitchell, P.L.; Rioux, L.E.; Hasan, F.; Jin, T.; Roblet, C.R.; Doyen, A.; Pilon, G.; St Pierre, P.; Lavigne, C.; et al. LowMolecular-Weight Peptides from Salmon Protein Prevent Obesity-Linked Glucose Intolerance, Inflammation, and Dyslipidemia in LDLR-/-/ApoB100/100 Mice. J. Nutr. 2015, 145, 1415–1422.
968. Samane, S.; Christon, R.; Dombrowski, L.; Turcotte, S.; Charrouf, Z.; Lavigne, C.; Levy, E.; Bachelard, H.; Amarouch, H.; Marette, A.; et al. Fish oil and argan oil intake differently modulate insulin resistance and glucose intolerance in a rat model of dietary-induced obesity. Metabolism 2009, 58, 909–919.
969. Steerenberg, P.A.; Beekhof, P.K.; Feskens, E.J.; Lips, C.J.; Höppener, J.W.; Beems, R.B. Long-term effect of fish oil diet on basal and stimulated plasma glucose and insulin levels in ob/ob mice. Diabetes Nutr. Metab. 2002, 15, 205–214.
970. Gao, X.; Xu, J.; Jiang, C.; Zhang, Y.; Xue, Y.; Li, Z.; Wang, J.; Xue, C.; Wang, Y. Fish oil ameliorates trimethylamine N-oxideexacerbated glucose intolerance in high-fat diet-fed mice. Food Funct. 2015, 6, 1117– 1125.
971. Fedor, D.; Kelley, D.S. Prevention of insulin resistance by n-3 polyunsaturated fatty acids. Curr. Opin. Clin. Nutr. Metab. Care 2009, 12, 138–146.
972. Baynes, H.W.; Mideksa, S.; Ambachew, S. The role of polyunsaturated fatty acids (n-3 PUFAs) on the pancreatic β-cells and insulin action. Adipocyte 2018, 7, 81–87.
973. Richards, P.; Pais, R.; Habib, A.M.; Brighton, C.A.; Yeo, G.S.; Reimann, F.; Gribble, F.M. High fat diet impairs the function of glucagon-like peptide-1 producing L-cells. Peptides 2016, 77, 21–27.
974. Grabauskas, G.; Wu, X.; Zhou, S.; Li, J.; Gao, J.; Owyang, C. High-fat diet-induced vagal afferent dysfunction via upregulation of 2-pore domain potassium TRESK channel. JCI Insight 2019, 4, e130402.
975. Grigorova, N.; Ivanova, Z.; Bjorndal, B.; Vachkova, E.; Penchev, G.; Berge, R.; Ribarski, S.; Georgieva, T.M.; Yonkova, P.; Georgiev, I.P. Effect of fish oil supplementation and restricted feeding on body fat distribution and blood lipid profile in a rabbit model of castration-induced obesity. Res. Vet. Sci. 2019, 124, 99–105.
976. Schubert, M.M.; Irwin, C.; Seay, R.F.; Clarke, H.E.; Allegro, D.; Desbrow, B. Caffeine, coffee, and appetite control: A review. Int. J. Food Sci. Nutr. 2017, 68, 901–912.

977. Gavrieli, A.; Karfopoulou, E.; Kardatou, E.; Spyreli, E.; Fragopoulou, E.; Mantzoros, C.S.; Yannakoulia, M. Effect of different amounts of coffee on dietary intake and appetite of normal-weight and overweight/obese individuals. Obesity 2013, 21, 1127–1132.
978. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
979. Choi, B.S.; Daoust, L.; Pilon, G.; Marette, A.; Tremblay, A. Potential therapeutic applications of the gut microbiome in obesity: From brain function to body detoxification. Int. J. Obes. 2020, 44, 1818–1831.
980. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. Eur. Rev. Med. Pharmacol. Sci. 2016, 20, 4742–4749.
981. Trefflich, I.; Jabakhanji, A.; Menzel, J.; Blaut, M.; Michalsen, A.; Lampen, A.; Abraham, K.; Weikert, C. Is a vegan or a vegetarian diet associated with the microbiota composition in the gut? Results of a new cross-sectional study and systematic review. Crit. Rev. Food Sci. Nutr. 2020, 60, 2990–3004.
982. Barengolts, E. Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: Review of randomized controlled trials. Endocr. Pract. 2016, 22, 1224–1234.
983. da Silva, T.F.; Casarotti, S.N.; de Oliveira, G.L.V.; Penna, A.L.B. The impact of probiotics, prebiotics, and synbiotics on the biochemical, clinical, and immunological markers, as well as on the gut microbiota of obese hosts. Crit. Rev. Food Sci. Nutr. 2021, 61, 337–355.
984. Aoun, A.; Darwish, F.; Hamod, N. The Influence of the Gut Microbiome on Obesity in Adults and the Role of Probiotics, Prebiotics, and Synbiotics for Weight Loss. Prev. Nutr. Food Sci. 2020, 25, 113–123.
985. Gual-Grau, A.; Guirro, M.; Mayneris-Perxachs, J.; Arola, L.; Boqué, N. Impact of different hypercaloric diets on obesity features in rats: A metagenomics and metabolomics integrative approach. J. Nutr. Biochem. 2019, 71, 122–131.
986. Jaiswal, S.; Libby, P. Clonal haematopoiesis: Connecting ageing and inflammation in cardiovascular disease. Nat. Rev. Cardiol. 2020, 17, 137–144.
987. Bektas, A.; Schurman, S.H.; Sen, R.; Ferrucci, L. Aging, inflammation and the environment. Exp. Gerontol. 2018, 105, 10–18.
988. Popa-Wagner, A.; Petcu, E.B.; Capitanescu, B.; Hermann, D.M.; Radu, E.; Gresita, A. Ageing as a risk factor for cerebral ischemia: Underlying mechanisms and therapy in animal models and in the clinic. Mech. Ageing Dev. 2020, 190, 111312.
989. Aspray, T.J.; Hill, T.R. Osteoporosis and the Ageing Skeleton. Subcell. Biochem. 2019, 91, 453–476.
990. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; et al. Oxidative stress, aging, and diseases. Clin. Interv. Aging 2018, 13, 757–772.
991. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. Nat. Rev. Neurol. 2019, 15, 565–581.
992. Kozakova, M.; Palombo, C. Vascular Ageing and Aerobic Exercise. Int. J. Environ. Res. Public Health 2021, 18, 666.
993. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. Am. Fam. Physician 2017, 95, 425–432.
994. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. Phys. Med. Rehabil. Clin. N. Am. 2017, 28, 659–669.
995. Ohayon, M.; Wickwire, E.M.; Hirshkowitz, M.; Albert, S.M.; Avidan, A.; Daly, F.J.; Dauvilliers, Y.; Ferri, R.; Fung, C.; Gozal, D.; et al. National Sleep Foundation's sleep quality recommendations: First report. Sleep Health 2017, 3, 6–19.
996. Flicker, L.; Lautenschlager, N.T.; Almeida, O.P. Healthy mental ageing. J. Br. Menopause Soc. 2006, 12, 92–96.
997. Luo, J.; Mills, K.; le Cessie, S.; Noordam, R.; van Heemst, D. Ageing, age-related diseases and oxidative stress: What to do next? Ageing. Res. Rev. 2020, 57, 100982.
998. Davies, K.J.A. Free radicals and redox regulation in ageing. Free Radic. Biol. Med. 2019, 134, 688–689.
999. Viña, J.; Borrás, C.; Miquel, J. Theories of ageing. IUBMB Life 2007, 59, 249–254.
1000. Pajares, M.; Cuadrado, A.; Engedal, N.; Jirsova, Z.; Cahova, M. The Role of Free Radicals in Autophagy Regulation: Implications for Ageing. Oxidative Med. Cell. Longev. 2018, 2018, 2450748.
1001. Dhanjal, D.S.; Bhardwaj, S.; Sharma, R.; Bhardwaj, K.; Kumar, D.; Chopra, C.; Nepovimova, E.; Singh, R.; Kuca, K. Plant Fortification of the Diet for Anti-Ageing Effects: A Review. Nutrients 2020, 12, 3008.

1002. Bramorska, A.; Zarzycka, W.; Podolecka, W.; Kuc, K.; Brzezicka, A. Age-Related Cognitive Decline May Be Moderated by Frequency of Specific Food Products Consumption. Nutrients 2021, 13, 2504.
1003. De la Fuente, M. Effects of antioxidants on immune system ageing. Eur. J. Clin. Nutr. 2002, 56 (Suppl. 3), S5–S8.
1004. Yin, Y.; Shan, H.Q.; Huang, W.; Wu, Y.M.; Lu, H.; Jin, Y. Bioinformatic analysis of miRNA expression patterns in TFF2 knock-out mice. Genet. Mol. Res. 2014, 13, 8502–8510.
1005. Aihara, E.; Engevik, K.A.; Montrose, M.H. Trefoil Factor Peptides and Gastrointestinal Function. Annu. Rev. Physiol. 2017, 79, 357–380.
1006. Thim, L.; May, F.E. Structure of mammalian trefoil factors and functional insights. Cell Mol. Life Sci. 2005, 62, 2956–2973.
1007. Katoh, M. Trefoil factors and human gastric cancer (review). Int. J. Mol. Med. 2003, 12, 3–9.
1008. Dignass, A.; Lynch-Devaney, K.; Kindon, H.; Thim, L.; Podolsky, D.K. Trefoil peptides promote epithelial migration through a transforming growth factor beta-independent pathway. J. Clin. Investig. 1994, 94, 376–383.
1009. Taupin, D.; Podolsky, D.K. Trefoil factors: Initiators of mucosal healing. Nat. Rev. Mol. Cell Biol. 2003, 4, 721–732.
1010. Shah, A.A.; Leidinger, P.; Keller, A.; Wendschlag, A.; Meese, E.; Blin, N. Altered miRNA expression patterns in Tff2 knock-out mice correlate with cellular pathways of neoplastic development and caloric metabolism. Int. J. Mol. Med. 2012, 29, 637–643.
1011. Braga Emidio, N.; Brierley, S.M.; Schroeder, C.I.; Muttenthaler, M. Structure, Function, and Therapeutic Potential of the Trefoil Factor Family in the Gastrointestinal Tract. ACS Pharm. Transl. Sci. 2020, 3, 583– 597.
1012. Xue, Y.; Shen, L.; Cui, Y.; Zhang, H.; Chen, Q.; Cui, A.; Fang, F.; Chang, Y. Tff3, as a novel peptide, regulates hepatic glucose metabolism. PLoS ONE 2013, 8, e75240.
1013. Arnold, P.; Rickert, U.; Helmers, A.K.; Spreu, J.; Schneppenheim, J.; Lucius, R. Trefoil factor 3 shows anti-inflammatory effects on activated microglia. Cell Tissue Res. 2016, 365, 3–11.
1014. Braga Emidio, N.; Meli, R.; Tran, H.N.T.; Baik, H.; Morisset-Lopez, S.; Elliott, A.G.; Blaskovich, M.A.T.; Spiller, S.; Beck-Sickinger, A.G.; Schroeder, C.I.; et al. Chemical Synthesis of TFF3 Reveals Novel Mechanistic Insights and a Gut-Stable Metabolite. J. Med. Chem. 2021, 64, 9484–9495.
1015. Belle, N.M.; Ji, Y.; Herbine, K.; Wei, Y.; Park, J.; Zullo, K.; Hung, L.Y.; Srivatsa, S.; Young, T.; Oniskey, T.; et al. TFF3 interacts with LINGO2 to regulate EGFR activation for protection against colitis and gastrointestinal helminths. Nat. Commun. 2019, 10, 4408.
1016. Ge, H.; Gardner, J.; Wu, X.; Rulifson, I.; Wang, J.; Xiong, Y.; Ye, J.; Belouski, E.; Cao, P.; Tang, J.; et al. Trefoil Factor 3 (TFF3) Is Regulated by Food Intake, Improves Glucose Tolerance and Induces Mucinous Metaplasia. PLoS ONE 2015, 10, e0126924.
1017. Bujak, M.; Bujak, I.T.; Sobo˘canec, S.; Mihalj, M.; Novak, S.; Cosi´c, A.; Levak, M.T.; Kopa˘cin, V.; Mihaljevi´c, B.; Balog, T.; et al. ´ Trefoil Factor 3 Deficiency Affects Liver Lipid Metabolism. Cell. Physiol.Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 2018, 47, 827–841.
1018. Wu, X.; Zheng, H.; Yang, R.; Luan, X.; Zhang, L.; Jin, Q.; Jin, Y.; Xue, J. Mouse trefoil factor 3 ameliorated high-fat-diet-induced hepatic steatosis via increasing peroxisome proliferator-activated receptor-α-mediated fatty acid oxidation. Am. J. Physiol. Endocrinol. Metab. 2019, 317, E436–E445.
1019. Judd, L.M.; Chalinor, H.V.; Walduck, A.; Pavlic, D.I.; Däbritz, J.; Dubeykovskaya, Z.; Wang, T.C.; Menheniott, T.R.; Giraud, A.S. TFF2 deficiency exacerbates weight loss and alters immune cell and cytokine profiles in DSS colitis, and this cannot be rescued by wild-type bone marrow. Am. J. Physiol. Gastrointest. Liver Physiol. 2015, 308, G12–G24.
1020. Kurt-Jones, E.A.; Cao, L.; Sandor, F.; Rogers, A.B.; Whary, M.T.; Nambiar, P.R.; Cerny, A.; Bowen, G.; Yan, J.; Takaishi, S.; et al. Trefoil family factor 2 is expressed in murine gastric and immune cells and controls both gastrointestinal inflammation and systemic immune responses. Infect. Immun. 2007, 75, 471– 480.
1021. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity 2013, 21, 1389–1395.
1022. Lebherz-Eichinger, D.; Tudor, B.; Ankersmit, H.J.; Reiter, T.; Haas, M.; Einwallner, E.; Roth-Walter, F.; Krenn, C.G.; Roth, G.A. Increased trefoil factor 2 levels in patients with chronic kidney disease. PLoS ONE 2017, 12, e0174551.

1023. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 (TFF2) as an InflammatoryInduced and AntiInflammatory Tissue Repair Factor. Animals 2020, 10, 1646.
1024. Ghanemi, A.; Yoshioka, M.; St-Amand, J. High-Fat Diet-Induced Trefoil Factor Family Member 2 (TFF2) to Counteract the Immune-Mediated Damage in Mice. Animals 2021, 11, 258.
1025. Baus-Loncar, M.; Kayademir, T.; Takaishi, S.; Wang, T. Trefoil factor family 2 deficiency and immune response. Cell. Mol. Life Sci. 2005, 62, 2947–2955.
1026. Yoshioka, M.; Bolduc, C.; Raymond, V.; St-Amand, J. High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity 2008, 16, 2302–2307.
1027. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
1028. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 Expression as an Indicator of the Severity of the High-Fat Diet-Induced Obesity. Genes 2021, 12, 1505.
1029. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
1030. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2: From a High-Fat-Induced Gene to a Potential Obesity Therapy Target. Metabolites 2021, 11, 536.
1031. Wu, B.; Chien, E.Y.; Mol, C.D.; Fenalti, G.; Liu, W.; Katritch, V.; Abagyan, R.; Brooun, A.; Wells, P.; Bi, F.C.; et al. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptideantagonists. Science 2010, 330, 1066–1071.
1032. Ghanemi, A. Targeting G protein coupled receptor-related pathways as emerging molecular therapies. Saudi Pharm. J. 2015, 23, 115–129.
1033. Dubeykovskaya, Z.; Dubeykovskiy, A.; Solal-Cohen, J.; Wang, T.C. Secreted trefoil factor 2 activates the CXCR4 receptor in epithelial and lymphocytic cancer cell lines. J. Biol. Chem. 2009, 284, 3650–3662.
1034. Hirata, K.; Kodama, S.; Nakano, Y.; Minaki-Nakagawa, Y.; Aoyama, Y.; Sakikubo, M.; Goto, T.; Yoshida, M.; Masui, T.; Yamamoto, T.; et al. Exocrine tissue-driven TFF2 prevents apoptotic cell death of endocrine lineage during pancreas organogenesis. Sci Rep. 2019, 9, 1636.
1035. Orime, K.; Shirakawa, J.; Togashi, Y.; Tajima, K.; Inoue, H.; Ito, Y.; Sato, K.; Nakamura, A.; Aoki, K.; Goshima, Y.; et al. Trefoil factor 2 promotes cell proliferation in pancreatic β-cells through CXCR-4 mediated ERK1/2 phosphorylation. Endocrinology 2013, 154, 54–64.
1036. Guo, Z.; Chen, W.; Dai, G.; Huang, Y. Cordycepin suppresses the migration and invasion of human liver cancer cells by downregulating the expression of CXCR4. Int. J. Mol. Med. 2020, 45, 141–150.
1037. Yao, L.; Heuser-Baker, J.; Herlea-Pana, O.; Zhang, N.; Szweda, L.I.; Griffin, T.M.; Barlic-Dicen, J. Deficiency in adipocyte chemokine receptor CXCR4 exacerbates obesity and compromises thermoregulatory responses of brown adipose tissue in a mouse model of diet-induced obesity. FASEB J. 2014, 28, 4534–4550.
1038. Mouhieddine, T.H.; Ahmad, Y.; Barlogie, B.; Jagannath, S.; Teruya-Feldstein, J.; Richter, J. Increased Muscle CXCR4 Expression in the Setting of Rare Muscle-invasive Multiple Myeloma. Clin. Lymphoma Myeloma Leuk. 2020, 20, e341–e344.
1039. Banisadr, G.; Fontanges, P.; Haour, F.; Kitabgi, P.; Rostène, W.; Mélik Parsadaniantz, S. Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. Eur. J. Neurosci. 2002, 16, 1661–1671.
1040. Jiang, Q.; Sun, Y.; Liu, X. CXCR4 as a prognostic biomarker in gastrointestinal cancer: A meta-analysis. Biomarkers 2019, 24, 510–516.
1041. Urosevic, J.; Blasco, M.T.; Llorente, A.; Bellmunt, A.; Berenguer-Llergo, A.; Guiu, M.; Cañellas, A.; Fernandez, E.; Burkov, I.; Clapés, M.; et al. ERK1/2 Signaling Induces Upregulation of ANGPT2 and CXCR4 to Mediate Liver Metastasis in Colon Cancer. Cancer Res. 2020, 80, 4668–4680.
1042. Shibata, M.; Banno, R.; Sugiyama, M.; Tominaga, T.; Onoue, T.; Tsunekawa, T.; Azuma, Y.; Hagiwara, D.; Lu, W.; Ito, Y.; et al. AgRP Neuron-Specific Deletion of Glucocorticoid Receptor Leads to Increased Energy Expenditure and Decreased Body Weight in Female Mice on a High-Fat Diet. Endocrinology 2016, 157, 1457–1466.
1043. Lu, X.Y. Role of central melanocortin signaling in eating disorders. Psychopharmacol. Bull. 2001, 35, 45–65.

1044. Benoit, S.; Schwartz, M.; Baskin, D.; Woods, S.C.; Seeley, R.J. CNS melanocortin system involvement in the regulation of food intake. Horm. Behav. 2000, 37, 299–305.
1045. Biebermann, H.; Kühnen, P.; Kleinau, G.; Krude, H. The neuroendocrine circuitry controlled by POMC, MSH, and AGRP. Handb. Exp. Pharm. 2012, 209, 47–75.
1046. Seeley, R.J.; Drazen, D.L.; Clegg, D.J. The critical role of the melanocortin system in the control of energy balance. Annu. Rev. Nutr. 2004, 24, 133–149.
1047. Farrell, J.J.; Taupin, D.; Koh, T.J.; Chen, D.; Zhao, C.M.; Podolsky, D.K.; Wang, T.C. TFF2/SP-deficient mice show decreased gastric proliferation, increased acid secretion, and increased susceptibility to NSAID injury. J. Clin. Investig. 2002, 109, 193–204.
1048. Scholven, J.; Taras, D.; Sharbati, S.; Schön, J.; Gabler, C.; Huber, O.; Meyer zum Büschenfelde, D.; Blin, N.; Einspanier, R. Intestinal expression of TFF and related genes during postnatal development in a piglet probiotic trial. Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 2009, 23, 143–156.
1049. Dossinger, V.; Kayademir, T.; Blin, N.; Gött, P. Down-regulation of TFF expression in gastrointestinal cell lines by cytokines and nuclear factors. Cell. Physiol. Biochem. 2002, 12, 197–206.
1050. Capuano, E.; Janssen, A.E.M. Food Matrix and Macronutrient Digestion. Annu. Rev. Food Sci. Technol. 2021, 12, 193–212.
1051. Nieva-Echevarría, B.; Goicoechea, E.; Guillén, M.D. Food lipid oxidation under gastrointestinal digestion conditions: A review. Crit. Rev. Food Sci. Nutr. 2020, 60, 461–478.
1052. Raka, F.; Farr, S.; Kelly, J.; Stoianov, A.; Adeli, K. Metabolic control via nutrient-sensing mechanisms: Role of taste receptors and the gut-brain neuroendocrine axis. Am. J. Physiol. Endocrinol. Metab. 2019, 317, E559–E572.
1053. Vettor, R.; Di Vincenzo, A.; Maffei, P.; Rossato, M. Regulation of energy intake and mechanisms of metabolic adaptation or maladaptation after caloric restriction. Rev. Endocr. Metab. Disord. 2020, 21, 399– 409.
1054. Jequier, E. Leptin signaling, adiposity, and energy balance. Ann. N. Y. Acad. Sci. 2002, 967, 379–388.
1055. Frühbeck, G.; Catalán, V.; Rodríguez, A.; Gómez-Ambrosi, J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte 2018, 7, 57–62.
1056. Rebello, C.J.; Kirwan, J.P.; Greenway, F.L. Obesity, the most common comorbidity in SARS-CoV-2: Is leptin the link? Int. J. Obes. 2020, 44, 1810–1817.
1057. Ishioka, K.; Hatai, H.; Komabayashi, K.; Soliman, M.M.; Shibata, H.; Honjoh, T.; Kimura, K.; Saito, M. Diurnal variations of serum leptin in dogs: Effects of fasting and re-feeding. Vet. J. 2005, 169, 85–90.
1058. Kiani, A. Temporal Changes in Plasma Concentration of Leptin, IGF-1, Insulin and Metabolites Under Extended Fasting and Re-Feeding Conditions in Growing Lambs. Int. J. Endocrinol. Metab. 2013, 11, 34–40.
1059. Lee, Y.C.; Ko, Y.H.; Hsu, Y.P.; Ho, L.T. Plasma leptin response to oral glucose tolerance and fasting/refeeding tests in rats with fructose-induced metabolic derangements. Life Sci. 2006, 78, 1155 1162.
1060. Ronis, M.J.J.; Pedersen, K.B.; Watt, J. Adverse Effects of Nutraceuticals and Dietary Supplements. Annu. Rev. Pharm. Toxicol. 2018, 58, 583–601.
1061. Ghanemi, A. Is mapping borders between pharmacology and toxicology a necessity? Saudi Pharm. J. 2014, 22, 489–490.
1062. Ghanemi, A. How to define a pharmacological or a toxic food? Alex. J. Med. 2015, 51, 359–360.
1063. Ghanemi, A. Cell cultures in drug development: Applications, challenges and limitations. Saudi Pharm. J. 2015, 23, 453–454.
1064. Thérien, A.; Cie´slak, A.; Verreault, M.; Perreault, M.; Trottier, J.; Gobeil, S.; Vohl, M.-C.; Barbier, O. Omega-3 Polyunsaturated Fatty Acid: A Pharmaco-Nutraceutical Approach to Improve the Responsiveness to Ursodeoxycholic Acid. Nutrients 2021, 13, 2617.
1065. Kalra, E.K. Nutraceutical–definition and introduction. AAPS Pharm. Sci. 2003, 5, E25.
1066. Agarwal, R., Agarwal, P., 2017. Targeting extracellular matrix remodeling in disease: Could resveratrol be a potential candidate? Exp. Biol. Med. (Maywood) 242, 374–383.

1067. Aoi, W., Naito, Y., Takagi, T., Tanimura, Y., Takanami, Y., Kawai, Y., Sakuma, K., Hang, L.P., Mizushima, K., Hirai, Y., et al., 2013. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 62, 882–889.
1068. Aratani, Y., Kitagawa, Y., 1988. Enhanced synthesis and secretion of type IV collagen and entactin during adipose conversion of 3T3-L1 cells and production of unorthodox laminin complex. J. Biol. Chem. 263, 16163–16169.
1069. Aseer, K.R., Silvester, A.J., Kumar, A., Choi, M.S., JW, Y., 2017. SPARC paucity alleviates superoxide-mediated oxidative stress, apoptosis, and autophagy in diabetogenic hepatocytes. Free Radic. Biol. Med. 108, 874–895.
1070. Atorrasagasti, C., Onorato, A., Gimeno, M.L., Andreone, L., Garcia, M., Malvicini, M., Fiore, E., Bayo, J., Perone, M.J., Mazzolini, G.D., 2019. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. Clin. Sci. 133, 351–365.
1071. Basu, A., Kligman, L.H., Samulewicz, S.J., CC, H., 2001. Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40). BMC Cell Biol. 2, 15.
1072. Berggren, J.R., Tanner, C.J., Koves, T.R., Muoio, D.M., Houmard, J.A., 2005. Glucose uptake in muscle cell cultures from endurance-trained men. Med. Sci. Sports Exerc. 37, 579–584.
1073. Biolo, G., Cederholm, T., Muscaritoli, M., 2014. Muscle contractile and metabolic dysfunction is a common feature of sarcopenia of aging and chronic diseases: from sarcopenic obesity to cachexia. Clin. Nutr. 33, 737–748.
1074. Boengler, K., Kosiol, M., Mayr, M., Schulz, R., Rohrbach, S., 2017. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. J. Cachexia Sarcopenia Muscle 8, 349–369.
1075. Bradshaw, A.D., Sage, E.H., 2001. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J. Clin. Invest. 107, 1049–1054.
1076. Bradshaw, A.D., Reed, M.J., EH, S., 2002. SPARC-null mice exhibit accelerated cutaneous wound closure. J. Histochem. Cytochem. 50, 1–10.
1077. Bradshaw, A.D., Graves, D.C., Motamed, K., EH, S., 2003a. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc. Natl. Acad. Sci. U. S. A. 100, 6045– 6050.
1078. Bradshaw, A.D., Graves, D.C., Motamed, K., EH, S., 2003b. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc. Natl. Acad. Sci. U. S. A. 100, 6045– 6050.
1079. Brekken, R.A., Sage, E.H., 2000. SPARC, a matricellular protein: at the crossroads of cellmatrix. Matrix Biol. 19, 569–580.
1080. Chavey, C., Boucher, J., Monthouel-Kartmann, M.N., Sage, E.H., Castan-Laurell, I., Valet, P., Tartare-Deckert, S., Van Obberghen, E., 2006. Regulation of secreted protein acidic and rich in cysteine during adipose conversion and adipose tissue hyperplasia. Obesity Silver Spring (Silver Spring) 14, 1890– 1897.
1081. Chen, P., Hou, X., Hu, G., Wei, L., Jiao, L., Wang, H., Chen, S., Wu, J., Bao, Y., W, J., 2018. Abdominal subcutaneous adipose tissue: a favorable adipose depot for diabetes? Cardiovasc. Diabetol. 17, 93.
1082. DeFronzo, R.A., Jacot, E., Jequier, E., Maeder, E., Wahren, J., Felber, J.P., 1981. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 30, 1000–1007.
1083. Delany, A.M., Kalajzic, I., Bradshaw, A.D., Sage, E.H., Canalis, E., 2003. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. Endocrinology 144, 2588–2596.
1084. Delany, A.M., Amling, M., Priemel, M., Howe, C., Baron, R., Canalis, E., 2000. Osteopenia and decreased bone formation in osteonectin-deficient mice. J. Clin. Invest. 105, 915–923.
1085. Despotovic, S.Z., Milicevic, N.M., Milosevic, D.P., Despotovic, N., Erceg, P., Svorcan, P., Schumacher, U., Ullrich, S., Mihajlovic, G., Kalem, D., et al., 2017. Remodeling of extracellular matrix of the lamina propria in the uninvolved human rectal mucosa 10 and 20 cm away from the malignant tumor. Tumour Biol. 39, 1010428317711654.
1086. Despres, J.P., 1992. Abdominal obesity and the risk of coronary artery disease. Can. J. Cardiol. 8, 561–562.
1087. Després, J.-P., 2012. Body fat distribution and risk of cardiovascular disease. Circulation126, 1301–1313.
1088. Despres, J.P., Lemieux, I., 2006. Abdominal obesity and metabolic syndrome. Nature 444, 881–887.

1089. Despres, J.P., Moorjani, S., Lupien, P.J., Tremblay, A., Nadeau, A., Bouchard, C., 1990. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis 10, 497–511.
1090. Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., Raptis, S.A., 2011. Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. 93, S52–S59.
1091. Drela, N., Kozdron, E., Szczypiorski, P., 2004. Moderate exercise may attenuate some aspects of immunosenescence. BMC Geriatr. 4, 8.
1092. Duarte, F.O., Gomes-Gatto, C.D.V., Oishi, J.C., Lino, A.D.S., Stotzer, U.S., Rodrigues, M.F.C., Gatti da Silva, G.H., Selistre-de-Araujo, H.S., 2017. Physical training improves visceral adipose tissue health by remodelling extracellular matrix in rats with estrogen absence: a gene expression analysis. Int. J. Exp. Pathol. 98, 203–213.
1093. Emanuel Anna, L., Meijer Rick, I., Muskiet Marcel, H.A., van Raalte Daniël, H., Eringa Etto, C., Serné Erik, H., 2017. Role of insulin-stimulated adipose tissue perfusion in the development of whole-body insulin resistance. Arterioscler. Thromb. Vasc. Biol. 37, 411–418.
1094. Erdogan, B., Webb, D.J., 2017. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem. Soc. Trans. 45, 229–236.
1095. Ford, A.J., Rajagopalan, P., 2018. Extracellular matrix remodeling in 3D: implications in tissue homeostasis and disease progression. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 10, e1503.
1096. Francki, A., Bradshaw, A.D., Bassuk, J.A., Howe, C.C., Couser, W.G., EH, S., 1999. SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. J. Biol. Chem. 274, 32145–32152.
1097. Francki, A., Motamed, K., McClure, T.D., Kaya, M., Murri, C., Blake, D.J., Carbon, J.G., EH, S., 2003. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. J. Cell. Biochem. 88, 802–811.
1098. Frontera, W.R., Ochala, J., 2015. Skeletal muscle: a brief review of structure and function. Calcif. Tissue Int. 96, 183–195.
1099. Ghanemi, A., St-Amand, J., 2018. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 55, 20–22.
1100. Ghanemi, A., Melouane, A., Mucunguzi, O., Yoshioka, M., St-Amand, J., 2018b. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 215, 190–197.
1101. Ghanemi, A., Yoshioka, M., St-Amand, J., 2018a. Broken energy homeostasis and obesity pathogenesis: the surrounding concepts. J. Clin. Med. 7, 453.
1102. Gilmour, D.T., Lyon, G.J., Carlton, M.B., Sanes, J.R., Cunningham, J.M., Anderson, J.R., Hogan, B.L., Evans, M.J., Colledge, W.H., 1998. Mice deficient for the secreted glycoprotein SPARC/osteonectin/BM40 develop normally but show severe age-onset cataract formation and disruption of the lens. EMBO J. 17, 1860–1870.
1103. Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543–546.
1104. Hardouin, P., Rharass, T., Lucas, S., 2016. Bone marrow adipose tissue: to Be or not to Be a typical adipose tissue? Front. Endocrinol. (Lausanne) 7, 85.
1105. Harris, B.A., 2005. The influence of endurance and resistance exercise on muscle capillarization in the elderly: a review. Acta Physiol. Scand. 185, 89–97.
1106. Health topics, 2019. Obesity. [http://www.who.int/topics/obesity/en/]
1107. Hocking, S.L., Chisholm, D.J., James, D.E., 2008. Studies of regional adipose transplantation reveal a unique and beneficial interaction between subcutaneous adipose tissue and the intra-abdominal compartment. Diabetologia 51, 900–902.
1108. Hsu, C.P., Lin, C.H., CY, K., 2018. Endothelial-cell inflammation and damage by reactive oxygen species are prevented by propofol via ABCA1-mediated cholesterol efflux. Int. J. Med. Sci. 15, 978–985.
1109. Islam, M.S., Ciavattini, A., Petraglia, F., Castellucci, M., Ciarmela, P., 2018. Extracellularmatrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics. Hum. Reprod. Update 24, 59–85.
1110. Ito, M., Ohno, K., 2018. Protein-anchoring therapy to target extracellular matrix proteins to their physiological destinations. Matrix Biol. 68–69, 628–636.
1111. Jackson, M.J., 2016. Reactive oxygen species in sarcopenia: should we focus on excess oxidative damage or defective redox signalling? Mol. Aspects Med. 50, 33–40.

1112. Jager, S., Handschin, C., St-Pierre, J., Spiegelman, B.M., 2007. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 104, 12017–12022.
1113. Jequier, E., 2002. Leptin signaling, adiposity, and energy balance. Ann. N. Y. Acad. Sci. 967, 379–388.
1114. JM, H., 1994. Physical activity, fitness, health and aging. In: Bouchard, C., Shephard, R.J., Stephens, T. (Eds.), Physical Activity, Fitness, and Health: International Proceedings and Consensus Statement. Human Kinetics Publishers Inc, Champaign, pp. 993–1005.
1115. Jorgensen, L.H., Jepsen, P.L., Boysen, A., Dalgaard, L.B., Hvid, L.G., Ortenblad, N., Ravn, D., Sellathurai, J., Moller-Jensen, J., Lochmuller, H., Schroder, H.D., 2017. SPARC interacts with actin in skeletal muscle in vitro and in vivo. Am. J. Pathol. 187, 457–474.
1116. Jorgensen, L.H., Petersson, S.J., Sellathurai, J., Andersen, D.C., Thayssen, S., Sant, D.J., Jensen, C.H., Schroder, H.D., 2009. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. J. Histochem. Cytochem. 57, 29–39.
1117. Kilov, D., G, K., 2018. Philosophical determinants of obesity as a disease. Obes. Rev. 19, 41–48.
1118. Korenevsky, A., Milyutina, Y., Kozina, L., Kerkeshko, G., Arutjunyan, A., 2017. Role of reactive oxygen species in premature ageing of the female reproductive function. Curr. Aging Sci. 10, 26–31.
1119. Kos, K., Wong, S., Tan, B., Gummesson, A., Jernas, M., Franck, N., Kerrigan, D., Nystrom, F.H., Carlsson, L.M., Randeva, H.S., et al., 2009. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. Diabetes 58, 1780–1788.
1120. Lane, T.F., Sage, E.H., 1994. The biology of SPARC, a protein that modulates cell-matrix interactions. FASEB J. 8, 163–173.
1121. Lin, C.T.H., Kang, L., 2016. Adipose extracellular matrix remodelling in obesity and insulin resistance. Biochem. Pharmacol. 119, 8–16.
1122. Lira, V.A., Benton, C.R., Yan, Z., Bonen, A., 2010. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. 299, E145–161.
1123. Mansergh, F.C., Wells, T., Elford, C., Evans, S.L., Perry, M.J., Evans, M.J., Evans, B.A., 2007. Osteopenia in Sparc (osteonectin)-deficient mice: characterization of phenotypic determinants of femoral strength and changes in gene expression. Physiol. Genomics 32, 64–73.
1124. Mariman, E.C., Wang, P., 2010. Adipocyte extracellular matrix composition, dynamics and role in obesity. Cell. Mol. Life Sci. 67, 1277–1292.
1125. Martinez-Huenchullan, S., McLennan, S.V., Verhoeven, A., Twigg, S.M., Tam, C.S., 2017. The emerging role of skeletal muscle extracellular matrix remodelling in obesity and exercise. Obes. Rev. 18, 776–790.
1126. Mason, I.J., Murphy, D., Munke, M., Francke, U., Elliott, R.W., Hogan, B.L., 1986a. Developmental and transformation-sensitive expression of the Sparc gene on mouse chromosome 11. EMBO J. 5, 1831–1837.
1127. Mason, I.J., Taylor, A., Williams, J.G., Sage, H., Hogan, B.L., 1986b. Evidence from molecular cloning that SPARC, a major product of mouse embryo parietal endoderm, is related to an endothelial cell'culture shock' glycoprotein of Mr 43,000. EMBO J. 5, 1465–1472.
1128. Masson, S., Arosio, B., Luvara, G., Gagliano, N., Fiordaliso, F., Santambrogio, D., Vergani, C., Latini, R., Annoni, G., 1998. Remodelling of cardiac extracellular matrix during beta-adrenergic stimulation: upregulation of SPARC in the myocardium of adult rats. J. Mol. Cell. Cardiol. 30, 1505–1514.
1129. McCurdy, S., Baicu, C.F., Heymans, S., Bradshaw, A.D., 2010. Cardiac extracellular matrix remodeling: fibrillar collagens and secreted Protein Acidic and Rich in Cysteine (SPARC). J. Mol. Cell. Cardiol. 48, 544–549.
1130. McMurray, F., Patten, D.A., Harper, M.E., 2016. Reactive oxygen species and oxidative stress in obesity recent findings and empirical approaches. Obesity (Silver Spring) 24, 2301–2310.
1131. Melouane, A., Yoshioka, M., Kanzaki, M., St-Amand, J., 2019. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 229, 277–287.

1132. Melouane, A., Carbonell, A., Yoshioka, M., Puymirat, J., St-Amand, J., 2018. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS One 13, e0192714.
1133. Miyata, S., Kitagawa, H., 2017. Formation and remodeling of the brain extracellular matrix in neural plasticity: roles of chondroitin sulfate and hyaluronan. Biochim. Biophys. Acta Gen. Subj. 1861, 2420–2434.
1134. Mori, S., Kiuchi, S., Ouchi, A., Hase, T., Murase, T., 2014. Characteristic expression of extracellular matrix in subcutaneous adipose tissue development and adipogenesis; comparison with visceral adipose tissue. Int. J. Biol. Sci. 10, 825–833.
1135. Morrissey, M.A., Jayadev, R., Miley, G.R., Blebea, C.A., Chi, Q., Ihara, S., Sherwood, D.R., 2016. SPARC promotes cell invasion in vivo by decreasing type IV collagen levels in the basement membrane. PLoS Genet. 12, e1005905.
1136. Moustafa, P.E., Abdelkader, N.F., El Awdan, S.A., El-Shabrawy, O.A., Zaki, H.F., 2018. Extracellular Matrix Remodeling and Modulation of Inflammation and Oxidative Stress by Sulforaphane in Experimental Diabetic Peripheral Neuropathy. Inflammation 41, 1460–1476.
1137. Mucunguzi, O., Melouane, A., Ghanemi, A., Yoshioka, M., Boivin, A., Calvo, E.L., J, S.-A., 2017. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. (Lond.) 14, 66.
1138. Myers Jr., M.G., Heymsfield, S.B., Haft, C., Kahn, B.B., Laughlin, M., Leibel, R.L., Tschöp, M.H., Yanovski, J.A., 2012. Challenges and opportunities of defining clinical leptin resistance. Cell Metab. 15, 150–156.
1139. Naczki, C., John, B., Patel, C., Lafferty, A., Ghoneum, A., Afify, H., White, M., Davis, A., Jin, G., Kridel, S., Said, N., 2018. SPARC inhibits metabolic plasticity in ovarian cancer. Cancers (Basel) 10.
1140. Nakamura, K., Nakano, S., Miyoshi, T., Yamanouchi, K., Matsuwaki, T., Nishihara, M., 2012. Agerelated resistance of skeletal muscle-derived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. Aging (Albany NY) 4, 40–48.
1141. Nakamura, K., Nakano, S., Miyoshi, T., Yamanouchi, K., Nishihara, M., 2013. Loss of SPARC in mouse skeletal muscle causes myofiber atrophy. Muscle Nerve 48, 791–799.
1142. Neeland, I.J., Ross, R., Despres, J.P., Matsuzawa, Y., Yamashita, S., Shai, I., Seidell, J., Magni, P., Santos, R.D., Arsenault, B., et al., 2019. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. Lancet Diabetes Endocrinol. 7 (9), 715–725.
1143. Neufer, P.D., Dohm, G.L., 1993. Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. Am. J. Physiol. 265, C1597–1603.
1144. Neuzillet, C., Tijeras-Raballand, A., Cros, J., Faivre, S., Hammel, P., Raymond, E., 2013. Stromal expression of SPARC in pancreatic adenocarcinoma. Cancer Metastasis Rev. 32, 585–602.
1145. Nie, J., Sage, E.H., 2009a. SPARC functions as an inhibitor of adipogenesis. J. Cell Commun. Signal. 3, 247–254.
1146. Nie, J., Sage, E.H., 2009b. SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling. J. Biol. Chem. 284, 1279–1290.
1147. Nie, J., Bradshaw, A.D., Delany, A.M., Sage, E.H., 2011. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. Connect. Tissue Res. 52, 99–108.
1148. Nishida, Y., Tanaka, H., Tobina, T., Murakami, K., Shono, N., Shindo, M., Ogawa, W., Yoshioka, M., St-Amand, J., 2010. Regulation of muscle genes by moderate exercise. Int. J. Sports Med. 31, 656–670.
1149. Norose, K., Clark, J.I., Syed, N.A., Basu, A., Heber-Katz, E., Sage, E.H., Howe, C.C., 1998. SPARC deficiency leads to early-onset cataractogenesis. Invest. Ophthalmol. Vis. Sci. 39, 2674–2680.
1150. Nuutila, P., Knuuti, M.J., Raitakari, M., Ruotsalainen, U., Teras, M., Voipio-Pulkki, L.M., Haaparanta, M., Solin, O., Wegelius, U., Yki-Jarvinen, H., 1994. Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans. Am. J. Physiol. 267, E941–946.
1151. Panigrahy, S.K., Bhatt, R., Kumar, A., 2017. Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. J. Drug Target. 25, 93–101.
1152. Park, H.S., Lee, K., 2005. Greater beneficial effects of visceral fat reduction compared with subcutaneous fat reduction on parameters of the metabolic syndrome: a study of weight reduction programmes in subjects with visceral and subcutaneous obesity. Diabet. Med. 22, 266–272.

1153. Petersson, S.J., Jorgensen, L.H., Andersen, D.C., Norgaard, R.C., Jensen, C.H., Schroder, H.D., 2013. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 28, 1451–1460.
1154. Polyzos, S.A., Margioris, A.N., 2018. Sarcopenic obesity. Hormones (Athens) 17, 321–331.
1155. Radom-Aizik, S., Hayek, S., Shahar, I., Rechavi, G., Kaminski, N., Ben-Dov, I., 2005. Effects of aerobic training on gene expression in skeletal muscle of elderly men. Med. Sci. Sports Exerc. 37, 1680–1696.
1156. Riedl, I., Yoshioka, M., Nishida, Y., Tobina, T., Paradis, R., Shono, N., Tanaka, H., StAmand, J., 2010. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 45, 896–903.
1157. Rosset, E.M., Bradshaw, A.D., 2016. SPARC/osteonectin in mineralized tissue. Matrix Biol. 52–54, 78–87.
1158. Sage, H., Vernon, R.B., Funk, S.E., Everitt, E.A., Angello, J., 1989. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. J. Cell Biol. 109, 341–356.
1159. Said, N., Socha, M.J., Olearczyk, J.J., Elmarakby, A.A., Imig, J.D., Motamed, K., 2007. Normalization of the ovarian cancer microenvironment by SPARC. Mol. Cancer Res. 5, 1015–1030.
1160. Sainz, N., Barrenetxe, J., Moreno-Aliaga, M.J., Martinez, J.A., 2015. Leptin resistance and dietinduced obesity: central and peripheral actions of leptin. Metabolism 64, 35–46.
1161. Schellings, M.W., Vanhoutte, D., Swinnen, M., Cleutjens, J.P., Debets, J., van Leeuwen, R.E., d'Hooge, J., Van de Werf, F., Carmeliet, P., Pinto, Y.M., et al., 2009. Absence of SPARC results in increased cardiac rupture and dysfunction after acute myocardial infarction. J. Exp. Med. 206, 113–123.
1162. Scime, A., Desrosiers, J., Trensz, F., Palidwor, G.A., Caron, A.Z., Andrade-Navarro, M.A., Grenier, G., 2010. Transcriptional profiling of skeletal muscle reveals factors that are necessary to maintain satellite cell integrity during ageing. Mech. Ageing Dev. 131, 9–20.
1163. Smas, C.M., Sul, H.S., 1995. Control of adipocyte differentiation. Biochem. J. 309 (Pt 3), 697–710.
1164. Smith, J.D., Borel, A.L., Nazare, J.A., Haffner, S.M., Balkau, B., Ross, R., Massien, C., Almeras, N., Despres, J.P., 2012. Visceral adipose tissue indicates the severity of cardiometabolic risk in patients with and without type 2 diabetes: results from the INSPIRE ME IAA study. J. Clin. Endocrinol. Metab. 97, 1517– 1525.
1165. So, B., Kim, H.J., Kim, J., Song, W., 2014. Exercise-induced myokines in health and metabolic diseases. Integr. Med. Res. 3, 172–179.
1166. Song, H., Guan, Y., Zhang, L., Li, K., Dong, C., 2010. SPARC interacts with AMPK and regulates GLUT4 expression. Biochem. Biophys. Res. Commun. 396, 961–966.
1167. St-Amand, J., Yoshioka, M., Nishida, Y., Tobina, T., Shono, N., Tanaka, H., 2012. Effects of mildexercise training cessation in human skeletal muscle. Eur. J. Appl. Physiol. 112, 853–869.
1168. St-Onge, M.P., Gallagher, D., 2010. Body composition changes with aging: the cause or the result of alterations in metabolic rate and macronutrient oxidation? Nutrition 26, 152–155.
1169. Swaroop, A., Hogan, B.L., Francke, U., 1988. Molecular analysis of the cDNA for human SPARC/osteonectin/BM-40: sequence, expression, and localization of the gene to chromosome 5q31-q33. Genomics 2, 37–47.
1170. Tai, I.T., Tang, M.J., 2008. SPARC in cancer biology: its role in cancer progression and potential for therapy. Drug Resist. Updat. 11, 231–246.
1171. Takahashi, M., Nagaretani, H., Funahashi, T., Nishizawa, H., Maeda, N., Kishida, K., Kuriyama, H., Shimomura, I., Maeda, K., Hotta, K., et al., 2001. The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease. Obes. Res. 9, 388–393.
1172. Tanaka, H.Y., Kitahara, K., Sasaki, N., Nakao, N., Sato, K., Narita, H., Shimoda, H., Matsusaki, M., Nishihara, H., Masamune, A., Kano, M.R., 2019. Pancreatic stellate cells derived from human pancreatic cancer demonstrate aberrant SPARC-dependent ECM remodeling in 3D engineered fibrotic tissue of clinically relevant thickness. Biomaterials 192, 355–367.
1173. Taneda, S., Pippin, J.W., Sage, E.H., Hudkins, K.L., Takeuchi, Y., Couser, W.G., Alpers, C.E., 2003. Amelioration of diabetic nephropathy in SPARC-null mice. J. Am. Soc. Nephrol. 14, 968–980.
1174. Tartare-Deckert, S., Chavey, C., Monthouel, M.N., Gautier, N., Van Obberghen, E., 2001. The matricellular protein SPARC/osteonectin as a newly identified factor up-regulated in obesity. J. Biol. Chem. 276, 22231 22237.

1175. Termine, J.D., Kleinman, H.K., Whitson, S.W., Conn, K.M., McGarvey, M.L., Martin, G.R., 1981. Osteonectin, a bone-specific protein linking mineral to collagen. Cell 26, 99–105.
1176. Theocharis, A.D., Skandalis, S.S., Gialeli, C., Karamanos, N.K., 2016. Extracellular matrix structure. Adv. Drug Deliv. Rev. 97, 4–27.
1177. Reynolds, T.H., Reid, P., Larkin, L.M., Dengel, D.R., 2004. Effects of aerobic exercise training on the protein kinase B (PKB)/mammalian target of rapamycin (mTOR) signaling pathway in aged skeletal muscle. Exp. Gerontol. 39, 379–385.
1178. Tomos, I.P., Tzouvelekis, A., Aidinis, V., Manali, E.D., Bouros, E., Bouros, D., Papiris, S.A., 2017. Extracellular matrix remodeling in idiopathic pulmonary fibrosis. It is the' bed' that counts and not' the sleepers'. Expert Rev. Respir. Med. 11, 299–309.
1179. Tran, T.T., Yamamoto, Y., Gesta, S., Kahn, C.R., 2008a. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 7, 410–420.
1180. Tran, T.T., Yamamoto, Y., Gesta, S., Kahn, C.R., 2008b. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 7, 410–420.
1181. van der Steen, S.C., Raave, R., Langerak, S., van Houdt, L., van Duijnhoven, S.M., van Lith, S.A., Massuger, L.F., Daamen, W.F., Leenders, W.P., van Kuppevelt, T.H., 2017. Targeting the extracellular matrix of ovarian cancer using functionalized, drug loaded lyophilisomes. Eur. J. Pharm. Biopharm. 113, 229–239.
1182. Vasilaki, A., Richardson, A., Van Remmen, H., Brooks, S.V., Larkin, L., McArdle, A., Jackson, M.J., 2017. Role of nerve-muscle interactions and reactive oxygen species in regulation of muscle proteostasis with ageing. J. Physiol. 595, 6409–6415.
1183. Wang, C.-Y., Liao, J.K., 2012. A mouse model of diet-induced obesity and insulin resistance. Methods Mol. Biol. (Clifton, NJ) 821, 421–433.
1184. Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C., Spiegelman, B.M., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.
1185. Yoshioka, M., Bolduc, C., Raymond, V., St-Amand, J., 2008. High-fat meal-induced changes in the duodenum mucosa transcriptome. Obesity (Silver Spring) 16, 2302–2307.
1186. Riazi, A.M.; Kwon, S.Y.; Stanford, W.L. Stem Cell Sources for Regenerative Medicine. Adv. Struct. Saf. Stud. 2009, 482, 55–90.
1187. Mizuno, H. Adipose-derived stem and stromal cells for cell-based therapy: Current status of preclinical studies and clinical trials. Curr. Opin. Mol. Ther. 2010, 12, 442–449.
1188. Aboushwareb, T.; Atala, A. Stem cells in urology. Nat. Clin. Pract. Urol. 2008, 5, 621–631.
1189. Horton, E.R.; Vallmajo-Martin, Q.; Martin, I.; Snedeker, J.G.; Ehrbar, M.; Blache, U. Extracellular Matrix Production by Mesenchymal Stromal Cells in Hydrogels Facilitates Cell Spreading and Is Inhibited by FGF-2. Adv. Heal. Mater. 2020, 1901669.
1190. Geckil, H.; Xu, F.; Zhang, X.; Moon, S.; Demirci, U. Engineering hydrogels as extracellular matrix mimics. Nanomedicine 2010, 5, 469–484.
1191. Saldin, L.T.; Cramer, M.C.; Velankar, S.S.; White, L.; Badylak, S.F. Extracellular matrix hydrogels from decellularized tissues: Structure and function. Acta Biomater. 2017, 49, 1–15.
1192. Swinehart, I.T.; Badylak, S.F. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. Dev. Dyn. 2016, 245, 351–360
1193. Theocharis, A.D.; Skandalis, S.S.; Gialeli, C.; Karamanos, N.K. Extracellular matrix structure. Adv. Drug Deliv. Rev. 2016, 97, 4–27.
1194. Vigetti, D.; Viola, M.; Karousou, E.; Deleonibus, S.; Karamanou, K.; De Luca, G.; Passi, A. Epigenetics in extracellular matrix remodeling and hyaluronan metabolism. FEBS J. 2014, 281, 4980–4992.
1195. Sonbol, H.S. Extracellular Matrix Remodeling in Human Disease. J. Microsc. Ultrastruct. 2018, 6, 123– 128.
1196. Rauch, U. Extracellular matrix components associated with remodeling processes in brain. Cell. Mol. Life Sci. 2004, 61, 2031–2045.
1197. Park, K.M.; Lewis, D.; Gerecht, S. Bioinspired Hydrogels to Engineer Cancer Microenvironments. Annu. Rev. Biomed. Eng. 2017, 19, 109–133.
1198. Termine, J.D.; Kleinman, H.K.; Whitson, S.; Conn, K.M.; McGarvey, M.L.; Martin, G.R. Osteonectin, a bone-specific protein linking mineral to collagen. Cell 1981, 26, 99–105.

1199. Bradshaw, A.D. The role of SPARC in extracellular matrix assembly. J. Cell Commun. Signal. 2009, 3, 239–246.
1200. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Boil. 2019, 117, 105627.
1201. Kelleher, C.M.; McLean, S.; Mecham, R.P. Vascular Extracellular Matrix and Aortic Development, in Current Topics in Developmental Biology; Academic Press: Cambridge, MA, USA, 2004; pp. 153–188.
1202. Maeda, H.; Wada, N.; Tomokiyo, A.; Monnouchi, S.; Akamine, A. Chapter Six-Prospective Potency of TGF-β1 on Maintenance and Regeneration of Periodontal Tissue, in International Review of Cell and Molecular Biology; Jeon, K.W., Ed.; Academic Press: Cambridge, MA, USA, 2013; pp. 283–367.
1203. McKee, D.M.; Cole, W.G. Chapter 2-Bone Matrix and Mineralization, in Pediatric Bone, 2nd ed.; Glorieux, F.H., Pettifor, J.M., Jüppner, H., Eds.; Academic Press: Cambridge, MA, USA, 2012; pp. 9–37.
1204. Workman, G.; Bradshaw, A.D. Chapter 19-Production and Purification of Recombinant Human SPARC, in Methods in Cell Biology; Mecham, R.P., Ed.; Academic Press: Cambridge, MA, USA, 2018; pp. 335–345.
1205. Humphrey, E.L.; Morris, G.E.; Fuller, H. Valproate reduces collagen and osteonectin in cultured bone cells. Epilepsy Res. 2013, 106, 446–450.
1206. Cerilli, A.L.; Wick, M.R. Chapter 3-Immunohistology of Soft Tissue and Osseous Neoplasms, in Diagnostic Immunohistochemistry, 2nd ed.; Dabbs, D.J., Ed.; Churchill Livingstone: London, UK, 2006; pp. 65–120.
1207. Heinegard, D.; Oldberg, A. Structure and biology of cartilage and bone matrix noncollagenous macromolecules. Faseb J. 1989, 3, 2042–2051.
1208. Jundt, G.; Termine, J.D.; Schulz, A. Osteonectin–a differentiation marker of bone cells. Cell Tissue Res. 1987, 248, 409–415.
1209. Badylak, S.F. The extracellular matrix as a scaffold for tissue reconstruction. Semin. Cell Dev. Boil. 2002, 13, 377–383.
1210. Hodde, J.P. Extracellular matrix as a bioactive material for soft tissue reconstruction. ANZ J. Surg. 2006, 76, 1096–1100.
1211. Spang, M.T.; Karen, C. Extracellular matrix hydrogel therapies: In vivo applications and development. Acta Biomater. 2018, 68, 1–14.
1212. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation andtissue response to injury. J. Clin. Investig. 2001, 107, 1049–1054.
1213. Brekken, R.A.; Sage, E. SPARC, a matricellular protein: At the crossroads of cell–matrix communication. Matrix Boil. 2001, 19, 815–827.
1214. Bella, J.; Hulmes, D.J. Fibrillar Collagens. Subcell Biochem. 2017, 82, 457–490.
1215. Mortimer, G.M.; Minchin, R.F. Cryptic epitopes and functional diversity in extracellular proteins. Int. J. Biochem. Cell Boil. 2016, 81, 112–120.
1216. Wong, S.L.; Sukkar, M.B. The SPARC protein: An overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease. Br. J. Pharmacol. 2016, 174, 3–14.
1217. Bonnans, C.; Chou, J.; Werb, Z. Remodelling the extracellular matrix in development and disease. Nat. Rev. Mol. Cell Boil. 2014, 15, 786–801.
1218. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. Matrix Boil. 2016, 52, 78–87.
1219. Holland, P.W.H.; Harper, S.J.; McVey, J.H.; Hogan, B.L. In vivo expression of mRNA for the Ca++-binding protein SPARC (osteonectin) revealed by in situ hybridization. J. Cell Boil. 1987, 105, 473–482.
1220. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
1221. Müller, M.J.; Lagerpusch, M.; Enderle, J.; Schautz, B.; Heller, M.; Bosy-Westphal, A. Beyond the body mass index: Tracking body composition in the pathogenesis of obesity and the metabolic syndrome. Obes. Rev. 2012, 13, 6–13.
1222. Goossens, G.H. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. Obes. Facts 2017, 10, 207–215.
1223. Després, J.-P.; Fias, F. Body Fat Distribution and Risk of Cardiovascular Disease. Circulation 2012, 126, 1301–1313.

1224. Bradshaw, A.D.; Graves, D.C.; Motamed, K.; Sage, E.H. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc. Natl. Acad. Sci. USA 2003, 100, 6045–6050.
1225. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
1226. Melouane, A.; Yoshioka, M.; St-Amand, J. Extracellular matrix/mitochondria pathway: A novel potential target for sarcopenia. Mitochondrion 2019, 50, 63–70.
1227. Romani, P.; Brian, I.; Santinon, G.; Pocaterra, A.; Audano, M.; Pedretti, S.; Mathieu, S.; Forcato, M.; Bicciato, S.; Manneville, J.-B.; et al. Extracellular matrix mechanical cues regulate lipid metabolism through Lipin-1 and SREBP. Nature 2019, 21, 338–347.
1228. Sullivan, W.J.; Mullen, P.J.; Schmid, E.W.; Flores, A.; Momcilovic, M.; Sharpley, M.S.; Jelinek, D.; Whiteley, A.E.; Maxwell, M.B.; Wilde, B.; et al. Extracellular Matrix Remodeling Regulates Glucose Metabolism through TXNIP Destabilization. Cell 2018, 175, 117–132.e21.
1229. Song, H.; Guan, Y.; Zhang, L.; Li, K.; Dong, C. SPARC interacts with AMPK and regulates GLUT4 expression. Biochem. Biophys. Res. Commun. 2010, 396, 961–966.
1230. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. J. VeterMed Sci. 2019, 81, 1649–1654.
1231. Riley, J.H.; Bradshaw, A.D. The Influence of the Extracellular Matrix in Inflammation: Findings from the SPARC-Null Mouse; Anat Rec: Hoboken, NJ, USA, 2019.
1232. Gilmour, D.T.; Lyon, G.J.; Carlton, M.B.; Sanes, J.R.; Cunningham, J.M.; Anderson, J.R.; Hogan, B.L.; Evans, M.J.; Colledge, W.H. Mice deficient for the secreted glycoprotein SPARC/osteonectin/BM40 develop normally but show severe age-onset cataract formation and disruption of the lens. EMBO J. 1998, 17, 1860–1870.
1233. Swaminathan, S.S.; Oh, D.-J.; Kang, M.H.; Ren, R.; Jin, R.; Gong, H.; Rhee, U.J. Secreted Protein Acidic and Rich in Cysteine (SPARC)-Null Mice Exhibit More Uniform Outflow. Investig. Opthalmol. Vis. Sci. 2013, 54, 2035–2047.
1234. Sangaletti, S.; Tripodo, C.; Cappetti, B.; Casalini, P.; Chiodoni, C.; Piconese, S.; Santangelo, A.; Parenza, M.; Arioli, I.; Miotti, S.; et al. SPARC Oppositely Regulates Inflammation and Fibrosis in BleomycinInduced Lung Damage. Am. J. Pathol. 2011, 179, 3000–3010.
1235. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. Genes Immun. 2007, 8, 262–274.
1236. Pichler, R.H.; Bassuk, J.A.; Hugo, C.; Reed, M.J.; Eng, E.; Gordon, K.L.; Pippin, J.; Alpers, C.E.; Couser, W.G.; Sage, E.H.; et al. SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growth-factor-medicated mesangial cell proliferation in vitro. Am. J. Pathol. 1996, 148, 1153–1167.
1237. Francki, A.; Motamed, K.; McClure, T.D.; Kaya, M.; Murri, C.; Blake, D.J.; Carbon, J.G.; Sage, E.H. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. J. Cell. Biochem. 2003, 88, 802–811.
1238. Ghanemi, A.; St-Amand, J. Interleukin-6 as a “metabolic hormone”. Cytokine 2018, 112, 132–136.
1239. Xu, Y.; Zhang, Y.; Ye, J. IL-6: A Potential Role in Cardiac Metabolic Homeostasis. Int. J. Mol. Sci. 2018, 19, 2474.
1240. Qu, D.; Liu, J.; Lau, C.W.; Huang, Y. IL-6 in diabetes and cardiovascular complications. Br. J. Pharmacol. 2014, 171, 3595–3603.
1241. Erdogan, B.; Webb, D.J. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem. Soc. Trans. 2017, 45, 229–236.
1242. Moriggi, M.; Giussani, M.; Torretta, E.; Capitanio, D.; Sandri, M.; Leone, R.; De Palma, S.; Vasso, M.; Vozzi, G.; Tagliabue, E.; et al. ECM Remodeling in Breast Cancer with Different Grade: Contribution of 2D DIGE Proteomics. Proteom. 2018, 18, 1800278.
1243. Yuzhalin, A.E.; Lim, S.Y.; Kutikhin, A.; Gordon-Weeks, A.N. Dynamic matrisome: ECM remodeling factors licensing cancer progression and metastasis. Biochim. Biophys. Acta (BBA) Bioenerg. 2018, 1870, 207–228.

1244. Yang, M.; Liu, P.; Huang, P. Cancer stem cells, metabolism, and therapeutic significance. Tumor Boil. 2016, 37, 5735–5742.
1245. Lyssiotis, C.A.; Kimmelman, A.C. Metabolic Interactions in the Tumor Microenvironment. Trends Cell Boil. 2017, 27, 863–875.
1246. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
1247. Irminger-Finger, I.; Kargul, J.; Laurent, G.J.; Laurent, G.J. Extra cellular matrix a modular soil for stem cells. Int. J. Biochem. Cell Boil. 2016, 81, 164.
1248. Murphy-Ullrich, J.E.; Sage, E.H. Revisiting the matricellular concept. Matrix Boil. 2014, 37, 1–14.
1249. P. Arulselvan, et al., Role of antioxidants and natural products in inflammation, Oxid. Med. Cell Longev. 2016 (2016) 5276130.
1250. G.W. Schmid-Schönbein, Analysis of inflammation, Annu. Rev. Biomed. Eng. 8 (1) (2006) 93–151.
1251. D.V. Kuprash, S.A. Nedospasov, Molecular and cellular mechanisms of inflammation, Biochemistry (Mosc) 81 (11) (2016) 1237–1239.
1252. W.G. Spector, D.A. Willoughby, Vasoactive amines in acute inflammation, Ann. N. Y. Acad. Sci. 116 (1964) 839–846.
1253. K.R. Patil, et al., Animal Models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals, Int. J. Mol. Sci. 20 (18) (2019).
1254. T. Ganz, Anemia of inflammation, N. Engl. J. Med. 381 (12) (2019) 1148–1157.
1255. A. Ghanemi, M. Yoshioka, J. St-Amand, Broken energy homeostasis and obesity pathogenesis: the surrounding concepts, J. Clin. Med. 7 (11) (2018).
1256. A. Ghanemi, J. St-Amand, Interleukin-6 as a "metabolic hormone", Cytokine 112 (2018) 132–136.
1257. A. Ghanemi, J. St-Amand, Redefining obesity toward classifying as a disease, Eur. J. Intern. Med. 55 (2018) 20–22.
1258. N.V. Zotova, et al., Integral estimation of systemic inflammatory response under sepsis, Gen. Physiol. Biophys. 39 (1) (2020) 13–26.
1259. Y. Zhu, et al., Changes of inflammatory mediators and oxidative stress indicators in children with Henoch-Schonlein purpura and clinical effects of hemoperfusion in the treatment of severe Henoch-Schonlein purpura with gastrointestinal involvement in children, BMC Pediatr 19 (1) (2019) 409.
1260. D.C. McMillan, K. Canna, C.S. McArdle, Systemic inflammatory response predicts survival following curative resection of colorectal cancer, Br. J. Surg. 90 (2) (2003) 215–219.
1261. H. Song, et al., MiR-29 family members interact with SPARC to regulate glucose metabolism, Biochem. Biophys. Res. Commun. 497 (2) (2018) 667–674.
1262. K. Kos, et al., Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose, Diabetes 58 (8) (2009) 1780–1788.
1263. A. Ghanemi, M. Yoshioka, J. St-Amand, Secreted protein acidic and rich in cysteine: metabolic and homeostatic properties beyond the extracellular matrix structure, Appl. Sci. 10 (7) (2020) 2388.
1264. A. Ghanemi, et al., Secreted protein acidic and rich in cysteine and bioenergetics: extracellular matrix, adipocytes remodeling and skeletal muscle metabolism, Int. J. Biochem. Cell Biol. 117 (2019) 105627.
1265. Y. Shen, et al., SPARC is over-expressed in adipose tissues of diet-induced obese rats and causes insulin resistance in 3T3-L1 adipocytes, Acta Histochem. 116 (1) (2014) 158–166.
1266. K. Kos, J.P. Wilding, SPARC: a key player in the pathologies associated with obesity and diabetes, Nat. Rev. Endocrinol. 6 (4) (2010) 225–235.
1267. S.A. Rempel, et al., Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response, Genes Immun. 8 (3) (2007) 262–274.
1268. A. Ghanemi, M. Yoshioka, J. St-Amand, Secreted protein acidic and rich in cysteine and cancer: a homeostatic hormone? Cytokine 127 (2020) 154996.
1269. G.P. Nagaraju, D. Sharma, Anti-cancer role of SPARC, an inhibitor of adipogenesis, Cancer Treat. Rev. 37 (7) (2011) 559–566.
1270. S.L.I. Wong, M.B. Sukkar, The SPARC protein: an overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease, Br. J. Pharmacol. 174 (1) (2017) 3–14.
1271. L. Ali, J.G. Schnitzler, J. Kroon, Metabolism: the road to inflammation and atherosclerosis, Curr. Opin. Lipidol. 29 (6) (2018) 474–480.
1272. J. Saravia, et al., Signaling networks in immunometabolism, Cell Res. (2020).

1273. L. Chen, et al., Mechanisms linking inflammation to insulin resistance, Int. J. Endocrinol. 2015 (2015) 508409.
1274. N. Singh, et al., Inflammation and cancer, Ann. Afr. Med. 18 (3) (2019) 121–126.
1275. M. Murata, Inflammation and cancer, Environ. Health Prev. Med. 23 (1) (2018) 50.
1276. S. Tsalamandris, et al., The role of inflammation in diabetes: current concepts and future perspectives, Eur. Cardiol. 14 (1) (2019) 50–59.
1277. P. Hunter, The inflammation theory of disease. The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment, EMBO Rep. 13 (11) (2012) 968–970.
1278. R.H. Pichler, et al., SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growth-factor-medicated mesangial cell proliferation in vitro, Am. J. Pathol. 148 (4) (1996) 1153–1167.
1279. N.A. Said, et al., SPARC ameliorates ovarian cancer-associated inflammation, Neoplasia 10 (10) (2008) 1092–1104.
1280. H.J. Riley, A.D. Bradshaw, The influence of the extracellular matrix in inflammation: findings from the SPARC-null mouse, Anatom. Rec. n/a (n/a).
1281. S. Sangaletti, et al., SPARC oppositely regulates inflammation and fibrosis in bleomycin-induced lung damage, Am. J. Pathol. 179 (6) (2011) 3000–3010.
1282. Z. Cao, M.E. Cooper, Role of angiotensin II in tubulointerstitial injury, Semin. Nephrol. 21 (6) (2001) 554–562.
1283. H. Toba, et al., Secreted protein acidic and rich in cysteine facilitates age-related cardiac inflammation and macrophage M1 polarization, Am. J. Physiol. Cell Physiol. 308 (12) (2015) C972–C982.
1284. M. Tanaka, et al., Secreted protein acidic and rich in cysteine functions in colitis via IL17A regulation in mucosal CD4(+) T cells, J. Gastroenterol. Hepatol. 33 (3) (2018) 671–680.
1285. M.J. Socha, et al., Secreted protein acidic and rich in cysteine deficiency ameliorates renal inflammation and fibrosis in angiotensin hypertension, Am. J. Pathol. 171 (4) (2007) 1104–1112.
1286. Y.L. Ng, et al., Secreted protein acidic and rich in cysteine (SPARC) exacerbates colonic inflammatory symptoms in dextran sodium sulphate-induced murine colitis, PLoS ONE 8 (10) (2013) e77575.
1287. C. Atorrasagasti, et al., Lack of the matricellular protein SPARC (secreted protein, acidic and rich in cysteine) attenuates liver fibrogenesis in mice, PLoS ONE 8 (2) (2013) e54962.
1288. G. Mazzolini, et al., SPARC expression is associated with hepatic injury in rodents and humans with non-alcoholic fatty liver disease, Sci. Rep. 8 (1) (2018) 725.
1289. G. Dimitriadis, et al., Insulin effects in muscle and adipose tissue, Diabetes Res. Clin. Pract. 93 (2011) S52–S59.
1290. D.S. Edgerton, et al., Insulin's direct effects on the liver dominate the control of hepatic glucose production, J. Clin. Invest. 116 (2) (2006) 521–527.
1291. V. Abella, et al., Leptin in the interplay of inflammation, metabolism and immune system disorders, Nat. Rev. Rheumatol. 13 (2) (2017) 100–109.
1292. J. Trombetta-Esilva, A.D. Bradshaw, The function of SPARC as a mediator of fibrosis, Open Rheumatol. J. 6 (2012) 146–155.
1293. A.D. Bradshaw, The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: does expression of SPARC by macrophages influence outcomes? J. Mol. Cell. Cardiol. 93 (2016) 156–161.
1294. B.S. Harris, et al., SPARC regulates collagen interaction with cardiac fibroblast cell surfaces, Am. J. Physiol. Heart Circ. Physiol. 301 (3) (2011) H841–H847.
1295. E. Frizell, et al., Expression of SPARC in normal and fibrotic livers, Hepatology 21 (3) (1995) 847–854.
1296. S. Omi, et al., Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice, J. Vet. Med. Sci. 81 (11) (2019) 1649–1654.
1297. L.T. McDonald, et al., Increased macrophage-derived SPARC precedes collagen deposition in myocardial fibrosis, Am. J. Physiol. Heart Circ. Physiol. 315 (1) (2018) H92–h100.
1298. A.D. Bradshaw, et al., Age-dependent alterations in fibrillar collagen content and myocardial diastolic function: role of SPARC in post-synthetic procollagen processing, Am. J. Physiol. Heart Circ. Physiol. 298 (2) (2010) H614–H622.

1299. W. Chang, et al., SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin, J. Biol. Chem. 285 (11) (2010) 8196–8206.
1300. M. Mack, Inflammation and fibrosis, Matrix Biol. 68–69 (2018) 106–121.
1301. C.J. Song, et al., Inflammation and fibrosis in polycystic kidney disease, Results Probl. Cell Differ. 60 (2017) 323–344.
1302. R. Kolb, F.S. Sutterwala, W. Zhang, Obesity and cancer: inflammation bridges the two, Curr. Opin. Pharmacol. 29 (2016) 77–89.
1303. C. Chiodoni, M.P. Colombo, S. Sangaletti, Matricellular proteins: from homeostasis to inflammation, cancer, and metastasis, Cancer Metastasis Rev. 29 (2) (2010) 295–307.
1304. A.S. Llera, et al., Matricellular proteins and inflammatory cells: a task force to promote or defeat cancer? Cytokine Growth Factor Rev. 21 (1) (2010) 67–76.
1305. I. Riedl, et al., Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity, Exp. Gerontol. 45 (11) (2010) 896–903.
1306. W. Aoi, et al., Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation, FASEB J. 33 (9) (2019) 10551–10562.
1307. F.A. Galle, D. Martella, G. Bresciani, Antioxidant and anti-inflammatory modulation of exercise during aging, Rev Esp Geriatr Gerontol 53 (5) (2018) 279–284.
1308. C.V. de Sousa, et al., The antioxidant effect of exercise: a systematic review and meta-analysis, Sports Med. 47 (2) (2017) 277–293.
1309. S. Krebs, A. Berling-Ernst, M. Halle, Physical activity and cancer, Sportverletz. Sportschaden 32 (2) (2018) 143–147.
1310. D.W. Jenkins, A. Jenks, Exercise and diabetes: a narrative review, J. Foot Ankle Surg. 56 (5) (2017) 968–974.
1311. M. Takahashi, et al., The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease, Obes. Res. 9 (7) (2001) 388–393.
1312. B. So, et al., Exercise-induced myokines in health and metabolic diseases, Integr. Med. Res 3 (4) (2014) 172–179.
1313. J. Vaz, et al., SPARC: a potential prognostic and therapeutic target in pancreatic cancer, Pancreas 44 (7) (2015) 1024–1035.
1314. M. Sinn, et al., SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study, Ann. Oncol. 25 (5) (2014) 1025–1032.
1315. S. Mukherjee, et al., Secreted protein acidic and rich in cysteine (SPARC) regulates thermogenesis in white and brown adipocytes, Mol. Cell. Endocrinol. 506 (2020) 110757.
1316. A. Munasinghe, et al., Fibronectin acts as a molecular switch to determine SPARC function in pancreatic cancer, Cancer Lett. 477 (2020) 88–96.
1317. T. Carvalheiro, et al., Extracellular SPARC cooperates with TGF-beta signalling to induce pro-fibrotic activation of systemic sclerosis patient dermal fibroblasts, Rheumatology (Oxford) (2019).
1318. P. Lu, et al., Extracellular matrix degradation and remodeling in development and disease, Cold Spring Harb. Perspect. Biol. 3 (12) (2011).
1319. C. Bonnans, J. Chou, Z. Werb, Remodelling the extracellular matrix in development and disease, Nat. Rev. Mol. Cell Biol. 15 (12) (2014) 786–801.
1320. A.D. Theocharis, et al., Extracellular matrix structure, Adv. Drug Deliv. Rev. 97 (2016) 4–27.
1321. J.D. Termine, et al., Osteonectin, a bone-specific protein linking mineral to collagen, Cell 26 (1 Pt 1) (1981) 99–105.
1322. I.T. Tai, M.J. Tang, SPARC in cancer biology: its role in cancer progression and potential for therapy, Drug Resist. Updat. 11 (6) (2008) 231–246.
1323. A.D. Bradshaw, E.H. Sage, SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury, J. Clin. Invest. 107 (9) (2001) 1049–1054.
1324. A. Basu, et al., Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40), BMC Cell Biol. 2 (2001) 15.
1325. E.M. Rosset, A.D. Bradshaw, SPARC/osteonectin in mineralized tissue, Matrix Biol. 52–54 (2016) 78–87.
1326. R.A. Brekken, E.H. Sage, SPARC, a matricellular protein: at the crossroads of cellmatrix, Matrix Biol. 19 (7) (2000) 569–580.
1327. I. Riedl, et al., Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity, Exp. Gerontol. 45 (11) (2010) 896–903.

1328. A. Melouane, et al., Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells, Life Sci. 229 (2019) 277–287.
1329. A. Ghanemi, et al., Secreted protein acidic and rich in cysteine and bioenergetics: extracellular matrix, adipocytes remodeling and skeletal muscle metabolism, Int. J. Biochem. Cell Biol. 117 (2019) 105627.
1330. C. Mattiuzzi, G. Lippi, Current cancer epidemiology, J. Epidemiol. Glob. Health 9 (4) (2019) 217–222.
1331. C. Naczki, et al., SPARC inhibits metabolic plasticity in ovarian cancer, Cancers (Basel) 10 (10) (2018).
1332. J. Ma, et al., SPARC inhibits breast cancer bone metastasis and may be a clinical therapeutic target, Oncol. Lett. 14 (5) (2017) 5876–5882.
1333. J. Nie, E.H. Sage, SPARC functions as an inhibitor of adipogenesis, J. Cell Commun. Signal. 3 (3–4) (2009) 247–254.
1334. J. Nie, E.H. Sage, SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling, J. Biol. Chem. 284 (2) (2009) 1279–1290.
1335. A. Ghanemi, M. Yoshioka, J. St-Amand, Broken energy homeostasis and obesity pathogenesis: the surrounding concepts, J. Clin. Med. (2018) 7(11).
1336. A. Zhu, et al., SPARC overexpression in primary tumors correlates with disease recurrence and overall survival in patients with triple negative breast cancer, Oncotarget 7 (47) (2016) 76628–76634.
1337. N.A. Said, et al., SPARC ameliorates ovarian cancer-associated inflammation, Neoplasia 10 (10) (2008) 1092–1104.
1338. N. Said, et al., Normalization of the ovarian cancer microenvironment by SPARC, Mol. Cancer Res. 5 (10) (2007) 1015–1030.
1339. G.K. Yiu, et al., SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells, Am. J. Pathol. 159 (2) (2001) 609–622.
1340. N. Said, K. Motamed, Absence of host-secreted protein acidic and rich in cysteine (SPARC) augments peritoneal ovarian carcinomatosis, Am. J. Pathol. 167 (6) (2005) 1739–1752.
1341. J. Chen, et al., SPARC is a key regulator of proliferation, apoptosis and invasion in human ovarian cancer, PLoS One 7 (8) (2012) e42413.
1342. C. Neuzillet, et al., Stromal expression of SPARC in pancreatic adenocarcinoma, Cancer Metastasis Rev. 32 (3–4) (2013) 585–602.
1343. O.L. Podhajcer, et al., The role of the matricellular protein SPARC in the dynamic interaction between the tumor and the host, Cancer Metastasis Rev. 27 (4) (2008) 691–705.
1344. M. Murakawa, et al., The impact of SPARC expression on the survival of pancreatic ductal adenocarcinoma patients after curative resection, J Cancer 10 (3) (2019) 627–633.
1345. Y. Huang, et al., SPARC expression and prognostic value in non-small cell lung cancer, Chinese J. Cancer 31 (11) (2012) 541–548.
1346. H. Alachkar, et al., SPARC promotes leukemic cell growth and predicts acute myeloid leukemia outcome, J. Clin. Invest. 124 (4) (2014) 1512–1524.
1347. W. Chang, et al., SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin, J. Biol. Chem. 285 (11) (2010) 8196–8206.
1348. Yun, M.H. Changes in Regenerative Capacity through Lifespan. Int. J. Mol. Sci. 2015, 16, 25392–25432.
1349. Zhang, Y.; Mignone, J.; MacLellan, W.R. Cardiac Regeneration and Stem Cells. Physiol. Rev. 2015, 95, 1189–1204.
1350. Sarker, M.D.; Naghieh, S.; McInnes, A.D.; Schreyer, D.J.; Chen, X.B. Regeneration of peripheral nerves by nerve guidance conduits: Influence of design, biopolymers, cells, growth factors, and physical stimuli. Prog. Neurobiol. 2018, 171, 125–150.
1351. Easterling, M.R.; Engbrecht, K.M.; Crespi, E.J. Endocrine Regulation of Epimorphic Regeneration. Endocrinology 2019, 160, 2969–2980.
1352. Wojda, S.J.; Donahue, S.W. Parathyroid hormone for bone regeneration. J. Orthop. Res. 2018, 36, 2586– 2594.
1353. Shen, Y.; Liu, W.C.; Lin, K.; Pan, H.; Darvell, B.; Peng, S.; Wen, C.; Deng, L.; Lu, W.W.; Chang, J. Interfacial pH: A Critical Factor for Osteoporotic Bone Regeneration. Langmuir 2011, 27, 2701–2708.

1354. Shen, Y.; Liu, W.; Wen, C.; Pan, H.; Wang, T.; Darvell, B.W.; Lu, W.W.; Huang, W. Bone regeneration: Importance of local pH—Strontium-doped borosilicate scaffold. J. Mater. Chem. 2012, 22, 8662–8670.
1355. Pibiri, M. Liver regeneration in aged mice: New insights. Aging 2018, 10, 1801–1824.
1356. Varela-Eirin, M.; Loureiro, J.; Fonseca, E.; Corrochano, S.; Caeiro, J.R.; Collado, M.; Mayan, M.D. Cartilage regeneration and ageing: Targeting cellular plasticity in osteoarthritis. Ageing Res. Rev. 2018, 42, 56–71.
1357. Li, H.; Ye, Z.-H. Microenvironment of liver regeneration in liver cancer. Chin. J. Integr. Med. 2017, 23, 555–560.
1358. Ambrosi, T.H.; Scialdone, A.; Graja, A.; Gohlke, S.; Jank, A.M.; Bocian, C.; Woelk, L.; Fan, H.; Logan, D.W.; Schürmann, A.; et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. Cell. Stem. Cell. 2017, 20, 771–784.e6.
1359. Cooke, J.P. Inflammation and Its Role in Regeneration and Repair. Circ. Res. 2019, 124, 1166–1168.
1360. Korolev, Y.N.; Geniatulina, M.S.; Mikhailik, L.V.; Nikulina, L.A. Intracellular regeneration of adrenocorticocytes in response to the prophylactic application of low-intensity electromagnetic radiation under the conditions of radiation (an experimental study). Vopr. Kurortol. Fizioter. Lech. Fiz. Kul'tury 2019, 96, 43–49.
1361. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. Matrix Biol. 2016, 54, 78–87.
1362. Maillard, C.; Malaval, L.; Delmas, P. Immunological screening of SPARC/Osteonectin in nonmineralized tissues. Bone 1992, 13, 257–264.
1363. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
1364. Motamed, K. SPARC (osteonectin/BM-40). Int. J. Biochem. Cell Biol. 1999, 31, 1363–1366.
1365. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
1366. Bradshaw, A.D. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? J. Mol. Cell. Cardiol. 2016, 93, 156–161.
1367. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). J. Mol. Cell. Cardiol. 2010, 48, 544–549.
1368. Yan, Q.; Sage, E.H. SPARC, a Matricellular Glycoprotein with Important Biological Functions. J. Histochem. Cytochem. 1999, 47, 1495–1505.
1369. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J. Clin. Investig. 2001, 107, 1049–1054.
1370. Deckx, S.; Johnson, D.M.; Rienks, M.; Carai, P.; Van Deel, E.; Van der Velden, J.; Sipido, K.R.; Heymans, S.; Papageorgiou, A.P. Extracellular SPARC increases cardiomyocyte contraction during health and disease. PLoS ONE 2019, 14, e0209534.
1371. Jørgensen, L.H.; Jepsen, P.L.; Boysen, A.; Dalgaard, L.B.; Hvid, L.G.; Ørtenblad, N.; Ravn, D.; Sellathurai, J.; Møller-Jensen, J.; Lochmuller, H.; et al. SPARC Interacts with Actin in Skeletal Muscle In Vitro and In Vivo. Am. J. Pathol. 2017, 187, 457–474.
1372. Shen, Y.; Zhao, Y.; Yuan, L.; Yi, W.; Zhao, R.; Yi, Q.; Yong, T. SPARC is over-expressed in adipose tissues of diet-induced obese rats and causes insulin resistance in 3T3-L1 adipocytes. Acta Histochem. 2014, 116, 158–166.
1373. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schroeder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 2013, 28, 1451–1460.
1374. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
1375. Scavelli, K.; Chatterjee, A.; Rhee, U.J. Secreted Protein Acidic and Rich in Cysteine in Ocular Tissue. J. Ocul. Pharmacol. Ther. 2015, 31, 396–405.
1376. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.

1377. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
1378. Niklason, L. Understanding the Extracellular Matrix to Enhance Stem Cell-Based Tissue Regeneration. Cell. Stem. Cell. 2018, 22, 302–305.
1379. Delany, A.M.; Hankenson, K. Thrombospondin-2 and SPARC/osteonectin are critical regulators of bone remodeling. J. Cell Commun. Signal. 2009, 3, 227–238.
1380. Caldez, M.J.; Van Hul, N.; Koh, H.W.; Teo, X.Q.; Fan, J.J.; Tan, P.Y.; Dewhurst, M.R.; Too, P.G.; Talib, S.Z.A.; Chiang, B.E.; et al. Metabolic Remodeling during Liver Regeneration. Dev. Cell 2018, 47, 425– 438.e5.
1381. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. FASEB J. 2019, 33, 10551–10562.
1382. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. Clin. Sci. 2019, 133, 351–365.
1383. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
1384. Melouane, A.; Yoshioka, M.; St-Amand, J. Extracellular matrix/mitochondria pathway: A novel potential target for sarcopenia. Mitochondrion 2020, 50, 63–70.
1385. Smith, G.M.; Gallo, G. The role of mitochondria in axon development and regeneration. Dev. Neurobiol. 2018, 78, 221–237.
1386. Han, S.M.; Baig, H.S.; Hammarlund, M. Mitochondria Localize to Injured Axons to Support Regeneration. Neuron 2016, 92, 1308–1323.
1387. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
1388. Nie, J.; Sage, E.H. SPARC inhibits adipogenesis by its enhancement of beta-catenin signaling. J. Biol. Chem. 2009, 284, 1279–1290.
1389. Nie, J.; Bradshaw, A.D.; Delany, A.M.; Sage, E.H. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. Connect. Tissue Res. 2011, 52, 99–108.
1390. Mukherjee, S.; Choi, M.J.; Kim, S.W.; Yun, J.-W. Secreted protein acidic and rich in cysteine (SPARC) regulates thermogenesis in white and brown adipocytes. Mol. Cell. Endocrinol. 2020, 506, 110757.
1391. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
1392. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
1393. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
1394. Hermetet, F.; Buffière, A.; Aznague, A.; De Barros, J.-P.P.; Bastie, J.-N.; Delva, L.; Quéré, R. High-fat diet disturbs lipid raft/TGF-β signaling-mediated maintenance of hematopoietic stem cells in mouse bone marrow. Nat. Commun. 2019, 10, 1–11.
1395. Wilkinson, A.C.; Yamazaki, S. The hematopoietic stem cell diet. Int. J. Hematol. 2018, 107, 634–641.
1396. Ribeiro, N.; Sousa, S.R.; Brekken, R.A.; Monteiro, F.J. Role of SPARC in bone remodeling and cancerrelated bone metastasis. J. Cell Biochem. 2014, 115, 17–26.
1397. Baloˇglu, M.; Özkorkmaz, E.G. Biochemical and immunohistochemical investigations on bone formation and remodelling in ovariectomised rats with tamoxifen citrate administration. Folia Morphol. 2019, 78, 789– 797.
1398. Delany, A.M.; McMahon, D.J.; Powell, J.S.; Greenberg, D.A.; Kurland, E.S. Osteonectin/SPARC polymorphisms in Caucasian men with idiopathic osteoporosis. Osteoporos. Int. 2007, 19, 969–978.
1399. Zurlo, F.; Larson, K.; Bogardus, C.; Ravussin, E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J. Clin. Investig. 1990, 86, 1423–1427.

1400. Di Girolamo, D.J.; Kiel, D.P.; Esser, K.A. Bone and Skeletal Muscle: Neighbors with Close Ties. J. Bone Miner. Res. 2013, 28, 1509–1518.
1401. Nagaraju, G.P.; Sharma, D. Anti-cancer role of SPARC, an inhibitor of adipogenesis. Cancer Treat. Rev. 2011, 37, 559–566.
1402. Chlenski, A.; Guerrero, L.J.; Peddinti, R.; Spitz, J.A.; Leonhardt, P.T.; Yang, Q.; Tian, Y.; Salwen, H.R.; Cohn, S.L.; Yang, Q. Anti-angiogenic SPARC peptides inhibit progression of neuroblastoma tumors. Mol. Cancer 2010, 9, 138.
1403. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
1404. Sangaletti, S.; Tripodo, C.; Cappetti, B.; Casalini, P.; Chiodoni, C.; Piconese, S.; Santangelo, A.; Parenza, M.; Arioli, I.; Miotti, S.; et al. SPARC Oppositely Regulates Inflammation and Fibrosis in BleomycinInduced Lung Damage. Am. J. Pathol. 2011, 179, 3000–3010.
1405. Rienks, M.; Carai, P.; van Teeffelen, J.; Eskens, B.; Verhesen, W.; Hemmeryckx, B.; Johnson, D.M.; van Leeuwen, R.; Jones, E.A.; Heymans, S.; et al. SPARC preserves endothelial glycocalyx integrity, and protects against adverse cardiac inflammation and injury during viral myocarditis. Matrix Biol. 2018, 74, 21–34.
1406. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. Genes Immun. 2007, 8, 262–274.
1407. Sass, F.A.; Fuchs, M.; Pumberger, M.; Geissler, S.; Duda, G.N.; Perka, C.; Schmidt-Bleek, K. Immunology Guides Skeletal Muscle Regeneration. Int. J. Mol. Sci. 2018, 19, 835.
1408. Muire, P.J.; Mangum, L.H.; Wenke, J.C. Time Course of Immune Response and Immunomodulation During Normal and Delayed Healing of Musculoskeletal Wounds. Front. Immunol. 2020, 11.
1409. Ahuja, P.; Sdek, P.; MacLellan, W.R. Cardiac Myocyte Cell Cycle Control in Development, Disease, and Regeneration. Physiol. Rev. 2007, 87, 521–544.
1410. Stary, M.; Pasteiner, W.; Summer, A.; Hrdina, A.; Eger, A.; Weitzer, G. Parietal endoderm secreted SPARC promotes early cardiomyogenesis in vitro. Exp. Cell Res. 2005, 310, 331–343.
1411. Riley, H.J.; Kelly, R.R.; Van Laer, A.O.; Neff, L.S.; Dasgupta, S.; Baicu, C.F.; McDonald, L.T.; LaRue, A.C.; Zile, M.R.; Bradshaw, A.D. SPARC Production by Bone Marrow-Derived Cells Contributes to Myocardial Fibrosis in Pressure-Overload. Am. J. Physiol. Heart Circ. Physiol. 2020.
1412. Avolio, E.; Mangialardi, G.; Slater, S.C.; Alvino, V.V.; Gu, Y.; Cathery, W.; Beltrami, A.P.; Katare, R.; Heesom, K.; Caputo, M.; et al. Secreted Protein Acidic and Cysteine Rich Matricellular Protein Is Enriched in the Bioactive Fraction of the Human Vascular Pericyte Secretome. Antioxid. Redox Signal. 2020.
1413. Benincasa, G.; Franzese, M.; Schiano, C.; Marfella, R.; Miceli, M.; Infante, T.; Sardu, C.; Zanfardino, M.; Affinito, O.; Mansueto, G.; et al. DNA methylation profiling of CD04(+)/CD08(+) T cells reveals pathogenic mechanisms in increasing hyperglycemia: PIRAMIDE pilot study. Annu. Med. Surg. 2020, 60, 218–226.
1414. West, M.D.; Sternberg, H.; Labat, I.; Janus, J.; Chapman, K.B.; Malik, N.N.; De Grey, A.D.; LaRocca, D. Toward a unified theory of aging and regeneration. Regen. Med. 2019, 14, 867–886.
1415. Chen, F.; Liu, Y.; Wong, N.-K.; Xiao, J.; So, K.-F. Oxidative Stress in Stem Cell Aging. Cell Transplant. 2017, 26, 1483–1495.
1416. Wang, J.; Wang, H. Oxidative Stress in Pancreatic Beta Cell Regeneration. Oxidative Med. Cell. Longev. 2017, 2017, 1930261.
1417. Brioche, T.; Lemoine-Morel, S. Oxidative Stress, Sarcopenia, Antioxidant Strategies and Exercise: Molecular Aspects. Curr. Pharm. Des. 2016, 22, 2664–2678.
1418. Hultman, E.; Spriet, L.L.; Söderlund, K. Biochemistry of muscle fatigue. Biomed. Biochim. Acta 1986, 45, S97–S106.
1419. Sahlin, K. Muscle fatigue and lactic acid accumulation. Acta Physiol. Scand. Suppl. 1986, 556, 83–91.
1420. Capuano, P.; Capasso, G. The importance of intracellular pH in the regulation of cell function. G Ital. Nfrol. 2003, 20, 139–150.
1421. Lönnqvist, S.; Emanuelsson, P.; Kratz, G. Influence of acidic pH on keratinocyte function and reepithelialisation of human in vitro wounds. J. Plast. Surg. Hand Surg. 2015, 49, 346–352.

1422. Kruse, C.R.; Singh, M.; Targosinski, S.; Sinha, I.; Sørensen, J.A.; Eriksson, E.; Nuutila, K. The effect of pH on cell viability, cell migration, cell proliferation, wound closure, and wound reepithelialization: In vitro and in vivo study. Wound Repair Regen. 2017, 25, 260–269.
1423. Shuster, S. Osteoporosis, like skin ageing, is caused by collagen loss which is reversible. J. R. Soc. Med. 2020, 113, 158–160.
1424. Ramu, I.; Buchholz, S.M.; Patzak, M.S.; Goetze, R.G.; Singh, S.K.; Richards, F.M.; Jodrell, D.I.; Sipos, B.; Ströbel, P.; Ellenrieder, V.; et al. SPARC dependent collagen deposition and gemcitabine delivery in a genetically engineered mouse model of pancreas cancer. EBioMedicine 2019, 48, 161–168.
1425. Bradshaw, A.D.; Puolakkainen, P.; Wight, T.N.; Helene, S.E.; Dasgupta, J.; Davidson, J.M. SPARC-Null Mice Display Abnormalities in the Dermis Characterized by Decreased Collagen Fibril Diameter and Reduced Tensile Strength. J. Investig. Dermatol. 2003, 120, 949–955.
1426. Francki, A.; Bradshaw, A.D.; Bassuk, J.A.; Howe, C.C.; Couser, W.G.; Sage, E.H. SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. J. Biol. Chem. 1999, 274, 32145–32152.
1427. Rentz, T.J.; Poobalarahi, F.; Bornstein, P.; Sage, E.H.; Bradshaw, A.D. SPARC regulates processing of procollagen I and collagen fibrillogenesis in dermal fibroblasts. J. Biol. Chem. 2007, 282, 22062–22071.
1428. Jura, M.; Kozak, L.P. Obesity and related consequences to ageing. Age (Dordrecht) 2016, 38, 1–18.
1429. Marzetti, E.; Calvani, R.; Tosato, M.; Cesari, M.; Di Bari, M.; Cherubini, A.; Collamati, A.; D'Angelo, E.; Pahor, M.; Bernabei, R.; et al. Sarcopenia: An overview. Aging Clin. Exp. Res. 2017, 29, 11–17.
1430. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res. 2019, 781, 175–185.
1431. Aspray, T.J.; Hill, T.R. Osteoporosis and the Ageing Skeleton. Subcell. Biochem. 2019, 91, 453–476.
1432. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
1433. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
1434. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schroeder, H.D. Secreted Protein Acidic and Rich in Cysteine (SPARC) in Human Skeletal Muscle. J. Histochem. Cytochem. 2008, 57, 29–39.
1435. Cheng, L.; Sun, X.; Guo, J.; Lu, G. SPARC support the expansion of cord blood stem cells in vitro. Cell Res. 2008, 18, S49.
1436. Zhu, J.; Wang, L.-Y.; Li, C.-Y.; Wu, J.-Y.; Zhang, Y.-T.; Pang, K.-P.; Wei, Y.; Du, L.-Q.; Liu, M.; Wu, X.-Y. SPARC promotes self-renewal of limbal epithelial stem cells and ocular surface restoration through JNK and p38-MAPK signaling pathways. Stem Cells 2019, 38, 134–145.
1437. Luo, Z.; Luo, P.; Yu, Y.; Zhao, Q.; Zhao, X.; Cheng, L. SPARC promotes the development of erythroid progenitors. Exp. Hematol. 2012, 40, 828–836.
1438. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. Matrix Biol. 2016, 52–54, 78–87.
1439. Bradshaw, A.D. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? J. Mol. Cell. Cardiol. 2016, 93, 156–161.
1440. Chlenski, A.; Guerrero, L.J.; Salwen, H.R.; Yang, Q.; Tian, Y.; Morales La Madrid, A.; Mirzoeva, S.; Bouyer, P.G.; Xu, D.; Walker, M.; et al. Secreted protein acidic and rich in cysteine is a matrix scavenger chaperone. PLoS ONE 2011, 6, e23880.
1441. Motamed, K. SPARC (osteonectin/BM-40). Int. J. Biochem. Cell. Biol. 1999, 31, 1363–1366.
1442. Wang, L.Y.; Zhang, Y.T.; Du, L.Q.; Wu, X.Y.; Zhu, J. The Effect of SPARC on the Proliferation and Migration of Limbal Epithelial Stem Cells During the Corneal Epithelial Wound Healing. Stem Cells Dev. 2021, 30, 301–308.
1443. Basu, A.; Kligman, L.H.; Samulewicz, S.J.; Howe, C.C. Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40). BMC Cell Biol. 2001, 2, 15.
1444. Mazzolini, G.; Atorrasagasti, C.; Onorato, A.; Peixoto, E.; Schlattjan, M.; Sowa, J.P.; Sydor, S.; Gerken, G.; Canbay, A. SPARC expression is associated with hepatic injury in rodents and humans with non-alcoholic fatty liver disease. Sci. Rep. 2018, 8, 725.

1445. Chen, S.; Zou, Q.; Chen, Y.; Kuang, X.; Wu, W.; Guo, M.; Cai, Y.; Li, Q. Regulation of SPARC family proteins in disorders of the central nervous system. Brain Res. Bull. 2020, 163, 178–189.
1446. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
1447. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
1448. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
1449. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G.D. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. Clin. Sci. (Lond.) 2019, 133, 351–365.
1450. Hu, L.; He, F.; Huang, M.; Zhao, Q.; Cheng, L.; Said, N.; Zhou, Z.; Liu, F.; Dai, Y.S. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. Sci. Rep. 2020, 10, 17581.
1451. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
1452. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
1453. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
1454. Riley, H.J.; Bradshaw, A.D. The Influence of the Extracellular Matrix in Inflammation: Findings from the SPARC-Null Mouse. Anat. Rec. (Hoboken) 2020, 303, 1624–1629.
1455. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
1456. Tanaka, M.; Takagi, T.; Naito, Y.; Uchiyama, K.; Hotta, Y.; Toyokawa, Y.; Ushiroda, C.; Hirai, Y.; Aoi, W.; Higashimura, Y.; et al. Secreted protein acidic and rich in cysteine functions in colitis via IL17A regulation in mucosal CD4(+) T cells. J. Gastroenterol. Hepatol. 2018, 33, 671–680.
1457. Framson, P.E.; Sage, E.H. SPARC and tumor growth: Where the seed meets the soil? J. Cell Biochem. 2004, 92, 679–690.
1458. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
1459. Wong, S.L.; Sukkar, M.B. The SPARC protein: An overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease. Br. J. Pharmacol. 2017, 174, 3–14.
1460. Bao, J.M.; Dang, Q.; Lin, C.J.; Lo, U.G.; Feldkoren, B.; Dang, A.; Hernandez, E.; Li, F.; Panwar, V.; Lee, C.F.; et al. SPARC is a key mediator of TGF-β-induced renal cancer metastasis. J. Cell. Physiol. 2021, 236, 1926–1938.
1461. Camacho, D.; Jesus, J.P.; Palma, A.M.; Martins, S.A.; Afonso, A.; Peixoto, M.L.; Pelham, C.J.; Moreno, E.; Gogna, R. SPARC-p53: The double agents of cancer. Adv. Cancer Res. 2020, 148, 171–199.
1462. Feng, J.; Tang, L. SPARC in Tumor Pathophysiology and as a Potential Therapeutic Target. Curr. Pharm. Des. 2014, 20, 6182–6190.
1463. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. Nat. Rev. Endocrinol. 2010, 6, 225–235.
1464. Whittal, M.C.; Molladavoodi, S.; Zwambag, D.P.; Millecamps, M.; Stone, L.S.; Gregory, D.E. Mechanical Consequence of Induced Intervertebral Disc Degeneration in the SPARC-Null Mouse. J. Biomech. Eng. 2021, 143, 024501.
1465. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). J. Mol. Cell. Cardiol. 2010, 48, 544–549.
1466. Onorato, M.A.; Fiore, E.; Bayo, J.; Casali, C.; Fernandez-Tomé, M.; Rodríguez, M.; Domínguez, L.; Argemi, J.; Hidalgo, F.; Favre, C.; et al. SPARC inhibition accelerates NAFLD-associated hepatocellular carcinoma development by dysregulating hepatic lipid metabolism. Liver Int. 2021, 41, 1677–1693.
1467. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. Genes Immun.2007, 8, 262–274.

1468. Rivera, L.B.; Bradshaw, A.D.; Brekken, R.A. The regulatory function of SPARC in vascular biology. Cell Mol. Life Sci. 2011, 68, 3165–3173.
1469. Mendis, D.B.; Ivy, G.O.; Brown, I.R. SPARC/osteonectin mRNA is induced in blood vessels following injury to the adult rat cerebral cortex. Neurochem. Res. 1998, 23, 1117–1123.
1470. Lee, S.H.; Lee, J.A.; Park, H.S.; Song, Y.S.; Jang, Y.J.; Kim, J.H.; Lee, Y.J.; Heo, Y. Associations among SPARC mRNA expression in adipose tissue, serum SPARC concentration and metabolic parameters in Korean women. Obesity (Silver Spring) 2013, 21, 2296–2302.
1471. Lee, Y.J.; Heo, Y.S.; Park, H.S.; Lee, S.H.; Lee, S.K.; Jang, Y.J. Serum SPARC and matrix metalloproteinase-2 and metalloproteinase-9 concentrations after bariatric surgery in obese adults. Obes. Surg. 2014, 24, 604–610.
1472. Kos, K.; Wong, S.; Tan, B.; Gummesson, A.; Jernas, M.; Franck, N.; Kerrigan, D.; Nystrom, F.H.; Carlsson, L.M.; Randeva, H.S.; et al. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. Diabetes 2009, 58, 1780–1788.
1473. Shi, D.; Jiang, K.; Fu, Y.; Fang, R.; Liu, X.I.; Chen, J. Overexpression of SPARC correlates with poor prognosis in patients with cervical carcinoma and regulates cancer cell epithelial-mesenchymal transition. Oncol. Lett. 2016, 11, 3251–3258.
1474. Zhong, M.E.; Chen, Y.; Xiao, Y.; Xu, L.; Zhang, G.; Lu, J.; Qiu, H.; Ge, W.; Wu, B. Serum extracellular vesicles contain SPARC and LRG1 as biomarkers of colon cancer and differ by tumour primary location. EBioMedicine 2019, 50, 211–223.
1475. Liu, Y.; Feng, Y.; Wang, X.; Yang, X.; Hu, Y.; Li, Y.; Zhang, Q.; Huang, Y.; Shi, K.; Ran, C.; et al. SPARC Negatively Correlates With Prognosis After Transarterial Chemoembolization and Facilitates Proliferation and Metastasis of Hepatocellular Carcinoma via ERK/MMP Signaling Pathways. Front. Oncol. 2020, 10, 813.
1476. Bloomston, M.; Ellison, E.C.; Muscarella, P.; Al-Saif, O.; Martin, E.W.; Melvin, W.S.; Frankel, W.L. Stromal osteonectin overexpression is associated with poor outcome in patients with ampullary cancer. Ann. Surg. Oncol. 2007, 14, 211–217.
1477. Ikuta, Y.; Nakatsura, T.; Kageshita, T.; Fukushima, S.; Ito, S.; Wakamatsu, K.; Baba, H.; Nishimura, Y. Highly sensitive detection of melanoma at an early stage based on the increased serum secreted protein acidic and rich in cysteine and glypican-3 levels. Clin. Cancer Res. 2005, 11, 8079–8088.
1478. Papapanagiotou, A.; Sgourakis, G.; Karkoulias, K.; Raptis, D.; Parkin, E.; Brotzakis, P.; Panchal, S.; Papavassiliou, A.G. Osteonectin as a screening marker for pancreatic cancer: A prospective study. J. Int. Med. Res. 2018, 46, 2769–2779.
1479. Wang, Z.; Song, H.Y.; An, M.M.; Zhu, L.L. Association of serum SPARC level with severity of coronary artery lesion in type 2 diabetic patients with coronary heart disease. Int. J. Clin. Exp. Med. 2015, 8, 19290– 19296.
1480. Wu, D.; Li, L.; Yang, M.; Liu, H.; Yang, G. Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus. Eur. J. Endocrinol. 2011, 165, 597–601.
1481. Pichler, R.H.; Bassuk, J.A.; Hugo, C.; Reed, M.J.; Eng, E.; Gordon, K.L.; Pippin, J.; Alpers, C.E.; Couser, W.G.; Sage, E.H.; et al. SPARC is expressed by mesangial cells in experimental mesangial proliferative nephritis and inhibits platelet-derived-growthfactor-medicated mesangial cell proliferation in vitro. Am. J. Pathol. 1996, 148, 1153–1167.
1482. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
1483. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 2013, 28, 1451–1460.
1484. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix communication. Matrix Biol. 2001, 19, 816–827.
1485. Hua, H.W.; Jiang, H.S.; Jia, L.; Jia, Y.P.; Yao, Y.L.; Chen, Y.W.; Jiang, F.; Lu, D.Q.; Zhou, Q.; Jiang, M.W.; et al. SPARC regulates ferroptosis induced by sorafenib in human hepatocellular carcinoma. Cancer Biomark. 2021, 1–9.
1486. Pedersen, B.K. The physiology of optimizing health with a focus on exercise as medicine. Annu. Rev.
Physiol. 2019, 81, 607–627.

1487. Aoi, W.; Sakuma, K. Skeletal muscle: Novel and intriguing characteristics as a secretory organ. BioDiscovery 2013, 7, e8942.
1488. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and high-fat diet in obesity: Functional genomics perspectives of two energy homeostasis pillars. Genes 2020, 11, 875.
1489. Berryhill, B.L.; Kane, B.; Stramer, B.M.; Fini, M.E.; Hassell, J.R. Increased SPARC accumulation during corneal repair. Exp. Eye Res. 2003, 77, 85–92.
1490. Phan, E.; Ahluwalia, A.; Tarnawski, A.S. Role of SPARC—Matricellular protein in pathophysiology and tissue injury healing. Implications for gastritis and gastric ulcers. Med. Sci. Monit. 2007, 13, RA25–RA30.
1491. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
1492. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
1493. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
1494. Matsuo, K.; Sato, K.; Suemoto, K.; Miyamoto-Mikami, E.; Fuku, N.; Higashida, K.; Tsuji, K.; Xu, Y.; Liu, X.; Iemitsu, M.; et al. A mechanism underlying preventive effect of high-intensity training on colon cancer. Med. Sci. Sports Exerc. 2017, 49, 1805–1816.
1495. Norheim, F.; Raastad, T.; Thiede, B.; Rustan, A.C.; Drevon, C.A.; Haugen, F. Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. Am. J. Physiol. Endocrinol. Metab. 2011, 301, E1013–E1021.
1496. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma myokine concentrations after acute exercise in non-obese and obese sedentary women. Front. Physiol. 2020, 11, 18.
1497. Billat, V.L.; Mouisel, E.; Roblot, N.; Melki, J. Inter- and intrastrain variation in mouse critical runningspeed. J. Appl. Physiol. (1985) 2005, 98, 1258–1263.
1498. Available online: https://insights.envigo.com/hubfs/resources/data-sheets/2018s-datasheet-0915.pdf (accessed on 9 August 2020).
1499. Norose, K.; Lo, W.K.; Clark, J.I.; Sage, E.H.; Howe, C.C. Lenses of SPARC-null mice exhibit an abnormal cell surface-basement membrane interface. Exp. Eye Res. 2000, 71, 295–307.
1500. Nie, J.; Bradshaw, A.D.; Delany, A.M.; Sage, E.H. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. Connect. Tissue Res. 2011, 52, 99–108.
1501. Faude, O.; Kindermann, W.; Meyer, T. Lactate threshold concepts: How valid are they? Sports Med. 2009, 39, 469–490.
1502. Schefer, V.; Talan, M.I. Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity. Exp. Gerontol. 1996, 31, 387–392.
1503. Nishida, Y.; Tokuyama, K.; Nagasaka, S.; Higaki, Y.; Shirai, Y.; Kiyonaga, A.; Shindo, M.; Kusaka, I.; Nakamura, T.; Ishibashi, S.; et al. Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartment labeled minimal model. Diabetes 2004, 53, 315–320.
1504. Tanaka, H.; Shindo, M. The benefits of the low intensity training. Ann. Physiol. Anthr. 1992, 11, 365–368.
1505. Motoyama, M.; Sunami, Y.; Kinoshita, F.; Kiyonaga, A.; Tanaka, H.; Shindo, M.; Irie, T.; Urata, H.; Sasaki, J.; Arakawa, K. Blood pressure lowering effect of low intensity aerobic training in elderly hypertensive patients. Med. Sci. Sports Exerc. 1998, 30, 818–823.
1506. Sunami, Y.; Motoyama, M.; Kinoshita, F.; Mizooka, Y.; Sueta, K.; Matsunaga, A.; Sasaki, J.; Tanaka, H.; Shindo, M. Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. Metabolism 1999, 48, 984–988.
1507. Kawanishi, N.; Yano, H.; Mizokami, T.; Takahashi, M.; Oyanagi, E.; Suzuki, K. Exercise training attenuates hepatic inflammation, fibrosis and macrophage infiltration during diet induced-obesity in mice. Brain Behav. Immun. 2012, 26, 931–941.
1508. Van Praag, H.; Shubert, T.; Zhao, C.; Gage, F.H. Exercise enhances learning and hippocampal neurogenesis in aged mice. J. Neurosci. 2005, 25, 8680–8685.

1509. Rowlatt, C.; Chesterman, F.C.; Sheriff, M.U. Lifespan, age changes and tumour incidence in an ageing C57BL mouse colony. Lab. Anim. 1976, 10, 419–442.
1510. Kunstyr, I.; Leuenberger, H.G. Gerontological data of C57BL/6J mice. I. Sex differences in survival curves. J. Gerontol. 1975, 30, 157–162.
1511. Smith, J.P.; Hicks, P.S.; Ortiz, L.R.; Martinez, M.J.; Mandler, R.N. Quantitative measurement of muscle strength in the mouse. J. Neurosci. Methods 1995, 62, 15–19.
1512. Takeshita, H.; Yamamoto, K.; Nozato, S.; Inagaki, T.; Tsuchimochi, H.; Shirai, M.; Yamamoto, R.; Imaizumi, Y.; Hongyo, K.; Yokoyama, S.; et al. Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice. Sci. Rep. 2017, 7, 42323.
1513. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
1514. Zong, S.; Wu, M.; Gu, J.; Liu, T.; Guo, R.; Yang, M. Structure of the intact 14 subunit human cytochrome c oxidase. Cell Res. 2018, 28, 1026–1034.
1515. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 2012, 9, 671–675.
1516. Taylor, S.C.; Berkelman, T.; Yadav, G.; Hammond, M. A defined methodology for reliable quantification of Western blot data. Mol. Biotechnol. 2013, 55, 217–226.
1517. Taylor, S.C.; Posch, A. The design of a quantitative western blot experiment. Biomed. Res. Int. 2014, 2014, 361590.
1518. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet-induced obesity. Life Sci. 2018, 215, 190–197.
1519. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; StAmand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity (Silver Spring) 2013, 21, 1389–1395.
1520. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J. Clin. Investig. 2001, 107, 1049–1054.
1521. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix communication. Matrix Biol. 2001, 19, 816–827.
1522. Lane, T.F.; Sage, E.H. The biology of SPARC, a protein that modulates cell-matrix interactions. FASEB J. 1994, 8, 163–173.
1523. Yan, Q.; Sage, E.H. SPARC, a matricellular glycoprotein with important biological functions. J. Histochem. Cytochem. 1999, 47, 1495–1506.
1524. Mason, I.J.; Murphy, D.; Münke, M.; Francke, U.; Elliott, R.W.; Hogan, B.L. Developmental and transformation-sensitive expression of the Sparc gene on mouse chromosome 11. EMBO J. 1986, 5, 1831 1837.
1525. Swaroop, A.; Hogan, B.L.; Francke, U. Molecular analysis of the cDNA for human SPARC/osteonectin/BM-40: Sequence, expression, and localization of the gene to chromosome 5q31-q33. Genomics 1988, 2, 37–47.
1526. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix. Matrix Biol. 2000, 19, 569–580.
1527. Francki, A.; Motamed, K.; McClure, T.D.; Kaya, M.; Murri, C.; Blake, D.J.; Carbon, J.G.; Sage, E.H. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. J. Cell. Biochem. 2003, 88, 802–811.
1528. Basu, A.; Kligman, L.H.; Samulewicz, S.J.; Howe, C.C. Impaired wound healing in mice deficient in a matricellular protein SPARC (osteonectin, BM-40). BMC Cell Biol. 2001, 2, 15.
1529. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
1530. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine: Metabolic and homeostatic properties beyond the extracellular matrix structure. Appl. Sci. 2020, 10, 2388.
1531. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
1532. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.

1533. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schrøder, H.D. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. J. Histochem. Cytochem. 2009, 57, 29–39.
1534. Holland, P.W.; Harper, S.J.; McVey, J.H.; Hogan, B.L. In vivo expression of mRNA for the Ca++ binding protein SPARC (osteonectin) revealed by in situ hybridization. J. Cell Biol. 1987, 105, 473–482.
1535. Sage, H.; Vernon, R.B.; Decker, J.; Funk, S.; Iruela-Arispe, M.L. Distribution of the calcium-binding protein SPARC in tissues of embryonic and adult mice. J. Histochem. Cytochem. 1989, 37, 819–829.
1536. Framson, P.E.; Sage, E.H. SPARC and tumor growth: Where the seed meets the soil? J. Cell. Biochem. 2004, 92, 679–690.
1537. Holloszy, J.O. Muscle metabolism during exercise. Arch. Phys. Med. Rehabil. 1982, 63, 231–234.
1538. Ferraro, E.; Giammarioli, A.M.; Chiandotto, S.; Spoletini, I.; Rosano, G. Exercise-induced skeletal muscle remodeling and metabolic adaptation: Redox signaling and role of autophagy. Antioxid. Redox Signal. 2014, 21, 154–176.
1539. Jørgensen, L.H.; Jepsen, P.L.; Boysen, A.; Dalgaard, L.B.; Hvid, L.G.; Ørtenblad, N.; Ravn, D.; Sellathurai, J.; Møller-Jensen, J.; Lochmüller, H.; et al. SPARC interacts with actin in skeletal muscle in vitro and in vivo. Am. J. Pathol. 2017, 187, 457–474.
1540. Chlenski, A.; Guerrero, L.J.; Salwen, H.R.; Yang, Q.; Tian, Y.; La Madrid, A.M.; Mirzoeva, S.; Bouyer, P.G.; Xu, D.; Walker, M.; et al. Secreted protein acidic and rich in cysteine is a matrix scavenger chaperone. PLoS ONE 2011, 6, e23880.
1541. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectin-deficient mice. J. Clin. Investig. 2000, 105, 915–923.
1542. Wang, H.; Fertala, A.; Ratner, B.D.; Sage, E.H.; Jiang, S. Identifying the SPARC binding sites on collagen I and procollagen I by atomic force microscopy. Anal. Chem. 2005, 77, 6765–6771.
1543. Giudici, C.; Raynal, N.; Wiedemann, H.; Cabral, W.A.; Marini, J.C.; Timpl, R.; Bächinger, H.P.; Farndale, R.W.; Sasaki, T.; Tenni, R. Mapping of SPARC/BM-40/osteonectin-binding sites on fibrillar collagens. J. Biol. Chem. 2008, 283, 19551–19560.
1544. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. J. Vet.
Med. Sci. 2019, 81, 1649–1654.
1545. Sage, H.; Vernon, R.B.; Funk, S.E.; Everitt, E.A.; Angello, J. SPARC, a secreted protein associated with cellular proliferation, inhibits cell spreading in vitro and exhibits Ca+2-dependent binding to the extracellular matrix. J. Cell Biol. 1989, 109, 341–356.
1546. Francki, A.; Bradshaw, A.D.; Bassuk, J.A.; Howe, C.C.; Couser, W.G.; Sage, E.H. SPARC regulates the expression of collagen type I and transforming growth factor-beta1 in mesangial cells. J. Biol. Chem. 1999, 274, 32145–32152.
1547. Bradshaw, A.D.; Reed, M.J.; Sage, E.H. SPARC-null mice exhibit accelerated cutaneous wound closure. J. Histochem. Cytochem. 2002, 50, 1–10.
1548. Melouane, A.; Yoshioka, M.; St-Amand, J. Extracellular matrix/mitochondria pathway: A novel potential target for sarcopenia. Mitochondrion 2020, 50, 63–70.
1549. Song, H.; Guan, Y.; Zhang, L.; Li, K.; Dong, C. SPARC interacts with AMPK and regulates GLUT4 expression. Biochem. Biophys. Res. Commun. 2010, 396, 961–966.
1550. Lira, V.A.; Benton, C.R.; Yan, Z.; Bonen, A. PGC-1alpha regulation by exercise training and its influences on muscle function and insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. 2010, 299, E145– E161.
1551. Jäger, S.; Handschin, C.; St-Pierre, J.; Spiegelman, B.M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc. Natl. Acad. Sci. USA 2007, 104, 12017– 12022.
1552. Wu, Z.; Puigserver, P.; Andersson, U.; Zhang, C.; Adelmant, G.; Mootha, V.; Troy, A.; Cinti, S.; Lowell, B.; Scarpulla, R.C.; et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999, 98, 115–124.
1553. Smith, G.M.; Gallo, G. The role of mitochondria in axon development and regeneration. Dev. Neurobiol. 2018, 78, 221–237.
1554. Han, S.M.; Baig, H.S.; Hammarlund, M. Mitochondria localize to injured axons to support regeneration. Neuron 2016, 92, 1308–1323.

1555. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. FASEB J. 2019, 33, 10551–10562.
1556. Bradshaw, A.D. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? J. Mol. Cell. Cardiol. 2016, 93, 156–161.
1557. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and secreted protein acidic and rich in cysteine (SPARC). J. Mol. Cell. Cardiol. 2010, 48, 544–549.
1558. Cheng, L.; Sun, X.; Guo, J.; Lu, G. SPARC support the expansion of cord blood stem cells in vitro. Cell Res. 2008, 18, S49.
1559. Zhu, J.; Wang, L.Y.; Li, C.Y.; Wu, J.Y.; Zhang, Y.T.; Pang, K.P.; Wei, Y.; Du, L.Q.; Liu, M.; Wu, X.Y. SPARC promotes self-renewal of limbal epithelial stem cells and ocular surface restoration through JNK and p38-MAPK signaling pathways. Stem Cells 2020, 38, 134–145.
1560. Rooney, K.; Trayhurn, P. Lactate and the GPR81 receptor in metabolic regulation: Implications for adipose tissue function and fatty acid utilisation by muscle during exercise. Br. J. Nutr. 2011, 106, 1310–1316.
1561. Gladden, L.B. Muscle as a consumer of lactate. Med. Sci. Sports Exerc. 2000, 32, 764–771.
1562. Gladden, L.B. Lactate metabolism: A new paradigm for the third millennium. J. Physiol. 2004, 558, 5–30.
1563. Hashimoto, T.; Hussien, R.; Oommen, S.; Gohil, K.; Brooks, G.A. Lactate sensitive transcription factor network in L6 cells: Activation of MCT1 and mitochondrial biogenesis. FASEB J. 2007, 21, 2602–2612.
1564. Hashimoto, T.; Brooks, G.A. Mitochondrial lactate oxidation complex and an adaptive role for lactate production. Med. Sci. Sports Exerc. 2008, 40, 486–494.
1565. Gladden, L.B. A lactatic perspective on metabolism. Med. Sci. Sports Exerc. 2008, 40, 477–485.
1566. Jacobs, I. Blood lactate. Sports Med. 1986, 3, 10–25.
1567. Campolongo, M.; Benedetti, L.; Podhajcer, O.L.; Pitossi, F.; Depino, A.M. Hippocampal SPARC regulates depression-related behavior. Genes Brain Behav. 2012, 11, 966–976.
1568. Leveille, G.A.; Chakrabarty, K. Diurnal variations in tissue glycogen and liver weight of meal-fed rats. J. Nutr. 1967, 93, 546–554.
1569. Kreitzman, S.N.; Coxon, A.Y.; Szaz, K.F. Glycogen storage: Illusions of easy weight loss, excessive weight regain, and distortions in estimates of body composition. Am. J. Clin. Nutr. 1992, 56 (Suppl. 1), 292s–293s.
1570. Ma, C.L.; Ma, X.T.; Wang, J.J.; Liu, H.; Chen, Y.F.; Yang, Y. Physical exercise induces hippocampal neurogenesis and prevents cognitive decline. Behav. Brain Res. 2017, 317, 332–339.
1571. Yuan, T.F.; Paes, F.; Arias-Carrión, O.; Ferreira Rocha, N.B.; de Sá Filho, A.S.; Machado, S. Neural mechanisms of exercise: Anti-depression, neurogenesis, and serotonin signaling. CNS Neurol. Disord. Drug Targets 2015, 14, 1307–1311.
1572. Uddin, M.S.; Hossain, M.F.; Mamun, A.A.; Shah, M.A.; Hasana, S.; Bulbul, I.J.; Sarwar, M.S.; Mansouri, R.A.; Ashraf, G.M.; Rauf, A.; et al. Exploring the multimodal role of phytochemicals in the modulation of cellular signaling pathways to combat age-related neurodegeneration. Sci. Total Environ. 2020, 725, 138313.
1573. Hung, C.W.; Chen, Y.C.; Hsieh, W.L.; Chiou, S.H.; Kao, C.L. Ageing and neurodegenerative diseases. Ageing Res. Rev. 2010, 9 (Suppl. 1), S36–S46.
1574. Mahalakshmi, B.; Maurya, N.; Lee, S.-D.; Bharath Kumar, V. Possible neuroprotective mechanisms of physical exercise in neurodegeneration. Int. J. Mol. Sci. 2020, 21, 5895.
1575. Ang, E.-T.; Tai, Y.-K.; Lo, S.-Q.; Seet, R.; Soong, T.-W. Neurodegenerative diseases: Exercising toward neurogenesis and neuroregeneration. Front. Aging Neurosci. 2010, 2, 25.
1576. Liu, Y.; Yan, T.; Chu, J.M.; Chen, Y.; Dunnett, S.; Ho, Y.S.; Wong, G.T.; Chang, R.C. The beneficial effects of physical exercise in the brain and related pathophysiological mechanisms in neurodegenerative diseases. Lab. Investig. 2019, 99, 943–957.
1577. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary running suppresses tumor growth

through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. Cell Metab. 2016, 23, 554–562.
1578. Wedell-Neergaard, A.S.; Lang Lehrskov, L.; Christensen, R.H.; Legaard, G.E.; Dorph, E.; Larsen, M.K.; Launbo, N.; Fagerlind, S.R.; Seide, S.K.; Nymand, S.; et al. Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: A randomized controlled trial. Cell Metab. 2019, 29, 844–855.e3.
1579. Ellingsgaard, H.; Hojman, P.; Pedersen, B.K. Exercise and health—Emerging roles of IL-6. Curr. Opin. Physiol. 2019, 10, 49–54.
1580. Sakuma, K.; Yamaguchi, A. Sarcopenic obesity and endocrinal adaptation with age. Int. J. Endocrinol. 2013, 2013, 204164.
1581. Candow, D.G.; Chilibeck, P.D. Differences in size, strength, and power of upper and lower body muscle groups in young and older men. J. Gerontol. A Biol. Sci. Med. Sci. 2005, 60, 148–156.
1582. Kalyani, R.R.; Corriere, M.; Ferrucci, L. Age-related and disease-related muscle loss: The effect of diabetes, obesity, and other diseases. Lancet Diabetes Endocrinol. 2014, 2, 819–829.
1583. Kim, T.N.; Choi, K.M. The implications of sarcopenia and sarcopenic obesity on cardiometabolic disease. J. Cell. Biochem. 2015, 116, 1171–1178.
1584. Dhingra, R.; Vasan, R.S. Age as a risk factor. Med. Clin. N. Am. 2012, 96, 87–91.
1585. Keenan, C.R.; White, R.H. Age as a risk factor for venous thromboembolism after major surgery. Curr. Opin. Pulm. Med. 2005, 11, 398–402.
1586. Koshiyama, M.; Tamaki, K.; Ohsawa, M. Age-specific incidence rates of atrial fibrillation and risk factors for the future development of atrial fibrillation in the Japanese general population. J. Cardiol. 2020, 77, 88–92.
1587. Zhang, H.; Rogers, K.; Sukkar, L.; Jun, M.; Kang, A.; Young, T.; Campain, A.; Cass, A.; Chow, C.K.; Comino, E.; et al. Prevalence, incidence and risk factors of diabetes in Australian adults aged ≥ 45 years: A cohort study using linked routinely-collected data. J. Clin. Transl. Endocrinol. 2020, 22, 100240.
1588. Mosher, C.L.; Weber, J.M.; Frankel, C.W.; Neely, M.L.; Palmer, S.M. Risk factors for mortality in lung transplant recipients aged ≥ 65 years: A retrospective cohort study of 5815 patients in the scientific registry of transplant recipients. J. Heart Lung Transplant. 2020.
1589. Norose, K.; Clark, J.I.; Syed, N.A.; Basu, A.; Heber-Katz, E.; Sage, E.H.; Howe, C.C. SPARC deficiency leads to early-onset cataractogenesis. Investig. Ophthalmol. Vis. Sci. 1998, 39, 2674–2680.
1590. Singh, M. Mood, food, and obesity. Front. Psychol. 2014, 5, 925.
1591. Ortolani, D.; Oyama, L.M.; Ferrari, E.M.; Melo, L.L.; Spadari-Bratfisch, R.C. Effects of comfort food on food intake, anxiety-like behavior and the stress response in rats. Physiol. Behav. 2011, 103, 487–492.
1592. Bradshaw, A.D.; Graves, D.C.; Motamed, K.; Sage, E.H. SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc. Natl. Acad. Sci. USA 2003, 100, 6045–6050.
1593. Sinclair, A.J.; Abdelhafiz, A.H. Cardiometabolic disease in the older person: Prediction and prevention for the generalist physician. Cardiovasc. Endocrinol. Metab. 2020, 9, 90–95.
1594. Islam, H.; Hood, D.A.; Gurd, B.J. Looking beyond PGC-1α: Emerging regulators of exercise-induced skeletal muscle mitochondrial biogenesis and their activation by dietary compounds. Appl. Physiol. Nutr. Metab. 2020, 45, 11–23.
1595. Su, J.; Chen, X.; Kanekura, T. A CD147-targeting siRNA inhibits the proliferation, invasiveness, and VEGF production of human malignant melanoma cells by down-regulating glycolysis. Cancer Lett. 2009, 273, 140–147.
1596. Gillies, R.J.; Raghunand, N.; Karczmar, G.S.; Bhujwalla, Z.M. MRI of the tumor microenvironment. J. Magn. Reason. Imaging 2002, 16, 430–450.
1597. Gladden, L.B. Current trends in lactate metabolism: Introduction. Med. Sci. Sports Exerc. 2008, 40, 475 476.
1598. Brooks, G.A. Lactate shuttles in nature. Biochem. Soc. Trans. 2002, 30, 258–264.
1599. Brooks, G.A. Current concepts in lactate exchange. Med. Sci. Sports Exerc. 1991, 23, 895–906.
1600. Sasaki, T.; Hohenester, E.; Göhring, W.; Timpl, R. Crystal structure and mapping by site-directed mutagenesis of the collagen-binding epitope of an activated form of BM-40/SPARC/osteonectin. EMBO J. 1998, 17, 1625–1634.
1601. Bailey, A.J. The role of collagen in the development of muscle and its relationship to eating quality. J. Anim. Sci. 1985, 60, 1580–1587.

1602. Au, E.; Richter, M.W.; Vincent, A.J.; Tetzlaff, W.; Aebersold, R.; Sage, E.H.; Roskams, A.J. SPARC from olfactory ensheathing cells stimulates Schwann cells to promote neurite outgrowth and enhances spinal cord repair. J. Neurosci. 2007, 27, 7208–7221.
1603. Lee, M.H.; Amin, N.D.; Venkatesan, A.; Wang, T.; Tyagi, R.; Pant, H.C.; Nath, A. Impaired neurogenesis and neurite outgrowth in an HIV-gp120 transgenic model is reversed by exercise via BDNF production and Cdk5 regulation. J. Neurovirol. 2013, 19, 418–431.
1604. Vincent, A.J.; Lau, P.W.; Roskams, A.J. SPARC is expressed by macroglia and microglia in the developing and mature nervous system. Dev. Dyn. 2008, 237, 1449–1462.
1605. Brekken, R.A.; Puolakkainen, P.; Graves, D.C.; Workman, G.; Lubkin, S.R.; Sage, E.H. Enhanced growth of tumors in SPARC null mice is associated with changes in the ECM. J. Clin. Investig. 2003, 111, 487–495.
1606. Puolakkainen, P.A.; Brekken, R.A.; Muneer, S.; Sage, E.H. Enhanced growth of pancreatic tumors in SPARC-null mice is associated with decreased deposition of extracellular matrix and reduced tumor cell apoptosis. Mol. Cancer Res. 2004, 2, 215–224.
1607. Said, N.; Frierson, H.F.; Sanchez-Carbayo, M.; Brekken, R.A.; Theodorescu, D. Loss of SPARC in bladder cancer enhances carcinogenesis and progression. J. Clin. Investig. 2013, 123, 751–766.
1608. Liu, Y.P.; Hsiao, M. Exercise-induced SPARC prevents tumorigenesis of colon cancer. Gut 2013, 62, 810–811.
1609. Aoi, W. Possibility of the novel myokine SPARC: A mechanistic approach to colon cancer prevention by physical exercise. Jpn. J. Phys. Fit. Sports Med. 2013, 62, 263–271.
1610. Konhilas, J.P.; Maass, A.H.; Luckey, S.W.; Stauffer, B.L.; Olson, E.N.; Leinwand, L.A. Sex modifies exercise and cardiac adaptation in mice. Am. J. Physiol. Heart Circ. Physiol. 2004, 287, H2768–H2776.
1611. Li, L.; Li, Y.; Wang, H.; Chen, W.; Liu, X. Effect of force level and gender on pinch force perception in healthy adults. Iperception 2020, 11, 2041669520927043.
1612. Sundberg, S. Maximal oxygen uptake in relation to age in blind and normal boys and girls. Acta Paediatr. Scand. 1982, 71, 603–608.
1613. Hafen, P.S.; Vehrs, P.R. Sex-related differences in the maximal lactate steady state. Sports 2018, 6, 154.
1614. Zhang, J.; Ji, L. Gender differences in peak blood lactate concentration and lactate removal. Ann. Sports Med. Res. 2016, 3, 1088.
1615. Green, H.J.; Fraser, I.G.; Ranney, D.A. Male and female differences in enzyme activities of energy metabolism in vastus lateralis muscle. J. Neurol. Sci. 1984, 65, 323–331.
1616. Yoshioka, M.; St-Pierre, S.; Suzuki, M.; Tremblay, A. Effects of red pepper added to high-fat and high carbohydrate meals on energy metabolism and substrate utilization in Japanese women. Br. J. Nutr. 1998, 80, 503–510.
1617. Yoshioka, M.; Lim, K.; Kikuzato, S.; Kiyonaga, A.; Tanaka, H.; Shindo, M.; Suzuki, M. Effects of red pepper diet on the energy metabolism in men. J. Nutr. Sci. Vitam. (Tokyo) 1995, 41, 647–656.
1618. Simoneau, J.A.; Bouchard, C. Human variation in skeletal muscle fiber-type proportion and enzyme activities. Am. J. Physiol. 1989, 257, E567–E572.
1619. Simoneau, J.A.; Lortie, G.; Boulay, M.R.; Thibault, M.C.; Thériault, G.; Bouchard, C. Skeletal muscle histochemical and biochemical characteristics in sedentary male and female subjects. Can. J. Physiol. Pharm. 1985, 63, 30–35.
1620. Di Luigi, L.; Romanelli, F.; Sgrò, P.; Lenzi, A. Andrological aspects of physical exercise and sport medicine. Endocrine 2012, 42, 278–284.
1621. Isacco, L.; Duché, P.; Boisseau, N. Influence of hormonal status on substrate utilization at rest and during exercise in the female population. Sports Med. 2012, 42, 327–342.
1622. Amelink, G.J.; Bär, P.R. Exercise-induced muscle protein leakage in the rat. Effects of hormonal manipulation. J. Neurol. Sci. 1986, 76, 61–68.
1623. Jura, M.; Kozak, L.P. Obesity and related consequences to ageing. Age 2016, 38, 23.
1624. Han, T.S.; Tajar, A.; Lean, M.E. Obesity and weight management in the elderly. Br. Med. Bull. 2011, 97, 169–196.
1625. Han, T.S.; Wu, F.C.; Lean, M.E. Obesity and weight management in the elderly: A focus on men. Best Pract. Res. Clin. Endocrinol. Metab. 2013, 27, 509–525.
1626. Batsis, J.A.; Villareal, D.T. Sarcopenic obesity in older adults: Aetiology, epidemiology and treatment strategies. Nat. Rev. Endocrinol. 2018, 14, 513–537.

1627. Polyzos, S.A.; Margioris, A.N. Sarcopenic obesity. Hormones 2018, 17, 321–331.
1628. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
1629. Goossens, G.H. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. Obes. Facts 2017, 10, 207–215.
1630. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
1631. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 999.
1632. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
1633. Škurla, M.; Rybáˇr, R. Obesity and reduced fertility of men. Ceska Gynekol. 2018, 83, 212–217.
1634. Best, D.; Bhattacharya, S. Obesity and fertility. Horm. Mol. Biol. Clin. Investig. 2015, 24, 5–10.
1635. Saad, M.J.; Santos, A.; Prada, P.O. Linking Gut Microbiota and Inflammation to Obesity and Insulin Resistance. Physiology 2016, 31, 283–293.
1636. Dye, L.; Boyle, N.B.; Champ, C.; Lawton, C. The relationship between obesity and cognitive health and decline. Proc. Nutr. Soc. 2017, 76, 443–454.
1637. Petrakis, D.; Margină, D.; Tsarouhas, K.; Tekos, F.; Stan, M.; Nikitovic, D.; Kouretas, D.; Spandidos, D.A.; Tsatsakis, A. Obesity—A risk factor for increased COVID-19 prevalence, severity and lethality (Review). Mol. Med. Rep. 2020, 22, 9–19.
1638. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
1639. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.
1640. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Post-Coronavirus Disease-2019 (COVID-19): Toward a Severe Multi-Level Health Crisis? Med. Sci. 2021, 9, 68.
1641. Rubio-Almanza, M.; Cámara-Gómez, R.; Merino-Torres, J.F. Obesity and type 2 diabetes: Also linked in therapeutic options. Endocrinol. Diabetes Nutr. 2019, 66, 140–149.
1642. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms andperspectives. Metabolism 2019, 92, 121–135.
1643. Koliaki, C.; Liatis, S.; Kokkinos, A. Obesity and cardiovascular disease: Revisiting an old relationship. Metabolism 2019, 92, 98–107.
1644. Dixon, A.E.; Peters, U. The effect of obesity on lung function. Expert Rev. Respir. Med. 2018, 12, 755 767.
1645. Barazzoni, R.; Gortan Cappellari, G.; Ragni, M.; Nisoli, E. Insulin resistance in obesity: An overview of fundamental alterations. Eat Weight Disord. 2018, 23, 149–157.
1646. Csige, I.; Ujvárosy, D.; Szabó, Z.; L″orincz, I.; Paragh, G.; Harangi, M.; Somodi, S. The Impact of Obesity on the Cardiovascular System. J. Diabetes Res. 2018, 2018, 3407306.
1647. De Mello, A.H.; Costa, A.B.; Engel, J.D.G.; Rezin, G.T. Mitochondrial dysfunction in obesity. Life Sci. 2018, 192, 26–32.
1648. Vekic, J.; Zeljkovic, A.; Stefanovic, A.; Jelic-Ivanovic, Z.; Spasojevic-Kalimanovska, V. Obesity and dyslipidemia. Metabolism 2019, 92, 71–81.
1649. Polyzos, S.A.; Kountouras, J.; Mantzoros, C.S. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. Metabolism 2019, 92, 82–97.
1650. Andersen, C.J.; Murphy, K.E.; Fernandez, M.L. Impact of Obesity and Metabolic Syndrome on Immunity. Adv. Nutr. 2016, 7, 66–75.
1651. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. Medicines 2021, 8, 57.
1652. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
1653. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. Cell 2013, 153, 1194–1217.
1654. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. Nat. Rev. Neurol. 2019, 15, 565–581.

1655. Bramorska, A.; Zarzycka, W.; Podolecka, W.; Kuc, K.; Brzezicka, A. Age-Related Cognitive Decline May Be Moderated by Frequency of Specific Food Products Consumption. Nutrients 2021, 13, 2504.
1656. Gallo Marin, B.; Aghagoli, G.; Lavine, K.; Yang, L.; Siff, E.J.; Chiang, S.S.; Salazar-Mather, T.P.; Dumenco, L.; Savaria, M.C.; Aung, S.N.; et al. Predictors of COVID-19 severity: A literature review. Rev. Med. Virol. 2021, 31, 1–10.
1657. Al-Sofiani, M.E.; Ganji, S.S.; Kalyani, R.R. Body composition changes in diabetes and aging. J. Diabetes Complicat. 2019, 33, 451–459.
1658. Park, S.W.; Goodpaster, B.H.; Lee, J.S.; Kuller, L.H.; Boudreau, R.; de Rekeneire, N.; Harris, T.B.; Kritchevsky, S.; Tylavsky, F.A.; Nevitt, M.; et al. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. Diabetes Care 2009, 32, 1993–1997.
1659. Fane, M.; Weeraratna, A.T. How the ageing microenvironment influences tumour progression. Nat. Rev. Cancer 2020, 20, 89–106.
1660. Costantino, S.; Paneni, F.; Cosentino, F. Ageing, metabolism and cardiovascular disease. J. Physiol. 2016, 594, 2061–2073.
1661. Barnes, P.J. Pulmonary Diseases and Ageing. Subcell. Biochem. 2019, 91, 45–74.
1662. Consitt, L.A.; Dudley, C.; Saxena, G. Impact of Endurance and Resistance Training on Skeletal Muscle Glucose Metabolism in Older Adults. Nutrients 2019, 11, 2636.
1663. Tyrrell, D.J.; Goldstein, D.R. Ageing and atherosclerosis: Vascular intrinsic and extrinsic factors and potential role of IL-6. Nat. Rev. Cardiol. 2021, 18, 58–68.
1664. Tyrrell, D.J.; Blin, M.G.; Song, J.; Wood, S.C.; Zhang, M.; Beard, D.A.; Goldstein, D.R. Age-Associated Mitochondrial Dysfunction Accelerates Atherogenesis. Circ. Res. 2020, 126, 298–314.
1665. Liu, H.H.; Li, J.J. Aging and dyslipidemia: A review of potential mechanisms. Ageing Res. Rev. 2015, 19, 43–52.
1666. Stahl, E.C.; Haschak, M.J.; Popovic, B.; Brown, B.N. Macrophages in the Aging Liver and Age-Related Liver Disease. Front. Immunol. 2018, 9, 2795.
1667. Almeida, S.; Rato, L.; Sousa, M.; Alves, M.G.; Oliveira, P.F. Fertility and Sperm Quality in the Aging Male. Curr. Pharm. Des. 2017, 23, 4429–4437.
1668. American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee. Female age-related fertility decline. Committee Opinion No. 589. Fertil. Steril. 2014, 101, 633– 634.
1669. Sadighi Akha, A.A. Aging and the immune system: An overview. J. Immunol. Methods 2018, 463, 21–26.
1670. Sousounis, K.; Baddour, J.A.; Tsonis, P.A. Aging and regeneration in vertebrates. Curr. Top Dev. Biol. 2014, 108, 217–246.
1671. Cox, A.J.; West, N.P.; Cripps, A.W. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol. 2015, 3, 207–215.
1672. Sendama, W. The effect of ageing on the resolution of inflammation. Ageing Res. Rev. 2020, 57, 101000.
1673. Pomatto, L.C.D.; Davies, K.J.A. Adaptive homeostasis and the free radical theory of ageing. Free Radic. Biol Med. 2018, 124, 420–430.
1674. Rani, V.; Deep, G.; Singh, R.K.; Palle, K.; Yadav, U.C. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sci. 2016, 148, 183–193.
1675. Torres-Fuentes, C.; Schellekens, H.; Dinan, T.G.; Cryan, J.F. The microbiota-gut-brain axis in obesity. Lancet Gastroenterol. Hepatol. 2017, 2, 747–756.
1676. Dinan, T.G.; Cryan, J.F. Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. J. Physiol. 2017, 595, 489–503.
1677. Fock, K.M.; Khoo, J. Diet and exercise in management of obesity and overweight. J. Gastroenterol. Hepatol. 2013, 28 (Suppl. S4), 59–63.
1678. Swift, D.L.; McGee, J.E.; Earnest, C.P.; Carlisle, E.; Nygard, M.; Johannsen, N.M. The Effects of Exercise and Physical Activity on Weight Loss and Maintenance. Prog. Cardiovasc. Dis. 2018, 61, 206 213.
1679. Papaioannou, K.G.; Nilsson, A.; Nilsson, L.M.; Kadi, F. Healthy Eating Is Associated with Sarcopenia Risk in Physically Active Older Adults. Nutrients 2021, 13, 2813.
1680. Mora, J.C.; Valencia, W.M. Exercise and Older Adults. Clin. Geriatr. Med. 2018, 34, 145–162.

1681. Stoner, L.; Beets, M.W.; Brazendale, K.; Moore, J.B.; Weaver, R.G. Exercise Dose and Weight Loss in Adolescents with Overweight Obesity: A Meta-Regression. Sports Med. 2019, 49, 83–94.
1682. Swift, D.L.; Johannsen, N.M.; Lavie, C.J.; Earnest, C.P.; Church, T.S. The role of exercise and physical activity in weight loss and maintenance. Prog. Cardiovasc. Dis. 2014, 56, 441–447.
1683. Elia, M. Obesity in the elderly. Obes. Res. 2001, 9 (Suppl. S4), 244s–248s.
1684. Fleg, J.L. Aerobic exercise in the elderly: A key to successful aging. Discov. Med. 2012, 13, 223 228.
1685. Ghanemi, A.; St-Amand, J. Interleukin-6 as a "metabolic hormone". Cytokine 2018, 112, 132–136.
1686. Idorn, M.; Thor Straten, P. Exercise and cancer: From "healthy" to "therapeutic"? Cancer Immunol. Immunother. 2017, 66, 667–671.
1687. Cattadori, G.; Segurini, C.; Picozzi, A.; Padeletti, L.; Anzà, C. Exercise and heart failure: An update. ESC Heart Fail. 2018, 5, 222–232.
1688. Fiuza-Luces, C.; Santos-Lozano, A.; Joyner, M.; Carrera-Bastos, P.; Picazo, O.; Zugaza, J.L.; Izquierdo, M.; Ruilope, L.M.; Lucia, A. Exercise benefits in cardiovascular disease: Beyond attenuation of traditional risk factors. Nat. Rev. Cardiol. 2018, 15, 731–743.
1689. Hansen, D.; Niebauer, J.; Cornelissen, V.; Barna, O.; Neunhäuserer, D.; Stettler, C.; Tonoli, C.; Greco, E.; Fagard, R.; Coninx, K.; et al. Exercise Prescription in Patients with Different Combinations of Cardiovascular Disease Risk Factors: A Consensus Statement from the EXPERT Working Group. Sports Med. 2018, 48, 1781–1797.
1690. Wang, J.; Liu, S.; Li, G.; Xiao, J. Exercise Regulates the Immune System. Adv. Exp. Med. Biol. 2020, 1228, 395–408.
1691. Whillier, S. Exercise and Insulin Resistance. Adv. Exp. Med. Biol. 2020, 1228, 137–150.
1692. De Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. Sports Med. 2017, 47, 277–293.
1693. Van der Windt, D.J.; Sud, V.; Zhang, H.; Tsung, A.; Huang, H. The Effects of Physical Exercise on Fatty Liver Disease. Gene Expr. 2018, 18, 89–101.
1694. Saito, Y.; Chikenji, T.S.; Matsumura, T.; Nakano, M.; Fujimiya, M. Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors. Nat. Commun. 2020, 11, 889.
1695. Schüttler, D.; Clauss, S.; Weckbach, L.T.; Brunner, S. Molecular Mechanisms of Cardiac Remodeling and Regeneration in Physical Exercise. Cells 2019, 8, 1128.
1696. Paneroni, M.; Simonelli, C.; Vitacca, M.; Ambrosino, N. Aerobic Exercise Training in Very Severe Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. Am. J. Phys. Med. Rehabil. 2017, 96, 541–548.
1697. Nolan, C.M.; Rochester, C.L. Exercise Training Modalities for People with Chronic Obstructive Pulmonary Disease. COPD 2019, 16, 378–389.
1698. Yang, J.; Cao, R.Y.; Gao, R.; Mi, Q.; Dai, Q.; Zhu, F. Physical Exercise Is a Potential "Medicine" for Atherosclerosis. Adv. Exp. Med. Biol. 2017, 999, 269–286.
1699. Gan, Z.; Fu, T.; Kelly, D.P.; Vega, R.B. Skeletal muscle mitochondrial remodeling in exercise and diseases. Cell Res. 2018, 28, 969–980.
1700. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
1701. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res. Rev. Mutat. Res. 2019, 781, 175–185.
1702. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
1703. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
1704. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G.D. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. Clin. Sci. 2019, 133, 351–365.
1705. Kwon, J.H.; Moon, K.M.; Min, K.W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. Healthcare 2020, 8, 378.

1706. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
1707. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. Genes 2021, 12, 1832.
1708. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. Biomolecules 2021, 11, 1689.
1709. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
1710. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
1711. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
1712. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
1713. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
1714. Morgan, A.E.; Davies, T.J.; Mc Auley, M.T. The role of DNA methylation in ageing and cancer. Proc. Nutr. Soc. 2018, 77, 412–422.
1715. Zhang, W.; Qu, J.; Liu, G.H.; Belmonte, J.C.I. The ageing epigenome and its rejuvenation. Nat. Rev. Mol. Cell Biol. 2020, 21, 137–150.
1716. Covarrubias, A.J.; Perrone, R.; Grozio, A.; Verdin, E. NAD(+) metabolism and its roles in cellular processes during ageing. Nat. Rev. Mol. Cell Biol. 2021, 22, 119–141.
1717. Booth, L.N.; Brunet, A. The Aging Epigenome. Mol. Cell. 2016, 62, 728–744.
1718. Thum, T. Non-coding RNAs in ageing. Ageing Res. Rev. 2014, 17, 1–2.
1719. Gupta, S.K.; Piccoli, M.T.; Thum, T. Non-coding RNAs in cardiovascular ageing. Ageing Res. Rev. 2014, 17, 79–85.
1720. Samblas, M.; Milagro, F.I.; Martínez, A. DNA methylation markers in obesity, metabolic syndrome, and weight loss. Epigenetics 2019, 14, 421–444.
1721. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. Cell Metab. 2019, 29, 1028–1044.
1722. Horikoshi, M.; Beaumont, R.N.; Day, F.R.; Warrington, N.M.; Kooijman, M.N.; Fernandez-Tajes, J.; Feenstra, B.; van Zuydam, N.R.; Gaulton, K.J.; Grarup, N.; et al. Genome-wide associations for birth weight and correlations with adult disease. Nature 2016, 538, 248–252.
1723. Eeckhoute, J.; Oger, F.; Staels, B.; Lefebvre, P. Coordinated Regulation of PPARγ Expression and Activity through Control of Chromatin Structure in Adipogenesis and Obesity. PPAR Res. 2012, 2012, 164140.
1724. Shi, Y.; Qu, J.; Gai, L.; Yuan, D.; Yuan, C. Long Non-coding RNAs in Metabolic and Inflammatory Pathways in Obesity. Curr. Pharm. Des. 2020, 26, 3317–3325.
1725. Ouni, M.; Schürmann, A. Epigenetic contribution to obesity. Mamm. Genome. 2020, 31, 134–145.
1726. Jung, M.; Pfeifer, G.P. Aging and DNA methylation. BMC Biol. 2015, 13, 7.
1727. Beerman, I.; Bock, C.; Garrison, B.S.; Smith, Z.D.; Gu, H.; Meissner, A.; Rossi, D.J. Proliferationdependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. Cell Stem. Cell 2013, 12, 413–425.
1728. Unnikrishnan, A.; Freeman, W.M.; Jackson, J.; Wren, J.D.; Porter, H.; Richardson, A. The role of DNA methylation in epigenetics of aging. Pharmacol. Ther. 2019, 195, 172–185.
1729. Sibbett, R.A.; Altschul, D.M.; Marioni, R.E.; Deary, I.J.; Starr, J.M.; Russ, T.C. DNA methylation-based measures of accelerated biological ageing and the risk of dementia in the oldest-old: A study of the Lothian Birth Cohort 1921. BMC Psychiatry 2020, 20, 91.
1730. Matías-García, P.R.; Ward-Caviness, C.K.; Raffield, L.M.; Gao, X.; Zhang, Y.; Wilson, R.; Gào, X.; Nano, J.; Bostom, A.; Colicino, E.; et al. DNAm-based signatures of accelerated aging and mortality in blood are associated with low renal function. Clin. Epigenetics 2021, 13, 121.
1731. Ambatipudi, S.; Horvath, S.; Perrier, F.; Cuenin, C.; Hernandez-Vargas, H.; Le Calvez-Kelm, F.; Durand, G.; Byrnes, G.; Ferrari, P.; Bouaoun, L.; et al. DNA methylome analysis identifies accelerated

epigenetic ageing associated with postmenopausal breast cancer susceptibility. Eur. J. Cancer 2017, 75, 299– 307.
1732. Peng, C.; Cardenas, A.; Rifas-Shiman, S.L.; Hivert, M.F.; Gold, D.R.; Platts-Mills, T.A.; Lin, X.; Oken, E.; Avila, L.; Celedón, J.C.; et al. Epigenetic age acceleration is associated with allergy and asthma in children in Project Viva. J. Allergy Clin. Immunol. 2019, 143, 2263–2270.e2214.
1733. Van Dijk, S.J.; Molloy, P.L.; Varinli, H.; Morrison, J.L.; Muhlhausler, B.S. Epigenetics and human obesity. Int. J. Obes. 2015, 39, 85–97.
1734. Ahrens, M.; Ammerpohl, O.; von Schönfels, W.; Kolarova, J.; Bens, S.; Itzel, T.; Teufel, A.; Herrmann, A.; Brosch, M.; Hinrichsen, H.; et al. DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. Cell Metab. 2013, 18, 296–302.
1735. Zhou, Y.; Hambly, B.D.; McLachlan, C.S. FTO associations with obesity and telomere length. J. Biomed. Sci. 2017, 24, 65.
1736. Gurung, R.L.; Liu, S.; Liu, J.J.; Chan, S.M.; Moh, M.C.; Ang, K.; Tang, W.E.; Sum, C.F.; Tavintharan, S.; Lim, S.C. Ethnic disparities in relationships of obesity indices with telomere length in Asians with type 2 diabetes. J. Diabetes 2019, 11, 386–393.
1737. Voisin, S.; Eynon, N.; Yan, X.; Bishop, D.J. Exercise training and DNA methylation in humans. Acta
Physiol. 2015, 213, 39–59.
1738. Fernandes, J.; Arida, R.M.; Gomez-Pinilla, F. Physical exercise as an epigenetic modulator of brain plasticity and cognition. Neurosci. Biobehav. Rev. 2017, 80, 443–456.
1739. Solagna, F.; Nogara, L.; Dyar, K.A.; Greulich, F.; Mir, A.A.; Türk, C.; Bock, T.; Geremia, A.; Baraldo, M.; Sartori, R.; et al. Exercisedependent increases in protein synthesis are accompanied by chromatin modifications and increased MRTF-SRF signalling. Acta Physiol. 2020, 230, e13496.
1740. Bonilauri, B.; Dallagiovanna, B. Long Non-coding RNAs Are Differentially Expressed after Different Exercise Training Programs. Front. Physiol. 2020, 11, 567614.
1741. Zhang, Y.; He, N.; Feng, B.; Ye, H. Exercise Mediates Heart Protection via Non-coding RNAs. Front. Cell Dev. Biol. 2020, 8, 182.
1742. Dawson, M.A.; Kouzarides, T. Cancer epigenetics: From mechanism to therapy. Cell 2012, 150, 12–27.
1743. Miranda Furtado, C.L.; Dos Santos Luciano, M.C.; Silva Santos, R.D.; Furtado, G.P.; Moraes, M.O.; Pessoa, C. Epidrugs: Targeting epigenetic marks in cancer treatment. Epigenetics 2019, 14, 1164–1176.
1744. Yong-Quan Ng, G.; Yang-Wei Fann, D.; Jo, D.G.; Sobey, C.G.; Arumugam, T.V. Dietary Restriction and Epigenetics: Part I. Cond. Med. 2019, 2, 284–299.
1745. Yong-Quan Ng, G.; Fann, D.Y.; Jo, D.G.; Sobey, C.G.; Arumugam, T.V. Epigenetic Regulation by Dietary Restriction: Part II. Cond. Med. 2019, 2, 300–310.
1746. Gray, N.; Le Bot, N.; Heemels, M.T. Regeneration. Nature 2018, 557, 321.
1747. Diehl, A.M. Liver regeneration. Front. Biosci. 2002, 7, e301–e314.
1748. Lin, A.; Makushok, T.; Diaz, U.; Marshall, W.F. Methods for the Study of Regeneration in Stentor. J. Vis. Exp. 2018, 136, 57759.
1749. Odelberg, S.J. Cellular plasticity in vertebrate regeneration. Anat. Rec. B New Anat. 2005, 287, 25–35.
1750. Majidinia, M.; Sadeghpour, A.; Yousefi, B. The roles of signaling pathways in bone repair and regeneration. J. Cell Physiol. 2018, 233, 2937–2948.
1751. Galliot, B.; Crescenzi, M.; Jacinto, A.; Tajbakhsh, S. Trends in tissue repair and regeneration. Development 2017, 144, 357–364.
1752. Wooff, Y.; Man, S.M.; Aggio-Bruce, R.; Natoli, R.; Fernando, N. IL-1 Family Members Mediate Cell Death, Inflammation and Angiogenesis in Retinal Degenerative Diseases. Front. Immunol. 2019, 10, 1618.
1753. Dugger, B.N.; Dickson, D.W. Pathology of Neurodegenerative Diseases. Cold Spring Harb. Perspect. Biol. 2017, 9, a028035.
1754. Vajkoczy, P.; Weidner, N. Degenerative spinal diseases. Der Nervenarzt 2018, 89, 619.
1755. Nandi, A.; Yan, L.J.; Jana, C.K.; Das, N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. Oxid. Med. Cell Longev. 2019, 2019, 9613090.
1756. Wu, C.L.; Harasymowicz, N.S.; Klimak, M.A.; Collins, K.H.; Guilak, F. The role of macrophages in osteoarthritis and cartilage repair. Osteoarthr. Cartil. 2020, 28, 544–554.

1757. Murphy, M.P.; Koepke, L.S.; Lopez, M.T.; Tong, X.; Ambrosi, T.H.; Gulati, G.S.; Marecic, O.; Wang, Y.; Ransom, R.C.; Hoover, M.Y.; et al. Articular cartilage regeneration by activated skeletal stem cells. Nat. Med. 2020, 26, 1583–1592.
1758. Jeon, S.; Oh, I.H. Regeneration of the retina: Toward stem cell therapy for degenerative retinal diseases. BMB Rep. 2015, 48, 193–199.
1759. Wang, J.; Li, X.; Chen, H. Organoid models in lung regeneration and cancer. Cancer Lett. 2020, 475, 129–135.
1760. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
1761. Roberts, S.; Colombier, P.; Sowman, A.; Mennan, C.; Rölfing, J.H.; Guicheux, J.; Edwards, J.R. Ageing in the musculoskeletal system. Acta Orthop. 2016, 87, 15–25.
1762. Yamakawa, H.; Kusumoto, D.; Hashimoto, H.; Yuasa, S. Stem Cell Aging in Skeletal Muscle Regeneration and Disease. Int. J. Mol. Sci. 2020, 21, 1830.
1763. Kennedy, J.M.; Zochodne, D.W. Impaired peripheral nerve regeneration in diabetes mellitus. J. Peripher. Nerv. Syst. 2005, 10, 144–157.
1764. Yokoyama, Y.; Nagino, M.; Nimura, Y. Mechanism of impaired hepatic regeneration in cholestatic liver. J. Hepatobiliary Pancreat Surg. 2007, 14, 159–166.
1765. Greenwood, H.L.; Singer, P.A.; Downey, G.P.; Martin, D.K.; Thorsteinsdóttir, H.; Daar, A.S. Regenerative medicine and the developing world. PLoS Med. 2006, 3, e381.
1766. Brokesh, A.M.; Gaharwar, A.K. Inorganic Biomaterials for Regenerative Medicine. ACS Appl. Mater. Interfaces 2020, 12, 5319–5344.
1767. Makarevich, P.I.; Efimenko, A.Y.; Tkachuk, V.A. Biochemical Regulation of Regenerative Processes by Growth Factors and Cytokines: Basic Mechanisms and Relevance for Regenerative Medicine. Biochemistry 2020, 85, 11–26.
1768. Cedar, S.H. The function of stem cells and their future roles in healthcare. Br. J. Nurs. 2006, 15, 104–107.
1769. Forbes, S.J. Recent advances in stem cells and regenerative medicine. Qjm 2014, 107, 251–252.
1770. Raspa, A.; Pugliese, R.; Maleki, M.; Gelain, F. Recent therapeutic approaches for spinal cord injury. Biotechnol. Bioeng. 2016, 113, 253–259.
1771. Salgado, A.J.; Oliveira, J.M.; Martins, A.; Teixeira, F.G.; Silva, N.A.; Neves, N.M.; Sousa, N.; Reis, R.L. Tissue engineering and regenerative medicine: Past, present, and future. Int. Rev. NeuroBiol. 2013, 108, 1–33.
1772. McPhail, M.J.; Janus, J.R.; Lott, D.G. Advances in regenerative medicine for otolaryngology/head and neck surgery. BMJ 2020, 369, m718.
1773. Edgar, L.; Pu, T.; Porter, B.; Aziz, J.M.; La Pointe, C.; Asthana, A.; Orlando, G. Regenerative medicine, organ bioengineering and transplantation. Br. J. Surg. 2020, 107, 793–800.
1774. Sahle, F.F.; Kim, S.; Niloy, K.K.; Tahia, F.; Fili, C.V.; Cooper, E.; Hamilton, D.J.; Lowe, T.L. Nanotechnology in regenerative ophthalmology. Adv. Drug Deliv. Rev. 2019, 148, 290–307.
1775. Jahangirian, H.; Azizi, S.; Rafiee-Moghaddam, R.; Baratvand, B.; Webster, T.J. Status of Plant ProteinBased Green Scaffolds for Regenerative Medicine Applications. Biomolecules 2019, 9, 619.
1776. Cable, J.; Fuchs, E.; Weissman, I.; Jasper, H.; Glass, D.; Rando, T.A.; Blau, H.; Debnath, S.; Oliva, A.; Park, S.; et al. Adult stem cells and regenerative medicine-a symposium report. Ann. N. Y. Acad. Sci. 2020, 1462, 27–36.
1777. Hosseini, S.; Taghiyar, L.; Safari, F.; Baghaban Eslaminejad, M. Regenerative Medicine Applications of Mesenchymal Stem Cells. Adv. Exp. Med. Biol. 2018, 1089, 115–141.
1778. Khan, A.; Wang, B.; Ni, Y. Chitosan-Nanocellulose Composites for Regenerative Medicine Applications. Curr. Med. Chem. 2020, 27, 4584–4592.
1779. Suman, S.; Domingues, A.; Ratajczak, J.; Ratajczak, M.Z. Potential Clinical Applications of Stem Cells in Regenerative Medicine. Adv. Exp. Med. Biol. 2019, 1201, 1–22.
1780. Nilforoushzadeh, M.A.; Sisakht, M.M.; Seifalian, A.M.; Amirkhani, M.A.; Banafshe, H.R.; Verdi, J.; Sharifzad, F.; Taghiabadi, E. Regenerative Medicine Applications in Wound Care. Curr. Stem Cell Res. Ther. 2017, 12, 658–674.
1781. Stocum, D.L. Regenerative biology and medicine. J. Musculoskelet. Neuronal. Interact. 2002, 2, 270–273.
1782. Hsu, M.N.; Chang, Y.H.; Truong, V.A.; Lai, P.L.; Nguyen, T.K.N.; Hu, Y.C. CRISPR technologies for stem cell engineering and regenerative medicine. Biotechnol. Adv. 2019, 37, 107447.

1783. Gouveia, C.; Huyser, C.; Egli, D.; Pepper, M.S. Lessons Learned from Somatic Cell Nuclear Transfer. Int. J. Mol. Sci. 2020, 21, 2314.
1784. Lowe, T.L.; Agrahari, V.; Kannan, R.M.; Kannan, S. Nanotechnology enabled regenerative medicine for neurological disorders. Adv. Drug Deliv. Rev. 2019, 148, 1–2.
1785. Fricain, J.C.; De Olivera, H.; Devillard, R.; Kalisky, J.; Remy, M.; Kériquel, V.; Le Nihounen, D.; Grémare, A.; Guduric, V.; Plaud, A.; et al. 3D bioprinting in regenerative medicine and tissue engineering. Med. Sci. 2017, 33, 52–59.
1786. Christ, G.J.; Saul, J.M.; Furth, M.E.; Andersson, K.E. The pharmacology of regenerative medicine. Pharmacol. Rev. 2013, 65, 1091–1133.
1787. Andersson, K.E.; Christ, G.J. Regenerative pharmacology: The future is now. Mol. Interv. 2007, 7, 79–86.
1788. Williams, J.K.; Andersson, K.E. Regenerative pharmacology: Recent developments and future perspectives. Regen. Med. 2016, 11, 859–870.
1789. Mahdavi, S.S.; Abdekhodaie, M.J.; Mashayekhan, S.; Baradaran-Rafii, A.; Djalilian, A.R. Bioengineering Approaches for Corneal Regenerative Medicine. Tissue Eng. Regen. Med. 2020, 17, 567–593.
1790. Craig, D.M.; Ashcroft, S.P.; Belew, M.Y.; Stocks, B.; Currell, K.; Baar, K.; Philp, A. Utilizing small nutrient compounds as enhancers of exercise-induced mitochondrial biogenesis. Front. Physiol. 2015, 6, 296.
1791. Booth, F.W.; Ruegsegger, G.N.; Toedebusch, R.G.; Yan, Z. Endurance Exercise and the Regulation of Skeletal Muscle Metabolism. Prog. Mol. Biol. Transl. Sci. 2015, 135, 129–151.
1792. McGee, S.L.; Hargreaves, M. Exercise and skeletal muscle glucose transporter 4 expression: Molecular mechanisms. Clin. Exp. Pharmacol. Physiol. 2006, 33, 395–399.
1793. Bo, B.; Zhou, Y.; Zheng, Q.; Wang, G.; Zhou, K.; Wei, J. The Molecular Mechanisms Associated with Aerobic Exercise-Induced Cardiac Regeneration. Biomolecules 2020, 11, 19.
1794. Fiuza-Luces, C.; Santos-Lozano, A.; Joyner, M.; Carrera-Bastos, P.; Picazo, O.; Zugaza, J.L.; Izquierdo, M.; Ruilope, L.M.; Lucia, A. Exercise benefits in cardiovascular disease: Beyond attenuation of traditional risk factors. Nat. Rev. Cardiol. 2018, 15, 731–743.
1795. Smith, J.K. Exercise as an Adjuvant to Cartilage Regeneration Therapy. Int. J. Mol. Sci. 2020, 21, 9471.
1796. Saito, Y.; Chikenji, T.S.; Matsumura, T.; Nakano, M.; Fujimiya, M. Exercise enhances skeletal muscle regeneration by promoting senescence in fibro-adipogenic progenitors. Nat. Commun. 2020, 11, 889.
1797. Schüttler, D.; Clauss, S.; Weckbach, L.T.; Brunner, S. Molecular Mechanisms of Cardiac Remodeling and Regeneration in Physical Exercise. Cells 2019, 8, 1128.
1798. He, N.; Ye, H. Exercise and Muscle Atrophy. Adv. Exp. Med. Biol. 2020, 1228, 255–267.
1799. Nalbandian, A.; Nguyen, C.; Katheria, V.; Llewellyn, K.J.; Badadani, M.; Caiozzo, V.; Kimonis, V.E. Exercise training reverses skeletal muscle atrophy in an experimental model of VCP disease. PLoS ONE 2013, 8, e76187.
1800. Perandini, L.A.; Chimin, P.; Lutkemeyer, D.D.S.; Câmara, N.O.S. Chronic inflammation in skeletal muscle impairs satellite cells function during regeneration: Can physical exercise restore the satellite cell niche? FEBS J. 2018, 285, 1973–1984.
1801. Valenti, M.T.; Dalle Carbonare, L.; Dorelli, G.; Mottes, M. Effects of physical exercise on the prevention of stem cells senescence. Stem Cell Rev. Rep. 2020, 16, 33–40.
1802. Gordon, T.; English, A.W. Strategies to promote peripheral nerve regeneration: Electrical stimulation and/or exercise. Eur. J. Neurosci. 2016, 43, 336–350.
1803. Larrick, J.W.; Mendelsohn, A.R. Exercise Partially Rejuvenates Muscle Stem Cells. Rejuvenation Res. 2020, 23, 262–265.
1804. Brett, J.O.; Arjona, M.; Ikeda, M.; Quarta, M.; de Morrée, A.; Egner, I.M.; Perandini, L.A.; Ishak, H.D.; Goshayeshi, A.; Benjamin, D.I.; et al. Exercise rejuvenates quiescent skeletal muscle stem cells in old mice through restoration of Cyclin D1. Nat. Metab. 2020, 2, 307–317.
1805. Son, J.S.; Chae, S.A.; Testroet, E.D.; Du, M.; Jun, H.P. Exercise-induced myokines: A brief review of controversial issues of this decade. Expert. Rev. Endocrinol. Metab. 2018, 13, 51–58.
1806. Liu, Y.P.; Hsiao, M. Exercise-induced SPARC prevents tumorigenesis of colon cancer. Gut 2013, 62, 810–811.

1807. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
1808. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. Biomolecules 2021, 11, 1689.
1809. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
1810. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
1811. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
1812. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
1813. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. BioChem. Cell Biol. 2019, 117, 105627.
1814. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. FASEB J. 2019, 33, 10551–10562.
1815. Ribeiro, N.; Sousa, S.R.; Brekken, R.A.; Monteiro, F.J. Role of SPARC in bone remodeling and cancerrelated bone metastasis. J. Cell BioChem. 2014, 115, 17–26.
1816. Chimal-Monroy, J.; Bravo-Ruíz, T.; Furuzawa-Carballeda, G.J.; Lira, J.M.; de la Cruz, J.C.; Almazán, A.; Krötzsch-Gómez, F.E.; Arrellín, G.; Díaz de León, L. Collagen-PVP accelerates new bone formation of experimentally induced bone defects in rat skull and promotes the expression of osteopontin and SPARC during bone repair of rat femora fractures. Ann. N. Y. Acad. Sci. 1998, 857, 232–236.
1817. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. Genes 2021, 12, 1832.
1818. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
1819. Kwon, J.H.; Moon, K.M.; Min, K.W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. Healthcare 2020, 8, 378.
1820. Nilsson, M.I.; Bourgeois, J.M.; Nederveen, J.P.; Leite, M.R.; Hettinga, B.P.; Bujak, A.L.; May, L.; Lin, E.; Crozier, M.; Rusiecki, D.R.; et al. Lifelong aerobic exercise protects against inflammaging and cancer. PLoS ONE 2019, 14, e0210863.
1821. de Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. Sports Med. 2017, 47, 277–293.
1822. Hojman, P.; Gehl, J.; Christensen, J.F.; Pedersen, B.K. Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. Cell Metab. 2018, 27, 10–21.
1823. Garatachea, N.; Pareja-Galeano, H.; Sanchis-Gomar, F.; Santos-Lozano, A.; Fiuza-Luces, C.; Morán, M.; Emanuele, E.; Joyner, M.J.; Lucia, A. Exercise attenuates the major hallmarks of aging. Rejuvenation Res. 2015, 18, 57–89.
1824. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Diet Impact on Obesity beyond Calories and Trefoil Factor Family 2 (TFF2) as an Illustration: Metabolic Implications and Potential Applications. Biomolecules 2021, 11, 1830.
1825. Cheng, C.W.; Yilmaz, Ö.H. 100 Years of Exploiting Diet and Nutrition for Tissue Regeneration. Cell Stem Cell 2021, 28, 370–373.
1826. Nencioni, A.; Caffa, I.; Cortellino, S.; Longo, V.D. Fasting and cancer: Molecular mechanisms and clinical application. Nat. Rev. Cancer 2018, 18, 707–719.
1827. Cornide-Petronio, M.E.; Álvarez-Mercado, A.I.; Jiménez-Castro, M.B.; Peralta, C. Current Knowledge about the Effect of Nutritional Status, Supplemented Nutrition Diet, and Gut Microbiota on Hepatic IschemiaReperfusion and Regeneration in Liver Surgery. Nutrients 2020, 12, 284.

1828. Wu, X.; Zhang, Y.; Xing, Y.; Zhao, B.; Zhou, C.; Wen, Y.; Xu, X. High-fat and high-glucose microenvironment decreases Runx2 and TAZ expression and inhibits bone regeneration in the mouse. J. Orthop. Surg. Res. 2019, 14, 55.
1829. Buranasin, P.; Mizutani, K.; Iwasaki, K.; Pawaputanon Na Mahasarakham, C.; Kido, D.; Takeda, K.; Izumi, Y. High glucose-induced oxidative stress impairs proliferation and migration of human gingival fibroblasts. PLoS ONE 2018, 13, e0201855.
1830. Abdel Nazeer, A.; Saito, S.; Sayed, S.; Hassan, L.; Askar, F.; Al-Jahdari, W.; Seki, T.; Hideaki, O. Normal glucose enhances neuronal regeneration after lidocaine-induced injury. Br. J. Anaesth. 2010, 104, 482–486.
1831. Camargo, W.A.; de Vries, R.; van Luijk, J.; Hoekstra, J.W.; Bronkhorst, E.M.; Jansen, J.A.; van den Beucken, J. Diabetes Mellitus and Bone Regeneration: A Systematic Review and Meta-Analysis of Animal Studies. Tissue Eng. Part B Rev. 2017, 23, 471–479.
1832. Mattson, M.P.; Longo, V.D.; Harvie, M. Impact of intermittent fasting on health and disease processes. Ageing Res. Rev. 2017, 39, 46–58.
1833. Rangan, P.; Choi, I.; Wei, M.; Navarrete, G.; Guen, E.; Brandhorst, S.; Enyati, N.; Pasia, G.; Maesincee, D.; Ocon, V.; et al. Fasting-Mimicking Diet Modulates Microbiota and Promotes Intestinal Regeneration to Reduce Inflammatory Bowel Disease Pathology. Cell Rep. 2019, 26, 2704–2719.e2706.
1834. Song, S.; Bai, M.; Ling, Z.; Lin, Y.; Wang, S.; Chen, Y. Intermittent administration of a fastingmimicking diet reduces intestinal inflammation and promotes repair to ameliorate inflammatory bowel disease in mice. J. Nutr. Biochem. 2021, 96, 108785.
1835. Cheng, C.W.; Adams, G.B.; Perin, L.; Wei, M.; Zhou, X.; Lam, B.S.; Da Sacco, S.; Mirisola, M.; Quinn, D.I.; Dorff, T.B.; et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell 2014, 14, 810–823.
1836. Cheng, C.W.; Villani, V.; Buono, R.; Wei, M.; Kumar, S.; Yilmaz, O.H.; Cohen, P.; Sneddon, J.B.; Perin, L.; Longo, V.D. FastingMimicking Diet Promotes Ngn3-Driven β-Cell Regeneration to Reverse Diabetes. Cell 2017, 168, 775–788.e712.
1837. Bradley, C.A. Diabetes: Fasting and β cell regeneration. Nat. Rev. Endocrinol. 2017, 13, 252.
1838. Arciero, P.J.; Edmonds, R.; He, F.; Ward, E.; Gumpricht, E.; Mohr, A.; Ormsbee, M.J.; Astrup, A. Protein-Pacing Caloric-Restriction Enhances Body Composition Similarly in Obese Men and Women during Weight Loss and Sustains Efficacy during Long-Term Weight Maintenance. Nutrients 2016, 8, 476.
1839. Ma, S.; Sun, S.; Geng, L.; Song, M.; Wang, W.; Ye, Y.; Ji, Q.; Zou, Z.; Wang, S.; He, X.; et al. Caloric Restriction Reprograms the Single-Cell Transcriptional Landscape of Rattus Norvegicus Aging. Cell 2020, 180, 984–1001.e1022.
1840. He, F.; Zuo, L.; Ward, E.; Arciero, P.J. Serum Polychlorinated Biphenyls Increase and Oxidative Stress Decreases with a ProteinPacing Caloric Restriction Diet in Obese Men and Women. Int. J. Environ. Res. Public Health 2017, 14, 59.
1841. Hayashi, T.; Fujita, K.; Nojima, S.; Hayashi, Y.; Nakano, K.; Ishizuya, Y.; Wang, C.; Yamamoto, Y.; Kinouchi, T.; Matsuzaki, K.; et al. High-Fat Diet-Induced Inflammation Accelerates Prostate Cancer Growth via IL6 Signaling. Clin. Cancer Res. 2018, 24, 4309–4318.
1842. Kim, J.D.; Yoon, N.A.; Jin, S.; Diano, S. Microglial UCP2 Mediates Inflammation and Obesity Induced by High-Fat Feeding. Cell Metab. 2019, 30, 952–962.e955.
1843. Fujita, K.; Hayashi, T.; Matsushita, M.; Uemura, M.; Nonomura, N. Obesity, Inflammation, and Prostate Cancer. J. Clin. Med. 2019, 8, 201.
1844. Cömert, E.D.; Gökmen, V. Evolution of food antioxidants as a core topic of food science for a century. Food Res. Int. 2018, 105, 76–93.
1845. Benzie, I.F.; Choi, S.W. Antioxidants in food: Content, measurement, significance, action, cautions, caveats, and research needs. Adv. Food Nutr. Res. 2014, 71, 1–53.
1846. Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. Crit. Rev. Food Sci. Nutr. 2018, 58, 1260–1270.
1847. Mohd Sahardi, N.F.N.; Makpol, S. Ginger (Zingiber officinale Roscoe) in the Prevention of Ageing and Degenerative Diseases: Review of Current Evidence. Evid.-Based Complement. Alternat. Med. 2019, 2019, 5054395.
1848. Marsman, H.A.; de Graaf, W.; Heger, M.; van Golen, R.F.; Ten Kate, F.J.; Bennink, R.; van Gulik, T.M. Hepatic regeneration and functional recovery following partial liver resection in an experimental model of hepatic steatosis treated with omega-3 fatty acids. Br. J. Surg. 2013, 100, 674–683.

1849. Sharples, A.P.; Hughes, D.C.; Deane, C.S.; Saini, A.; Selman, C.; Stewart, C.E. Longevity and skeletal muscle mass: The role of IGF signalling, the sirtuins, dietary restriction and protein intake. Aging Cell 2015, 14, 511–523.
1850. Bibbò, S.; Ianiro, G.; Giorgio, V.; Scaldaferri, F.; Masucci, L.; Gasbarrini, A.; Cammarota, G. The role of diet on gut microbiota composition. Eur. Rev. Med. Pharmacol. Sci. 2016, 20, 4742–4749.
1851. Shavandi, A.; Saeedi, P.; Gérard, P.; Jalalvandi, E.; Cannella, D.; Bekhit, A.E. The role of microbiota in tissue repair and regeneration. J. Tissue Eng. Regen. Med. 2020, 14, 539–555.
1852. Eugene, A.R.; Masiak, J. The Neuroprotective Aspects of Sleep. MEDtube Sci. 2015, 3, 35–40.
1853. Elkhenany, H.; AlOkda, A.; El-Badawy, A.; El-Badri, N. Tissue regeneration: Impact of sleep on stem cell regenerative capacity. Life Sci. 2018, 214, 51–61.
1854. Dierickx, P.; Van Laake, L.W.; Geijsen, N. Circadian clocks: From stem cells to tissue homeostasis and regeneration. EMBO Rep. 2018, 19, 18–28.
1855. Brown, S.A. Circadian clock-mediated control of stem cell division and differentiation: Beyond night and day. Development 2014, 141, 3105–3111.
1856. Majidinia, M.; Reiter, R.J.; Shakouri, S.K.; Mohebbi, I.; Rastegar, M.; Kaviani, M.; Darband, S.G.; Jahanban-Esfahlan, R.; Nabavi, S.M.; Yousefi, B. The multiple functions of melatonin in regenerative medicine. Ageing Res. Rev. 2018, 45, 33–52.
1857. Pandi-Perumal, S.R.; BaHammam, A.S.; Brown, G.M.; Spence, D.W.; Bharti, V.K.; Kaur, C.; Hardeland, R.; Cardinali, D.P. Melatonin antioxidative defense: Therapeutical implications for aging and neurodegenerative processes. Neurotox. Res. 2013, 23, 267–300.
1858. Chen, B.; You, W.; Shan, T. The regulatory role of melatonin in skeletal muscle. J. Muscle Res. Cell Motil. 2020, 41, 191–198.
1859. Pourabbas, M.; Bagheri, R.; Hooshmand Moghadam, B.; Willoughby, D.S.; Candow, D.G.; Elliott, B.T.; Forbes, S.C.; Ashtary-Larky, D.; Eskandari, M.; Wong, A.; et al. Strategic Ingestion of High-Protein Dairy Milk during a Resistance Training Program Increases Lean Mass, Strength, and Power in Trained Young Males. Nutrients 2021, 13, 948
1860. Saracino, P.G.; Saylor, H.E.; Hanna, B.R.; Hickner, R.C.; Kim, J.S.; Ormsbee, M.J. Effects of Pre-Sleep Whey vs. Plant-Based Protein Consumption on Muscle Recovery Following Damaging Morning Exercise. Nutrients 2020, 12, 2049.
1861. Mônico-Neto, M.; Dáttilo, M.; Ribeiro, D.A.; Lee, K.S.; de Mello, M.T.; Tufik, S.; Antunes, H.K.M. REM sleep deprivation impairs muscle regeneration in rats. Growth Factors 2017, 35, 12–18.
1862. Chen, P.; Yao, H.; Su, W.; He, Y.; Cheng, K.; Wang, Y.; Peng, W.; Li, P. Sleep deprivation worsened oral ulcers and delayed healing process in an experimental rat model. Life Sci. 2019, 232, 116594.
1863. Apovian, C.M. Obesity: Definition, comorbidities, causes, and burden. Am. J. Manag. Care 2016, 22, s176–s185.
1864. Steptoe, A.; Deaton, A.; Stone, A.A. Subjective wellbeing, health, and ageing. Lancet 2015, 385, 640– 648.
1865. Tam, B.T.; Morais, J.A.; Santosa, S. Obesity and ageing: Two sides of the same coin. Obes. Rev. 2020, 21, e12991.
1866. Ghosh, S.; Sinha, J.K.; Raghunath, M. 'Obesageing': Linking obesity & ageing. Indian J. Med. Res. 2019, 149, 610–615.
1867. Ambrosi, T.H.; Scialdone, A.; Graja, A.; Gohlke, S.; Jank, A.M.; Bocian, C.; Woelk, L.; Fan, H.; Logan, D.W.; Schürmann, A.; et al. Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration. Cell Stem Cell 2017, 20, 771–784.e776.
1868. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. Diseases 2021, 9, 87.
1869. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
1870. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
1871. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
1872. Conway, B.; Rene, A. Obesity as a disease: No lightweight matter. Obes. Rev. 2004, 5, 145–151.
1873. Christensen, S. Recognizing obesity as a disease. J. Am. Assoc. Nurse Pract. 2020, 32, 497–503.

1874. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
1875. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
1876. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.
1877. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. Medicines 2021, 8, 57.
1878. Aunan, J.R.; Watson, M.M.; Hagland, H.R.; Søreide, K. Molecular and biological hallmarks of ageing. Br. J. Surg. 2016, 103, e29–e46.
1879. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. Nat. Rev. Neurol. 2019, 15, 565–581.
1880. Melzer, D.; Pilling, L.C.; Ferrucci, L. The genetics of human ageing. Nat. Rev. Genet. 2020, 21, 88–101.
1881. van den Beld, A.W.; Kaufman, J.M.; Zillikens, M.C.; Lamberts, S.W.J.; Egan, J.M.; van der Lely, A.J. The physiology of endocrine systems with ageing. Lancet Diabetes Endocrinol. 2018, 6, 647–658.
1882. Horvath, S.; Raj, K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat. Rev. Genet. 2018, 19, 371–384.
1883. Bacalini, M.G.; D'Aquila, P.; Marasco, E.; Nardini, C.; Montesanto, A.; Franceschi, C.; Passarino, G.; Garagnani, P.; Bellizzi, D. The methylation of nuclear and mitochondrial DNA in ageing phenotypes and longevity. Mech. Ageing Dev. 2017, 165, 156–161.
1884. Geiger, A.E.; Daughtry, M.R.; Yen, C.N.; Kirkpatrick, L.T.; Shi, H.; Gerrard, D.E. Dual effects of obesity on satellite cells and muscle regeneration. Physiol. Rep. 2020, 8, e14511.
1885. Conboy, I.M.; Rando, T.A. Aging, stem cells and tissue regeneration: Lessons from muscle. Cell Cycle 2005, 4, 407–410.
1886. Yun, M.H. Changes in Regenerative Capacity through Lifespan. Int. J. Mol. Sci. 2015, 16, 25392–25432.
1887. Blau, H.M.; Cosgrove, B.D.; Ho, A.T. The central role of muscle stem cells in regenerative failure with aging. Nat. Med. 2015, 21, 854–862.
1888. Walsh, S.K.; Schneider, S.E.; Amundson, L.A.; Neu, C.P.; Henak, C.R. Maturity-dependent cartilage cell plasticity and sensitivity to external perturbation. J. Mech. Behav. BioMed. Mater. 2020, 106, 103732.
1889. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. Metabolism 2019, 92, 163– 169.
1890. Swift, D.L.; McGee, J.E.; Earnest, C.P.; Carlisle, E.; Nygard, M.; Johannsen, N.M. The Effects of Exercise and Physical Activity on Weight Loss and Maintenance. Prog. Cardiovasc. Dis. 2018, 61, 206–213.
1891. Pedersen, B.K. Which type of exercise keeps you young? Curr. Opin. Clin. Nutr. Metab. Care 2019, 22, 167–173.
1892. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. Phys. Med. Rehabil. Clin. N. Am. 2017, 28, 659–669.
1893. Mora, J.C.; Valencia, W.M. Exercise and Older Adults. Clin. Geriatr. Med. 2018, 34, 145–162.
1894. Reddy, R.; Wittenberg, A.; Castle, J.R.; El Youssef, J.; Winters-Stone, K.; Gillingham, M.; Jacobs, P.G. Effect of Aerobic and Resistance Exercise on Glycemic Control in Adults With Type 1 Diabetes. Can. J. Diabetes 2019, 43, 406–414.e401.
1895. Savikj, M.; Gabriel, B.M.; Alm, P.S.; Smith, J.; Caidahl, K.; Björnholm, M.; Fritz, T.; Krook, A.; Zierath, J.R.; Wallberg-Henriksson, H. Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: A randomised crossover trial. Diabetologia 2019, 62, 233–237.
1896. Gleeson, M.; Bishop, N.C.; Stensel, D.J.; Lindley, M.R.; Mastana, S.S.; Nimmo, M.A. The antiinflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. Nat. Rev. Immunol. 2011, 11, 607–615.
1897. Collao, N.; Rada, I.; Francaux, M.; Deldicque, L.; Zbinden-Foncea, H. Anti-Inflammatory Effect of Exercise Mediated by Toll-Like Receptor Regulation in Innate Immune Cells—A Review. Int. Rev. Immunol. 2020, 39, 39–52.

1898. Ho, S.S.; Dhaliwal, S.S.; Hills, A.P.; Pal, S. The effect of 12 weeks of aerobic, resistance or combination exercise training on cardiovascular risk factors in the overweight and obese in a randomized trial. BMC Public Health 2012, 12, 704.
1899. Wang, J.; Liu, S.; Li, G.; Xiao, J. Exercise Regulates the Immune System. Adv. Exp. Med. Biol. 2020, 1228, 395–408.
1900. Plikus, M.V.; Van Spyk, E.N.; Pham, K.; Geyfman, M.; Kumar, V.; Takahashi, J.S.; Andersen, B. The circadian clock in skin: Implications for adult stem cells, tissue regeneration, cancer, aging, and immunity. J. Biol. Rhythm. 2015, 30, 163–182.
1901. Zhang, Y.; Mignone, J.; MacLellan, W.R. Cardiac Regeneration and Stem Cells. Physiol. Rev. 2015, 95, 1189–1204.
1902. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
1903. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
1904. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res. Rev. Mutat. Res. 2019, 781, 175–185.
1905. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
1906. Swindell, W.R. Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. BMC Genom. 2009, 10, 585.
1907. Linford, N.J.; Beyer, R.P.; Gollahon, K.; Krajcik, R.A.; Malloy, V.L.; Demas, V.; Burmer, G.C.; Rabinovitch, P.S. Transcriptional response to aging and caloric restriction in heart and adipose tissue. Aging Cell 2007, 6, 673–688.
1908. Wanders, D.; Ghosh, S.; Stone, K.P.; Van, N.T.; Gettys, T.W. Transcriptional impact of dietary methionine restriction on systemic inflammation: Relevance to biomarkers of metabolic disease during aging. Biofactors 2014, 40, 13–26.
1909. Franco, C.; Soares, R.; Pires, E.; Koci, K.; Almeida, A.M.; Santos, R.; Coelho, A.V. Understanding regeneration through proteomics. Proteomics 2013, 13, 686–709.
1910. Stoick-Cooper, C.L.; Moon, R.T.; Weidinger, G. Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. Genes Dev. 2007, 21, 1292–1315.
1911. Partridge, L.; Deelen, J.; Slagboom, P.E. Facing up to the global challenges of ageing. Nature 2018, 561, 45–56.
1912. Lifestyle Management: Standards of Medical Care in Diabetes-2018. Diabetes Care 2018, 41, S38–S50.
1913. El-Sherif, A.; El-Sherif, S.; Taylor, A.H.; Ayakannu, T. Ovarian Cancer: Lifestyle, Diet and Nutrition. Nutr. Cancer 2021, 73, 1092–1107.
1914. Walsh, R. Lifestyle and mental health. Am. Psychol. 2011, 66, 579–592.
1915. Lu, K.D.; Forno, E. Exercise and lifestyle changes in pediatric asthma. Curr. Opin. Pulm. Med. 2020, 26, 103–111.
1916. Sharma, R.; Biedenharn, K.R.; Fedor, J.M.; Agarwal, A. Lifestyle factors and reproductive health: Taking control of your fertility. Reprod. Biol. Endocrinol. 2013, 11, 66.
1917. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
1918. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
1919. Williams, E.P.; Mesidor, M.; Winters, K.; Dubbert, P.M.; Wyatt, S.B. Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. Curr. Obes. Rep. 2015, 4, 363–370.
1920. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
1921. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
1922. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.

1923. Albashir, A.A.D. The potential impacts of obesity on COVID-19. Clin. Med. 2020, 20, e109–e113.
1924. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.
1925. Chaplin, D.D. Overview of the immune response. J. Allergy Clin. Immunol. 2010, 125, S3–S23.
1926. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
1927. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.
1928. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
1929. Green, W.D.; Beck, M.A. Obesity Impairs the Adaptive Immune Response to Influenza Virus. Ann. Am. Thorac. Soc. 2017, 14, S406–S409.
1930. Honce, R.; Schultz-Cherry, S. Influenza in obese travellers: Increased risk and complications, decreased vaccine effectiveness. J. Travel Med. 2019, 26, taz020.
1931. Sheridan, P.A.; Paich, H.A.; Handy, J.; Karlsson, E.A.; Hudgens, M.G.; Sammon, A.B.; Holland, L.A.; Weir, S.; Noah, T.L.; Beck, M.A. Obesity is associated with impaired immune response to influenza vaccination in humans. Int. J. Obes. 2012, 36, 1072–1077.
1932. Honce, R.; Schultz-Cherry, S. Impact of Obesity on Influenza A Virus Pathogenesis, Immune Response, and Evolution. Front. Immunol. 2019, 10, 1071.
1933. Ovsyannikova, I.G.; White, S.J.; Larrabee, B.; Grill, D.E.; Jacobson, R.M.; Poland, G.A. Leptin and leptin-related gene polymorphisms, obesity, and influenza A/H1N1 vaccine-induced immune responses in older individuals. Vaccine 2013, 32, 881–887.
1934. Watanabe, M.; Balena, A.; Tuccinardi, D.; Tozzi, R.; Risi, R.; Masi, D.; Caputi, A.; Rossetti, R.; Spoltore, M.E.; Filippi, V.; et al. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. Diabetes Metab. Res. Rev. 2021, e3465.
1935. Esser, N.; Legrand-Poels, S.; Piette, J.; Scheen, A.J.; Paquot, N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res. Clin. Pract. 2014, 105, 141–150.
1936. Cox, A.J.; West, N.P.; Cripps, A.W. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol. 2015, 3, 207–215.
1937. de Heredia, F.P.; Gómez-Martínez, S.; Marcos, A. Obesity, inflammation and the immune system. Proc. Nutr. Soc. 2012, 71, 332–338.
1938. Lee, B.C.; Lee, J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim. Biophys. Acta 2014, 1842, 446–462.
1939. Karczewski, J.; Sledzi´nska, E.; Baturo, A.; Jo´nczyk, I.; Maleszko, A.; Samborski, P.; Begier-Krasi´nska, B.; Dobrowolska, A. Obesity and inflammation. Eur. Cytokine. Netw. 2018, 29, 83–94.
1940. Sun, K.; Kusminski, C.M.; Scherer, P.E. Adipose tissue remodeling and obesity. J. Clin. Investig. 2011, 121, 2094–2101.
1941. Kucharska, A.M.; Pyrzak, B.; Demkow, U. Regulatory T Cells in Obesity. ˙ Adv. Exp. Med. Biol. 2015, 866, 35–40.
1942. Li, C.; Xu, M.M.; Wang, K.; Adler, A.J.; Vella, A.T.; Zhou, B. Macrophage polarization and metainflammation. Transl. Res. 2018, 191, 29–44.
1943. Thomas, D.; Apovian, C. Macrophage functions in lean and obese adipose tissue. Metabolism 2017, 72, 120–143.
1944. Engin, A.B. Adipocyte-Macrophage Cross-Talk in Obesity. Adv. Exp. Med. Biol. 2017, 960, 327–343.
1945. Beck, M.A. Influenza and obesity: Will vaccines and antivirals protect? J. Infect. Dis. 2012, 205, 172– 173.
1946. Petridou, A.; Siopi, A.; Mougios, V. Exercise in the management of obesity. Metabolism 2019, 92, 163– 169.
1947. Hsu, K.J.; Liao, C.D.; Tsai, M.W.; Chen, C.N. Effects of Exercise and Nutritional Intervention on Body Composition, Metabolic Health, and Physical Performance in Adults with Sarcopenic Obesity: A MetaAnalysis. Nutrients 2019, 11, 2163.
1948. Bray, G.A.; Frühbeck, G.; Ryan, D.H.; Wilding, J.P. Management of obesity. Lancet 2016, 387, 1947– 1956.

1949. da Silveira, M.P.; da Silva Fagundes, K.K.; Bizuti, M.R.; Starck, É.; Rossi, R.C.; de Resende, E.S.D.T. Physical exercise as a tool to help the immune system against COVID-19: An integrative review of the current literature. Clin. Exp. Med. 2021, 21, 15–28.
1950. Alawna, M.; Amro, M.; Mohamed, A.A. Aerobic exercises recommendations and specifications for patients with COVID-19: A systematic review. Eur. Rev. Med. Pharmacol. Sci. 2020, 24, 13049–13055.
1951. Suzuki, K.; Tagami, K. Voluntary wheel-running exercise enhances antigen-specific antibody-producing splenic B cell response and prolongs IgG half-life in the blood. Eur. J. Appl. Physiol. 2005, 94, 514–519.
1952. Fragala, M.S.; Kraemer, W.J.; Mastro, A.M.; Denegar, C.R.; Volek, J.S.; Kupchak, B.R.; Häkkinen, K.; Anderson, J.M.; Maresh, C.M. Glucocorticoid receptor expression on human B cells in response to acute heavy resistance exercise. Neuroimmunomodulation 2011, 18, 156–164.
1953. Ghanemi, A.; St-Amand, J. Interleukin-6 as a "metabolic hormone". Cytokine 2018, 112, 132–136.
1954. de Sousa, C.V.; Sales, M.M.; Rosa, T.S.; Lewis, J.E.; de Andrade, R.V.; Simões, H.G. The Antioxidant Effect of Exercise: A Systematic Review and Meta-Analysis. Sports Med. 2017, 47, 277–293.
1955. Smith, J.K. Exercise as an Adjuvant to Cartilage Regeneration Therapy. Int. J. Mol. Sci. 2020, 21, 9471.
1956. Schüttler, D.; Clauss, S.; Weckbach, L.T.; Brunner, S. Molecular Mechanisms of Cardiac Remodeling and Regeneration in Physical Exercise. Cells 2019, 8, 1128.
1957. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
1958. Scheffer, D.D.L.; Latini, A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochim. Biophys. Acta Mol. Basis Dis. 2020, 1866, 165823.
1959. Kawanishi, N.; Yano, H.; Yokogawa, Y.; Suzuki, K. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exerc. Immunol. Rev. 2010, 16, 105–118.
1960. Johnson, N.A.; Sachinwalla, T.; Walton, D.W.; Smith, K.; Armstrong, A.; Thompson, M.W.; George, J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. Hepatology 2009, 50, 1105–1112.
1961. Samouda, H.; de Beaufort, C.; Stranges, S.; Guinhouya, B.C.; Gilson, G.; Hirsch, M.; Jacobs, J.; Leite, S.; Vaillant, M.; Dadoun, F. Adding anthropometric measures of regional adiposity to BMI improves prediction of cardiometabolic, inflammatory and adipokines profiles in youths: A cross-sectional study. BMC Pediatr. 2015, 15, 168.
1962. Singh, S.; Sharma, A.N.; Murad, M.H.; Buttar, N.S.; El-Serag, H.B.; Katzka, D.A.; Iyer, P.G. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: A systematic review and meta-analysis. Clin. Gastroenterol. Hepatol. 2013, 11, 1399–1412.
1963. Liu, D.; Li, Q.; Dong, J.; Li, D.; Xu, X.; Xing, W.; Zhang, X.; Cao, W.; Hou, H.; Wang, H.; et al. The Association Between Normal BMI With Central Adiposity and Proinflammatory Potential Immunoglobulin G N-Glycosylation. Diabetes Metab. Syndr. Obesity Targets Ther. 2019, 12, 2373–2385.
1964. Ali, O.; Cerjak, D.; Kent, J.W.; James, R.; Blangero, J.; Zhang, Y. Obesity, central adiposity and cardiometabolic risk factors in children and adolescents: A family-based study. Pediatr. Obes. 2014, 9, e58– e62.
1965. Shang, X.; Scott, D.; Hodge, A.; Khan, B.; Khan, N.; English, D.R.; Giles, G.G.; Ebeling, P.R.; Sanders, K.M. Adiposity assessed by anthropometric measures has a similar or greater predictive ability than dualenergy X-ray absorptiometry measures for abdominal aortic calcification in community-dwelling older adults. Int. J. Cardiovasc. Imaging 2016, 32, 1451–1460.
1966. Zimta, A.A.; Tigu, A.B.; Muntean, M.; Cenariu, D.; Slaby, O.; Berindan-Neagoe, I. Molecular Links between Central Obesity and Breast Cancer. Int. J. Mol. Sci. 2019, 20, 5364.
1967. Lin, T.Y.; Hung, S.C.; Lim, P.S. Central obesity and incident atherosclerotic cardiovascular disease events in hemodialysis patients. Nutr. Metab. Cardiovasc. Dis. 2020, 30, 500–507.
1968. Shinkai, S.; Shore, S.; Shek, P.N.; Shephard, R.J. Acute exercise and immune function. Relationship between lymphocyte activity and changes in subset counts. Int. J. Sports Med. 1992, 13, 452–461.
1969. Perna, F.M.; Schneiderman, N.; LaPerriere, A. Psychological stress, exercise and immunity. Int. J. Sports Med. 1997, 18, S78–S83.

1970. Timmons, B.W.; Cieslak, T. Human natural killer cell subsets and acute exercise: A brief review. Exerc. Immunol. Rev. 2008, 14, 8–23.
1971. Horohov, D.W.; Dimock, A.; Guirnalda, P.; Folsom, R.W.; McKeever, K.H.; Malinowski, K. Effect of exercise on the immune response of young and old horses. Am. J. Vet. Res. 1999, 60, 643–647.
1972. Lin, Y.S.; Jan, M.S.; Chen, H.I. The effect of chronic and acute exercise on immunity in rats. Int. J. Sports Med. 1993, 14, 86–92.
1973. Frisina, J.P.; Gaudieri, S.; Cable, T.; Keast, D.; Palmer, T.N. Effects of acute exercise on lymphocyte subsets and metabolic activity. Int. J. Sports Med. 1994, 15, 36–41.
1974. Tiller, T. Single B cell antibody technologies. N. Biotechnol. 2011, 28, 453–457.
1975. Rashidian, J.; Lloyd, J. Single B Cell Cloning and Production of Rabbit Monoclonal Antibodies. Methods Mol. Biol. 2020, 2070, 423–441.
1976. Nicholas, D.A.; Nikolajczyk, B.S. B cells shed light on diminished vaccine responses in obesity. Obesity 2016, 24, 551.
1977. Shaikh, S.R.; Haas, K.M.; Beck, M.A.; Teague, H. The effects of diet-induced obesity on B cell function. Clin. Exp. Immunol. 2015, 179, 90–99.
1978. Frasca, D.; Ferracci, F.; Diaz, A.; Romero, M.; Lechner, S.; Blomberg, B.B. Obesity decreases B cell responses in young and elderly individuals. Obesity 2016, 24, 615–625.
1979. Wang, J.; Liu, S.; Li, G.; Xiao, J. Exercise Regulates the Immune System. Adv. Exp. Med. Biol. 2020, 1228, 395–408.
1980. Goedecke, J.H.; Micklesfield, L.K. The effect of exercise on obesity, body fat distribution and risk for type 2 diabetes. Med. Sport Sci. 2014, 60, 82–93.
1981. Lee, S.; Kuk, J.L.; Davidson, L.E.; Hudson, R.; Kilpatrick, K.; Graham, T.E.; Ross, R. Exercise without weight loss is an effective strategy for obesity reduction in obese individuals with and without Type 2 diabetes. J. Appl. Physiol. 2005, 99, 1220–1225.
1982. Alpsoy, ¸S. Exercise and Hypertension. Adv. Exp. Med. Biol. 2020, 1228, 153–167.
1983. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
1984. Kirwan, J.P.; Sacks, J.; Nieuwoudt, S. The essential role of exercise in the management of type 2 diabetes. Cleve Clin. J. Med. 2017, 84, S15–S21.
1985. Keating, S.E.; Hackett, D.A.; Parker, H.M.; O'Connor, H.T.; Gerofi, J.A.; Sainsbury, A.; Baker, M.K.; Chuter, V.H.; Caterson, I.D.; George, J.; et al. Effect of aerobic exercise training dose on liver fat and visceral adiposity. J. Hepatol. 2015, 63, 174–182.
1986. Lemura, L.M.; Von Duvillard, S.P.; Andreacci, J.; Klebez, J.M.; Chelland, S.A.; Russo, J. Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic and combination training in young women. Graefe's Arch. Clin. Exp. Ophthalmol. 2000, 82, 451–458.
1987. Piché, M.E.; Tchernof, A.; Després, J.P. Obesity Phenotypes, Diabetes, and Cardiovascular Diseases. Circ. Res. 2020, 126, 1477–1500.
1988. Ross, R.; Neeland, I.J.; Yamashita, S.; Shai, I.; Seidell, J.; Magni, P.; Santos, R.D.; Arsenault, B.; Cuevas, A.; Hu, F.B.; et al. Waist circumference as a vital sign in clinical practice: A Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat. Rev. Endocrinol. 2020, 16, 177–189.
1989. Blüher, M. Metabolically Healthy Obesity. Endocr. Rev. 2020, 41, 405–420.
1990. Iacobini, C.; Pugliese, G.; Blasetti Fantauzzi, C.; Federici, M.; Menini, S. Metabolically healthy versus metabolically unhealthy obesity. Metabolism 2019, 92, 51–60.
1991. Dekker, M.J.; Lee, S.; Hudson, R.; Kilpatrick, K.; Graham, T.E.; Ross, R.; Robinson, L.E. An exercise intervention without weight loss decreases circulating interleukin-6 in lean and obese men with and without type 2 diabetes mellitus. Metabolism 2007, 56, 332–338.
1992. Duncan, G.E.; Perri, M.G.; Theriaque, U.W.; Hutson, A.D.; Eckel, R.H.; Stacpoole, P.W. Exercise Training, Without Weight Loss, Increases Insulin Sensitivity and Postheparin Plasma Lipase Activity in Previously Sedentary Adults. Diabetes Care 2003, 26, 557–562.
1993. Mestek, M.L.; Westby, C.M.; Van Guilder, G.P.; Greiner, J.J.; Stauffer, B.; DeSouza, C.A. Regular Aerobic Exercise, Without Weight Loss, Improves Endothelium-dependent Vasodilation in Overweight and Obese Adults. Obesity 2010, 18, 1667–1669.

1994. Chia, C.W.; Egan, J.M.; Ferrucci, L. Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk. Circ. Res. 2018, 123, 886–904.
1995. Cartee, G.D. Influence of age on skeletal muscle glucose transport and glycogen metabolism. Med. Sci. Sports Exerc. 1994, 26, 577–585.
1996. Fleg, J.L. Aerobic exercise in the elderly: A key to successful aging. Discov. Med. 2012, 13, 223–228.
1997. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. Phys. Med. Rehabil. Clin. N. Am. 2017, 28, 659–669.
1998. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. Am. Fam. Physician 2017, 95, 425–432.
1999. Consitt, L.A.; Dudley, C.; Saxena, G. Impact of Endurance and Resistance Training on Skeletal Muscle Glucose Metabolism in Older Adults. Nutrients 2019, 11, 2636.
2000. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
2001. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
2002. Aoi, W.; Sakuma, K. Skeletal muscle: Novel and intriguing characteristics as a secretory organ. BioDiscovery 2013, 7, e8942.
2003. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
2004. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
2005. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. Genes 2021, 12, 1832.
2006. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
2007. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. Front. Physiol. 2020, 11, 18.
2008. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. Aging Cell 2014, 13, 175–184.
2009. Delany, A.M.; Kalajzic, I.; Bradshaw, A.D.; Sage, E.H.; Canalis, E. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. Endocrinology 2003, 144, 2588–2596.
2010. Motamed, K. SPARC (osteonectin/BM-40). Int. J. Biochem. Cell Biol. 1999, 31, 1363–1366.
2011. Scavelli, K.; Chatterjee, A.; Rhee, D.J. Secreted Protein Acidic and Rich in Cysteine in Ocular Tissue. J. Ocul. Pharmacol. Ther. 2015, 31, 396–405.
2012. Sage, H.; Johnson, C.; Bornstein, P. Characterization of a novel serum albumin-binding glycoprotein secreted by endothelial cells in culture. J. Biol. Chem. 1984, 259, 3993–4007.
2013. Brekken, R.A.; Sage, E.H. SPARC, a matricellular protein: At the crossroads of cell-matrix. Matrix Biol. 2000, 19, 569–580.
2014. Norose, K.; Clark, J.I.; Syed, N.A.; Basu, A.; Heber-Katz, E.; Sage, E.H.; Howe, C.C. SPARC deficiency leads to early-onset cataractogenesis. Investig. Ophthalmol. Vis. Sci. 1998, 39, 2674–2680.
2015. Yan, Q.; Sage, E.H. SPARC, a matricellular glycoprotein with important biological functions. J. Histochem. Cytochem. 1999, 47, 1495–1506.
2016. Zhu, J.; Wang, L.Y.; Li, C.Y.; Wu, J.Y.; Zhang, Y.T.; Pang, K.P.; Wei, Y.; Du, L.Q.; Liu, M.; Wu, X.Y. SPARC promotes self-renewal of limbal epithelial stem cells and ocular surface restoration through JNK and p38-MAPK signaling pathways. Stem Cells 2020, 38, 134–145.

2017. Alachkar, H.; Santhanam, R.; Maharry, K.; Metzeler, K.H.; Huang, X.; Kohlschmidt, J.; Mendler, J.H.; Benito, J.M.; Hickey, C.; Neviani, P.; et al. SPARC promotes leukemic cell growth and predicts acute myeloid leukemia outcome. J. Clin. Investig. 2014, 124, 1512–1524.
2018. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectindeficient mice. J. Clin. Investig. 2000, 105, 915–923.
2019. Barker, T.H.; Baneyx, G.; Cardó-Vila, M.; Workman, G.A.; Weaver, M.; Menon, P.M.; Dedhar, S.; Rempel, S.A.; Arap, W.; Pasqualini, R.; et al. SPARC regulates extracellular matrix organization through its modulation of integrin-linked kinase activity. J. Biol. Chem. 2005, 280, 36483–36493.
2020. Bradshaw, A.D. The role of SPARC in extracellular matrix assembly. J. Cell Commun. Signal. 2009, 3, 239–246.
2021. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
2022. Onorato, A.M.; Fiore, E.; Bayo, J.; Casali, C.; Fernandez-Tomé, M.; Rodríguez, M.; Domínguez, L.; Argemi, J.; Hidalgo, F.; Favre, C.; et al. SPARC inhibition accelerates NAFLD-associated hepatocellular carcinoma development by dysregulating hepatic lipid metabolism. Liver Int. 2021, 41, 1677–1693.
2023. Song, H.; Ding, L.; Zhang, S.; Wang, W. MiR-29 family members interact with SPARC to regulate glucose metabolism. Biochem. Biophys. Res. Commun. 2018, 497, 667–674.
2024. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
2025. McCurdy, S.; Baicu, C.F.; Heymans, S.; Bradshaw, A.D. Cardiac extracellular matrix remodeling: Fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). J. Mol. Cell. Cardiol. 2010, 48, 544–549.
2026. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 2013, 28, 1451–1460.
2027. Bradshaw, A.D.; Sage, E.H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J. Clin. Investig. 2001, 107, 1049–1054.
2028. Kim, J.S.; Galvão, D.A.; Newton, R.U.; Gray, E.; Taaffe, D.R. Exercise-induced myokines and their effect on prostate cancer. Nat. Rev. Urol. 2021, 18, 519–542.
2029. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. Biomolecules 2021, 11, 1689.
2030. Frontera, W.R.; Ochala, J. Skeletal muscle: A brief review of structure and function. Calcif. Tissue Int. 2015, 96, 183–195.
2031. Barbalho, S.M.; Flato, U.A.P.; Tofano, R.J.; Goulart, R.A.; Guiguer, E.L.; Detregiachi, C.R.P.; Buchaim, D.V.; Araújo, A.C.; Buchaim, R.L.; Reina, F.T.R.; et al. Physical Exercise and Myokines: Relationships with Sarcopenia and Cardiovascular Complications. Int. J. Mol. Sci. 2020, 21, 3607.
2032. Lee, J.H.; Jun, H.S. Role of Myokines in Regulating Skeletal Muscle Mass and Function. Front. Physiol. 2019, 10, 42.
2033. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. Cell 2013, 153, 1194–1217.
2034. Distefano, G.; Goodpaster, B.H. Effects of Exercise and Aging on Skeletal Muscle. Cold Spring Harb. Perspect. Med. 2018, 8, a029785.
2035. Stegeman, R.; Weake, V.M. Transcriptional Signatures of Aging. J. Mol. Biol. 2017, 429, 2427–2437.
2036. Dhingra, R.; Vasan, R.S. Age as a risk factor. Med. Clin. N. Am. 2012, 96, 87–91.
2037. Cheitlin, M.D. Cardiovascular physiology-changes with aging. Am. J. Geriatr. Cardiol. 2003, 12, 9 13.
2038. Kõks, S.; Dogan, S.; Tuna, B.G.; González-Navarro, H.; Potter, P.; Vandenbroucke, R.E. Mouse models of ageing and their relevance to disease. Mech. Ageing Dev. 2016, 160, 41–53.
2039. Vanhooren, V.; Libert, C. The mouse as a model organism in aging research: Usefulness, pitfalls and possibilities. Ageing Res. Rev. 2013, 12, 8–21.
2040. Azzu, V.; Valencak, T.G. Energy Metabolism and Ageing in the Mouse: A Mini-Review. Gerontology 2017, 63, 327–336.
2041. Azman, K.F.; Zakaria, R. D-Galactose-induced accelerated aging model: An overview. Biogerontology 2019, 20, 763–782.

2042. Santilli, V.; Bernetti, A.; Mangone, M.; Paoloni, M. Clinical definition of sarcopenia. Clin. Cases Miner. Bone Metab. 2014, 11, 177–180.
2043. Jørgensen, L.H.; Jepsen, P.L.; Boysen, A.; Dalgaard, L.B.; Hvid, L.G.; Ørtenblad, N.; Ravn, D.; Sellathurai, J.; Møller-Jensen, J.; Lochmüller, H.; et al. SPARC Interacts with Actin in Skeletal Muscle in Vitro and in Vivo. Am. J. Pathol. 2017, 187, 457–474.
2044. Kjær, M. Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. Physiol. Rev. 2004, 84, 649–698.
2045. Gelse, K.; Pöschl, E.; Aigner, T. Collagens—Structure, function, and biosynthesis. Adv. Drug Deliv. Rev. 2003, 55, 1531–1546.
2046. Wang, T.; Wagner, A.; Gehwolf, R.; Yan, W.; Passini, F.S.; Thien, C.; Weissenbacher, N.; Lin, Z.; Lehner, C.; Teng, H.; et al. Load-induced regulation of tendon homeostasis by SPARC, a genetic predisposition factor for tendon and ligament injuries. Sci. Transl. Med. 2021, 13, eabe5738.
2047. Gruber, J.; Schaffer, S.; Halliwell, B. The mitochondrial free radical theory of ageing–where do we stand? Front. Biosci. 2008, 13, 6554–6579.
2048. DeFronzo, R.A. Glucose Intolerance and Aging. Diabetes Care 1981, 4, 493–501.
2049. Kos, K.; Wong, S.; Tan, B.; Gummesson, A.; Jernas, M.; Franck, N.; Kerrigan, D.; Nystrom, F.H.; Carlsson, L.M.; Randeva, H.S.; et al. Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. Diabetes 2009, 58, 1780–1788.
2050. Lee, S.H.; Lee, J.A.; Park, H.S.; Song, Y.S.; Jang, Y.J.; Kim, J.H.; Lee, Y.J.; Heo, Y. Associations among SPARC mRNA expression in adipose tissue, serum SPARC concentration and metabolic parameters in Korean women. Obesity 2013, 21, 2296–2302.
2051. Mukherjee, S.; Choi, M.J.; Kim, S.W.; Yun, J.W. Secreted protein acidic and rich in cysteine (SPARC) regulates thermogenesis in white and brown adipocytes. Mol. Cell. Endocrinol. 2020, 506, 110757.
2052. Guo, S.S.; Zeller, C.; Chumlea, W.C.; Siervogel, R.M. Aging, body composition, and lifestyle: The Fels Longitudinal Study. Am. J. Clin. Nutr. 1999, 70, 405–411.
2053. Sackmann-Sala, L.; Berryman, D.E.; Munn, R.D.; Lubbers, E.R.; Kopchick, J.J. Heterogeneity among white adipose tissue depots in male C57BL/6J mice. Obesity 2012, 20, 101–111.
2054. Jo, J.; Gavrilova, O.; Pack, S.; Jou, W.; Mullen, S.; Sumner, A.E.; Cushman, S.W.; Periwal, V. Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. PLoS Comput. Biol. 2009, 5, e1000324.
2055. Nie, J.; Bradshaw, A.D.; Delany, A.M.; Sage, E.H. Inactivation of SPARC enhances high-fat diet-induced obesity in mice. Connect. Tissue Res. 2011, 52, 99–108.
2056. Carosio, S.; Berardinelli, M.G.; Aucello, M.; Musarò, A. Impact of ageing on muscle cell regeneration. Ageing Res. Rev. 2011, 10, 35–42.
2057. Hwang, A.B.; Brack, A.S. Muscle Stem Cells and Aging. Curr. Top. Dev. Biol. 2018, 126, 299–322.
2058. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
2059. Mansergh, F.C.; Wells, T.; Elford, C.; Evans, S.L.; Perry, M.J.; Evans, M.J.; Evans, B.A. Osteopenia in Sparc (osteonectin)-deficient mice: Characterization of phenotypic determinants of femoral strength and changes in gene expression. Physiol. Genom. 2007, 32, 64–73.
2060. Mansergh, F.C.; Wride, M.A.; Walker, V.E.; Adams, S.; Hunter, S.M.; Evans, M.J. Gene expression changes during cataract progression in Sparc null mice: Differential regulation of mouse globins in the lens. Mol. Vis. 2004, 10, 490–511.
2061. Rempel, S.A.; Hawley, R.C.; Gutiérrez, J.A.; Mouzon, E.; Bobbitt, K.R.; Lemke, N.; Schultz, C.R.; Schultz, L.R.; Golembieski, W.; Koblinski, J.; et al. Splenic and immune alterations of the Sparc-null mouse accompany a lack of immune response. Genes Immun. 2007, 8, 262–274.
2062. Whittal, M.C.; Molladavoodi, S.; Zwambag, D.P.; Millecamps, M.; Stone, L.S.; Gregory, D.E. Mechanical Consequence of Induced Intervertebral Disc Degeneration in the SPARC-Null Mouse. J. Biomech. Eng. 2021, 143, 024501.
2063. Gruber, H.E.; Sage, E.H.; Norton, H.J.; Funk, S.; Ingram, J.; Hanley, E.N., Jr. Targeted deletion of the SPARC gene accelerates disc degeneration in the aging mouse. J. Histochem. Cytochem. 2005, 53, 1131– 1138.

2064. Atorrasagasti, C.; Onorato, A.; Gimeno, M.L.; Andreone, L.; Garcia, M.; Malvicini, M.; Fiore, E.; Bayo, J.; Perone, M.J.; Mazzolini, G.D. SPARC is required for the maintenance of glucose homeostasis and insulin secretion in mice. Clin. Sci. 2019, 133, 351–365.
2065. Harries, L.W.; McCulloch, L.J.; Holley, J.E.; Rawling, T.J.; Welters, H.J.; Kos, K. A role for SPARC in the moderation of human insulin secretion. PLoS ONE 2013, 8, e68253.
2066. Hu, L.; He, F.; Huang, M.; Zhao, Q.; Cheng, L.; Said, N.; Zhou, Z.; Liu, F.; Dai, Y.S. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. Sci. Rep. 2020, 10, 17581.
2067. Kahn, S.E.; Hull, R.L.; Utzschneider, K.M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006, 444, 840–846.
2068. Andrikopoulos, S.; Blair, A.R.; Deluca, N.; Fam, B.C.; Proietto, J. Evaluating the glucose tolerance test in mice. Am. J. Physiol. Endocrinol. Metab. 2008, 295, E1323–E1332.
2069. Songsorn, P.; Ruffino, J.; Vollaard, N.B. No effect of acute and chronic supramaximal exercise on circulating levels of the myokine SPARC. Eur. J. Sport Sci. 2017, 17, 447–452.
2070. Batsis, J.A.; Villareal, D.T. Sarcopenic obesity in older adults: Aetiology, epidemiology and treatment strategies. Nat. Rev. Endocrinol. 2018, 14, 513–537.
2071. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
2072. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. Diseases 2021, 9, 87.
2073. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
2074. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. FASEB J. 2019, 33, 10551–10562.
2075. Richter, E.A.; Hargreaves, M. Exercise, GLUT4, and skeletal muscle glucose uptake. Physiol. Rev. 2013, 93, 993–1017.
2076. Pearson, A.M. Muscle growth and exercise. Crit. Rev. Food Sci. Nutr. 1990, 29, 167–196.
2077. Liberman, K.; Forti, L.N.; Beyer, I.; Bautmans, I. The effects of exercise on muscle strength, body composition, physical functioning and the inflammatory profile of older adults: A systematic review. Curr. Opin. Clin. Nutr. Metab. Care 2017, 20, 30–53.
2078. Willis, L.H.; Slentz, C.A.; Bateman, L.A.; Shields, A.T.; Piner, L.W.; Bales, C.W.; Houmard, J.A.; Kraus, W.E. Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults. J. Appl. Physiol. 2012, 113, 1831–1837.
2079. Francaux, M.; Deldicque, L. Exercise and the control of muscle mass in human. Pflug. Arch.-Eur. J. Physiol. 2019, 471, 397–411.
2080. Bishop, D.J.; Botella, J.; Genders, A.J.; Lee, M.J.; Saner, N.J.; Kuang, J.; Yan, X.; Granata, C. HighIntensity Exercise and Mitochondrial Biogenesis: Current Controversies and Future Research Directions. Physiology 2019, 34, 56–70.
2081. Kwon, J.H.; Moon, K.M.; Min, K.W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. Healthcare 2020, 8, 378.
2082. Huh, J.Y. The role of exercise-induced myokines in regulating metabolism. Arch. Pharmacal Res. 2018, 41, 14–29.
2083. Pedersen, L.; Idorn, M.; Olofsson, G.H.; Lauenborg, B.; Nookaew, I.; Hansen, R.H.; Johannesen, H.H.; Becker, J.C.; Pedersen, K.S.; Dethlefsen, C.; et al. Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. Cell Metab. 2016, 23, 554–562.
2084. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
2085. Rowlatt, C.; Chesterman, F.C.; Sheriff, M.U. Lifespan, age changes and tumour incidence in an ageing C57BL mouse colony. Lab. Anim. 1976, 10, 419–442.
2086. Kunstyr, I.; Leuenberger, H.G. Gerontological data of C57BL/6J mice. I. Sex differences in survival curves. J. Gerontol. 1975, 30, 157–162.
2087. Billat, V.L.; Mouisel, E.; Roblot, N.; Melki, J. Inter- and intrastrain variation in mouse critical running speed. J. Appl. Physiol. 2005, 98, 1258–1263.

2088. Schefer, V.; Talan, M.I. Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity. Exp. Gerontol. 1996, 31, 387–392.
2089. Teklad Global 18% Protein Rodent Diet (Sterilizable). Available online: https://insights.envigo.com/hubfs/resources/datasheets/2018s-datasheet-0915.pdf (accessed on 13 December 2020).
2090. Norose, K.; Lo, W.K.; Clark, J.I.; Sage, E.H.; Howe, C.C. Lenses of SPARC-null mice exhibit an abnormal cell surface-basement membrane interface. Exp. Eye Res. 2000, 71, 295–307.
2091. Nishida, Y.; Tokuyama, K.; Nagasaka, S.; Higaki, Y.; Shirai, Y.; Kiyonaga, A.; Shindo, M.; Kusaka, I.; Nakamura, T.; Ishibashi, S.; et al. Effect of moderate exercise training on peripheral glucose effectiveness, insulin sensitivity, and endogenous glucose production in healthy humans estimated by a two-compartmentlabeled minimal model. Diabetes 2004, 53, 315–320.
2092. Tanaka, H.; Shindo, M. The benefits of the low intensity training. Ann. Physiol. Anthr. 1992, 11, 365–368.
2093. Motoyama, M.; Sunami, Y.; Kinoshita, F.; Kiyonaga, A.; Tanaka, H.; Shindo, M.; Irie, T.; Urata, H.; Sasaki, J.; Arakawa, K. Blood pressure lowering effect of low intensity aerobic training in elderly hypertensive patients. Med. Sci. Sports Exerc. 1998, 30, 818–823.
2094. Sunami, Y.; Motoyama, M.; Kinoshita, F.; Mizooka, Y.; Sueta, K.; Matsunaga, A.; Sasaki, J.; Tanaka, H.; Shindo, M. Effects of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. Metabolism 1999, 48, 984–988.
2095. Frontera, W.R.; Hughes, V.A.; Fielding, R.A.; Fiatarone, M.A.; Evans, W.J.; Roubenoff, R. Aging of skeletal muscle: A 12-yr longitudinal study. J. Appl. Physiol. 2000, 88, 1321–1326.
2096. Yamada, M.; Moriguch, Y.; Mitani, T.; Aoyama, T.; Arai, H. Age-dependent changes in skeletal muscle mass and visceral fat area in Japanese adults from 40 to 79 years-of-age. Geriatr. Gerontol. Int. 2014, 14 (Suppl. S1), 8–14.
2097. Miyamoto, T.; Shimizu, Y.; Matsuo, Y.; Otaru, T.; Kanzawa, Y.; Miyamae, N.; Yamada, E.; Katsuno, T. Effects of exercise intensity and duration on a myokine, secreted protein acidic and rich in cysteine. Eur. J. Sport Sci. 2021, 1–10.
2098. Potteiger, J.A.; Jacobsen, D.J.; Donnelly, J.E. A comparison of methods for analyzing glucose and insulin areas under the curve following nine months of exercise in overweight adults. Int. J. Obes. Relat. Metab. Disord. 2002, 26, 87–89.
2099. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 2012, 9, 671–675.
2100. Taylor, S.C.; Berkelman, T.; Yadav, G.; Hammond, M. A defined methodology for reliable quantification of Western blot data. Mol. Biotechnol. 2013, 55, 217–226.
2101. Taylor, S.C.; Posch, A. The design of a quantitative western blot experiment. BioMed Res. Int. 2014, 2014, 361590.
2102. Ghanemi, A.; Melouane, A.; Mucunguzi, O.; Yoshioka, M.; St-Amand, J. Energy and metabolic pathways in trefoil factor family member 2 (Tff2) KO mice beyond the protection from high-fat diet induced obesity. Life Sci. 2018, 215, 190–197.
2103. Lo, S.; Russell, J.C.; Taylor, A.W. Determination of glycogen in small tissue samples. J. Appl. Physiol. 1970, 28, 234–236.
2104. Parlee, S.D.; Lentz, S.I.; Mori, H.; MacDougald, O.A. Quantifying size and number of adipocytes in adipose tissue. Methods Enzymol. 2014, 537, 93–122.
2105. De Giorgio, M.R.; Yoshioka, M.; Riedl, I.; Moreault, O.; Cherizol, R.G.; Shah, A.A.; Blin, N.; Richard, D.; St-Amand, J. Trefoil factor family member 2 (Tff2) KO mice are protected from high-fat diet-induced obesity. Obesity 2013, 21, 1389–1395.
2106. Available online: https://www.cyagen.com/us/en/service/piggybac-transgenic-mouse.html (accessed on 15 December 2020).
2107. Luu-The, V.; Paquet, N.; Calvo, E.; Cumps, J. Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction. BioTechniques 2005, 38, 287–293.
2108. Bustin, S.A.; Benes, V.; Garson, J.A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M.W.; Shipley, G.L.; et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 2009, 55, 611–622.

2109. Bustin, S.A.; Beaulieu, J.F.; Huggett, J.; Jaggi, R.; Kibenge, F.S.; Olsvik, P.A.; Penning, L.C.; Toegel, S. MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. BMC Mol. Biol. 2010, 11, 74.
2110. Smith, J.P.; Hicks, P.S.; Ortiz, L.R.; Martinez, M.J.; Mandler, R.N. Quantitative measurement of muscle strength in the mouse. J. Neurosci. Methods 1995, 62, 15–19.
2111. Takeshita, H.; Yamamoto, K.; Nozato, S.; Inagaki, T.; Tsuchimochi, H.; Shirai, M.; Yamamoto, R.; Imaizumi, Y.; Hongyo, K.; Yokoyama, S.; et al. Modified forelimb grip strength test detects aging-associated physiological decline in skeletal muscle function in male mice. Sci. Rep. 2017, 7, 42323.
2112. Capaldi, R.A.; Malatesta, F.; Darley-Usmar, V.M. Structure of cytochrome c oxidase. Biochim. Biophys. Acta 1983, 726, 135–148.
2113. García-Horsman, J.A.; Barquera, B.; Rumbley, J.; Ma, J.; Gennis, R.B. The superfamily of heme copper respiratory oxidases. J. Bacteriol. 1994, 176, 5587–5600.
2114. Saraste, M. Structure and evolution of cytochrome oxidase. Antonie Leeuwenhoek 1994, 65, 285–287.
2115. Azzi, A. Cytochrome c oxidase. Towards a clarification of its structure, interactions and mechanism. Biochim. Biophys. Acta 1980, 594, 231–252.
2116. Available online: https://www.ncbi.nlm.nih.gov/gene/17709 (accessed on 16 January 2022).
2117. Dittmer, A.; Dittmer, J. Beta-actin is not a reliable loading control in Western blot analysis. Electrophoresis 2006, 27, 2844–2845.
2118. Bao, J.M.; Dang, Q.; Lin, C.J.; Lo, U.G.; Feldkoren, B.; Dang, A.; Hernandez, E.; Li, F.; Panwar, V.; Lee, C.F.; et al. SPARC is a key mediator of TGF-β-induced renal cancer metastasis. J. Cell. Physiol. 2021, 236, 1926–1938.
2119. Chang, W.; Wei, K.; Jacobs, S.S.; Upadhyay, D.; Weill, D.; Rosen, G.D. SPARC suppresses apoptosis of idiopathic pulmonary fibrosis fibroblasts through constitutive activation of beta-catenin. J. Biol. Chem. 2010, 285, 8196–8206.
2120. Shen, Y.; Zhao, Y.; Yuan, L.; Yi, W.; Zhao, R.; Yi, Q.; Yong, T. SPARC is over-expressed in adipose tissues of diet-induced obese rats and causes insulin resistance in 3T3-L1 adipocytes. Acta Histochem. 2014, 116, 158–166.
2121. Wu, D.; Li, L.; Yang, M.; Liu, H.; Yang, G. Elevated plasma levels of SPARC in patients with newly diagnosed type 2 diabetes mellitus. Eur. J. Endocrinol. 2011, 165, 597–601.
2122. Yiu, G.K.; Chan, W.Y.; Ng, S.W.; Chan, P.S.; Cheung, K.K.; Berkowitz, R.S.; Mok, S.C. SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells. Am. J. Pathol. 2001, 159, 609–622.
2123. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Matsuwaki, T.; Nishihara, M. Age-related resistance of skeletal muscle-derived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. Aging 2012, 4, 40–48.
2124. Nie, J.; Chang, B.; Traktuev, D.O.; Sun, J.; March, K.; Chan, L.; Sage, E.H.; Pasqualini, R.; Arap, W.; Kolonin, M.G. IFATS collection: Combinatorial peptides identify alpha5beta1 integrin as a receptor for the matricellular protein SPARC on adipose stromal cells. Stem Cells 2008, 26, 2735–2745.
2125. Jacunski, M.; Melville, P.; Currie, G.P. Exercise: The panacea in management of many ills. Now is the time to engage. J. R. Coll. Physicians Edinb. 2021, 51, 120–122.
2126. Hosker, D.K.; Elkins, R.M.; Potter, M.P. Promoting Mental Health and Wellness in Youth Through Physical Activity, Nutrition, and Sleep. Child. Adolesc. Psychiatr. Clin. N. Am. 2019, 28, 171–193.
2127. Peluso, M.A.; de Andrade, L.H.G. Physical activity and mental health: The association between exercise and mood. Clinics 2005, 60, 61–70
2128. Myers, J.; McAuley, P.; Lavie, C.J.; Despres, J.P.; Arena, R.; Kokkinos, P. Physical activity and cardiorespiratory fitness as major markers of cardiovascular risk: Their independent and interwoven importance to health status. Prog. Cardiovasc. Dis. 2015, 57, 306–314.
2129. Katzmarzyk, P.T.; Ross, R.; Blair, S.N.; Després, J.P. Should we target increased physical activity or less sedentary behavior in the battle against cardiovascular disease risk development? Atherosclerosis 2020, 311, 107–115.
2130. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obese Animals as Models for Numerous Diseases: Advantages and Applications. Medicina 2021, 57, 399.
2131. Ghanemi, A.; St-Amand, J. Redefining obesity toward classifying as a disease. Eur. J. Intern. Med. 2018, 55, 20–22.

2132. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Broken Energy Homeostasis and Obesity Pathogenesis: The Surrounding Concepts. J. Clin. Med. 2018, 7, 453.
2133. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Obesity as a Neuroendocrine Reprogramming. Medicina 2021, 57, 66.
2134. Owen, P.J.; Miller, C.T.; Mundell, N.L.; Verswijveren, S.; Tagliaferri, S.D.; Brisby, H.; Bowe, S.J.; Belavy, D.L. Which specific modes of exercise training are most effective for treating low back pain? Network meta-analysis. Br. J. Sports Med. 2020, 54, 1279–1287.
2135. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
2136. Wilkinson, T.J.; Shur, N.F.; Smith, A.C. "Exercise as medicine" in chronic kidney disease. Scand. J. Med. Sci. Sports 2016, 26, 985–988.
2137. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Regeneration during Obesity: An Impaired Homeostasis. Animals 2020, 10, 2344.
2138. Idorn, M.; Straten, P.T. Exercise and cancer: From "healthy" to "therapeutic"? Cancer Immunol. Immunother. 2017, 66, 667–671.
2139. Balducci, S.; Sacchetti, M.; Haxhi, J.; Orlando, G.; D'Errico, V.; Fallucca, S.; Menini, S.; Pugliese, G. Physical exercise as therapy for type 2 diabetes mellitus. Diabetes Metab. Res. Rev. 2014, 30, 13–23.
2140. Simpson, R.J.; Kunz, H.; Agha, N.; Graff, R. Exercise and the Regulation of Immune Functions. Prog. Mol. Biol. Transl. Sci. 2015, 135, 355–380.
2141. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology 2021, 10, 545.
2142. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Will an obesity pandemic replace the coronavirus disease-2019 (COVID-19) pandemic? Med. Hypotheses 2020, 144, 110042.
2143. Sharman, J.E.; La Gerche, A.; Coombes, J.S. Exercise and cardiovascular risk in patients with hypertension. Am. J. Hypertens. 2015, 28, 147–158.
2144. Benedetti, M.G.; Furlini, G.; Zati, A.; Mauro, G.L. The Effectiveness of Physical Exercise on Bone Density in Osteoporotic Patients. Biomed. Res. Int. 2018, 2018, 4840531.
2145. Metsios, G.S.; Moe, R.H.; Kitas, G.D. Exercise and inflammation. Best Pract. Res. Clin. Rheumatol. 2020, 34, 101504.
2146. Ströhle, A. Sports psychiatry: Mental health and mental disorders in athletes and exercise treatment of mental disorders. Eur. Arch. Psychiatry Clin. Neurosci. 2019, 269, 485–498.
2147. Zhang, Y.; Zhang, H.; Ma, X.; Di, Q. Mental Health Problems during the COVID-19 Pandemics and the Mitigation Effects of Exercise: A Longitudinal Study of College Students in China. Int. J. Environ. Res. Public Health 2020, 17, 3722.
2148. Mikkelsen, K.; Stojanovska, L.; Polenakovic, M.; Bosevski, M.; Apostolopoulos, V. Exercise and mental health. Maturitas 2017, 106, 48–56.
2149. Jurado-Fasoli, L.; De-la, O.A.; Molina-Hidalgo, C.; Migueles, J.H.; Castillo, M.J.; Amaro-Gahete, F.J. Exercise training improves sleep quality: A randomized controlled trial. Eur. J. Clin. Investig. 2020, 50, e13202.
2150. Yang, P.Y.; Ho, K.H.; Chen, H.C.; Chien, M.Y. Exercise training improves sleep quality in middle-aged and older adults with sleep problems: A systematic review. J. Physiother. 2012, 58, 157–163.
2151. Kwon, J.H.; Moon, K.M.; Min, K.-W. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. Healthcare 2020, 8, 378.
2152. Aoi, W.; Sakuma, K. Skeletal muscle: Novel and intriguing characteristics as a secretory organ. BioDiscovery 2013, 7.
2153. Aoi, W.; Hirano, N.; Lassiter, D.G.; Björnholm, M.; Chibalin, A.V.; Sakuma, K.; Tanimura, Y.; Mizushima, K.; Takagi, T.; Naito, Y.; et al. Secreted protein acidic and rich in cysteine (SPARC) improves glucose tolerance via AMP-activated protein kinase activation. Faseb J. 2019, 33, 10551–10562.
2154. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
2155. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
2156. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res. Rev. Mutat. Res. 2019, 781, 175–185.

2157. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
2158. Scheler, M.; Irmler, M.; Lehr, S.; Hartwig, S.; Staiger, H.; Al-Hasani, H.; Beckers, J.; de Angelis, M.H.; Häring, H.U.; Weigert, C. Cytokine response of primary human myotubes in an in vitro exercise model. Am. J. Physiol. Cell Physiol. 2013, 305, C877–C886.
2159. Nikoli´c, N.; Bakke, S.S.; Kase, E.T.; Rudberg, I.; Halle, I.F.; Rustan, A.C.; Thoresen, G.H.; Aas, V. Correction: Electrical Pulse Stimulation of Cultured Human Skeletal Muscle Cells as an In Vitro Model of Exercise. PLoS ONE 2013, 8, e33203.
2160. Tarum, J.; Folkesson, M.; Atherton, P.J.; Kadi, F. Electrical pulse stimulation: An in vitro exercise model for the induction of human skeletal muscle cell hypertrophy. A proof-of-concept study. Exp. Physiol. 2017, 102, 1405–1413.
2161. Feng, Y.Z.; Nikoli´c, N.; Bakke, S.S.; Kase, E.T.; Guderud, K.; Hjelmesæth, J.; Aas, V.; Rustan, A.C.; Thoresen, G.H. Myotubes from lean and severely obese subjects with and without type 2 diabetes respond differently to an in vitro model of exercise. Am. J. Physiol. Cell. Physiol. 2015, 308, C548–C556.
2162. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
2163. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
2164. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
2165. Phan, E.; Ahluwalia, A.; Tarnawski, A.S. Role of SPARC—Matricellular protein in pathophysiology and tissue injury healing. Implications for gastritis and gastric ulcers. Med. Sci. Monit. 2007, 13, RA25–RA30.
2166. Harries, L.W.; McCulloch, L.J.; Holley, J.E.; Rawling, T.J.; Welters, H.J.; Kos, K. A role for SPARC in the moderation of human insulin secretion. PLoS ONE 2013, 8, e68253.
2167. Hu, L.; He, F.; Huang, M.; Zhao, Q.; Cheng, L.; Said, N.; Zhou, Z.; Liu, F.; Dai, Y.S. SPARC promotes insulin secretion through down-regulation of RGS4 protein in pancreatic β cells. Sci. Rep. 2020, 10, 17581.
2168. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
2169. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
2170. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. Matrix Biol. 2016, 52–54, 78– 87.
2171. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
2172. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 2013, 28, 1451–1460.
2173. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schrøder, H.D. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. J. Histochem. Cytochem. 2009, 57, 29–39.
2174. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
2175. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
2176. Lee, J.H.; Jun, H.S. Role of Myokines in Regulating Skeletal Muscle Mass and Function. Front. Physiol. 2019, 10, 42.
2177. Kim, J.S.; Galvão, D.A.; Newton, R.U.; Gray, E.; Taaffe, D.R. Exercise-induced myokines and their effect on prostate cancer. Nat. Rev. Urol. 2021, 18, 519–542.

2178. Joyce, N.C.; Oskarsson, B.; Jin, L.W. Muscle biopsy evaluation in neuromuscular disorders. Phys. Med. Rehabil. Clin. N. Am. 2012, 23, 609–631.
2179. Pearl, G.S.; Ghatak, N.R. Muscle biopsy. Arch. Pathol. Lab. Med. 1995, 119, 303–306.
2180. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. Front. Physiol. 2020, 11, 18.
2181. Brown, J.C.; Damjanov, N.; Courneya, K.S.; Troxel, A.B.; Zemel, B.S.; Rickels, M.R.; Ky, B.; Rhim, A.D.; Rustgi, A.K.; Schmitz, K.H. A randomized dose-response trial of aerobic exercise and health-related quality of life in colon cancer survivors. Psychooncology 2018, 27, 1221–1228.
2182. Dunn, A.L.; Trivedi, M.H.; Kampert, J.B.; Clark, C.G. Chambliss HO: Exercise treatment for depression: Efficacy and dose response. Am. J. Prev. Med. 2005, 28, 1–8.
2183. Malliaras, P.; Johnston, R.; Street, G.; Littlewood, C.; Bennell, K.; Haines, T.; Buchbinder, R. The Efficacy of Higher Versus Lower Dose Exercise in Rotator Cuff Tendinopathy: A Systematic Review of Randomized Controlled Trials. Arch. Phys. Med. Rehabil. 2020, 101, 1822–1834.
2184. Miyamoto, T.; Shimizu, Y.; Matsuo, Y.; Otaru, T.; Kanzawa, Y.; Miyamae, N.; Yamada, E.; Katsuno, T. Effects of exercise intensity and duration on a myokine, secreted protein acidic and rich in cysteine. Eur. J. Sport Sci. 2021, 8, 1–10.
2185. Songsorn, P.; Ruffino, J.; Vollaard, N.B. No effect of acute and chronic supramaximal exercise on circulating levels of the myokine SPARC. Eur. J. Sport Sci. 2017, 17, 447–452.
2186. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. Phys. Med. Rehabil. Clin. N. Am. 2017, 28, 659–669.
2187. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. Am. Fam. Physician. 2017, 95, 425–432.
2188. Baker, M.K.; Atlantis, E.; Singh, M.A.F. Multi-modal exercise programs for older adults. Age Ageing 2007, 36, 375–381.
2189. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Transdisciplinary Approach for Sarcopenia. Molecular mechanism of sarcopenia: The role of skeletal muscle niche component SPARC in the regulation of myogenesis and adipogenesis and its alteration with age. Clin Calcium 2014, 24, 1471–1478.
2190. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. J. Vet. Med. Sci. 2019, 81, 1649–1654.
2191. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. Aging Cell 2014, 13, 175–184.
2192. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Matsuwaki, T.; Nishihara, M. Age-related resistance of skeletal muscle-derived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. Aging (Albany NY) 2012, 4, 40–48.
2193. Song, H.; Ding, L.; Zhang, S.; Wang, W. MiR-29 family members interact with SPARC to regulate glucose metabolism. Biochem. Biophys. Res. Commun. 2018, 497, 667–674.
2194. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. Nat. Rev. Endocrinol. 2010, 6, 225–235.
2195. Pierrakos, C.; Vincent, J.L. Sepsis biomarkers: A review. Crit Care 2010, 14, R15.
2196. Capecchi, R.; Puxeddu, I.; Pratesi, F.; Migliorini, P. New biomarkers in SLE: From bench to bedside. Rheumatology (Oxford) 2020, 59, v12–v18.
2197. Kunc, P.; Fabry, J.; Lucanska, M.; Pecova, R. Biomarkers of Bronchial Asthma. Physiol. Res. 2020, 69, S29–S34.
2198. Aronson, J.K.; Ferner, R.E. Biomarkers-A General Review. Curr. Protoc. Pharmacol. 2017, 76, 9–23.
2199. Sandokji, I.; Greenberg, J.H. Novel biomarkers of acute kidney injury in children: An update on recent findings. Curr. Opin. Pediatr. 2020, 32, 354–359.
2200. Kawata, K.; Tierney, R.; Langford, D. Blood and cerebrospinal fluid biomarkers. Handb. Clin. Neurol. 2018, 158, 217–233.
2201. Avramouli, A.; Vlamos, P. Buccal Mucosa Biomarkers in Alzheimer's Disease. Adv. Exp. Med. Biol. 2020, 1195, 49–56.
2202. Zhou, L.T.; Lv, L.L.; Liu, B.C. Urinary Biomarkers of Renal Fibrosis. Adv. Exp. Med. Biol. 2019, 1165, 607–623.

2203. Shen, L.; Liu, X.; Zhang, H.; Lin, J.; Feng, C.; Iqbal, J. Biomarkers in autism spectrum disorders: Current progress. Clin. Chim. Acta. 2020, 502, 41–54.
2204. Steckl, A.J.; Ray, P. Stress Biomarkers in Biological Fluids and Their Point-of-Use Detection. ACS Sens. 2018, 3, 2025–2044.
2205. Gururajan, A.; Clarke, G.; Dinan, T.G.; Cryan, J.F. Molecular biomarkers of depression. Neurosci. Biobehav. Rev. 2016, 64, 101–133.
2206. Watt, F.E. Osteoarthritis biomarkers: Year in review. Osteoarthritis Cartilage 2018, 26, 312–318.
2207. Chen, X.H.; Huang, S.; Kerr, D. Biomarkers in clinical medicine. IARC Sci. Publ. 2011, 303–322.
2208. Jing, J.; Gao, Y. Urine biomarkers in the early stages of diseases: Current status and perspective. Discov. Med. 2018, 25, 57–65.
2209. Agah, E.; Saleh, F.; Sanjari, M.H.; Saghazadeh, A.; Tafakhori, A.; Rezaei, N. CSF and blood biomarkers in amyotrophic lateral sclerosis: Protocol for a systematic review and meta-analysis. Syst. Rev. 2018, 7, 237.
2210. Bhide, A.A.; Cartwright, R.; Khullar, V.; Digesu, G.A. Biomarkers in overactive bladder. Int. Urogynecol. J. 2013, 24, 1065–1072.
2211. Ahn, M.H.; Kim, H.A. Biomarkers for adult-onset still's disease: Up-to-date. Expert. Rev. Mol. Diagn. 2019, 19, 655–657.
2212. Zhao, X.; Modur, V.; Carayannopoulos, L.N.; Laterza, O.F. Biomarkers in Pharmaceutical Research. Clin. Chem. 2015, 61, 1343–1353.
2213. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. Biomolecules 2021, 11, 1689.
2214. Curigliano, G. Personalizing medicine through personalized communication: Individuality of the patient across borders and cultures. J. Natl. Compr. Canc. Netw. 2010, 8, 277–278.
2215. Ziegelstein, R.C. Perspectives in Primary Care: Knowing the Patient as a Person in the Precision Medicine Era. Ann. Fam. Med. 2018, 16, 4–5.
2216. Erden, A. Personalized Medicine: How Studying Individual Differences Can Shed Light on Treating Thousands of Patients: An Interview with Richard Lifton, PhD. Yale. J. Biol. Med. 2015, 88, 407–411.
2217. Abettan, C.; Welie, J.V.M. The impact of twenty-first century personalized medicine versus twenty-first century medicine's impact on personalization. Philos. Ethics Humanit Med. 2020, 15, 11.
2218. Boersma, G.J.; Benthem, L.; van Beek, A.P.; van Dijk, G.; Scheurink, A.J. Personality, a key factor in personalized medicine? Eur. J. Pharmacol. 2011, 667, 23–25.
2219. Khatri, V.P.; Petrelli, N.J. Precision Medicine. Surg. Oncol. Clin. N. Am. 2020, 29, xv–xvi.
2220. Ramaswami, R.; Bayer, R.; Galea, S. Precision Medicine from a Public Health Perspective. Annu. Rev. Public Health 2018, 39, 153–168.
2221. Beckmann, J.S.; Lew, D. Reconciling evidence-based medicine and precision medicine in the era of big data: Challenges and opportunities. Genome Med. 2016, 8, 134.
2222. Antman, E.M.; Loscalzo, J. Precision medicine in cardiology. Nat. Rev. Cardiol. 2016, 13, 591–602.
2223. Weil, A.R. Precision Medicine. Health Aff. (Millwood) 2018, 37, 687.
2224. Xie, F.; Chan, J.C.; Ma, R.C. Precision medicine in diabetes prevention, classification and management. J. Diabetes Investig. 2018, 9, 998–1015.
2225. Forrest, S.J.; Geoerger, B.; Janeway, K.A. Precision medicine in pediatric oncology. Curr. Opin. Pediatr. 2018, 30, 17–24.
2226. König, I.R.; Fuchs, O.; Hansen, G.; von Mutius, E.; Kopp, M.V. What is precision medicine? Eur. Respir. J. 2017, 50.
2227. Yardley, J.E.; Campbell, M.D. Moving Toward Precision Medicine with Diabetes, Exercise and Physical Activity. Can. J. Diabetes 2020, 44, 679.
2228. Bonafiglia, J.T.; Ramic, L.; Islam, H. Challenges to personalized exercise medicine: Lack of repeatability and influence of genetics. J. Physiol. 2020, 598, 5021–5023.
2229. Buford TW, Roberts MD, Church TS: Toward exercise as personalized medicine. Sports Med. 2013, 43, 157–165.
2230. Garner, S.; Fenton, T.; Martin, L.; Creaser, C.; Johns, C.; Barnabe, C. Personalized diet and exercise recommendations in early rheumatoid arthritis: A feasibility trial. Musculoskeletal Care 2018, 16, 167–172.
2231. Kostek, M. Precision Medicine and Exercise Therapy in Duchenne Muscular Dystrophy. Sports (Basel) 2019, 7, 64.

2232. Ross, R.; Goodpaster, B.H.; Koch, L.G.; Sarzynski, M.A.; Kohrt, W.M.; Johannsen, N.M.; Skinner, J.S.; Castro, A.; Irving, B.A.; Noland, R.C.; et al. Precision exercise medicine: Understanding exercise response variability. Br. J. Sports Med. 2019, 53, 1141–1153.
2233. Wong, H.J.; Morra, D. Excellent hospital care for all: Open and operating 24/7. J. Gen. Intern. Med. 2011, 26, 1050–1052.
2234. Lopez, M.A.; Hall, M.; Auger, K.A.; Bettenhausen, J.L.; Colvin, J.D.; Cutler, G.J.; Fieldston, E.; Macy, M.L.; Morse, R.; Raphael, J.L.; et al. Care of Pediatric High-Cost Hospitalizations Across Hospital Types. Hosp. Pediatr. 2020, 10, 206–213.
2235. Copnell, B.; Hagger, V.; Wilson, S.G.; Evans, S.M.; Sprivulis, P.C.; Cameron, P.A. Measuring the quality of hospital care: An inventory of indicators. Intern. Med. J. 2009, 39, 352–360.
2236. Buttigieg, S.C.; Abela, L.; Pace, A. Variables affecting hospital length of stay: A scoping review. J. Health Organ. Manag. 2018, 32, 463–493.
2237. Hua, M.; Lu, Y.; Ma, X.; Morrison, R.S.; Li, G.; Wunsch, H. Association Between the Implementation of Hospital-Based Palliative Care and Use of Intensive Care During Terminal Hospitalizations. JAMA Netw. Open 2020, 3, e1918675.
2238. Liu, J.Y.; Dickter, J.K. Nosocomial Infections: A History of Hospital-Acquired Infections. Gastrointest. Endosc. N. Am. 2020, 30, 637–652.
2239. Alwasel, A.; Alossimi, B.; Alsadun, M.; Alhussaini, K. Bedsores Management: Efficiency Simulation of a New Mattress Design. Healthcare 2021, 9, 1701.
2240. Bluestein, D.; Javaheri, A. Pressure ulcers: Prevention, evaluation, and management. Am. Fam. Physician 2008, 78, 1186–1194.
2241. Buso, A.; Comelli, M.; Picco, R.; Isola, M.; Magnesa, B.; Pišot, R.; Rittweger, J.; Salvadego, D.; Šimuniˇc, B.; Grassi, B.; et al. Mitochondrial Adaptations in Elderly and Young Men Skeletal Muscle Following 2 Weeks of Bed Rest and Rehabilitation. Front. Physiol. 2019, 10, 474.
2242. LeBlanc, A.; Gogia, P.; Schneider, V.; Krebs, J.; Schonfeld, E.; Evans, H. Calf muscle area and strength changes after five weeks of horizontal bed rest. Am. J. Sports Med. 1988, 16, 624–629.
2243. Mahmassani, Z.S.; Reidy, P.T.; McKenzie, A.I.; Stubben, C.; Howard, M.T.; Drummond, M.J. Agedependent skeletal muscle transcriptome response to bed rest-induced atrophy. J. Appl. Physiol. 2019, 126, 894–902.
2244. Dirks, M.L.; Smeets, J.S.J.; Holwerda, A.M.; Kouw, I.W.K.; Marzuca-Nassr, G.N.; Gijsen, A.P.; Holloway, G.P.; Verdijk, L.B.; van Loon, L.J.C. Dietary feeding pattern does not modulate the loss of muscle mass or the decline in metabolic health during short-term bed rest. Am. J. Physiol. Endocrinol. Metab. 2019, 316, E536–E545.
2245. Bloomfield, S.A. Changes in musculoskeletal structure and function with prolonged bed rest. Med. Sci. Sports Exerc. 1997, 29, 197–206.
2246. Perhonen, M.A.; Franco, F.; Lane, L.D.; Buckey, J.C.; Blomqvist, C.G.; Zerwekh, J.E.; Peshock, R.M.; Weatherall, P.T.; Levine, B.D. Cardiac atrophy after bed rest and spaceflight. J. Appl. Physiol. 2001, 91, 645– 653.
2247. Dittmer, D.K.; Teasell, R. Complications of immobilization and bed rest. Part 1: Musculoskeletal and cardiovascular complications. Can. Fam. Physician 1993, 39, 1428–1432, 1435–1437.
2248. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Secreted protein acidic and rich in cysteine internalization and its age-related alterations in skeletal muscle progenitor cells. Aging Cell 2014, 13, 175–184.
2249. Sousa-Victor, P.; Muñoz-Cánoves, P. Regenerative decline of stem cells in sarcopenia. Mol. Asp. Med. 2016, 50, 109–117.
2250. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
2251. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res. Rev. Mutat. Res. 2019, 781, 175–185.
2252. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. Diseases 2021, 9, 87.
2253. Rosa-Caldwell, M.E.; Greene, N.P. Muscle metabolism and atrophy: Let's talk about sex. Biol. Sex Differ. 2019, 10, 43.
2254. Nakamura, K.; Yamanouchi, K.; Nishihara, M. Transdisciplinary Approach for Sarcopenia. Molecular mechanism of sarcopenia: The role of skeletal muscle niche component SPARC in the

regulation of myogenesis and adipogenesis and its alteration with age. Clin. Calcium. 2014, 24, 1471–1478.
2255. Li, H.; Hastings, M.H.; Rhee, J.; Trager, L.E.; Roh, J.D.; Rosenzweig, A. Targeting Age-Related Pathways in Heart Failure. Circ. Res. 2020, 126, 533–551.
2256. Easter, M.; Bollenbecker, S.; Barnes, J.W.; Krick, S. Targeting Aging Pathways in Chronic Obstructive Pulmonary Disease. Int. J. Mol. Sci. 2020, 21, 6924.
2257. Qaisar, R.; Karim, A.; Muhammad, T.; Shah, I.; Khan, J. Prediction of sarcopenia using a battery of circulating biomarkers. Sci. Rep. 2021, 11, 8632.
2258. Baek, K.W.; Jung, Y.K.; Kim, J.S.; Park, J.S.; Hah, Y.S.; Kim, S.J.; Yoo, J.I. Rodent Model of Muscular Atrophy for Sarcopenia Study. J. Bone Metab. 2020, 27, 97–110.
2259. Bonaldo, P.; Sandri, M. Cellular and molecular mechanisms of muscle atrophy. Dis. Mech. 2013, 6, 25– 39.
2260. Ji, L.L.; Yeo, D. Mitochondrial dysregulation and muscle disuse atrophy. F1000Res 2019, 8, F1000.
2261. Tuttle, C.S.L.; Thang, L.A.N.; Maier, A.B. Markers of inflammation and their association with muscle strength and mass: A systematic review and meta-analysis. Ageing Res. Rev. 2020, 64, 101185.
2262. Kwak, J.Y.; Hwang, H.; Kim, S.K.; Choi, J.Y.; Lee, S.M.; Bang, H.; Kwon, E.S.; Lee, K.P.; Chung, S.G.; Kwon, K.S. Prediction of sarcopenia using a combination of multiple serum biomarkers. Sci. Rep. 2018, 8, 8574.
2263. Miokovic, T.; Armbrecht, G.; Gast, U.; Rawer, R.; Roth, H.J.; Runge, M.; Felsenberg, D.; Belavý, D.L. Muscle atrophy, pain, and damage in bed rest reduced by resistive (vibration) exercise. Med. Sci. Sports Exerc. 2014, 46, 1506–1516.
2264. Leblanc, A.D.; Schneider, V.S.; Evans, H.J.; Engelbretson, D.A.; Krebs, J.M. Bone mineral loss and recovery after 17 weeks of bed rest. J. Bone Min. Res. 1990, 5, 843–850.
2265. Landi, F.; Marzetti, E.; Martone, A.M.; Bernabei, R.; Onder, G. Exercise as a remedy for sarcopenia. Curr. Opin. Clin. Nutr. Metab. Care 2014, 17, 25–31.
2266. Spaulding, H.R.; Selsby, J.T. Is Exercise the Right Medicine for Dystrophic Muscle? Med. Sci. Sports Exerc. 2018, 50, 1723–1732.
2267. Phu, S.; Boersma, D.; Duque, G. Exercise and Sarcopenia. J. Clin. Densitom. 2015, 18, 488–492.
2268. Marzetti, E.; Calvani, R.; Tosato, M.; Cesari, M.; Di Bari, M.; Cherubini, A.; Broccatelli, M.; Savera, G.; D'Elia, M.; Pahor, M.; et al. Physical activity and exercise as countermeasures to physical frailty and sarcopenia. Aging Clin. Exp. Res. 2017, 29, 35–42.
2269. He, N.; Ye, H. Exercise and Muscle Atrophy. Adv. Exp. Med. Biol. 2020, 1228, 255–267.
2270. Theilen, N.T.; Kunkel, G.H.; Tyagi, S.C. The Role of Exercise and TFAM in Preventing Skeletal Muscle Atrophy. J. Cell Physiol. 2017, 232, 2348–2358.
2271. Salles, J.I.; Guimarães, J.M.; Filho, G.M.; Morrissey, D. Effect of a specific exercise strategy on strength and proprioception in volleyball players with infraspinatus muscle atrophy. Scand. J. Med. Sci. Sports 2018, 28, 2093–2099.
2272. Czerwinski, S.M.; Kurowski, T.G.; O'Neill, T.M.; Hickson, R.C. Initiating regular exercise protects against muscle atrophy from glucocorticoids. J. Appl. Physiol. 1987, 63, 1504–1510.
2273. Baldi, J.C.; Jackson, R.D.; Moraille, R.; Mysiw, W.J. Muscle atrophy is prevented in patients with acute spinal cord injury using functional electrical stimulation. Spinal Cord 1998, 36, 463–469.
2274. Lake, D.A. Neuromuscular electrical stimulation. An overview and its application in the treatment of sports injuries. Sports Med. 1992, 13, 320–336.
2275. Granic, A.; Sayer, A.A.; Robinson, S.M. Dietary Patterns, Skeletal Muscle Health, and Sarcopenia in Older Adults. Nutrients 2019, 11, 745.
2276. Anton, S.D.; Hida, A.; Mankowski, R.; Layne, A.; Solberg, L.M.; Mainous, A.G.; Buford, T. Nutrition and Exercise in Sarcopenia. Curr. Protein. Pept. Sci. 2018, 19, 649–667.
2277. Urso, M.L. Disuse atrophy of human skeletal muscle: Cell signaling and potential interventions. Med. Sci. Sports Exerc. 2009, 41, 1860–1868.
2278. Guasconi, V.; Puri, P.L. Epigenetic drugs in the treatment of skeletal muscle atrophy. Curr. Opin. Clin. Nutr. Metab. Care 2008, 11, 233–241.
2279. Ding, S.; Dai, Q.; Huang, H.; Xu, Y.; Zhong, C. An Overview of Muscle Atrophy. Adv. Exp. Med. Biol. 2018, 1088, 3–19.
2280. Son, J.S.; Kim, J.H.; Kim, H.J.; Yoon, D.H.; Kim, J.S.; Song, H.S.; Song, W. Effect of resistance ladder training on sparc expression in skeletal muscle of hindlimb immobilized rats. Muscle Nerve 2016, 53, 951– 957.

2281. Caron, A.Z.; Drouin, G.; Desrosiers, J.; Trensz, F.; Grenier, G. A novel hindlimb immobilization procedure for studying skeletal muscle atrophy and recovery in mouse. J. Appl. Physiol. 2009, 106, 2049– 2059.
2282. Herbert, R.D.; Balnave, R.J. The effect of position of immobilisation on resting length, resting stiffness, and weight of the soleus muscle of the rabbit. J. Orthop. Res. 1993, 11, 358–366.
2283. Yuan, R.; Peters, L.L.; Paigen, B. Mice as a mammalian model for research on the genetics of aging. Ilar. J. 2011, 52, 4–15.
2284. Yuan, R.; Tsaih, S.W.; Petkova, S.B.; Marin de Evsikova, C.; Xing, S.; Marion, M.A.; Bogue, M.A.; Mills, K.D.; Peters, L.L.; Bult, C.J.; et al. Aging in inbred strains of mice: Study design and interim report on median lifespans and circulating IGF1 levels. Aging Cell 2009, 8, 277–287.
2285. Barreto, G.; Huang, T.T.; Giffard, R.G. Age-related defects in sensorimotor activity, spatial learning, and memory in C57BL/6 mice. J. Neurosurg. Anesth. 2010, 22, 214–219.
2286. Graber, T.G.; Ferguson-Stegall, L.; Kim, J.H.; Thompson, L.V. C57BL/6 neuromuscular healthspan scoring system. J. Gerontol. A Biol. Sci. Med. Sci. 2013, 68, 1326–1336.
2287. Parks, R.J.; Fares, E.; Macdonald, J.K.; Ernst, M.C.; Sinal, C.J.; Rockwood, K.; Howlett, S.E. A procedure for creating a frailty index based on deficit accumulation in aging mice. J. Gerontol. A Biol. Sci. Med. Sci. 2012, 67, 217–227.
2288. Palus, S.; Springer, J.I.; Doehner, W.; von Haehling, S.; Anker, M.; Anker, S.D.; Springer, J. Models of sarcopenia: Short review. Int. J. Cardiol. 2017, 238, 19–21.
2289. St-Amand, J.; Okamura, K.; Matsumoto, K.; Shimizu, S.; Sogawa, Y. Characterization of control and immobilized skeletal muscle: An overview from genetic engineering. Faseb J. 2001, 15, 684–692.
2290. Friedman, M.A.; Zhang, Y.; Wayne, J.S.; Farber, C.R.; Donahue, H.J. Single limb immobilization model for bone loss from unloading. J. Biomech. 2019, 83, 181–189.
2291. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Nishihara, M. Loss of SPARC in mouse skeletal muscle causes myofiber atrophy. Muscle Nerve 2013, 48, 791–799.
2292. Krawiec, B.J.; Frost, R.A.; Vary, T.C.; Jefferson, L.S.; Lang, C.H. Hindlimb casting decreases muscle mass in part by proteasomedependent proteolysis but independent of protein synthesis. Am. J. Physiol. Endocrinol. Metab. 2005, 289, E969–E980.
2293. Kelleher, A.R.; Pereira, S.L.; Jefferson, L.S.; Kimball, S.R. REDD2 expression in rat skeletal muscle correlates with nutrient-induced activation of mTORC1: Responses to aging, immobilization, and remobilization. Am. J. Physiol. Endocrinol. Metab. 2015, 308, E122–E129.
2294. Yoshihara, T.; Natsume, T.; Tsuzuki, T.; Chang, S.W.; Kakigi, R.; Sugiura, T.; Naito, H. Sex differences in forkhead box O3a signaling response to hindlimb unloading in rat soleus muscle. J. Physiol. Sci. 2019, 69, 235–244.
2295. Rosset, E.M.; Bradshaw, A.D. SPARC/osteonectin in mineralized tissue. Matrix Biol. 2016, 52–54, 78– 87.
2296. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. Biomolecules 2021, 11, 1689.
2297. Yan, Q.; Sage, E.H. SPARC, a matricellular glycoprotein with important biological functions. J. Histochem. Cytochem. 1999, 47, 1495–1506.
2298. Kao, S.C.; Kirschner, M.B.; Cooper, W.A.; Tran, T.; Burgers, S.; Wright, C.; Korse, T.; van den Broek, D.; Edelman, J.; Vallely, M.; et al. A proteomics-based approach identifies secreted protein acidic and rich in cysteine as a prognostic biomarker in malignant pleural mesothelioma. Br. J. Cancer 2016, 114, 524–531.
2299. Delany, A.M.; Kalajzic, I.; Bradshaw, A.D.; Sage, E.H.; Canalis, E. Osteonectin-null mutation compromises osteoblast formation, maturation, and survival. Endocrinology 2003, 144, 2588–2596.
2300. Sage, H.; Johnson, C.; Bornstein, P. Characterization of a novel serum albumin-binding glycoprotein secreted by endothelial cells in culture. J. Biol. Chem. 1984, 259, 3993–4007.
2301. Scavelli, K.; Chatterjee, A.; Rhee, D.J. Secreted Protein Acidic and Rich in Cysteine in Ocular Tissue. J. Ocul. Pharm. 2015, 31, 396–405.
2302. Nakamura, S.; Kamihagi, K.; Satakeda, H.; Katayama, M.; Pan, H.; Okamoto, H.; Noshiro, M.; Takahashi, K.; Yoshihara, Y.; Shimmei, M.; et al. Enhancement of SPARC (osteonectin) synthesis in arthritic cartilage. Increased levels in synovial fluids from patients with rheumatoid arthritis and regulation by growth factors and cytokines in chondrocyte cultures. Arthritis Rheum. 1996, 39, 539–551.

2303. Chandrasekhar, S.; Harvey, A.K.; Johnson, M.G.; Becker, G.W. Osteonectin/SPARC is a product of articular chondrocytes/cartilage and is regulated by cytokines and growth factors. Biochim. Biophys. Acta 1994, 1221, 7–14.
2304. Fujita, T.; Shiba, H.; Van Dyke, T.E.; Kurihara, H. Differential effects of growth factors and cytokines on the synthesis of SPARC DNA fibronectin and alkaline phosphatase activity in human periodontal ligament cells. Cell Biol. Int. 2004, 28, 281–286.
2305. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
2306. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.
2307. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
2308. Petersson, S.J.; Jørgensen, L.H.; Andersen, D.C.; Nørgaard, R.C.; Jensen, C.H.; Schrøder, H.D. SPARC is up-regulated during skeletal muscle regeneration and inhibits myoblast differentiation. Histol. Histopathol. 2013, 28, 1451–1460.
2309. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Leads to an Accelerated Ageing Phenotype Which Is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice. Metabolites 2022, 12, 125.
2310. Jørgensen, L.H.; Petersson, S.J.; Sellathurai, J.; Andersen, D.C.; Thayssen, S.; Sant, D.J.; Jensen, C.H.; Schrøder, H.D. Secreted protein acidic and rich in cysteine (SPARC) in human skeletal muscle. J. Histochem. Cytochem. 2009, 57, 29–39.
2311. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
2312. Omi, S.; Yamanouchi, K.; Nakamura, K.; Matsuwaki, T.; Nishihara, M. Reduced fibrillar collagen accumulation in skeletal muscle of secreted protein acidic and rich in cysteine (SPARC)-null mice. J. Vet. Med. Sci. 2019, 81, 1649–1654.
2313. Cho, W.J.; Kim, E.J.; Lee, S.J.; Kim, H.D.; Shin, H.J.; Lim, W.K. Involvement of SPARC in in vitro differentiation of skeletal myoblasts. Biochem. Biophys. Res. Commun. 2000, 271, 630–634.
2314. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
2315. Balachandran, A.; Krawczyk, S.N.; Potiaumpai, M.; Signorile, J.F. High-speed circuit training vs hypertrophy training to improve physical function in sarcopenic obese adults: A randomized controlled trial. Exp. Gerontol. 2014, 60, 64–71.
2316. Nakamura, K.; Nakano, S.; Miyoshi, T.; Yamanouchi, K.; Matsuwaki, T.; Nishihara, M. Age-related resistance of skeletal musclederived progenitor cells to SPARC may explain a shift from myogenesis to adipogenesis. Aging 2012, 4, 40–48.
2317. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
2318. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
2319. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
2320. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
2321. Harris, B.S.; Zhang, Y.; Card, L.; Rivera, L.B.; Brekken, R.A.; Bradshaw, A.D. SPARC regulates collagen interaction with cardiac fibroblast cell surfaces. Am. J. Physiol. Heart Circ. Physiol. 2011, 301, H841–H847.
2322. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
2323. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Exercise, Diet and Sleeping as Regenerative Medicine Adjuvants: Obesity and Ageing as Illustrations. Medicines 2022, 9, 7.

2324. Mansergh, F.C.; Wells, T.; Elford, C.; Evans, S.L.; Perry, M.J.; Evans, M.J.; Evans, B.A. Osteopenia in Sparc (osteonectin)-deficient mice: Characterization of phenotypic determinants of femoral strength and changes in gene expression. Physiol. Genom. 2007, 32, 64–73.
2325. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectindeficient mice. J. Clin. Investig. 2000, 105, 915–923.
2326. Luo, Z.; Zhou, Y.; Luo, P.; Zhao, Q.; Xiao, N.; Yu, Y.; Yan, Q.; Lu, G.; Cheng, L. SPARC deficiency affects bone marrow stromal function, resulting in impaired B lymphopoiesis. J. Leukoc. Biol. 2014, 96, 73– 82.
2327. Ribeiro, N.; Sousa, S.R.; Brekken, R.A.; Monteiro, F.J. Role of SPARC in bone remodeling and cancer-related bone metastasis. J. Cell Biochem. 2014, 115, 17–26.
2328. Sodek, J.; Zhu, B.; Huynh, M.H.; Brown, T.J.; Ringuette, M. Novel functions of the matricellular proteins osteopontin and osteonectin/SPARC. Connect. Tissue Res. 2002, 43, 308–319.
2329. Rowe, D.W. Chapter 61—Osteogenesis imperfecta. In Principles of Bone Biology, 4th ed.; Bilezikian, J.P., Martin, T.J., Clemens, T.L., Rosen, C.J., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 1489– 1505.
2330. Trombetta, J.M.; Bradshaw, A.D. SPARC/osteonectin functions to maintain homeostasis of the collagenous extracellular matrix in the periodontal ligament. J. Histochem. Cytochem. 2010, 58, 871–879.
2331. Rosset, E.M.; Trombetta-eSilva, J.; Hepfer, G.; Yao, H.; Bradshaw, A.D. SPARC and the N-propeptide of collagen I influence fibroblast proliferation and collagen assembly in the periodontal ligament. PLoS ONE 2017, 12, e0173209.
2332. Gehwolf, R.; Wagner, A.; Lehner, C.; Bradshaw, A.D.; Scharler, C.; Niestrawska, J.A.; Holzapfel, G.A.; Bauer, H.C.; Tempfer, H.; Traweger, A. Pleiotropic roles of the matricellular protein Sparc in tendon maturation and ageing. Sci. Rep. 2016, 6, 32635.
2333. Wang, T.; Wagner, A.; Gehwolf, R.; Yan, W.; Passini, F.S.; Thien, C.; Weissenbacher, N.; Lin, Z.; Lehner, C.; Teng, H.; et al. Load-induced regulation of tendon homeostasis by SPARC, a genetic predisposition factor for tendon and ligament injuries. Sci. Transl. Med. 2021, 13, eabe5738.
2334. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. Nat. Rev. Endocrinol. 2010, 6, 225–235.
2335. Chang, C.-H.; Yen, M.-C.; Liao, S.-H.; Hsu, Y.-L.; Lai, C.-S.; Chang, K.-P.; Hsu, Y.-L. Secreted Protein Acidic and Rich in Cysteine (SPARC) Enhances Cell Proliferation, Migration, and Epithelial Mesenchymal Transition, and SPARC Expression is Associated with Tumor Grade in Head and Neck Cancer. Int. J. Mol. Sci. 2017, 18, 1556.
2336. Takahashi, M.; Nagaretani, H.; Funahashi, T.; Nishizawa, H.; Maeda, N.; Kishida, K.; Kuriyama, H.; Shimomura, I.; Maeda, K.; Hotta, K.; et al. The expression of SPARC in adipose tissue and its increased plasma concentration in patients with coronary artery disease. Obes. Res. 2001, 9, 388–393.
2337. Tan, X.; Li, T.; Zhu, S.; Zhong, W.; Li, F.; Wang, Y. Induction of SPARC on Oxidative Stress, Inflammatory Phenotype Transformation, and Apoptosis of Human Brain Smooth Muscle Cells Via TGF-β1- NOX4 Pathway. J. Mol. Neurosci. 2020, 70, 1728–1741.
2338. Ma, J.; Gao, S.; Xie, X.; Sun, E.; Zhang, M.; Zhou, Q.; Lu, C. SPARC inhibits breast cancer bone metastasis and may be a clinical therapeutic target. Oncol. Lett. 2017, 14, 5876–5882.
2339. Droppert, P.M. A review of muscle atrophy in microgravity and during prolonged bed rest. J. Br. Interplanet. Soc. 1993, 46, 83–86.
2340. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. Genes 2021, 12, 1832.
2341. Ghanemi A, Yoshioka M, St-Amand J: Genetic Expression between Ageing and Exercise: Secreted Protein Acidic and Rich in Cysteine as a Potential "Exercise Substitute" Antiageing Therapy. Genes 2022, 13, 950.
2342. López-Otín, C.; Blasco, M.A.; Partridge, L.; Serrano, M.; Kroemer, G. The hallmarks of aging. Cell 2013, 153, 1194–1217.
2343. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Ageing and Obesity Shared Patterns: From Molecular Pathogenesis to Epigenetics. Diseases 2021, 9, 87.
2344. Covarrubias, A.J.; Kale, A.; Perrone, R.; Lopez-Dominguez, J.A.; Pisco, A.O.; Kasler, H.G.; Schmidt, M.S.; Heckenbach, I.; Kwok, R.; Wiley, C.D.; et al. Senescent cells promote tissue NAD (+) decline during ageing via the activation of CD38(+) macrophages. Nat. Metab. 2020, 2, 1265–1283.

2345. Wilkinson, D.J.; Piasecki, M.; Atherton, P.J. The age-related loss of skeletal muscle mass and function: Measurement and physiology of muscle fibre atrophy and muscle fibre loss in humans. Ageing Res. Rev. 2018, 47, 123–132.
2346. Liu, Z.; Wu, K.K.L.; Jiang, X.; Xu, A.; Cheng, K.K.Y. The role of adipose tissue senescence in obesityand ageing-related metabolic disorders. Clin. Sci. 2020, 1343, 315–330.
2347. Spinelli, R.; Parrillo, L.; Longo, M.; Florese, P.; Desiderio, A.; Zatterale, F.; Miele, C.; Raciti, G.A.; Beguinot, F. Molecular basis of ageing in chronic metabolic diseases. J. Endocrinol. Investig. 2020, 43, 1373– 1389.
2348. Konar, A.; Singh, P.; Thakur, M.K. Age-associated Cognitive Decline: Insights into Molecular Switches and Recovery Avenues. Aging Dis. 2016, 7, 121–129.
2349. Pawelec, G. Immunity and ageing in man. Exp. Gerontol. 2006, 41, 1239–1242.
2350. Hopkins, E.W. The Fountain of Youth. J. Am. Orient. Soc. 1905, 26, 1–67.
2351. Sims, J. Our ageing society: A view from the streets. Australas. J. Ageing 2018, 37, 168.
2352. Galloza, J.; Castillo, B.; Micheo, W. Benefits of Exercise in the Older Population. Phys. Med. Rehabil. Clin. N. Am. 2017, 28, 659–669.
2353. Mora, J.C.; Valencia, W.M. Exercise and Older Adults. Clin. Geriatr. Med. 2018, 34, 145–162.
2354. López-Lluch, G.; Navas, P. Calorie restriction as an intervention in ageing. J. Physiol. 2016, 594, 2043– 2060.
2355. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Impact of Adiposity and Fat Distribution, Rather Than Obesity, on Antibodies as an Illustration of Weight-Loss-Independent Exercise Benefits. Medicines 2021, 8, 57.
2356. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Exercise, Diet and Sleeping as Regenerative Medicine Adjuvants: Obesity and Ageing as Illustrations. Medicines 2022, 9, 7.
2357. Lee, P.G.; Jackson, E.A.; Richardson, C.R. Exercise Prescriptions in Older Adults. Am. Fam. Physician 2017, 95, 425–432.
2358. Han, P.; Zhang, W.; Kang, L.; Ma, Y.; Fu, L.; Jia, L.; Yu, H.; Chen, X.; Hou, L.; Wang, L.; et al. Clinical Evidence of Exercise Benefits for Stroke. Adv. Exp. Med. Biol. 2017, 1000, 131–151.
2359. Halabchi, F.; Alizadeh, Z.; Sahraian, M.A.; Abolhasani, M. Exercise prescription for patients with multiple sclerosis; potential benefits and practical recommendations. BMC Neurol. 2017, 17, 185.
2360. Rosenfeldt, A.B.; Linder, S.M.; Davidson, S.; Clark, C.; Zimmerman, N.M.; Lee, J.J.; Alberts, J.L. Combined Aerobic Exercise and Task Practice Improve Health-Related Quality of Life Poststroke: A Preliminary Analysis. Arch. Phys. Med. Rehabil. 2019, 100, 923–930.
2361. Garneau, L.; Parsons, S.A.; Smith, S.R.; Mulvihill, E.E.; Sparks, L.M.; Aguer, C. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. Front. Physiol. 2020, 11, 18.
2362. Aoi, W.; Naito, Y.; Takagi, T.; Tanimura, Y.; Takanami, Y.; Kawai, Y.; Sakuma, K.; Hang, L.P.; Mizushima, K.; Hirai, Y.; et al. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2013, 62, 882–889.
2363. Melouane, A.; Yoshioka, M.; Kanzaki, M.; St-Amand, J. Sparc, an EPS-induced gene, modulates the extracellular matrix and mitochondrial function via ILK/AMPK pathways in C2C12 cells. Life Sci. 2019, 229, 277–287.
2364. Distefano, G.; Goodpaster, B.H. Effects of Exercise and Aging on Skeletal Muscle. Cold Spring Harb. Perspect. Med. 2018, 8, a029785.
2365. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Leads to an Accelerated Ageing Phenotype Which Is Improved by Exercise Whereas SPARC Overexpression Mimics Exercise Effects in Mice. Metabolites 2022, 12, 125.
2366. Melouane, A.; Carbonell, A.; Yoshioka, M.; Puymirat, J.; St-Amand, J. Implication of SPARC in the modulation of the extracellular matrix and mitochondrial function in muscle cells. PLoS ONE 2018, 13, e0192714.
2367. Deane, C.S.; Ames, R.M.; Phillips, B.E.; Weedon, M.N.; Willis, C.R.G.; Boereboom, C.; Abdulla, H.; Bukhari, S.S.I.; Lund, J.N.; Williams, J.P.; et al. The acute transcriptional response to resistance exercise: Impact of age and contraction mode. Aging 2019, 11, 2111–2126.
2368. Pillon, N.J.; Gabriel, B.M.; Dollet, L.; Smith, J.A.B.; Sardón Puig, L.; Botella, J.; Bishop, D.J.; Krook, A.; Zierath, J.R. Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. Nat. Commun. 2020, 11, 470.

2369. Riedl, I.; Yoshioka, M.; Nishida, Y.; Tobina, T.; Paradis, R.; Shono, N.; Tanaka, H.; St-Amand, J. Regulation of skeletal muscle transcriptome in elderly men after 6 weeks of endurance training at lactate threshold intensity. Exp. Gerontol. 2010, 45, 896–903.
2370. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as a Molecular Physiological and Pathological Biomarker. Biomolecules 2021, 11, 1689.
2371. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Measuring Exercise-Induced Secreted Protein Acidic and Rich in Cysteine Expression as a Molecular Tool to Optimize Personalized Medicine. Genes 2021, 12, 1832.
2372. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise Training of Secreted Protein Acidic and Rich in Cysteine (Sparc) KO Mice Suggests That Exercise-Induced Muscle Phenotype Changes Are SPARC-Dependent. Appl. Sci. 2020, 10, 9108.
2373. Gruber, H.E.; Sage, E.H.; Norton, H.J.; Funk, S.; Ingram, J.; Hanley, E.N., Jr. Targeted deletion of the SPARC gene accelerates disc degeneration in the aging mouse. J. Histochem. Cytochem. 2005, 53, 1131– 1138.
2374. Whittal, M.C.; Molladavoodi, S.; Zwambag, D.P.; Millecamps, M.; Stone, L.S.; Gregory, D.E. Mechanical Consequence of Induced Intervertebral Disc Degeneration in the SPARC-Null Mouse. J. Biomech. Eng. 2021, 143, 024501.
2375. Mansergh, F.C.; Wells, T.; Elford, C.; Evans, S.L.; Perry, M.J.; Evans, M.J.; Evans, B.A. Osteopenia in Sparc (osteonectin)-deficient mice: Characterization of phenotypic determinants of femoral strength and changes in gene expression. Physiol. Genom. 2007, 32, 64–73.
2376. Norose, K.; Clark, J.I.; Syed, N.A.; Basu, A.; Heber-Katz, E.; Sage, E.H.; Howe, C.C. SPARC deficiency leads to early-onset cataractogenesis. Investig. Ophthalmol. Vis. Sci. 1998, 39, 2674–2680.
2377. Mansergh, F.C.; Wride, M.A.; Walker, V.E.; Adams, S.; Hunter, S.M.; Evans, M.J. Gene expression changes during cataract progression in Sparc null mice: Differential regulation of mouse globins in the lens. Mol. Vis. 2004, 10, 490–511.
2378. Luo, Z.; Zhou, Y.; Luo, P.; Zhao, Q.; Xiao, N.; Yu, Y.; Yan, Q.; Lu, G.; Cheng, L. SPARC deficiency affects bone marrow stromal function, resulting in impaired B lymphopoiesis. J. Leukoc. Biol. 2014, 96, 73– 82.
2379. Delany, A.M.; Amling, M.; Priemel, M.; Howe, C.; Baron, R.; Canalis, E. Osteopenia and decreased bone formation in osteonectindeficient mice. J. Clin. Investig. 2000, 105, 915–923.
2380. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and inflammation: Another homeostatic property? Cytokine 2020, 133, 155179.
2381. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and cancer: A homeostatic hormone? Cytokine 2020, 127, 154996.
2382. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine as A Regeneration Factor: Beyond the Tissue Repair. Life 2021, 11, 38.
2383. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Secreted Protein Acidic and Rich in Cysteine: Metabolic and Homeostatic Properties beyond the Extracellular Matrix Structure. Appl. Sci. 2020, 10, 2388.
2384. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Secreted protein acidic and rich in cysteine and bioenergetics: Extracellular matrix, adipocytes remodeling and skeletal muscle metabolism. Int. J. Biochem. Cell Biol. 2019, 117, 105627.
2385. Kos, K.; Wilding, J.P. SPARC: A key player in the pathologies associated with obesity and diabetes. Nat. Rev. Endocrinol. 2010, 6, 225–235.
2386. Mucunguzi, O.; Melouane, A.; Ghanemi, A.; Yoshioka, M.; Boivin, A.; Calvo, E.L.; St-Amand, J. Identification of the principal transcriptional regulators for low-fat and high-fat meal responsive genes in small intestine. Nutr. Metab. 2017, 14, 66.
2387. Ghanemi, A.; Yoshioka, M.; St-Amand, J. Trefoil Factor Family Member 2 Expression as an Indicator of the Severity of the High-Fat Diet-Induced Obesity. Genes 2021, 12, 1505.
2388. Melouane, A.; Ghanemi, A.; Aubé, S.; Yoshioka, M.; St-Amand, J. Differential gene expression analysis in ageing muscle and drug discovery perspectives. Ageing Res. Rev. 2018, 41, 53–63.
2389. Melouane, A.; Ghanemi, A.; Yoshioka, M.; St-Amand, J. Functional genomics applications and therapeutic implications in sarcopenia. Mutat. Res./Rev. Mutat. Res. 2019, 781, 175–185.
2390. Ghanemi, A.; Melouane, A.; Yoshioka, M.; St-Amand, J. Exercise and High-Fat Diet in Obesity: Functional Genomics Perspectives of Two Energy Homeostasis Pillars. Genes 2020, 11, 875.

2391. Ge H, Gardner J, Wu X, Rulifson I, Wang J, Xiong Y, Ye J, Belouski E, Cao P, Tang J, et al: Trefoil Factor 3 (TFF3) Is Regulated by Food Intake, Improves Glucose Tolerance and Induces Mucinous Metaplasia. PLoS One 2015, 10: e0126924.
2392. Workman G, Bradshaw AD: Production and purification of recombinant human SPARC. Methods Cell Biol 2018, 143:335-345.
2393. Gadde KM, Pritham Raj Y: Pharmacotherapy of Obesity: Clinical Trials to Clinical Practice. Curr Diab Rep 2017, 17:34.
2394. Ioannides-Demos LL, Piccenna L, McNeil JJ: Pharmacotherapies for obesity: past, current, and future therapies. J Obes 2011, 2011:179674.