Interventions nutritionnelles en contexte d'obésité - lumière sur le microbiote intestinal

Thèse

Béatrice Choi

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

Québec, Canada

Interventions nutritionnelles en contexte d'obésité – lumière sur le microbiote intestinal

Thèse

Béatrice So-Yun Choi

Sous la direction de :

André Marette, directeur de recherche
Angelo Tremblay, codirecteur de recherche

Résumé

La prévalence des maladies chroniques, dont les maladies cardio-métaboliques font partie, ne cesse d'augmenter mondialement. Les avancées dans le domaine du microbiote intestinal dans les dernières années montrent des liens de causalité entre les bactéries qui colonisent l'intestin et les métabolites qu'elles produisent. Le microbiote intestinal est donc une cible potentielle pour la prévention ou le traitement des comorbidités associées à l'obésité.

La perte rapide de masse grasse peut conduire à la libération endogène de molécules liposolubles aux effets potentiellement nocifs, comme les polluants organiques persistants (POP). L'objectif de la première étude était d'évaluer l'impact d'un extrait prébiotique de canneberge riche en polyphénols sur la libération de POP pendant la perte de poids. Malgré la perte de graisse plus importante chez les souris traitées à l'extrait de canneberge, leurs taux circulants de POP n'ont pas augmenté et leur homéostasie du glucose s'est améliorée par rapport aux souris traitées avec le véhicule. L'extrait a également induit des changements dans le microbiote intestinal, y compris la prolifération de *Parvibacter*, un membre de la famille des *Coriobacteriaceae* qui a un rôle potentiel dans le métabolisme des xénobiotiques.

L'objectif de la deuxième étude était de déterminer l'impact de l'hébergement de souris axéniques colonisées avec le microbiote intestinal de souris traitées à l'extrait de canneberge enrichi en proanthocyanidines ou de leurs homologues traitées avec un véhicule. Nous avons observé un phénotype hépatique opposé entre les deux secteurs d'hébergement, conventionnel ou axénique-gnotobiotique, ainsi qu'un impact sélectif sur la colonisation des taxons microbiens ainsi que sur le profil des métabolites fécaux. Ces résultats suggèrent que l'environnement dans lequel les souris gnotobiotiques sont logées influence fortement la composition et la fonction du microbiote intestinal et peut conduire à des phénotypes distincts après la colonisation. Une meilleure standardisation de l'utilisation des souris gnotobiotiques est nécessaire et des conditions d'hébergement strictes devraient être suivies.

Les modèles animaux de pathologies humaines sont classiquement nourris avec des diètes purifiés contenant de la caséine comme unique source de protéines. L'objectif de la troisième étude est de montrer que la source de protéine consommée influence le développement de

l'obésité et de la résistance à l'insuline induites par la diète. En effet, un mélange de protéines représentatif de la consommation humaine a augmenté le gain de poids, l'hyperinsulinémie et la voie de signalisation hépatique mTORC1/S6K1 par rapport à la caséine seule. Ces effets impliquent des altérations majeures de la composition microbiote intestinal et de la production microbienne d'acides gras à chaîne ramifiée, qui, dans les hépatocytes en culture, augmentent la production de glucose et activent la voie mTORC1/S6K1. Ces travaux montrent l'importance de considérer les sources de protéines dans les diètes animales et proposent des mécanismes d'actions potentiels des protéines alimentaires sur la santé métabolique.

L'objectif de la quatrième et dernière étude était de déterminer si la supplémentation en *Lacticaseibacillus rhamnosus* HA-114 accentuait l'impact bénéfique de la perte de poids sur la santé métabolique et cognitive. Cet essai de 12 semaines, randomisé, en double aveugle et contrôlé par placebo, a été effectué chez 152 adultes avec un surpoids suivant une intervention nutritionnelle pour induire une perte de poids contrôlée. Bien que la supplémentation en probiotiques n'ait pas potentialisé la réduction du poids corporel ou de la masse grasse, une diminution significative de l'insuline plasmatique, de l'HOMA-IR, du cholestérol-LDL et des triglycérides a été observée dans le groupe supplémenté en probiotiques uniquement. En ce qui concerne les traits liés à l'alimentation et à l'humeur, les effets bénéfiques ont été observés uniquement dans le groupe recevant la supplémentation en probiotiques ou étaient significativement plus importants dans ce groupe, y compris la diminution des tendances à la l'hyperphagie, la désinhibition et les envies de manger. Ce projet démontre la pertinence clinique de la supplémentation en probiotiques pour induire des résultats métaboliques et psychologiques bénéfiques chez les personnes en surpoids en cours de perte de poids.

Collectivement, ces études confirment la validité de cibler le microbiote intestinal afin d'atténuer les comorbidités liées à l'obésité. En outre, elles auront certainement un impact sur différents aspects de la prévention et du traitement de l'obésité et des troubles métaboliques en utilisant tout le potentiel du microbiote intestinal, pour lequel il reste encore beaucoup à comprendre et à découvrir.

Abstract

The prevalence of chronic diseases, of which cardiometabolic diseases are part of continues to increase worldwide. Following advances in the field of gut microbiota in recent years, causal links between the bacteria that colonize the gut and the metabolites they produce have been demonstrated. The gut microbiota is therefore a potential target for the prevention or treatment of obesity-related comorbidities.

Rapid fat loss can lead to the endogenous release of fat-soluble molecules with potentially harmful effects, such as persistent organic pollutants (POPs). The objective of the first study was to evaluate the impact of a prebiotic polyphenol-rich cranberry extract on the release of POPs during weight loss. Despite the greater fat loss in cranberry extract-treated mice, their circulating POP levels did not increase, and their glucose homeostasis improved following weight loss compared to vehicle-treated mice. The extract also induced changes in the gut microbiota, including the proliferation of *Parvibacter*, a member of the *Coriobacteriaceae* family that has a potential role in xenobiotic metabolism.

The objective of the second project was to determine the impact of housing axenic mice colonized with the gut microbiota of mice treated with proanthocyanidin-riched cranberry extract or their vehicle-treated counterparts. We observed an opposite liver phenotype between the two housing sectors, conventional or axenic-gnotobiotic, as well as a selective impact on the colonization of microbial taxa and on the fecal metabolite profile. These results suggest the environment in which gnotobiotic mice are housed strongly influences the composition and function of the gut microbiota and may lead to distinct phenotypes after colonization. Better standardization of the use of gnotobiotic mice is needed and strict housing conditions should be followed.

Animal models of human pathologies are classically fed with purified diets containing casein as the sole protein source. The objective of chapter 3 is to show that the source of protein consumed influences the development of obesity and insulin resistance induced by the diet. Indeed, a protein mixture representative of human consumption increased weight gain, hyperinsulinemia, and hepatic mTORC1/S6K1 signaling compared with casein alone. These

effects involve major alterations in gut microbiota composition and microbial production of branched chain fatty acids, which, in cultured hepatocytes, increase glucose production and activate the mTORC1/S6K1 pathway. This work demonstrates the importance of considering protein sources in animal diets and proposes potential mechanisms of action of dietary proteins on metabolic health.

The objective of the fourth and final project was to determine whether supplementation with *Lacticaseibacillus rhamnosus* HA-114 enhanced the beneficial impact of weight loss on metabolic and cognitive health. This 12-week, randomized, double-blind, placebo-controlled trial was conducted in 152 overweight adults with a nutritional intervention to induce controlled weight loss. Although probiotic supplementation did not potentiate reduction in body weight or fat mass, a significant decrease in plasma insulin, HOMA-IR, LDL cholesterol, and triglycerides was observed in the probiotic-supplemented group only. With respect to eating and mood-related traits, beneficial effects were observed only in the probiotic supplementation group or were significantly greater in this group, including decreased binge eating tendencies, disinhibition, and food cravings. This study demonstrates the clinical relevance of probiotic supplementation to induce beneficial metabolic and psychological outcomes in overweight individuals undergoing weight loss.

Collectively, these studies confirm the relevance of targeting the gut microbiota to alleviate obesity-related comorbidities. Furthermore, they will certainly have an impact on different aspects of prevention and treatment of obesity and metabolic disorders by using the full potential of the gut microbiota, for which there is still much to understand and discover.

Table des matières

Liste des figures

Liste des tableaux

Liste des abréviations

3-HIB – 3-hydroxyisobutyrate
AA – acides aminés
AC – acylcarnitine
AhR – aryl hydrocarbon receptor
ALT – alanine aminotransferase
AMP – peptides antimicrobiens
ANOVA – analysis of variance
AST – aspartate transaminase
AUC – area under the curve
BAT – brown adipose tissue
BBB – blood-brain barrier
BCAA – branched-chain amino acids
BCFA – branched-chain fatty acids
BCKA – branched-chain alpha-keto acids
BMI – body mass index
CAT – catalase
CE – cranberry extract
CFU – colony forming unit
CPAUL – comité de protection des animaux de l'Université Laval
DAG – diacylglycerol
DAT – dopamine transporter
DDT – dichlorodiphenyltrichloroethane
FCQ – food cravings questionnaire
FDR – false discovery rate
FMT – fecal microbiota transfer
GABA – gamma-aminobutyric acid
GC-MS/MS – chromatography coupled with tandem mass spectrometry
GEB – genetically engineered bacteria
GF – germ-free
GPC – gel permeation chromatography
GPx – glutathione peroxidase
GSIS – glucose stimulated insulin secretion
HDL – high-density lipoprotein
HF – high-fat
HFD – high-fat diet
HFHS – high-fat high-sucrose
HOMA-IR – homeostatic model assessment of insulin resistance
IgA – immunoglobuline A
IMC – indice de masse corporel
ImP – imidazole propionate
IRS1 – insulin receptor substrate 1
ISAPP – international scientific association for probiotics and prebiotics

IVC – individually ventilated cage
LC-MS – liquid chromatography coupled with mass spectrometry
LDA – linear discriminant analysis
LDL – low-density lipoprotein
LefSe – linear discriminant analysis effect size
LFD – low-fat diet
LPS – lipopolysaccharide
MDD – major depressive disorders
MDP – muramyl dipepdide
MeHg – methylmercury
mTOR/S6K1 – mechanistic target of rapamycin/S6 kinase 1
NAcc – nucleus accumbens
NAFLD – non-alcoholic fatty liver disease
OGTT – oral glucose tolerance test
OMS – Organisme Mondial de la Santé
PAC – proanthocyanidins
PBDE – polybrominated diphenyl ethers
PCB – polychlorinated biphenyl
PM – protein mix
PNG – peptidoglycan
POP – persistent organic pollutant
PRR – patterns recognition receptors
qNMR – quantitative nuclear magnetic resonance
Reb A – rebaudioside A
RMR – resting metabolic rate
SCFA – short-chain fatty acids
SD – standard deviation
SEM – standard error of the mean
SMR – sleeping metabolic rate
SOD – superoxide dismutase
SPF – specific pathogen free
T2D – type 2 diabetes
TCA – tricarboxylic acid
TCDD – 2,3,7,8-tetrachlorodibenzodioxin
TFEQ – three factor eating questionnaire
TMA – trimethylamine
TMAO – triméthylamine N-oxide
VAS – visual analog scale

À ma maman, Nathalie,
une femme exceptionnelle, dévouée et forte.

Remerciements

Tout au long de ma thèse, j'ai eu la chance d'avoir un entourage professionnel et personnel incomparable, pour lequel je suis éternellement reconnaissante. Les mots sur les prochaines pages ne font pas justice à tout le support que j'ai eu la chance d'avoir, mais j'espère qu'ils laisseront leur marque. Donc un énorme merci à :

André, pour m'avoir fait confiance et cru en moi dès le début, pour m'avoir fait découvrir les nombreuses facettes de la recherche en me donnant toujours de nouveaux défis qui m'ont permis de me dépasser et de démontrer mon potentiel au maximum. Angelo, dans un monde ou tout va vite et où on est souvent trop pressés, vous m'avez rappelé qu'il faut parfois prendre un moment de recul, réévaluer ses priorités et toujours avoir du plaisir dans ce qu'on fait puisque comme vous le dites si bien, la vie de chercheur est une vie d'artiste.

Phil, à l'origine mon chargé de projet, au fil des années tu es devenu un ami et un confident incroyable, merci pour tout. Geneviève et Bruno, je ne sais pas comment j'aurais réussi à passer à travers mes études graduées sans toutes les discussions, fous rires et le réconfort que vous m'avez apporté.

Laurence, ma binôme, tout le monde devrait avoir la chance d'avoir une inséparable comme toi pendant son doctorat. On a partagé tous les hauts et les bas que les études graduées nous ont fait vivre du début à la fin et je garderai tellement de bons souvenirs de toutes nos péripéties. Noë, ma « grande sœur », on a surmonté de beaux défis ensemble et ton support n'a jamais diminué malgré ton départ/la pandémie, je l'apprécie énormément. Les Françaises, Mélanie, Cécile, Marjorie et Pauline, merci pour vos encouragements, votre franchise et toutes les soirées.

Benjamin, Marianne and Ida thank you for all the family dinners, the diverse and honest discussions, and the perspective you gave me. You have changed me both as a scientist and as a person and I will be forever grateful for it. *Mange tak*.

Les merveilleuses techniciennes en santé animale avec lesquelles j'ai eu la chance de travailler, Val, Christine (Dion), Christine (Cric), Joanie, Jacinthe, Jenny et Geneviève, le labo Marette ne serait pas du tout le même sans vous. Tous les membres du laboratoire qui ont diversifié mon quotidien, Patricia/Trish, merci pour tous les délicieux desserts, ta disponibilité et tes conseils. Arianne pour nos nombreuses discussions, Adia pour ta curiosité naturelle et l'aide que tu apportes au labo, Fred, pour tous les échanges scientifiques stimulants, Marie-Julie, Jean-Philippe, Anne-Laure, Roxanne et Amélie pour les lunchs, discussions de corridor et autres échanges qui ajoutaient de la joie dans mes

journées. Amit, Beisy and Michael, thank you for your kindness and sense of devotion towards science and others. Renato, Lia, Lais, Vanessa V. et Bruno P., pour votre joie de vivre et votre positivisme hors du commun, *obrigada*! Camomille et Alice, qui ne sont pas restées longtemps dans le laboratoire, mais qui ont laissé leur marque sur plusieurs d'entre nous, toutes les deux à la fois fonceuses et attentionnées. Alex et Justin à l'animalerie, ce fut toujours un plaisir de travailler avec vous et de discuter dans les corridors, l'animalerie aura été une deuxième maison pour moi pendant plusieurs années! Les anciens qui sont partis depuis déjà quelques années, mais sur qui j'ai toujours pu compter, en particulier Fernando, Michaël et Geneviève C. Finalement, tous les autres modèles incroyables avec qui j'ai eu la chance de travailler de près ou de loin dont Jon D. Schertzer, Fredrik Bäckhed, Mathieu Laplante, Francine Durocher, Phillip J. White, Vanessa P. Houde, Benoit Arsenault, Denis Boudreau, Lucie Brunelle et Marie-Ève Chouinard.

Mes amies MC, Ali, Karo et Steph, qui me suivent depuis le début du baccalauréat en biologie et Val, Mass et Bine à mes côtés depuis encore plus longtemps. Cathou, mon ancienne coloc préférée, toujours là pour moi depuis maintenant plus de 10 ans. Nos soirées en présentiel ou en virtuel m'ont toujours fait le plus grand bien, merci pour tout votre support.

Maria, Alex, Tatiana, Apolinar, Roberto y Tati, *gracias por todo, por vuestro apoyo, energía y cariño, tengo la suerte de poder llamaros familia.*

Merci à mes cousines, Bénédicte, Adèle et Héloïse, mes complices et l'unique cousin Jean-Christophe. À Danièle et Claude, pour leur support moral extraordinaire. À Renée et Guy, des modèles, qui dévouent leur quotidien à améliorer la santé de leurs patients. À mamie, Lise, une des premières femmes au Québec à graduer du baccalauréat en agronomie et à grand-papa, Ghislain, ancien professeur et directeur du département d'agronomie, pour m'avoir transmis leur passion pour le savoir. À ma maman, Nathalie, le meilleur modèle que j'aurais pu avoir. Merci pour tout ce que tu as fait pour moi. Je suis très fière de devenir officiellement la troisième génération de *philosophiæ doctor* de la famille.

Victor, déjà 8 ans à tes côtés, tant de projets, tant de voyages et tellement plus à venir. Merci d'être qui tu es, de rendre la vie aussi belle, d'avoir été là depuis le début de cette aventure et d'avoir toujours cru en moi. Je n'aurais pas pu avoir un meilleur partenaire pour passer à travers cette thèse. *Gracias mi amor.*

Avant-propos

Dans cette thèse, un total de cinq articles sont insérés, le premier, un article de revue, étant la dernière partie de l'introduction et les quatre autres sont les articles insérés comme chapitres. De ces articles, trois sont publiés et deux sont en révision.

Introduction: *Potential therapeutic applications of the gut microbiome in obesity: from brain function to body detoxification,* publié le 20 juin 2020, dans *International Journal of Obesity*. Les seuls ajustements effectués sont au niveau du format, pour respecter l'uniformité à travers la thèse. Pour cet article, je suis première auteure, statut partagé avec ma co-première auteure Laurence Daoust, qui a soutenu sa thèse en Nutrition le 23 septembre 2021. Avec la supervision des autres co-auteurs, Laurence et moi avons établi la structure de l'article, effectué la revue de littérature, rédigé le manuscrit et créé les figures synthèses.

Référence complète de l'article :
Choi BS, 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 (Lond). 2020;44(9):1818-31.

Chapitre 1: *A polyphenol-rich cranberry extract protects against endogenous exposure to persistent organic pollutants during weight loss in mice*, publié le 28 October 2020, dans *Food and Chemical Toxicology.* Les seuls ajustements effectués sont au niveau du format, pour respecter l'uniformité à travers la thèse. Pour cet article, je suis première auteure et sous la supervision des co-auteurs, j'ai participé à l'élaboration du protocole expérimental, effectué le protocole animal et les analyses subséquentes, excluant l'analyse du microbiote intestinal. J'ai ensuite fait l'analyse des données et rédigé le manuscrit.

Référence complète de l'article: Choi BS, Varin TV, 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.

Chapitre 2: *Gnotobiotic mice housing conditions critically influence the phenotype associated with the transfer of fecal microbiota in a context of obesity* à été soumis le 2 novembre 2021 à *Gut*. Les seuls ajustements effectués sont au niveau du format, pour respecter l'uniformité à travers la thèse.

Pour cet article, je suis co-première auteure, avec Laurence Daoust. Avec la supervision des autres co-auteurs, Laurence et moi avons élaboré le protocole expérimental, effectué le protocole animal et une partie des analyses subséquentes. Nous avons ensuite fait l'analyse des données et rédigé le manuscrit.

Liste complète des auteurs : Laurence Daoust, Béatrice S-Y Choi, Anne-Laure Agrinier, Adia Ouellette, Thibault V. Varin, Geneviève Pilon, Emile Levy, Yves Desjardins, Fernando F. Anhê, Vanessa P. Houde & André Marette

Chapitre 3 : Feeding diversified protein sources exacerbates hepatic insulin resistance via increased gut microbial branched-chain fatty acids and mTORC1 signaling in obese mice, publié le 7 juin 2021 dans *Nature Communications.* Les seuls ajustements effectués sont au niveau du format, pour respecter l'uniformité à travers la thèse. Pour cet article, je suis première auteure, statut partagé avec ma co-première auteure Noëmie Daniel, qui a soutenu sa thèse en Nutrition le 13 mars 2020. Avec la supervision des autres co-auteurs, Noëmie et moi avons élaboré le protocole expérimental, effectué le protocole animal et une partie des analyses subséquentes. Nous avons ensuite fait l'analyse des données et rédigé le manuscrit.

Référence complète de l'article: Choi BS, Daniel N, Houde VP, Ouellette A, Marcotte B, Varin TV, Vors C, Feutry P, Ilkayeva O, Ståhlman M, St-Pierre P, Bäckhed F, Tremblay A, White PJ, Marette A. 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(1):3377.

Chapitre 4: *Lacticaseibacillus rhamnosus HA-114 improves eating and mood-related behaviors in adults with overweight during weight loss: a randomized controlled trial,* a été soumis le 26 octobre 2021, dans *Nutritional Neuroscience*. Les seuls ajustements effectués sont au niveau du format, pour respecter l'uniformité à travers la thèse. Pour cet article, je suis première auteure. Avec la supervision des autres co-auteurs, j'ai participé aux différentes phases de recrutement et de suivi des participants, j'ai fait une partie des analyses subséquentes, j'ai interprété les données et rédigé le manuscrit.

Liste complète des auteurs : Béatrice S.-Y. Choi, Lucie Brunelle, Geneviève Pilon, Brunella Gonzalez Cautela, Thomas A. Tompkins, Vicky Drapeau, André Marette & Angelo Tremblay

Au cours de ma thèse, j'ai également contribué aux projets d'autres étudiantes du laboratoire, Laurence Daoust et Ida Søgaard Larsen et à ceux de collaborateurs lors de mes stages dans les laboratoires des Dr. Schertzer à l'Université Mcmaster, et Dr. Bäcked à l'université de Göteborg, en Suède. De ces articles, trois sont publiés, l'un est en processus de révision et deux encore en préparation.

Fernando F. Anhê, Béatrice S.-Y. Choi, Jason R.B. Dyck, Jonathan D. Schertzer et André Marette. Host-Microbe Interplay in the Cardiometabolic Benefits of Dietary Polyphenols. Trends Endocrinol Metab. 2019 Jun;30(6):384-395. doi: 10.1016/j.tem.2019.04.002

Ida Søgaard Larsen, Benjamin A. H. Jensen, Erica Bonazzi, Béatrice S.-Y. Choi, Nanna Ny Kristensen, Esben Gjerløff Wedebye Schmidt, Annika Suenderhauf, Laurence Morin, Peter Bjarke Olsen, Lea Benedicte Skov Hansen, Torsten Schröder, Christian Sina, Benoît Chassaing, and André Marette. Fungal lysozyme leverages the gut microbiota to curb DSS-induced colitis. Gut Microbes. 2021 Jan-Dec;13(1):1988836. doi: 10.1080/19490976.2021.1988836.

Laurence Daoust, Béatrice S.-Y. Choi, Sébastien Lacroix, Vanessa Vilela, Thibault Varin, Stéphanie Dudonné, Geneviève Pilon, Denis Roy, Émile Lévy, Yves Desjardins, Benoit Chassaing, André Marette. Postnatal environment impacts the development of sex-specific metabolic and gut microbiota outcomes in the offspring. Gut Microbes. 2021 Jan-Dec;13(1):2004070. doi: 10.1080/19490976.2021.2004070.

Fernando F Anhê, Soumaya Zlitni, Cassandra Y. Chen, Kevin P. Foley, Nicole G. Barra, Béatrice S.-Y. Choi, Michael G. Surette, Laurent Biertho, Denis Richard, André Tchernof, André Marette, Jonathan D. Schertzer. Human gut microbiota after bariatric surgery alters intestinal morphology and glucose absorption in mice independent of obesity, under revision in *Gut*

Introduction

1. L'obésité – Une maladie chronique unique

1.1 Définition de l'obésité

Selon les lignes directrices 2020 du réseau Obésité Canada, l'obésité se définit comme « une maladie chronique complexe caractérisée par une accumulation anormale ou excessive de graisses corporelles qui est nuisible à la santé » [1]. Biologiquement parlant, les mammifères ont évolué pour permettre l'accumulation de réserves d'énergie, dans le but d'éviter de mourir de faim lorsque les ressources se font plus rares. Le tissu adipeux est la plus grande réserve énergétique du corps, mais il a également été démontré que ce tissu est un organe actif jouant de nombreux rôles dans différents systèmes. En effet, c'est notamment une protection thermique et physique, un organe sécrétoire ainsi qu'un endroit de stockage pour des xénobiotiques tels que les polluants liposolubles [2]. Au fil de l'histoire, dans de nombreuses cultures, un surplus de tissu adipeux était vu comme un avantage, un signe de richesse. Par contre, l'industrialisation de l'agriculture causant l'abondance d'aliments dans la majorité des pays industrialisés, combiné à une multitude d'autres facteurs, débalancent l'équilibre énergétique et causent une augmentation de l'accumulation de tissu adipeux et ce, au niveau populationnel dans de nombreux pays. Cette augmentation du poids corporel peut devenir problématique pour la santé et est considérée parmi les conditions liées avec la majorité des décès mondiaux selon l' Organisation mondiale de la santé (OMS) [3].

La corpulence est souvent catégorisée en utilisant l'indice de masse corporelle (IMC), qui consiste en le poids (kg) divisé par la taille (m) au carré. À ce jour, la définition de l'obésité reconnue internationalement est celle de l'OMS et est basée sur l'IMC. Un indice de masse corporelle entre 25 et 30 est considéré comme un état de surpoids et supérieur à 30 considéré comme un état d'obésité. Par contre, la formule mesurant l'IMC omet de prendre en considération de nombreux paramètres qui peuvent avoir un impact sur la densité corporelle, notamment, le sexe, l'âge, l'origine ethnique, la musculature, etc. Donc malgré

l'utilité au niveau épidémiologique et en recherche, cet outil est de plus en plus controversé comme paramètre diagnostic au niveau individuel, puisqu'un excès de tissu adipeux menant à des troubles de santé n'est pas au même niveau d'adiposité pour tous [4].

En raison du risque pour la santé, associé à une forte adiposité, des paramètres diagnostics doivent être en place pour faciliter le diagnostic et le traitement de l'obésité. Plusieurs nouveaux outils ont été proposés afin d'être en mesure d'évaluer le risque individuel lié à l'excès d'adiposité de façon plus spécifique. Ces outils qui tentent de remplacer l'IMC, qui est une mesure non-invasive qui requiert peu de matériel ou d'expertise sont notamment la mesure du tour de taille [5] et le ratio tour de hanche-tour de taille [6]. Lorsqu'on ajoute des prélèvements sanguins, on peut utiliser des indices encore plus précis pour déterminer un risque sur la santé de l'excès de poids, comme la taille hypertriglycéridémiante [7] et l'indice d'adiposité viscérale [8]. Ces outils ont tous en commun une chose, ils portent une attention particulière à l'adiposité viscérale, puisqu'il a été démontré qu'il existe différents types de tissus adipeux avec des fonctions diverses et que tous les types de tissus adipeux ne contribuent pas de la même façon au développement de complications cardio-métaboliques [9].

1.2 Caractérisation des tissus adipeux

Une première façon de catégoriser les tissus adipeux est en fonction de leur couleur, qui varie selon le taux de mitochondries. En effet, le tissu adipeux blanc est le plus présent dans l'organisme et est caractérisé par une grande gouttelette lipidique et peu de mitochondries. Ce tissu blanc peut également être divisé en plusieurs catégories aux fonctions très distinctes, notamment le tissu adipeux sous-cutané, viscéral et ectopique. Le tissu adipeux brun quant à lui est caractérisé par une multitude de petites gouttelettes lipidiques et est très riche en mitochondries, ce qui lui donne sa couleur plus foncée [10].

En contexte de débalancement énergétique positif, le tissu adipeux sain peut prendre de l'expansion de deux façons, la multiplication des adipocytes, nommée hyperplasie et l'expansion des adipocytes déjà présents, nommée hypertrophie. Par contre, lorsque le tissu adipeux sous-cutané n'est plus en mesure de croître sainement, un débordement de lipides

se retrouve dans le système circulatoire [11] et peut aller se loger dans d'autres organes de façon ectopique ou dans le tissu adipeux viscéral [12].

Le tissue adipeux viscéral est l'accumulation de gras qui se retrouve dans la cavité abdominale. La façon de le quantifier de façon précise est par des techniques d'imagerie [13], et il est particulièrement d'intérêt dans l'étude des maladies cardio-métaboliques puisqu'il est beaucoup plus fortement corrélé aux comorbidités que l'IMC [14] ou le tissu adipeux sous-cutané [15]. Le tissu adipeux ectopique quant à lui est un tissu qui s'accumule en périphérie et au sein d'autres organes, comme le foie, le cœur et les reins.

Le tissu adipeux brun est très différent en termes de fonction par rapport au tissu adipeux blanc, principalement par son un grand potentiel thermogénique [16]. Chez l'humain adulte, le potentiel du tissu adipeux brun a longtemps été peu étudié en raison de sa présence limitée comparativement à ce qui est retrouvé chez le nouveau-né [17]. Cependant, il a été démontré que la fonction du tissu adipeux brun peut être fortement stimulée par l'exposition au froid [18], suggérant un potentiel thérapeutique en contexte d'obésité. De plus, certains adipocytes du tissu adipeux blanc peuvent acquérir certaines caractéristiques des adipocytes bruns lorsqu'exposés à certaines conditions telles que l'acclimatation chronique au froid, l'activité physique, certains médicaments, etc. [17]. Ces adipocytes transformés sont alors appelés adipocytes beiges.

Outre le type de tissu adipeux, l'étude de différents processus tels que l'adipogénèse, la sécrétion d'adipokines et la régulation des cellules immunitaires au sein du tissu adipeux amène une compréhension nouvelle des fonctions diverses de ce tissu. En contexte d'obésité, une inflammation persistante communément appelée inflammation de bas grade a été observée à de maintes reprises [19]. Une augmentation trop rapide ou incontrôlée des adipocytes peut créer de l'apoptose et une infiltration de macrophages, caractérisée par des structures de type couronne, autour des cellules mortes [20]. L'expansion rapide engendre également un hypoxie, qui augmente l'utilisation du glucose et le développement de résistance à l'insuline dans les adipocytes [21]. Ces phénomènes contribuent au développement d'inflammation de bas grade, comme d'autres facteurs tels que

l'endotoxémie métabolique causée par une augmentation de la perméabilité intestinale associée à une dysbiose [22], qui sera discutée plus loin (Section 2 : Le microbiote intestinal – Un écosystème puissant).

1.3 Développement de l'obésité

À l'exception de quelques formes monogéniques rares, l'étiologie de l'obésité est très complexe et multifactorielle [23]. Elle peut être influencée par de nombreux facteurs génétiques et environnementaux (Figure 1).

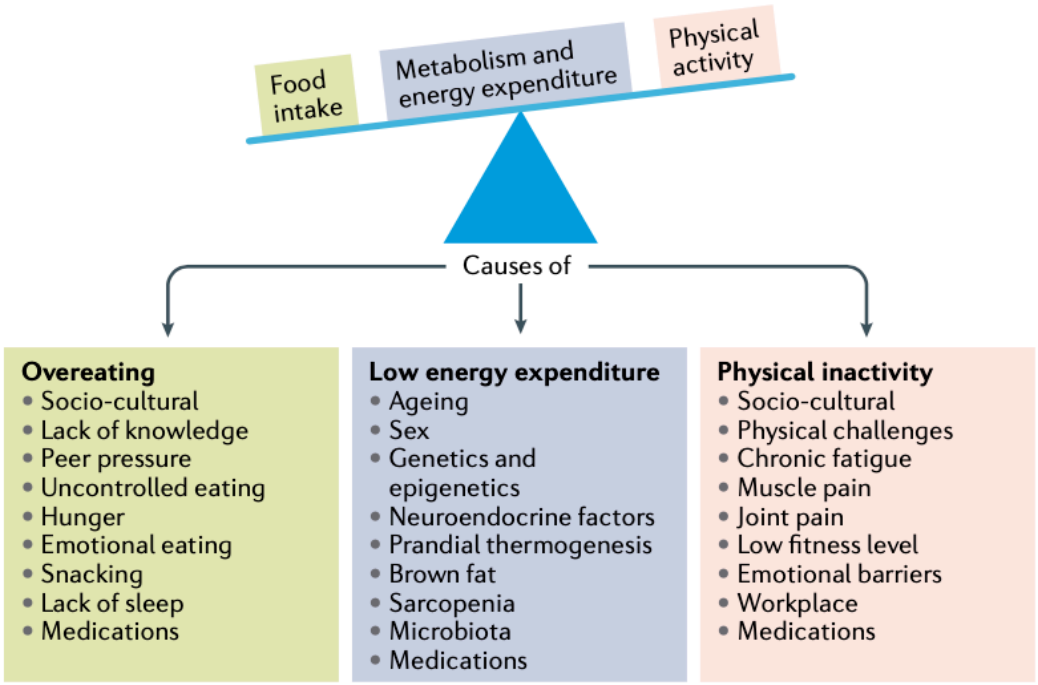


Figure 1. Tirée de Bluher, M. Obesity: global epidemiology and pathogenesis. Nat. Rev. Endocrinol. 15, 288-298 (2019). Les facteurs qui peuvent influencer une balance énergétique positive chronique, menant à l'obésité.

1.3.1 Facteurs génétiques et épigénétiques influençant le développement de l'obésité

Alors qu'initialement les études génétiques visaient à identifier des formes monogéniques de l'obésité, telles qu'une mutation au niveau du gène *MC4R,* qui a pour effet de provoquer une hyperphagie par manque de sensation de satiété [24], il est maintenant connu que dans la majorité des cas, l'héritabilité génétique de l'obésité est multigénique [25].

Pour déterminer l'impact de la génétique sur le poids corporel et d'autres paramètres de santé métabolique, plusieurs études ont été effectuées sur des jumeaux à la suite de démonstration que les taux de concordance pour différents degrés de surpoids étaient deux fois plus élevés chez les jumeaux monozygotes que chez les jumeaux dizygotes [26]. Lors d'études d'interventions, une réponse similaire entre deux jumeaux malgré une grande différence entre les paires de jumeaux confirme un impact de la génétique. Cela a été démontré entre autre en contexte d'excès calorique sur les paramètres anthropométriques [27] et en contexte de réponse à un protocole de perte de poids [28].

Outre la génétique et l'environnement, il a également été montré que la transmission de la susceptibilité à l'obésité peut résulter d'une programmation développementale [29]. Ce concept suggère que l'environnement rencontré au moment de la conception et pendant la vie fœtale et néonatale influence de façon permanente la structure, la fonction et le métabolisme des organes clés. L'un des facteurs impliqués dans cette programmation est l'héritabilité de modifications épigénétiques, que ce soit de la mère ou du père, car comme les changements génétiques peuvent être transmis d'un parent à un descendant lorsque présents au sein des cellules germinales. Les changements épigénétiques sont des modifications qui n'affectent pas directement le génome, mais bien la transcription de certains gènes. L'impact de l'épigénome sur le développement de l'obésité et la santé métabolique est de plus en plus étudié [30, 31] suite à des démonstrations de son importance sur des modèles tels que la drosophile [32] et le rat [33].

En plus de l'impact intergénérationnel, des changements épigénétiques, ceux-ci peuvent jouer un rôle important sur la santé d'un individu et être répertoriés dans les tissus métaboliques tels que les tissus adipeux, le foie, le muscle et le pancréas [30]. En contexte d'obésité, l'impact de l'alimentation sur les changements épigénétiques est d'un grand intérêt, puisque certains facteurs affectant la méthylation de l'ADN tels que des donneurs de groupes méthyles, peuvent provenir directement des aliments consommés [34, 35].

1.3.2 Facteurs environnementaux influençant le développement de l'obésité

L'environnement regroupe un ensemble de facteurs spécifiques affectant le développement de l'obésité, dont les facteurs caractérisant une bonne hygiène de vie, notamment une saine alimentation, un mode de vie actif, l'absence de la consommation de tabac, suffisamment de sommeil, un niveau de stress sain, etc. Les recommandations générales pouvant paraître simples, certaines sont complexes, comme la définition d'une saine alimentation.

Le guide alimentaire canadien 2019 a changé drastiquement par rapport à celui de 2007 notamment en mettant de l'avant non seulement des choix, mais aussi des habitudes alimentaires saines [36]. De plus, il utilise le concept de proportions au lieu de portions pour les recommandations alimentaires, donnant une plus grande place à la flexibilité des besoins individuels. La diète méditerranéenne est sans doute l'un des profils alimentaires les plus étudiés en lien avec la santé cardio-métabolique [37, 38]. De plus, il a été démontré à de nombreuses reprises qu'une alimentation peu transformée de source principalement végétale a également un impact favorable sur la santé [39]. Cependant, le concept de diète personnalisée a émergé dans les dernières années et des études ont montré qu'un même aliment peut avoir un effet très variable sur la réponse glycémique d'un individu à l'autre [40]. L'effet de certaines composantes de l'alimentation sera discuté plus loin (Section 3 : Aliments et suppléments : protéines alimentaires, prébiotiques et probiotiques**).**

Au niveau de l'activité physique, cette composante de l'hygiène de vie peut avoir un impact sur le bilan énergétique, mais a montré avoir des bienfaits sur la santé cardio-métabolique indépendamment d'une perte de poids. En effet, une augmentation de la condition cardio-respiratoire par une augmentation du niveau d'activité physique hebdomadaire a de nombreux bienfaits sur la santé comparativement à un mode de vie sédentaire même en contexte de prédisposition génétique à l'obésité [41].

D'autres facteurs tels que le microbiote intestinal [42-45] et l'exposition à des contaminants environnementaux [2, 46, 47] sont aussi à considérer en contexte d'obésité. En effet, il a été démontré que l'obésité pouvait se transmettre chez l'animal par une transplantation fécale [48], démontrant le rôle important que cette communauté de microorganismes peut avoir sur le métabolisme énergétique. Pour ce qui est de la pollution, plusieurs composés

xénobiotiques produits par l'industrialisation se retrouvent dans l'environnement et dans les aliments et peuvent avoir des effets néfastes sur la régulation hormonale en tant que perturbateurs endocriniens, affectant notamment la santé métabolique [49-51].

1.4 Comorbidités de l'obésité

Tel que mentionné plus tôt, l'obésité se définit par un excès d'adiposité ayant des effets néfastes sur la santé. Ces comorbidités peuvent être séparées en deux catégories; physiologiques et psychologiques.

1.4.1 Comorbidités physiologiques

Les comorbidités biologiques sont celles qui ont eu le plus d'attention dans les dernières décennies, puisqu'elles sont plus facilement mesurables et ont un impact direct sur l'espérance de vie et les coûts du système de santé. On y retrouve entre autres le syndrome métabolique [52], les maladies cardiovasculaires [53], la résistance à l'insuline,le diabète [54] ainsi que la stéatose hépatique non-alcoolique [55].

Le concept de syndrome métabolique existe depuis environ une centaine d'années [52]. La définition du syndrome métabolique a évolué au fil du temps. Fortement associé à l'obésité, ce syndrome est souvent prédicteur de désordres plus sévères, comme le diabète de type 2 ou des problèmes cardiovasculaires. Les critères les plus récents d'identification de ce syndrome sont la présence de trois des cinq critères suivants, dont les valeurs exactes sont relatives au sexe pour tous les paramètres et à l'origine ethnique pour le facteur tour de taille seulement [56]:

1. Une obésité abdominale (tour de taille)
2. Un niveau élevé de triglycérides circulants
3. Un niveau faible de HDL-cholestérol circulant
4. De l'hypertension
5. Une glycémie à jeun élevée

Une autre comorbidité de l'obésité qui est commune et souvent associée au syndrome métabolique est la résistance à l'insuline, caractérisée par une diminution du contrôle du

métabolisme du glucose par l'insuline [57]. Cette condition est souvent associée au développement du diabète de type 2 si elle n'est pas prise en charge, et est associée à des changements au niveau du foie, du tissu adipeux ainsi que du muscle, dont une augmentation de l'inflammation [58]. Lors de résistance à l'insuline au sein du muscle, une diminution du transport du glucose est observée puisque le processus de signalisation de l'insuline n'est pas en mesure de déclencher la translocation de vésicules contenant des transporteurs de glucose vers la membrane [57]. Au niveau du tissu adipeux, c'est majoritairement une mauvaise inhibition de la lipolyse qui est observée alors qu'au niveau du foie, une diminution de la répression de la production hépatique de glucose est observée. Il faut donc une hyperinsulinémie, davantage d'insuline, pour permettre la captation d'une même quantité de glucose, ce qui, à la longue, va aussi contribuer à désensibiliser les tissus à l'insuline.

Au niveau cellulaire, la résistance à l'insuline peut passer par différents mécanismes, notamment par une activation de mTORC1. Ce composant couple la stimulation du récepteur de l'insuline et la disponibilité des nutriments avec la synthèse des protéines via l'activation et la phosphorylation de la protéine ribosomale S6 [59]. En effet, la voie de signalisation mTORC1/S6K1 est un important régulateur de la taille des cellules qui coordonne l'activité de la machinerie de croissance cellulaire avec les niveaux d'énergie et de nutriments [60, 61], notamment par la phosphorylation de la sérine 1101 d'IRS1 [62].

Plusieurs facteurs génétiques et environnementaux peuvent avoir un impact sur le développement de la résistance à l'insuline, dont l'alimentation et la pratique de l'activité physique [57]. L'abondance de certains métabolites en circulation, tels que les acides aminés ramifiés (BCAA, de l'anglais *branched-chain amino acids*) [63-65] et les acylcarnitines [66, 67] a également été corrélée à la résistance à l'insuline. Plus récemment, le rôle d'autres tissus tels que l'intestin et le microbiote intestinal dans la régulation des fonctions de l'insuline a été montré [68], ce qui permet une meilleure compréhension du contrôle complexe et multifactoriel de l'homéostasie du glucose.

Suite à l'établissement d'une résistance à l'insuline au niveau de certains tissus et si celle-ci n'est pas prise en charge, elle peut mener au diabète de type 2. En effet, à long terme, une résistance à l'insuline engendrera une augmentation de la production d'insuline par le pancréas qui après un certain temps, se fatigue. À ce moment, la quantité d'insuline en circulation diminue et n'est plus suffisante pour contrôler les niveaux circulants de glucose, qui augmentent. C'est à ce stade qu'on commence à parler de diabète de type 2 [57]. Cette diminution de l'insuline a des effets directs sur certains tissus notamment le cerveau, le foie, le muscle et le tissu adipeux (Figure 2) [54].

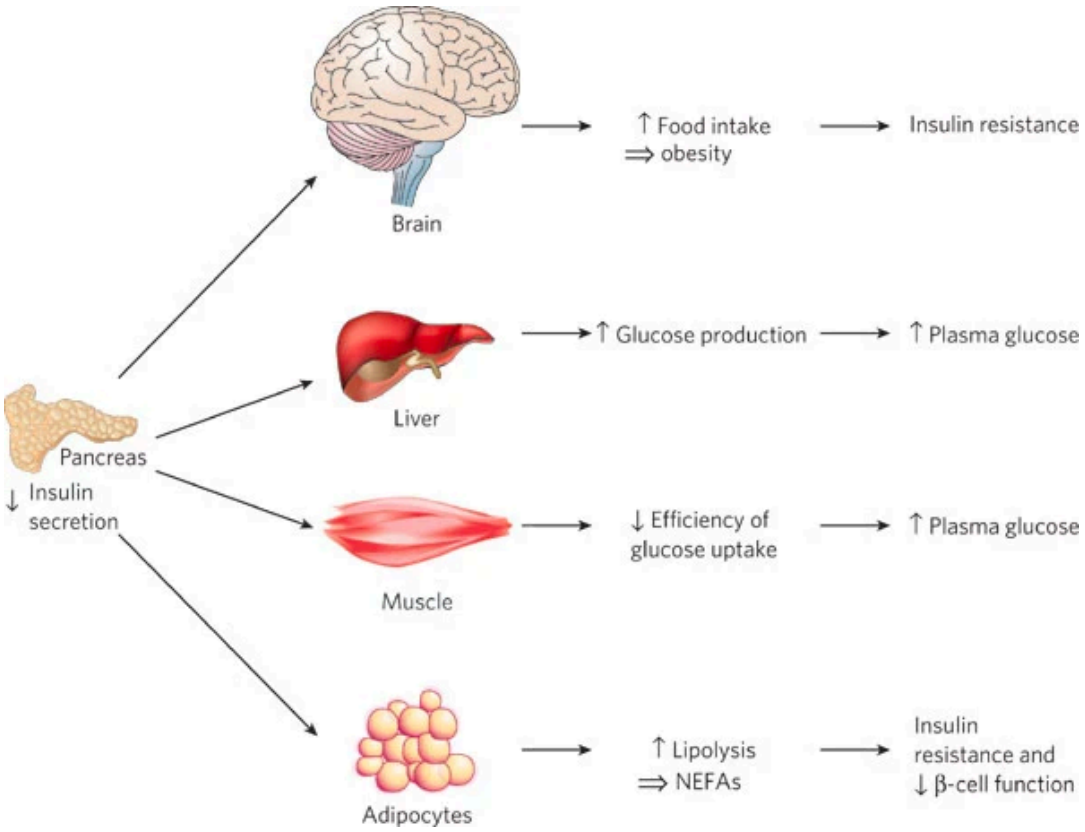


Figure 2. Tirée de Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840-6. Modèle du rôle critique de l'altération de la sécrétion d'insuline dans le lien entre l'obésité, la résistance à l'insuline et le diabète de type 2.

La stéatose hépatique non-alcoolique (NAFLD, de l'anglais *non-alcoholic fatty liver disease*) est un spectre de maladies hépatiques pouvant se rendre jusqu'au carcinome hépatocellulaire (Figure 3). Les trois premiers stades sont les plus fréquents, soit la

stéatose; une accumulation de lipides ectopiques au niveau des hépatocytes, la stéatohépatite; caractérisée par une augmentation de l'inflammation due à l'excès de lipides causant de la lipotoxicité, la dégénérescence d'hépatocytes et l'accumulation de macrophages, et finalement la fibrose caractérisée par une accumulation de tissu fibrotique [69].

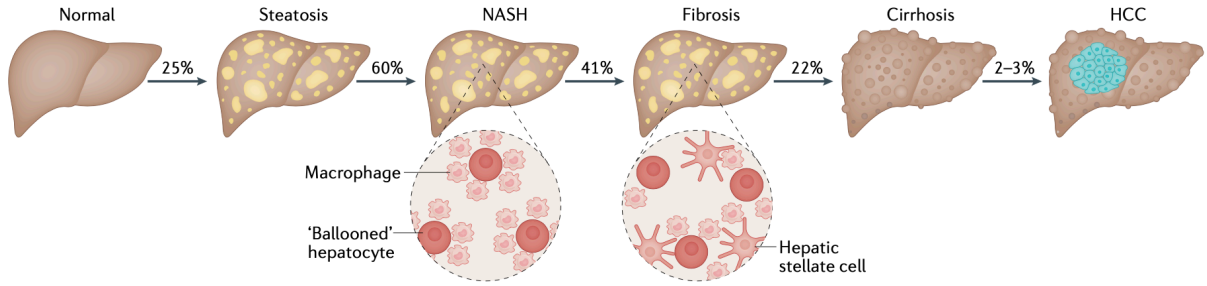


Figure 3. Tirée de Ferguson D, Finck BN. Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus. Nat Rev Endocrinol. 2021. Le spectre pathologique de la stéatose hépatique non alcoolique.

À l'instar de l'obésité, l'étiologie de la stéatose hépatique non-alcoolique, est complexe et multifactorielle. De plus, bien que l'obésité soit un facteur de risque du développement de ces désordres hépatiques, des patients minces en sont également atteints et cette prévalence a été observée dans différentes populations à travers le monde [55]. En effet, une consommation élevée de fructose, un sucre retrouvé en grande quantité dans les boissons sucrées augmente la prévalence de NAFLD [70] et ce, indépendamment du poids corporel [71].

En plus des trois conditions mentionnées ci-haut, il existe de nombreuses autres comorbidités physiologiques et physiques de l'obésité, telles que l'augmentation du risque de maladies cardiovasculaires [72], de problèmes rénaux [73], de certains cancers [74] ainsi que d'une dysfonction du système reproducteur [75].

1.4.2 Comorbidités psychologiques

Les comorbidités psychologiques de l'obésité sont moins bien comprises et plus difficilement quantifiables, mais la recherche dans ce domaine évolue grandement. On y

retrouve notamment les troubles de comportements alimentaires ainsi que des problèmes de l'humeur (anxiété, dépression, stress, faible estime corporelle, etc.), qui peuvent être aggravés par la discrimination et le jugement que les personnes avec obésité peuvent vivre [76].

Au niveau des comportements alimentaires, un dérèglement des signaux physiques ou psychologiques en lien avec la satiété et la consommation de nourriture peut avoir des effets indésirables. Certains troubles alimentaires, tels que l'hyperphagie et la dépendance à la nourriture sont plus présents chez les personnes avec un surpoids [77].

Les désordres cognitifs associés à l'humeur en contexte d'obésité sont fréquents, qui peuvent être causés par des débalancements hormonaux et autres problèmes biologiques. L'estime de soi et l'humeur peuvent aussi être affectées par le jugement de leur personne basé sur leur image corporelle, allant jusqu'à la discrimination [78, 79], par des proches, dans un milieu de travail ou des professionnels de la santé. D'ailleurs, la perception de discrimination par rapport au poids a été corrélée positivement avec la quantité de cortisol, l'hormone du stress [80].

1.5 Gestion de l'obésité

Selon les recommandations d'Obésité Canada, les approches en termes de gestion de l'obésité se basent sur trois piliers, soit une approche psychologique, une approche pharmacologique et la chirurgie bariatrique [81]. L'approche psychologique est celle qui englobe le plus d'éléments, soit tous les aspects de l'hygiène de vie, donc la gestion du sommeil et du stress, mais aussi la modification d'autres comportements dont l'augmentation de la pratique d'activité physique ainsi que des changements alimentaires, nommée thérapie nutritionnelle.

Lors d'études d'interventions nutritionnelles, la moyenne des participants observe souvent un effet favorable de l'intervention sur la perte de poids, mais il est tout de même important de prendre en considération plusieurs autres éléments. Par exemple, la majorité des études ne suivent pas à long terme les participants, donc qu'une perte de poids sur quelques mois

peut avoir peu d'impact sur la santé des participants, mais que si on était capable de continuer ou maintenir la perte de poids à long terme, des effets pourraient être observés. De plus, il y a toujours des participants qui sont résistants aux interventions et à la perte de poids même avec des diètes faibles en gras ou faibles en sucre [82]. Lorsque plusieurs programmes de perte de poids sont comparés (Atkins, Ornish, Weight Watchers and Zone Diets),l'adhésion au programme nutritionnel semble être le meilleur prédicteur de perte de poids et de réduction de complications cardiovasculaires à long terme [83].

Au niveau de l'approche pharmacologique, de nombreux médicaments sont en développement [84]. De plus, les analogues de GLP-1 qui peuvent être combinés à d'autres interventions sont maintenant couramment utilisés en pratique clinique puisqu'ils donnent des résultats significatifs au niveau de la perte de poids [85].

Pour ce qui est de l'approche chirurgicale, différents types de chirurgies dites bariatriques ou encore métaboliques sont pratiquées pour induire une diminution de poids corporel plus substantielle que celle obtenue avec les autres méthodes ainsi qu'une résolution de plusieurs comorbidités, tels que le diabète de type 2 [86]. En effet, il a été montré qu'à la suite d'une chirurgie bariatrique, la rémission du diabète de type 2 est possible, ou du moins une diminution drastique de l'utilisation de médicaments tels que l'insuline [87].

1.6 Conclusions générales sur la prévention et le traitement de l'obésité

Au niveau populationnel, des programmes mettant l'emphase sur la prévention, comme la taxation des boissons sucrées ont eu un impact sur la diminution de leur consommation dans certains pays [88]. Des indicateurs tels que le Nutri-Score, un système visuel composé de lettres et de couleurs pour déterminer la qualité nutritionnelle d'aliments préparés [89], sont aussi des façons de simplifier les choix pour un consommateur et d'influencer les industries à mettre des produits de meilleure qualité nutritionnelle sur la marché. Également, une meilleure éducation dans les écoles sur l'alimentation et l'activité physique et d'autres facteurs comme la gestion du stress, l'importance du sommeil, sont des mesures qui pourraient avoir des impacts populationnels importants. Un exemple concret de ce type de programme au Québec est le Grand défi Pierre Lavoie, qui vise l'adoption de saines

habitudes de vie et qui organise une multitude d'activités, particulièrement dans les écoles, pour promouvoir celles-ci. De plus, un élément qui est présentement en changement est la déconstruction au niveau de la société que la beauté est directement liée à la minceur et que les personnes avec obésité le sont par choix, par paresse, etc. Ces images véhiculées entre autres par l'industrie à l'ère numérique actuelle bombardent de messages les consommateurs, ce qui peut à la longue créer des biais, conscients ou inconscients. La diminution de la stigmatisation des enfants ou des adultes vivant avec l'obésité permettrait de favoriser une meilleure santé psychologique grâce à une plus grande ouverture d'esprit et une diminution de la discrimination dont ils peuvent subir, un concept véhiculé de plus en plus sous le nom de grossophobie.

Au niveau individuel, les personnes vivant avec l'obésité ont besoin d'interventions fondées sur des données probantes, y compris la thérapie nutritionnelle, l'activité physique, les approches psychothérapeutiques, la pharmacothérapie et la chirurgie. Puisqu'il ne semble pas y avoir de solution universelle à long terme, les approches personnalisées et multidisciplinaires semblent être plus bénéfiques pour la santé des individus vivant avec l'obésité [90] et devraient donc être favorisées.

2. Le microbiote intestinal – Un écosystème puissant

2.1 Définition

Le terme microbiote intestinal regroupe tous les microorganismes présents dans l'intestin, que ce soit dans un modèle animal comme la souris ou chez l'humain. Le nombre d'organismes ainsi que le nombre de gènes suggèrent l'importance de cet écosystème et le potentiel que celui-ci peut avoir sur son hôte. En effet, il est estimé qu'à elles seules, les bactéries sont aussi nombreuses que le nombre de cellules eucaryotes animales du corps humain [91] alors que le nombres de gènes dans le génome bactérien du microbiote intestinal serait 100x supérieur à la quantité de gènes contenue dans les 23 paires de chromosomes humains [92]. Le potentiel du microbiote est donc principalement dû à la diversité des fonctions possibles ainsi qu'à son adaptation rapide [93].

Malgré la présence des bactéries dans les différentes parties du tube gastro-intestinal, la grande majorité d'entre elles se situent dans le colon, là où la plus grande proportion de fermentation a lieu [94]. Outre le nombre, le type de bactéries retrouvées entre le petit intestin et le colon diverge grandement et ces communautés réagissent différemment en réponse à des changements nutritionnels [95-97]. De plus, la couche de mucus qui tapisse l'intestin peut également être colonisée par plusieurs bactéries, qui ne sont pas les mêmes que celles dans la lumière de l'intestin et qui ont aussi des fonctions différentes [98]. Il est aussi important de considérer que le microbiote intestinal n'est pas uniquement constitué de bactéries. On y retrouve également des cellules du domaine des eucaryotes appartenant à la famille des champignons, appelé le mycobiome [99, 100] ainsi que de nombreux virus, majoritairement des bactériophages, appelé le virome [101, 102].

La dysbiose, c'est-à-dire un état déséquilibré de la communauté microbienne, a été associée à de nombreuses pathologies [103-105], de plus en plus de chercheurs s'intéressent aux facteurs qui l'influence et à son développement. La composition exacte du microbiote varie même au sein d'un individu dans une courte période, entre autres selon les aliments consommés [44, 106] et le rythme circadien [107, 108]. Due à la grande variation et au renouvellement des bactéries, aux nombreux facteurs qui peuvent les influencer, à la grande variabilité interindividuelle en termes de génétique, comportements, environnement et besoins [109], il est impossible de définir un microbiote intestinal parfait universel, ce qui renforce le concept d'un besoin pour de la nutrition et de la médecine personnalisées [110]. En revanche, l'impact de certains facteurs sur le développement et la modulation du microbiote intestinal sont de mieux en mieux compris.

2.2 Le développement du microbiote intestinal et les facteurs qui l'influencent

Des données récentes ont montré qu'il n'était pas possible de détecter un microbiote dans le méconium fœtal avant la naissance [111], donc que le fœtus serait stérile. Par contre, un méconium avec un métabolome riche a été associé avec la composition du microbiote intestinal en bas âge et une diminution de la sensibilité aux allergies [112], suggérant qu'il est possible d'avoir un impact prénatal sur le développement du microbiote intestinal. De plus, il a récemment été démontré qu'à l'âge d'un an, les facteurs principaux sur sa

composition sont le mode de naissance ainsi que l'allaitement [113] et qu'à 5 ans, le microbiote intestinal est encore en développement, mais que le mode de naissance ne semble plus être un facteur pour déterminer sa composition [114]. La génétique et l'épigénétique sont également des facteurs qui influencent le microbiote intestinal, mais l'environnement semblerait avoir un impact plus fort sur celui-ci [115].

Outre le mode de naissance et l'allaitement, les facteurs environnementaux ayant le plus d'impacts sur la composition et la fonction du microbiote intestinal sont la diète, l'exercice, certaines maladies, le vieillissement, la prise de médicaments et d'antibiotiques ainsi que la géographie, qui englobe d'autres paramètres, tels que l'exposition à la pollution (Figure 4).

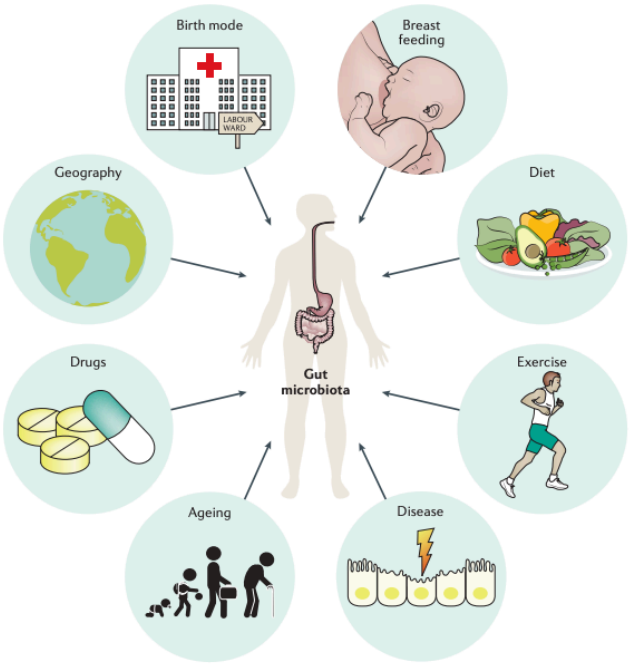


Figure 4. Tirée de Quigley, E.M.M. Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet? Nat. Rev. Gastroenterol. Hepatol. 14, 315-320 (2017). Les facteurs pouvant influencer la composition et la fonction du microbiote intestinal humain.

Comme le microbiote intestinal est directement en contact avec la nourriture consommée et que les bactéries ont également besoin de nutriments pour survivre et se reproduire,

l'alimentation est l'une des façons les plus efficaces de modifier le microbiote intestinal. De ce fait, différentes études ont tenté de mieux comprendre l'impact de l'alimentation sur le microbiote intestinal et la santé de l'hôte. Pour ce faire, de nombreuses interventions nutritionnelles ou supplémentations ont été effectuées chez l'animal et l'humain dans un contexte contrôlé, c'est-à-dire en tentant de garder tous les autres paramètres constants et en modifiant uniquement la diète. Des exemples spécifiques dont les sources de protéines, les prébiotiques et les probiotiques seront discutés plus loin (Section 4 : Aliments et suppléments : protéines alimentaires, prébiotiques et probiotiques). Outre la composition de l'alimentation, la quantité semble aussi avoir un impact sur le microbiote intestinal [116].

Une autre façon de mieux comprendre l'impact de la diète sur le métabolisme et le microbiote est l'étude de populations et de leurs habitudes alimentaires tels que les études de *Asnicar et al*. [110], et *Zeevi et al.* [40], qui requiert des cohortes de centaines de participants pour effectuer des analyses bio-informatiques avancées et une confirmation des modèles sur une cohorte de reproduction. Ce type d'étude de grande envergure permet non seulement de relier l'importance du rôle du microbiote intestinal sur la santé métabolique, mais aussi d'identifier certains groupes de bactéries, fonctions ou métabolites qui pourraient expliquer les phénotypes observés.

Récemment, afin de mieux comprendre les différences en termes de composition et de fonction bactérienne du microbiote intestinal, une équipe a séquencé le métagénome d'échantillons fécaux fossilisés datant de 1000-2000 ans, des paléofèces. Comparativement à des échantillons du temps présent, les bactéries des paléofèces sont plus diversifiées et plus similaires au microbiote intestinal des humains vivant dans des milieux non-industrialisés [117]. Ce type d'étude permet une meilleure compréhension de l'évolution du microbiote intestinal et de la dynamique entre l'hôte et son microbiote.

2.3 Liens de causalité entre le microbiote intestinal et certaines pathologies

Des associations ou corrélations de changements au niveau du microbiote intestinal avec certains symptômes ou certaines maladies, tels que l'obésité, ont été montrées à plusieurs reprises. Par contre, démontrer un lien de causalité est souvent plus difficile.

Chez l'animal, différentes techniques sont mises au point et utilisées de plus en plus couramment pour montrer un lien de cause à effet et non seulement une corrélation. Parmi ces techniques, on retrouve les protocoles de transplantation fécale, les protocoles en l'absence de microbiote et l'adoption croisée. Cependant, ces manipulations étant impossibles chez l'humain, les techniques pour démontrer un rôle causal sont principalement basées sur l'identification de métabolites microbiens ayant un impact spécifique ou encore sur des démonstrations bio-informatiques avancées, telles que les études de randomisation mendélienne.

Les protocoles de transplantation fécale (FMT, de l'anglais *fecal microbiota transfer*), que ce soit de fèces humaines à souris ou encore de souris à souris, permettent de démontrer qu'un phénotype est transférable à travers le microbiote, donc que le microbiote est suffisant pour induire celui-ci. Cette technique est effectuée de plus en plus fréquemment, mais très peu standardisée. Le contexte le plus contrôlé pour effectuer une FMT est dans un environnement stérile, sur des souris axéniques, c'est-à-dire dépourvues de bactéries [118]. De cette manière, la seule variable entre les groupes comparés est la source de matière fécale. Comme méthode alternative plus accessible techniquement et moins couteuse, des protocoles de FMT sont aussi réalisés sur des souris conventionnelles à la suite d'une administration d'antibiotiques et/ou de laxatif, pour retirer le plus possible le microbiote qui colonisait déjà ces animaux et assurer que celui qui sera transféré sera en mesure de coloniser le système digestif [119].

Un peu moins connue, la technique d'adoption croisée, qui consiste en l'échange de nouveaux nés avec des mères qui ont eu de bébés elles-mêmes récemment ou qui sont prêtes à allaiter [120] peut aussi être utilisée pour démontrer un effet causal du microbiote intestinal. Par rapport au protocole de FMT, l'adoption croisée prend aussi en compte le stade de développement auquel un animal est colonisé, c'est-à-dire à la naissance, ainsi qu'un développement normal du système immunitaire.

Une autre méthode pour établir un lien de causalité lorsque beaucoup de données provenant d'études observationnelles repose sur la randomisation mendélienne. Cette approche

épidémiologique se base sur l'étude de variants génétiques comme variables instrumentales pour déterminer les facteurs de risque modifiables qui affectent la santé de la population [121]. Avec l'augmentation de séquençage non seulement du génome, mais également du microbiome, des premières études de randomisation mendélienne ont été publiées, notamment en contexte de maladies métaboliques [122], renforçant la démonstration de l'impact important du microbiote intestinal et de ses métabolites sur la santé métabolique.

La démonstration d'un mécanisme d'action impliquant un métabolite d'origine microbienne est également une façon de confirmer que le microbiote peut contribuer au développement d'une pathologie [43]. Des exemples spécifiques au contexte d'obésité et de désordres métaboliques seront présentés dans les prochaines pages.

2.4 Microbiote intestinal en contexte d'obésité et de désordres métaboliques associés

Le rôle du microbiote intestinal sur le métabolisme de l'hôte, en particulier en contexte d'obésité et de désordres métaboliques a été grandement étudié et a fait l'objet de plusieurs revues de littérature de grande envergure dans les dernières années [42-44, 94, 123]. En effet, la relation entre le microbiote intestinal et son hôte est complexe et implique de nombreux mécanismes, certains encore inconnus. Cependant, des mécanismes tels que le rôle sur l'endotoxémie métabolique, les acides biliaires, la production d'hormones intestinales et de métabolites microbiens ont été démontrés (Figure 5) [124].

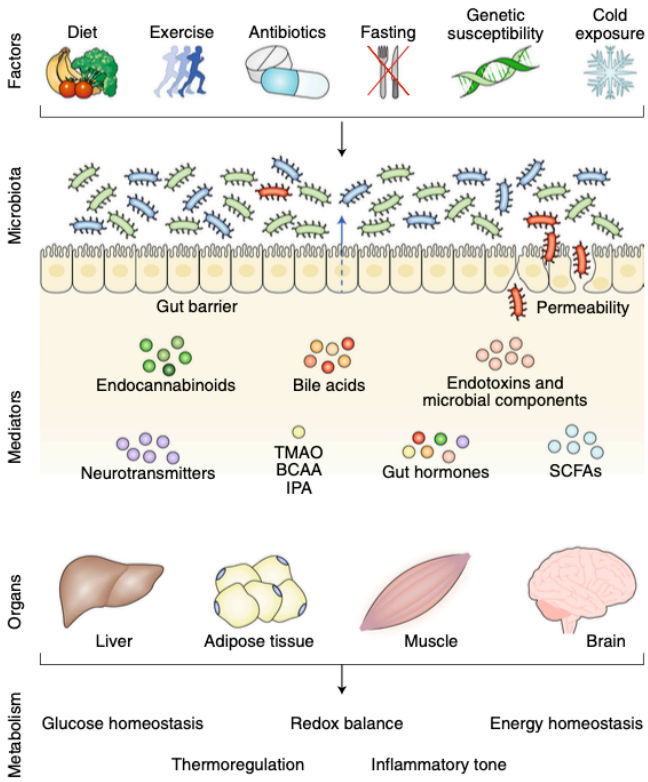


Figure 5. Tirée de Cani, P.D., et al. Microbial regulation of organismal energy homeostasis. Nat Metab 1, 34-46 (2019). L'interaction entre le microbiote intestinal et l'hôte et sa régulation du métabolisme

Le lien entre certains organes et le microbiote intestinal a particulièrement été étudié, notamment l'axe intestin-cerveau et l'axe intestin-foie. L'intestin et le cerveau sont les deux endroits avec le plus de connections nerveuses et sont directement reliés par le nerf vague [125, 126] et indirectement par la production de neurotransmetteurs par le microbiote intestinal [127]. Le foie, quant à lui, est un organe métabolique ayant plusieurs fonctions vitales et étant directement lié à l'intestin par la veine porte, il peut être fortement affecté par les métabolites produits par le microbiote, tels que les acides gras à chaine courtes (SCFA, de l'anglais *short-chain fatty acids*) et les changements d'abondance de certains nutriments [94].

Les SCFA sont des métabolites microbiens principalement dérivés de la fermentation des fibres et ont grandement été étudiés en contexte de santé métabolique [128]. Les SCFA sont des inhibiteurs des histones désacétylases et des ligands des récepteurs couplés aux

protéines G, et agissent donc comme des molécules de signalisation qui ont une activité anti-inflammatoire permettant de conserver l'homéostasie immunitaire [129]. Les trois composés majeurs de cette catégorie sont l'acétate, le butyrate et le propionate sont réputés pour avoir des effets favorables sur la santé de l'hôte, notamment sur certains organes tels que le tissu adipeux [130] et le foie [94]. Par exemple, en contexte de cirrhose hépatique, le butyrate est associé avec un meilleur pronostic, qui semble passer par une diminution de l'inflammation [131]. De plus, ces métabolites pourraient aussi être des régulateurs du métabolisme et de la fonction du muscle squelettique [132]. Leurs effets positifs sur le contrôle du poids corporel et de la sensitivité à l'insuline ont également été démontrés [130], principalement à travers leur rôle comme une source de communication entre l'intestin et le cerveau [133]. L'acétate en particulier peut traverser la barrière hémato-encéphalique et avoir un impact sur le cerveau, notamment au niveau du contrôle de l'appétit [134] et l'homéostasie énergétique [135]. Cependant, le contrôle de l'appétit n'est pas le seul facteur qui pourrait être affecté par les SCFA au niveau du cerveau, d'autres comportements et même des conditions neurogénératives seraient également affectées [125, 127, 136]. Finalement, bien que plusieurs études montrent un impact bénéfique des SCFA, dans certains contextes, dont un type de cancer du foie, ils peuvent avoir un impact néfaste, démontrant l'importance du contexte et de la santé de l'hôte dans l'étude du microbiote intestinal et de ses métabolites [137].

D'autres métabolites ont été identifiés comme potentiels biomarqueurs puisqu'ils étaient élevés en circulation chez des sujets diabétiques par rapport à des sujets ayant une régulation normale du glucose [138]. Koh *et al*. ont démontré que l'un d'entre eux, l'imidazole propionate, est produit par le microbiote intestinal à partir de l'acide aminé histidine et qu'il altère directement la tolérance au glucose et la signalisation de l'insuline [138]. Sur une autre cohorte, l'élévation en circulation de ce métabolite chez les diabétiques a été notée à nouveau et a été associée à des paramètres du microbiote, tels qu'une faible richesse bactérienne et un entérotype, c'est-à-dire un regroupement de taxa bactériens défini [139] précédemment associé à l'obésité [140]. De plus, la consommation d'histidine dans la diète ne semble pas avoir d'effet sur la quantité d'imidazole propionate retrouvée en circulation, donc ce serait une altération de son métabolisme par le microbiote qui serait

en cause [140]. Il a également été montré que cette molécule affecte l'effet de la metformine, un médicament couramment utilisé pour le traitement du diabète [141]. L'effet bénéfique de ce médicament est d'ailleurs lié, au moins en partie, à son effet sur le microbiote intestinal [142].

L'abondance de certaines bactéries peut être aussi corrélée avec des phénotypes positifs ou négatifs sur le métabolisme. Plusieurs bactéries commensales ont donc été étudiées pour comprendre si elles étaient la cause ou la conséquence d'un phénotype et comment elles pouvaient être augmentées lorsqu'elles sont favorables. *Akkermansia muciniphila* est un cas particulièrement célèbre dans le contexte de l'obésité et des désordres métaboliques. En effet, cette bactérie découverte au début des années 2000 [143] a fait l'objet de nombreuses études et est considérée comme probiotique pour aider au traitement et à la prévention de maladies métaboliques [144]. La relation entre une meilleure santé métabolique et l'augmentation d'*Akkermansia* a été démontrée à plusieurs reprises [145, 146]. L'effet bénéfique de sa supplémentation sous sa forme vivante ou pasteurisée, chez la souris ou l'humain, a également été montrée [147, 148]. Reconnue comme une bactérie dégradant le mucus, un des mécanisme potentiel des effets positifs d'*Akkermansia* est la stimulation et le renouvellement de la production de mucines, donc l'amélioration de la barrière intestinale, un effet protecteur en contexte d'inflammation de bas grade [149]. Par contre, les mêmes caractéristiques pourraient faciliter les effets néfastes lors d'une inflammation intestinale plus sévère, comme observé dans la colite [150].

D'autres bactéries prometteuses, telles que *Dysosmobacter welbionis* [151, 152], récemment découverte ou *Faecalibacterium prausnitzii* [153] sont aussi d'intérêt comme probiotiques potentiels pour aider à contrer les désordres métaboliques et certaines, au contraire, sembles êtres néfastes, tels que *Prevotella Copri* [154], bien qu'il ne semble pas y avoir de consensus définitif [110] .

Le contexte et l'environnement dans lequel une bactérie est étudiée reste donc des facteurs primordiaux pour déterminer si cette bactérie semble avoir un impact positif ou négatif sur son hôte, ajoutant à la complexité de l'étude de taxa bactériens spécifiques. De plus, des

corrélations entre certaines bactéries et composantes métaboliques peuvent varier selon l'origine ethnique et le statut socioculturel [155], démontrant l'importance de la diversification des cohortes étudiées et d'une reproductibilité des données dans des cohortes provenant de différents pays et continents. Avec les avancées technologiques qui rendent le séquençage plus accessible et le développement de la bio-informatique et de l'intelligence artificielle, il a été démontré qu'un consortium de bactéries est nécessaire pour bien prédire ou comprendre la relation hôte-microbiote, tel que la réponse glycémique à certains aliments [40], comme différents types de pains [156].

De ce fait, la découverte de marqueurs de la fonction globale du microbiote intestinal au lieu d'uniquement sa composition est mise de l'avant, puisque ces marqueurs pourraient être plus précis que l'abondance d'une bactérie et sont plus accessibles que le séquençage en profondeur du microbiote intestinal suivi d'analyses complexes. L'étude du métabolome et des meilleurs facteurs prédicteurs révèle que la diète et le microbiote sont les paramètres qui expliquent le mieux le métabolome, en contraste à la génétique, des paramètres cliniques, les mesures anthropométriques et les habitudes de vie [157]. De nombreuses associations spécifiques ont été faites entre des familles ou genres microbiens et des métabolites pouvant avoir un rôle sur la santé de l'hôte, tels que les lipoprotéines permettent aussi d'identifier de nouvelles cibles thérapeutiques [158]. Cependant, il a récemment été démontré qu'une différence de seulement quelques gènes dans des bactéries d'une même espèce peut avoir un impact critique sur le phénotype de l'hôte, remettant l'emphase sur l'importance de la fonction du microbiote et non seulement de l'abondance de certaines bactéries [159].

Un autre paramètre sur lequel le microbiote semble jouer un rôle important est la perte de poids qu'elle soit induite par la diète [160], par traitement pharmacologique [161] ou encore par chirurgie bariatrique [162]. Une méta-analyse a d'ailleurs montré que tous types d'intervention visant la perte de poids a un impact sur la composition du microbiote intestinal, mais que cet impact n'est pas toujours corrélé avec la quantité de poids perdu et que la composition initiale du microbiote intestinal peut influencer les réponses individuelles aux interventions de perte de poids [163]. De plus, certaines bactéries, tels

que *Bacteroides thetaiotaomicron* et certains métabolites, tels que le glutamate, semblent être directement associés avec l'obésité et la perte de poids [164].

L'impact du microbiote sur les maladies métaboliques est un domaine qui a énormément évolué dans les dernières décennies [93], permettant de prendre avantage des connaissances actuelles pour contribuer aux avenues thérapeutiques de certaines pathologies. Des applications du microbiote intestinal en contexte d'obésité seront discutées dans la section 4 : *Potential therapeutic applications of the gut microbiome in obesity: from brain function to body detoxification*.

2.5 Inflammation et perméabilité intestinale

Tel qu'introduit plus tôt (Section 1 : L'obésité – Une maladie chronique unique), l'obésité et les désordres métaboliques sont caractérisés par une inflammation de bas grade chronique, qui est souvent associée à l'endotoxémie métabolique. L'association entre l'endotoxémie métabolique et les désordres métaboliques a initialement été étudiée chez la souris, démontrant que chez les groupes avec obésité, une augmentation de lipopolysaccharide (LPS) était présente et que lorsque des souris étaient exposées de façon prolongée à des injections de LPS, l'homéostasie du glucose était affectée [165]. Par la suite, la même équipe a démontré que l'endotoxémie métabolique et l'augmentation de l'inflammation chez des souris obèses était liées au microbiote intestinal et que celui-ci était nécessaire pour observer ces effets [166]. Cette augmentation en circulation de LPS en contexte d'obésité et de risque cardiovasculaire a également été observée chez l'humain [167] et depuis de nombreuses études se sont penchées sur la relation entre le LPS, l'activation de l'inflammation et les risques métaboliques, notamment le diabète de type 2 [168]. L'endotoxémie métabolique devient donc une cible thérapeutique intéressante [169].

Cependant, il a récemment été montré que le potentiel endotoxémique d'une molécule de LPS serait spécifique à sa bactérie d'origine. Anhê *et al.* ont démontré que le LPS d'*E. coli*, qui est le plus étudié, altère la barrière intestinale et détériore le contrôle de la glycémie chez la souris, mais que des doses égales de LPS provenant d'autres bactéries, tels que *R. sphaeroides* n'avaient pas ces mêmes effets néfastes et pouvaient même restaurer les

paramètres affectés négativement par le LPS d'*E. coli* [170]. Ces données montrent que des techniques plus précises de mesure d'entoxémie métabolique sont nécessaires pour bien comprendre le risque individuel sur la santé.

Fortement liée à l'endotoxémie métabolique, la perméabilité intestinale est aussi un facteur étudié en contexte d'obésité et de désordres cardio-métaboliques, puisqu'une moins bonne barrière intestinale induit une augmentation de l'inflammation métabolique [171]. Cette barrière intestinale est composée de plusieurs composantes physiques, telles que le mucus recouvrant la surface de l'intestin, les jonctions épithéliales qui gardent les cellules proches les unes des autres et de composantes immunitaires, notamment les peptides antimicrobiens (AMP) et les immunoglobulines A (IgA) [172]. Par contre, en contexte de dysbiose, cette barrière peut être moins efficace [173].

Plusieurs métabolites dérivés ou transformés par le microbiote intestinal peuvent jouer un rôle sur la modulation du système immunitaire. En plus des SCFA discutés précédemment, les acides biliaires secondaires et les ligands du récepteur arylhydrocarboné (AhR, de l'anglais *aryl hydrocarbon receptor*) sont des exemples bien documentés [174]. Outre le rôle de métabolites, plusieurs composantes bactériennes ou modèles moléculaires associés à des agents pathogènes, tels que le LPS, la flagellin et le peptidoglycane peuvent entamer une réponse pro-inflammatoire [173]. Cette réponse débutant non seulement lorsque ces composés sont détectés dans l'intestin, mais est encore plus forte lorsqu'ils réussissent à passer la barrière intestinale, se retrouvent en circulation et potentiellement dans d'autres tissus, un concept nommé translocation (Figure 6).

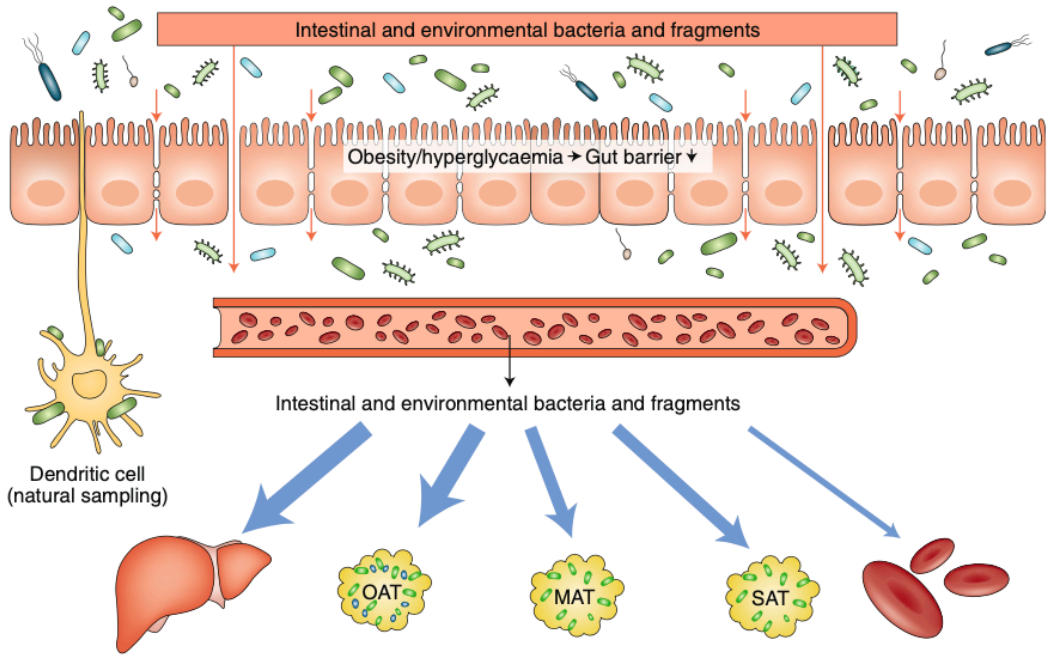


Figure 6 : Adaptée de Cani, P.D. & Van Hul, M. Microbial signatures in metabolic tissues: a novel paradigm for obesity and diabetes? *Nat Metab* **2**, 211-212 (2020). La compartimentation bactérienne chez les personnes obèses et diabétiques.

En contexte métabolique chez l'humain, ce n'est que très récemment qu'il a été prouvé que des fragments bactériens et même des bactéries entières sont présentes dans plusieurs tissus et qu'ils sont différents entre un groupe d'individus avec obésité comparativement à un groupe d'individus avec obésité et diabète de type 2 [175, 176]. De plus, Suppli *et al.* ont montré la présence d'ADNr bactérien au sein du foie chez des sujets sans obésité et que comparativement aux sujets avec obésité, les taxa bactériens des biopsies hépatiques étaient différents [177]. De plus, dans un contexte d'inflammation intestinale plus sévère, la maladie de Crohn, des bactéries viables ont été détectées dans la couche de tissu adipeux mésentérique, caractéristique de cette maladie [178]. Ces récentes découvertes montrent que non seulement des petites composantes bactériennes comme le LPS peuvent passer la barrière intestinale, mais qu'il serait possible que des bactéries entières et capables de se diviser puissent passer de la lumière de l'intestin au reste du corps, ouvrant un champ d'étude complet sur l'étude de ces bactéries et le potentiel qu'elles ont sur la santé de l'hôte [179].

3. Aliments et suppléments : protéines alimentaires, prébiotiques et probiotiques

Fondamentalement, la composition des aliments, principalement les macro- et micronutriments, est l'élément le plus étudié qui détermine si leur consommation est recommandée ou non. Par contre, les valeurs nutritionnelles ne sont pas le seul facteur ayant un impact sur la santé, la matrice étant un autre facteur significatif. À titre d'exemple, une méta-analyse a révélé que l'impact du fructose sur la production d'acide urique, un métabolite néfaste pour la santé cardiométabolique, est davantage influencé par la matrice de l'aliment consommé contenant du fructose, que par la quantité totale qu'il contient [180]. Une étude montre que la forme du sucre et que les propriétés physiques et chimiques de la matrice alimentaire ont un effet primordial sur l'absorption et les effets physiologiques [181].

Outre les nutriments, d'autres molécules se retrouvent également dans les aliments sans nécessairement apparaître sur les étiquettes nutritionnelles, telles que des polluants ainsi que des produits ajoutés lors de la transformation de certains aliments, pour différents buts, tels que prolonger la conservation, modifier la texture, rehausser le goût. Les émulsifiants alimentaires, présents dans de nombreux produits ultra-transformés favorisent le développement de la colite et du syndrome métabolique, de façon dépendante du microbiote intestinal [182] sont un bon exemple.

La somme des nutriments, leur structure, la matrice, la présence d'éléments ajoutés en industrie et de contaminants sont des composantes qui déterminent l'impact d'un aliment sur la santé et sur le microbiote intestinal. De plus, comme mentionné précédemment, un même aliment peut générer une réponse différente sur certains paramètres, dont la réponse glycémique selon l'individu et son microbiote [156].

3.1 Impact des protéines sur la santé métabolique en contexte d'obésité

En contexte d'obésité et de maladies cardio-métaboliques, les interventions nutritionnelles sont effectuées principalement à deux niveaux. Tout d'abord au niveau de la quantité

d’aliments consommés et ensuite au niveau de leur qualité. Les deux sources de macronutriments qui constituent la grande majorité de l’apport calorique journalier sont les glucides et les lipides. De ce fait, ceux-ci ont grandement été étudiés et divers régimes proposés pour diminuer les conséquences de l'obésité misent sur la réduction des quantités et des types spécifiques de lipides, notamment les gras trans et les gras saturés et de glucides, par exemple le sucrose et le fructose, retrouvé en grande quantité dans les boissons sucrées. Cependant, les protéines alimentaires ont également un impact sur la prise de poids et le risque de développer des maladies métaboliques.

Les protéines sont composées d’acides aminés (AA) et la nature d’une protéine se définit par sa structure. En nutrition, les AA sont regroupés en deux grandes catégories, les AA essentiels : le tryptophane, la lysine, la méthionine, la phénylalanine, la thréonine, la valine, la leucine, l'isoleucine et l'histidine ainsi que les AA non-essentiels, l’alanine, l'arginine, l'asparagine, l'acide aspartique, la cystéine, l'acide glutamique, la glutamine, la glycine, la proline, la sérine et la tyrosine. La différence entre ces deux catégories est que les AA essentiels ne peuvent être synthétisés par l’organisme ou sont produits en quantité insuffisante pour les besoins, donc doivent provenir de l’alimentation.

Bien que la majorité des études portent sur la quantité de protéines [183, 184], quelques études utilisant des modèles animaux ont montré que la source de protéine alimentaire influence directement le gain de poids [185, 186] et le métabolisme du glucose [187, 188]. Par exemple, le remplacement de la caséine, une protéine laitière, par des protéines de morue ou de soya peut améliorer la sensibilité à l'insuline et la tolérance au glucose [189-191] en plus de prévenir l'inflammation de bas grade, alors que les protéines de viande semblent avoir un effet plus délétère [185, 192]. Les effets bénéfiques de la protéine de morue sur la sensibilité à l'insuline et l'inflammation ont également été validés dans des études humaines [193, 194]. Ces premières études ont ouvert la voie au concept selon lequel les protéines alimentaires modulent les caractéristiques du syndrome métabolique indépendamment des lipides et des glucides alimentaires. Une étude récente démontre également une corrélation entre la quantité de protéine totale ainsi que la quantité de

protéine animale consommée avec le diabète de type 2, mais que la quantité de protéine végétale elle n'était pas corrélée [195].

Bien que l'impact de la source protéique sur le développement de maladies métaboliques soit connu, la plupart des diètes obésogènes commerciales utilisées sur les modèles animaux prennent rarement cela en compte. En effet, la très grande majorité des diètes purifiées utilisent la caséine comme unique source de protéine alimentaire, puisque c'est une source complète (donc elle contient tous les acides aminés essentiels), accessible, peu coûteuse, facile à extraire et à purifier. De plus, toutes les lignes directrices sur les besoins en nutriments des animaux de laboratoire, qui servent de cadre de référence aux producteurs de régimes pour rongeurs, sont basées sur la caséine uniquement [196]. Par contre, la caséine n'a pas la même composition que les sources végétales et autres sources animales de protéines qui sont abondantes dans la plupart des régimes alimentaires humains, et elle peut réduire la prise de poids corporel, améliorer le métabolisme du glucose et réduire l'inflammation par rapport à d'autres sources de protéines [185, 192].

3.1.1 Les AA en contexte métabolique

Les AA sont des modulateurs importants de la sensibilité à l'insuline, du métabolisme du glucose et de l'oxydation des lipides [197]. Une exposition accrue des cellules musculaires aux AA peut inhiber l'action métabolique de l'insuline par l'activation de mTORC1 et de sa cible en aval S6K1, ce qui déclenche une boucle de rétroaction négative qui régule à la baisse la signalisation de l'insuline [198, 199]. De plus, la résistance à l'insuline induite par une hyperinsulinémie chronique est exacerbée par une surcharge d'AA, observée dans les tissus de modèles murins d'obésité [62, 199] et dans les adipocytes humains traités aux AA [200]. Également, certains acides aminés, dont la leucine, ont un rôle sécrétagogue et augmentent la libération d'insuline [59].

Des études utilisant la métabolomique ciblée ont également révélé que des AA individuels ou des signatures spécifiques liées aux AA peuvent être corrélés au métabolisme énergétique et à la sensibilité à l'insuline. De plus, les perturbations de leur métabolisme peuvent être détectées très tôt, et ceux-ci peuvent être utilisés comme biomarqueurs [201].

Le meilleur exemple est la signature bien documentée des BCAA, la leucine, l'isoleucine et la valine, qui sont en circulation l'un des meilleurs prédicteurs de la résistance à l'insuline et du risque de diabète de type 2 chez les personnes avec obésité et même dans la population générale [63-65, 201, 202]. Une étude récente a également révélé que des niveaux élevés de BCAA peuvent déclencher l'utilisation de la glycine, qui agit comme donneur de carbone dans le cycle pyruvate-alanine, pour améliorer l'homéostasie lipidique, jouant ainsi un rôle protecteur en contexte de maladies cardio-métaboliques [203].

L'élévation des BCAA dans l'obésité est également impliquée dans la promotion des maladies cardiovasculaires, en partie par le biais des α-cétoacides (BCKA, de l'anglais *branched-chain alpha-keto acids*) qui entraînent une hypertrophie cardiaque pathologique [204]. De plus, le glutamate, un acide aminé provenant du catabolisme des BCAA a également été corrélé avec l'adiposité viscérale [205, 206] et l'athérosclérose [207]. Les acides aminés aromatiques (phénylalanine, tryptophane, tyrosine) ont également été associés positivement à un poids corporel plus élevé. Une des hypothèses proposées est que comme les BCAA et les acides aminés aromatiques sont en compétition pour le *L-type amino acid transporter* LAT1 (SLC7A5) [208], les acides aminés aromatiques s'accumulent en circulation puisqu'ils ne sont pas en mesure d'entrer dans les cellules [201]. Sans être des acides aminés, les acylcarnitines, des métabolites oxydatifs intermédiaires constitués d'un acide gras estérifié relié à une molécule de carnitine [209] ont aussi été associés au développement de maladies cardio-métaboliques [210, 211]. Certains acylcarnitines à courte chaîne, C3 et C5, sont aussi corrélés aux désordres métaboliques et sont des dérivés du catabolisme des BCAA [63]. Globalement, ces données suggèrent que ce n'est pas nécessairement le niveau des BCAA en circulation qui soit la cause des impacts néfastes sur le métabolisme, mais bien l'altération de la voie catabolique des BCAA [63] ayant de multiples conséquences.

3.1.2 Les dérivés microbiens des AA

Malgré le fait que la majeure partie de l'absorption des protéines alimentaires se fait dans le petit intestin, des données plus récentes montrent qu'une partie des effets des AA sur le métabolisme pourrait passer par leur métabolisme au niveau du microbiote intestinal. En

effet, contrairement à la fermentation des glucides, tels que les fibres, généralement associées à un phénotype positif, la fermentation des protéines est associée à un phénotype négatif [123]. L'imidazole propionate, discuté dans la section 2, est un exemple concret de cette relation de causalité, comme c'est un métabolite microbien dérivé de l'AA essentiel histidine, associé au diabète de type 2 [138, 140].

Les AA aromatiques, soit la tyrosine, la phénylalanine et le tryptophane peuvent être transformés par des enzymes bactériennes en plusieurs composés ayant des impacts importants sur leur hôte, notamment au niveau de l'immunité et de la perméabilité intestinale [212]. En contexte d'obésité, le métabolisme des AA aromatiques par le microbiote a également été associé à des changements au niveau du cerveau, notamment à une diminution de la mémoire [213] ainsi qu'une altération de certains comportements comme le contrôle de l'inhibition [214].

De plus, contrairement aux principaux SCFA qui sont produits lors de la fermentation des fibres, les acides gras à chaînes courtes ramifiés (BCFA, de l'anglais *branched-chain fatty acids*), sont dérivés de la fermentation des protéines, plus précisément des BCAA et sont généralement associés à un phénotype métabolique néfaste (Figure 7) [94]. En effet, lorsque mesurés dans des échantillons fécaux humains, les BCFA ont été corrélés à l'obésité [215], la stéatose hépatique non-alcoolique [103] ainsi que l'hypercholestérolémie [216].

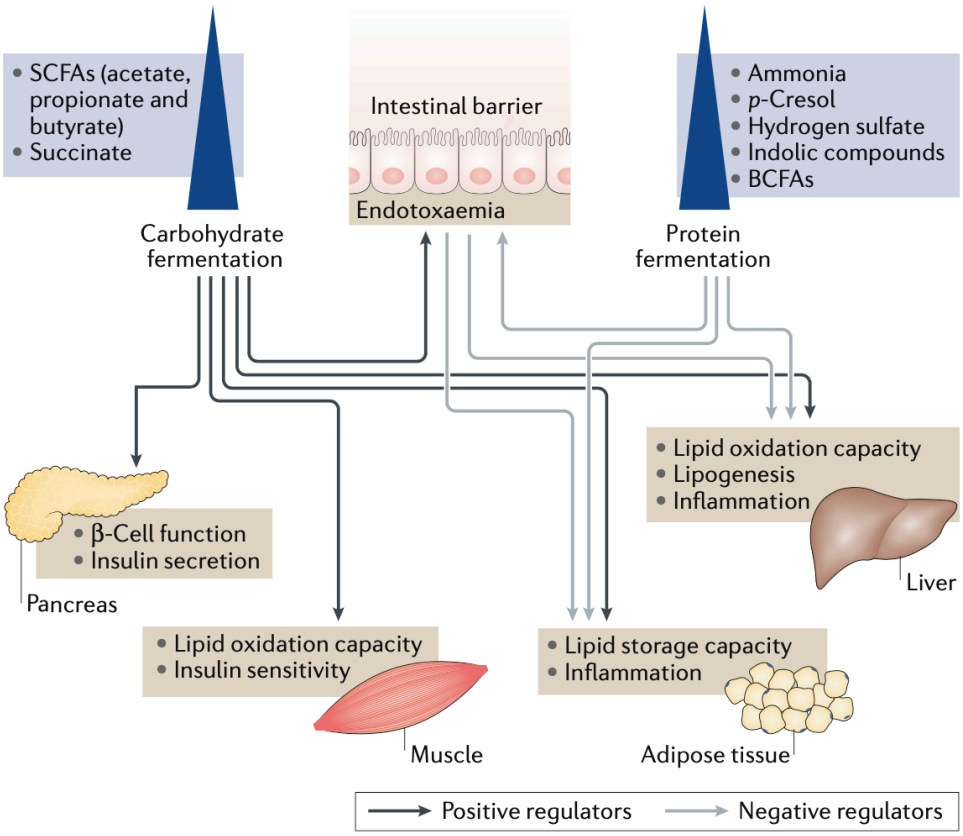


Figure 7. Tirée de Canfora EE, Meex RCR, Venema K, Blaak EE. Gut microbial metabolites in obesity, NAFLD and T2DM. Nat Rev Endocrinol. 2019;15(5):261-73. La relation entre les métabolites dérivés de la fermentation des protéines et des glucides et l'interaction inter-organes avec la résistance à l'insuline et le diabète de type 2.

Outre les AA, d'autres composés provenant des protéines alimentaires peuvent jouer un rôle sur la santé cardio-métabolique. Par exemple, le triméthylamine (TMA), produit par le microbiote intestinal à partir de la phosphatidylcholine, la choline, la L-carnitine et la bétaïne retrouvés principalement dans la viande rouge, est métabolisé en triméthylamine N-oxide (TMAO) par le foie, un métabolite associé au développement d'athérosclérose et à la survenue de maladies cardiovasculaires [217].

3.2 Les prébiotiques, plus particulièrement les polyphénols

Les aliments de sources végétales sont reconnus pour contenir différentes molécules ayant des bénéfices pour la santé, notamment les fibres alimentaires. Ces fibres ne peuvent être digérées sans l'aide de microorganismes, principalement au niveau du colon, elles

favorisent donc certaines bactéries et sont les prébiotiques les plus connus. La définition d'un prébiotique, provenant et traduite du consensus de l'association scientifique internationale pour les probiotiques et prébiotiques (ISAPP) est : « un substrat qui est utilisé sélectivement par des microorganismes de l'hôte, conférant un avantage pour la santé » [218]. Dans cette catégorie, en plus des oligosaccharides, de certains types d'acides gras, on retrouve également les composés phénoliques et phytochimiques, dont les polyphénols font maintenant partie.

Les polyphénols sont des antioxydants qu'on retrouve surtout dans les fruits et légumes, ainsi que dans le thé, le vin et le chocolat. De nombreuses études ont démontré leurs bienfaits par association [219] ou par supplémentation à l'aide de modèles précliniques [220-223] et d'études cliniques [224, 225]. L'impact favorable des polyphénols sur la santé métabolique serait médié par différents mécanismes, certains dépendants du microbiote intestinal (Figure 8) [226, 227].

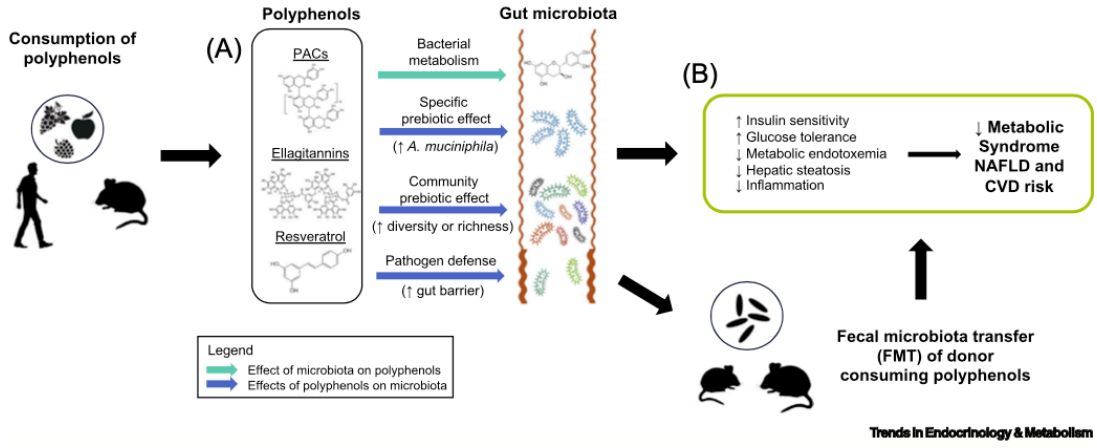


Figure 8. Tirée de Anhe, F.F., Choi, B.S.Y., Dyck, J.R.B., Schertzer, J.D. & Marette, A. Host-Microbe Interplay in the Cardiometabolic Benefits of Dietary Polyphenols. Trends Endocrinol. Metab. 30, 384-395 (2019). Modifications du microbiote intestinal médiées par les polyphénols et avantages associés au métabolisme. (A) Le métabolisme des polyphénols par les bactéries produit des métabolites et influence la composition et la fonction des bactéries du microbiote intestinal. (B) Les effets bénéfiques cardio-métaboliques de la consommation de polyphénols sont reproduits dans des souris colonisées avec le microbiote fécal des souris qui ont consommé les polyphénols

Tout d'abord ce sont des antioxydants, c'est-à-dire qu'ils permettent de réduire les dommages liés aux radicaux libres [228]. De plus, certains polyphénols peuvent se lier à des composés, comme le mercure, et empêcher leur biodisponibilité et leur absorption, donc favoriser leur excrétion [229].

Lors de leur fermentation par le microbiote intestinal, les polyphénols seront catabolisés et transformés pour produire des composés absorbables au niveau de l'intestin. Ces métabolites peuvent alors avoir des effets sur différents tissus [230]. Par exemple, les valérolactones sont les métabolites les plus communs retrouvés du métabolisme des proanthocyanidines (PAC) et contribuent aux bénéfices de ces derniers sur la santé [226].

Les polyphénols favorisent l'augmentation de la diversité et la richesse de la communauté bactérienne qui compose le microbiote intestinal et peuvent également favoriser des bactéries spécifiques ayant des propriétés bénéfiques, tels que *Akkermansia muciniphila* [220, 231]. Finalement, ils peuvent contribuer à la stimulation de l'intégrité de la barrière intestinale comme ils sont aussi des composés anti-inflammatoires et certains d'entre eux ont des propriétés antimicrobiennes, qui peuvent protéger contre l'établissement de microorganismes néfastes comme *Helicobacter pylori,* qui est à l'origine d'ulcères [226].

La démonstration des effets bénéfiques des polyphénols sur la santé métabolique par leur modification du microbiote a été démontrée à l'aide de protocoles de transplantation fécale [227], notamment d'un extrait riche en PAC et en ellagitannins provenant de camu camu [232] ainsi que des fractions de PAC et d'anthocyanines de bleuet [223].

3.3 Les probiotiques et les psychobiotiques

Outre les prébiotiques, l'autre catégorie de modulateurs de santé intestinale la plus documentée est celle des probiotiques. Définis comme des « microorganismes vivants qui, lorsqu'administrés en quantités adéquates, confèrent un avantage pour la santé de l'hôte » [233], de nombreux probiotiques sont disponibles en vente libre dans les pharmacies et retrouvés dans des produits alimentaires comme des yogourts, des jus, etc. L'utilisation des probiotiques est variée, que ce soit pour les troubles de digestion ou encore pour contribuer

au traitement de désordres métaboliques et il est important de rappeler que l'effet d'un probiotique est spécifique [233].

En contexte métabolique, les modes d'actions des probiotiques sont principalement une modulation du métabolisme énergétique, une meilleure barrière intestinale, une diminution de l'inflammation ainsi que des interactions avec le microbiote intestinal (Figure 9) [234].

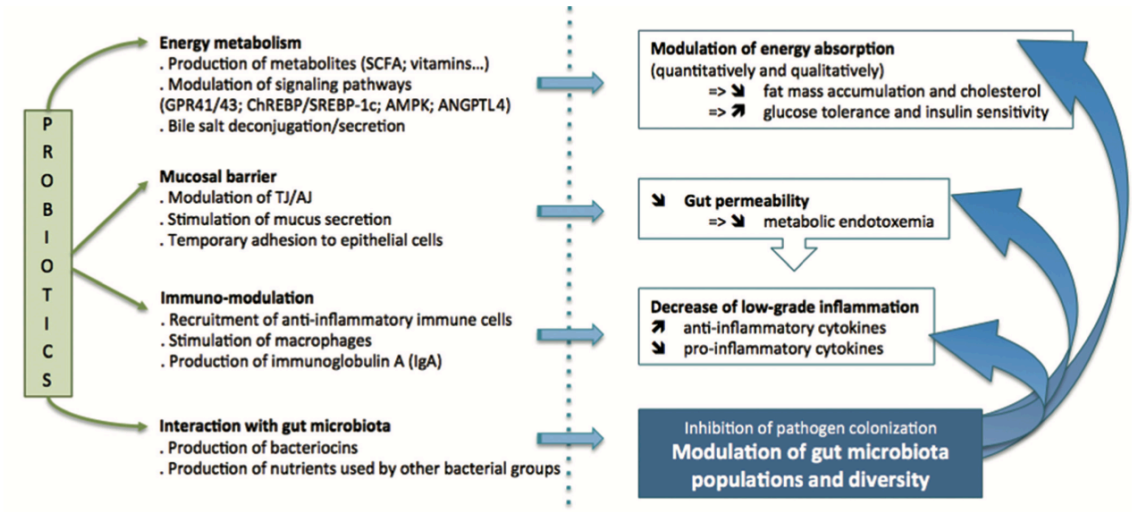


Figure 9. Tirée de Le Barz, M., *et al.* Probiotics as Complementary Treatment for Metabolic Disorders. *Diabetes Metab. J.* **39**, 291-303 (2015). Effets bénéfiques potentiels de la supplémentation en probiotique contre les désordres métaboliques.

Au niveau du métabolisme énergétique, les interactions des probiotiques peuvent se faire à travers la production de métabolites ayant un impact sur la prise alimentaire, tels que les SCFA [235], moduler certaines voies de signalisation [236] ou encore modifier la transformation secondaire des acides biliaires et augmenter leur excrétion [237].

De plus, les probiotiques peuvent agir au niveau de la modulation de la barrière intestinale [238], dont l'immunité intestinale, permettant une diminution de l'inflammation et même de translocation bactérienne [239]. Certaines souches peuvent induire une augmentation des jonctions serrées entre les cellules épithéliales [240] ou encore avoir une activité anti-inflammatoire à travers la protéine *Nucleotide-binding oligomerization domain 2*, NOD2 [241]. De plus, parfois le probiotique entier n'est pas nécessaire, puisqu'une fraction de la paroi cellulaire bactérienne, le muramyl dipepdide (MDP) est suffisante [242] et a des effets significatifs sur le développement de l'obésité et de la résistance à l'insuline. De

façon similaire, la protéine membranaire Amuc_1100, provenant de *Akkermansia muciniphila*, aurait des effets bénéfiques sur la barrière intestinale et expliquerait, en partie, les effets bénéfiques de la bactérie [148]. De ce fait, une version pasteurisée de la bactérie serait plus sécuritaire et facile à administrer sous forme de suppléments ou d'ingrédient dans certains aliments.

Un autre mode d'action des probiotiques est une interaction avec le microbiote intestinal commensal [243], mais il a également été montré que des probiotiques peuvent avoir des effets importants sans coloniser l'intestin ou générer des changements majeurs au niveau de la composition du microbiote intestinal [244]. L'impact sur l'épigénome du microbiote intestinal étant de plus en plus documenté, le cas particulier des changements épigénétiques induits par les probiotiques pourrait également expliquer certains des phénotypes observés [245, 246]. Ces changements auraient un impact sur l'individu qui consomme le probiotique, notamment sur l'immunité intestinale [247], mais étalement au niveau intergénérationnel, tel que la susceptibilité à développer l'obésité [248].

Plus récemment, le concept de psychobiotiques a également émergé, initialement limité aux probiotiques, qui, lorsqu'ingérés « confèrent un effet bénéfique sur la santé mentale à travers une interaction avec les bactéries commensales de l'intestin », mais a été mis à jour pour également inclure certains prébiotiques, [249] et symbiotiques, postbiotiques, aliments fermentés etc. [250]. Les psychobiotiques représentent de nouvelles avenues thérapeutiques [250] dans une multitude de contextes, dont des désordres neuropsychiatriques comme le stress et la dépression [251] et des maladies neurodégénératives [252].

La prochaine et dernière section de l'introduction sera sous forme de l'intégration d'un article conceptuel rédigé et publié pendant mon doctorat. Cette revue de littérature porte sur les applications thérapeutiques potentielles du microbiote intestinal, en particulier deux exemples, soit l'impact sur les comportements alimentaires et l'humeur ainsi que la diminution de la contamination corporelle de polluants lipophiles.

4. Potential therapeutic applications of the gut microbiome in obesity: from brain function to body detoxification

Béatrice S.-Y. Choi[1,2] Laurence Daoust[1,2] Geneviève Pilon[1,2] André Marette[1,2] and Angelo Tremblay[1,2,3]

1: Quebec Heart and Lung Institute, and Department of Medicine, Université Laval, Québec, QC, Canada

2: Institute of Nutrition and Functional Foods, Université Laval, Québec, QC, Canada

3: Department of Kinesiology, Université Laval, Québec, QC, Canada

These authors contributed equally: Béatrice S.-Y. Choi, Laurence Daoust

Corresponding author : Angelo Tremblay angelo.tremblay@kin.ulaval.ca

Adapté pour des raisons de format de l'article suivant [253] :

Choi, B.SY., Daoust, L., Pilon, G. *et al.* Potential therapeutic applications of the gut microbiome in obesity: from brain function to body detoxification. *Int J Obes* **44,** 1818–1831 (2020). https://doi.org/10.1038/s41366-020-0618-3

Résumé

La prévalence de l'obésité augmente chaque année et les comorbidités associées, telles que les maladies cardiovasculaires, figurent parmi les principales causes de décès dans le monde. Le microbiote intestinal est récemment apparu comme une cible potentielle pour des applications thérapeutiques visant à prévenir et à traiter ces comorbidités. Dans cette revue, nous nous concentrons sur trois conditions liées à l'obésité pour lesquelles l'utilisation de modulateurs du microbiote intestinal pourrait être bénéfique : les troubles de l'humeur, les comportements alimentaires et la détoxification de polluants organiques persistants (POP). D'une part, la modulation des signaux dérivés de l'intestin vers le cerveau dans un contexte d'obésité est impliquée dans le développement de la neuroinflammation et peut ensuite altérer les comportements. Un microbiote intestinal altéré pourrait modifier ces signaux et en atténuer les conséquences. D'autre part, l'obésité est associée à une accumulation accrue de contaminants lipophiles, tels que les POP. Cibler le microbiote

pourrait aider à la détoxication de l'organisme en réduisant leur biodisponibilité, en améliorant leur dégradation par bioremédiation ou leur excrétion par la circulation entéro-hépatique. Ainsi, une supplémentation en prébiotiques, probiotiques ou synbiotiques pourrait représenter une stratégie complémentaire aux stratégies actuelles, telles que les médicaments et les modifications du mode de vie, pour réduire la dépression, modifier les comportements alimentaires et diminuer la charge corporelle de polluants, compte tenu de l'épidémie d'obésité à laquelle notre société est confrontée.

Abstract

The prevalence of obesity is rising every year and associated comorbidities such as cardiovascular diseases are among the leading causes of death worldwide. The gut microbiota has recently emerged as a potential target for therapeutic applications to prevent and treat those comorbidities. In this review, we focus on three conditions related to obesity in which the use of gut microbiota modulators could have benefits; mood disorders, eating behaviors and body detoxification of persistent organic pollutants (POPs). On one hand, modulation of gut-derived signals to the brain in a context of obesity is involved in the development of neuroinflammation and can subsequently alter behaviors. An altered gut microbiome could change these signals and alleviate their consequences. On the other hand, obesity is associated with an increase accumulation of lipophilic contaminants, such as POPs. Targeting the microbiota could help body detoxication by reducing bioavailability, enhancing degradation by bioremediation or their excretion through the enterohepatic circulation. Thus, a supplementation of prebiotics, probiotics or synbiotics could represent a complementary strategy to current ones, such as medication and lifestyle modifications, to decrease depression, alter eating behaviors and lower body burden of pollutants considering the actual obesity epidemic our society is facing.

Introduction

Obesity research and clinical practice have significantly enriched knowledge and skills pertaining to obesity management over the last decades. These conceptual gains have however not been sufficient to reverse or even stabilize the prevalence of obesity in most countries of the world. One potential explanation of this growing gap between obesity-

related understanding and its growing prevalence is partly related to the traditional non-consideration of some relevant mechanisms and determinants. This is the case for the profile of microbiome, which has been shown to differ between normal-weight animals or humans and those displaying obesity [45]. In fact, gut microbiota transplant experiments have confirmed that an unfavorable microbial profile may promote obesity [48]. Beyond its impact on energy balance and metabolic regulation, it appears that the microbiome is involved in variations of non-traditional determinants of obesity such as food and emotion-related behaviors as well as body chemical pollution [254, 255]. In this paper, we discuss recent research observations that document a relationship between these factors and the profile of the gut microbiome. Additionally, relevant literature is reviewed with the intent to derive clinical applications in the management of obesity.

Novel therapeutic applications targeting gut microbiota to reduce obesity and associated comorbidities

Dietary interventions and the use of nutraceuticals to modulate the composition and function of the gut microbiota has gained in popularity as non-invasive, with minimal secondary effects to achieve weight loss or lower comorbidities associated to obesity. Dietary interventions that are subjected to extensive research include supplementation with either prebiotics, probiotics, synbiotics or postbiotics. The definition of prebiotics has been reconsidered in 2016 by an expert panel as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” [218]. This new consensus statement includes different types of oligosaccharides, but also recognizes phenolic and phytochemical compounds as prebiotics [218]. Indeed, many groups have demonstrated the positive impact of polyphenols on metabolic health through gut microbiota-dependent mechanisms [227]. More recently, studies have focused on the effect of specific types of polyphenols that could be extracted from polyphenol-rich foods, purified and concentrated to maximise their effects. High molecular weight polyphenols such as proanthocyanidins gained a lot of attention because of their low availability and therefore their ability to reach the colon and modulate the composition of the gut microbiota [256]. Other candidates are ellagitannins and ellagic acid that can be metabolized by some bacterial species in urolithin A, which is strongly associated with protection against cardiometabolic risk factors [257].

Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”[233]. The use of probiotics for different purposes has enhanced over the last years, since their positive impacts on metabolic health have been demonstrated many times. The use of probiotics in a context of obesity, either in preclinical or in clinical studies, has been shown to exert a positive effect by decreasing global inflammation through various mechanisms such as decreasing gut permeability to reduce metabolic endotoxemia [234]. More recently, a promising therapeutic approach involving genetically engineered bacteria (GEB) has emerged. The use of these GEB is also of great interest for their ability to bypass digestion and secrete drugs or therapeutic factors at the site of absorption. One notable example is the work of Chen et al. who have administered *E.coli Nissle 1917* bacteria expressing N-acyl-phosphatidylethanolamines to high-fat (HF) fed mice and observed reduced food intake and body weight gain [258]. Another way to enhance the effectiveness of both pre and probiotics is to combine them with each other to increase their beneficial potential on host health, a combination that has been coined synbiotics [259].

Numerous studies have demonstrated the role of *Akkermansia muciniphila* in preventing obesity-associated disorders in both humans and animal models [260-262]. Lately, two research teams highlighted potential mechanisms by which *Akkermansia muciniphila* promotes a healthy metabolic profile, through the protein Amuc_1100 and extracellular vesicles. Plovier et al. showed that the administration of the pasteurization form of this bacteria in high-fat high-sucrose (HFHS) fed mice can not only replicate the known protective effects associated with the administration of the live bacteria but in some case can also enhance them [263]. More interestingly, they reproduced the same results by isolating a single protein from the outer membrane of this bacteria, Amuc_1100, that is not affected by pasteurization. The activation of Toll-like receptor 2 by Amuc_1100 has been proposed as a potential signaling pathway contributing to the beneficial effects of this protein [263]. In a similar way, *Akkermansia*-derived extracellular vesicles were shown to enhance the beneficial effects associated with the administration of the live form of this bacteria, particularly on body weight gain [264]. These results strongly suggest that the

viability of a given bacterium is not the only criterion to define its health benefits to the host. This leads to the concept of postbiotics, which comprise microbial metabolites derived from host-microbial interactions (enzymes, lipids, proteins) and bacterial cell components (e.g. peptidoglycan, polysaccharides, lipoteichoic acids), that are now considered as an alternative to the use of live bacteria [263, 265]. Moreover, the growing interest associated with the use of *Akkemansia muciniphila* has led to other important findings. Indeed, the beneficial effect on glucose homeostasis of metformin, which is known as the most prescribed antidiabetic drug worldwide [266] has been associated to the abundance of *Akkermansia* spp. in HF fed mice [267] . Increased *Akkermansia muciniphila* abundance was also detected in diabetic patients taking metformin [268]. Both studies suggest that the effects are mediated by the gut microbiota and dependent on the presence and abundance of this bacterial genus. This concept has however been recently challenged by a study demonstrating that the beneficial effects of metformin are not dependent on the presence of the gut microbiota. In both GF and antibiotic-treated mice fed a HFD, the administration of metformin was associated with an anti-inflammatory response [269].

Fecal microbiota transplantation (FMT) is now getting more attention as it is the most effective treatment for recurrent *C. difficile* infection [270]. It is now considered as potential treatment in many diseases involving gut microbiome dysbiosis such as neurological, autoimmune, infectious and gastrointestinal disorders [271]. As obesity and cardiometabolic diseases are also associated with a dysbiosis, FMT as a possible treatment is now getting more consideration [272]. Although no change in body mass index (BMI) has been observed in most studies [273], receiving a FMT from a lean donor has been shown to improve insulin sensitivity in obese patients [274], but responses were transient in nature, highly variable and dependent of basal gut microbiota of receiver patients. These results may be very encouraging but a few concerns still remain, especially in terms of regulatory and safety issues [270], thus limiting the implementation of this procedure for clinical use. Gundling et al. [275] recently reported that around two thirds of the obese patients that were consulted through a questionnaire were willing to undergo FMT, provided that it was from a healthy and anonymous donor, which points towards the increased willingness of the patients to consider microbiome-derived therapeutic options.

Bariatric surgery is another approach used to treat obesity-related comorbidities. The main goal of these restrictive or malabsorptive surgeries is to induce drastic weight loss and therefore induce a better global health. It is actually an effective way for remission of type 2 diabetes, which explains why they are now being called metabolic surgeries [276]. It has been reported that metabolic improvements associated with those surgeries might be driven at least in part by the gut microbiota [162, 277]. Although this approach does not primarily targets the gut microbiome, it has an important effect on the microorganisms themselves since it impacts the digestive system and therefore the microbial environment [278]. It should be kept in mind that the gut microbiota might play a key role as a mediator of the benefits of bariatric surgery and therefore should be more studied.

Gut-brain axis and its impact on behaviors

Mechanism of action of the gut-brain axis in a context of obesity

The weight gain associated with the consumption of a diet enriched in fat and sugar has been linked with many metabolic disorders such as type 2 diabetes, dyslipidemia, non-alcoholic liver diseases and cardiovascular diseases. The low-grade chronic inflammation state that characterizes obese patients has an important causal role in the development of those disorders [279]. However, this inflammatory state is not limited to the peripheral tissues and the circulatory system but is also induced in different regions of the brain known as neuroinflammation. This process is strongly associated with neuronal damages [280]. Following the consumption of a high-fat diet (HFD), neuroinflammation appears long before subsequent weight gain [281]. It has been showed that a 24h exposition to a HFD (60% from fat) in mice is sufficient to induce an inflammatory response in the hypothalamus, as it is also observed in other metabolically active tissues including the gut, the liver and the white adipose tissue [282]. Transfer of gut-derived inflammatory signals to the hypothalamus through vagal afferents is probably involved in the onset of inflammation. Indeed, celiac vagotomy in 24h HF-fed mice prevents the increased mRNA expression of inflammatory markers (IL-6, TNF-α, Iba1) in the hypothalamus as compared to their sham counterparts [282]. Increased permeability of the blood-brain barrier (BBB) in obese mice models could also potentially contribute to the infiltration of immune cells

in different brain regions, eventually leading to behavioral issues [283]. The administration of an inhibitor of the protein kinase Cβ which is known to reduce BBB permeability in genetically obese mice prevents memory deficits to a similar extent as wild-type mice [283]. Thus, in both rodent animals and humans, neuronal injuries in the hypothalamic and mesolimbic dopamine system areas caused by the consumption of an HFD can have important consequences. Indeed, these regions of the brain are particularly sensitive to the impact of an obesogenic diet because of their implication in energy intake and body weight regulation [281, 284].

A growing body of evidence suggested an important bi-directional connection between the gut microbiota and the brain implicated in various neurological processes [285, 286]. However, the underlying mechanisms implicated in the regulation of brain functions by the gut microbiota are still poorly understood. It is however suggested that this relationship is in part mediated through gut-derived microbial metabolites [287]. Bacteria can generate and regulate multiple neuroactive compounds including gamma-aminobutyric acid (GABA) [288], catecholamines (norepinephrine and dopamine) [289], and serotonin [290] which can then influence many neurological processes. For instance, *Bacteroides* ssp. can produce important amount of GABA. Using functional magnetic resonance imaging and 16S rRNA sequencing, Strandwitz et al. showed a negative correlation between fecal levels of *Bacteroides* and brain characteristics associated with major depressive disorders (MDD) in 23 patients suffering from these mood disorders [288].

Short-chain fatty acids (SCFAs) (the most abundant: butyrate, propionate, acetate) that are produced by colonic bacteria through fermentation of carbohydrates have also been shown to act as important mediators of the gut-brain axis [133]. SCFAs do not only influence energy homeostasis through their well-documented ability to promote anorexigenic hormones but can also act directly on the central nervous system by crossing the BBB [134]. Frost et al. detected the presence of $^{13}C_2$ acetate derived from colonic fermentation of inulin in different brain regions in mice, but at higher concentrations in the hypothalamus, a region known for its implication in food intake regulation. The direct effect of acetate on hypothalamic acetyl-CoA carboxylase and AMP-activated protein

kinase activities and subsequent change of the Agouti-related peptide expression has been proposed as a potential mechanism for the reduced weight gain and energy intake observed in mice fed a HFD and supplemented with inulin, independently of the secretion of gut hormones [134].

Activation of patterns recognition receptors (PRR) in the brain by microbial products has also emerged as a pathway connecting the gut and the brain. Bacterial peptidoglycan (PNG) derived from the gut microbiota can cross the BBB and be recognized by the PNG-recognition proteins and nucleotide oligomerization domain (NOD)-like receptors under normal conditions. Altered social behaviors were observed in mice lacking the PGN-recognition protein 2, a PRR that recognizes PNG [291]. This pathway could be interesting to explore in a context of obesity-associated neuropsychiatric disorders.

Implication of the gut-brain axis in mood disorders

Obesity has been associated with increased risk of developing MDD. The inverse relationship which suggests that patients suffering from MDD are at risk of developing obesity is also true. However, data supporting this association are less consistent [292]. Growing body of evidence suggests that change in the composition and function of the gut microbiota is involved in the development of neuroinflammation and subsequent mood disorders [293, 294]. In normal weight individuals, multiple studies have demonstrated that patients with MDD have an altered gut microbiota composition [285, 286, 295, 296]. Recently, Valles-Colomer et al. identified two strains of bacteria which were found to be depleted in people suffering from depression, *Dialister* and *Coprococcus* spp [285]. Lin et al. identified *Prevotella* and *Klebsiella* porportions of the fecal microbial communities as important markers to be considered in the diagnosis and treatment of MDD [286]. Decreased relative adundance of *Faecalibacterium* has also been linked to MDD [285, 295] Yet, there is no consensus on the gut microbiota profile associated with those disorders. An overall imbalance of the microbial communities might be more representative of what is observed in MDD patients than an altered abundance of specific taxa [297].

To demonstrate the causal role of the gut microbiota in the onset of those disorders, studies involving FMT have been performed in germ-free (GF) mice [293, 298]. The absence of a bacterial community in these models has been associated with reduced depression-like behaviors compared to conventional mice, suggesting a strong implication of the gut microbiota in the development of these behaviors [293]. Humanized FMT from depressed patients to GF mice showed increased depression-like behaviors assessed by different behavioral tests only one week following the FMT [293]. Mechanistically, an impaired carbohydrate and amino acid metabolism by the microbiome and host metabolites have been observed in humanized depressed mice [293]. Using comparative metaproteomics analysis, Chen et al. found a disturbed bacterial proteins pathway of the fecal microbiome related to glucose metabolism, amino acid metabolism and carbon metabolism in patients suffering from MDD compared to healthy controls [297]. These results highlight the altered functions of the microbiome associated with behavioral disorders.

Both MDD and obesity are characterized by an inflammatory component. In an animal model, the consumption of a HFD has been associated with a depression-like phenotype accompanied with increased Firmicutes to Bacteroidetes ratio [287]. Dysbiosis is associated with increased production of bacterial endotoxins lipopolysaccharides (LPS) in association with increased gut permeability, promoting systemic inflammation [166]. Transplantation of a HF shaped microbiota to non-obese conventional-housed mice by antibiotic microbiota depletion impaired behavioral performances in relation with disrupted intestinal tight junction proteins and circulating endotoxin. These results point toward a role of the imbalance of gut microbiota in promoting neuroinflammation through increased intestinal permeability [294].

Many other potential mechanisms involving the gut-brain axis have also been proposed (Figure 1). The microbiome has been shown to modulate the expression of miRNA in the hippocampus thereby playing a role in regulating gene expression [299]. Impaired insulin sensitivity in the brain of obese mice could also trigger depressive-like behaviors through a gut-derived mechanism [300]. Disruption of the gut microbiota in HFHS-fed mice upon treatment with vancomycin or metronidazole showed reduced anxiety and stress behaviors

associated with increased insulin sensitivity in the brain [300]. Additionally, there is evidence suggesting impaired BBB functions in depressive disorders [301]. In animal models, the consumption of a western diet is associated with disrupted hippocampal BBB permeability [302, 303]. Based on the concentration of sodium fluorescein in different regions of the brain, Davidson et al. showed impaired integrity of the BBB in the hippocampus of high energy diet-induced obese rats as compared to their obesity-resistant and chow-fed counterparts [303]. Interestingly, the gut microbiota can also influence the permeability of the BBB promoting inflammatory mediators in the brain. The expression of tight junction proteins (e.g. occludin, claudin-5) that act as a structural basis for the BBB was significantly reduced in GF mice compared to conventional mice. Monocolonization of these mice with SCFA-producing bacteria (*C.tyrobutyricum* or *B.Thetaiotaomicron*) was associated with reduced BBB permeability to a level similar as conventional mice [304].

Implication of the gut-brain axis in eating behaviors

Recent evidence suggests that bacteria populating the gut could manipulate host eating behaviors through different pathways leading to body weight gain [305] (Figure 10). Nutrients that are absorbed can generate multiple signals throughout the entire intestinal track that will communicate with the brain. The low-grade chronic inflammation associated with diet-induced obesity could interfere with this highly regulated process promoting hyperphagia and cravings for energy-dense foods. Hence, altered gut-brain signaling through nutrient sensing in the gut could be implicated in the onset of obesity. Decreased expression of fatty acid receptors (GPR40, GPR41, GPR43, GPR120) and satiety peptide hormones (GLP-1, PYY, CCK) have been observed in GF mice. Indeed, these mice showed increased preference for fat and increased caloric intake [306]. Using a germ-free mice model, Swartz observed an increased mRNA and protein expression of the protein type 1 taste receptor 3, a receptor implicated in the detection of the sweet stimuli, and of the Na-glucose luminal transporter-1 in the small intestine. However, these mice did not show increased preference for a sugary solution [307] suggesting that postoral mechanisms are in place to stimulate the «absolute intake of carbohydrate» defined as the acceptance [308]. Overall, these results suggest that the absence of a microbe in the intestine affects signaling pathways implicated in nutrient detection, potentially involved in eating behaviors.

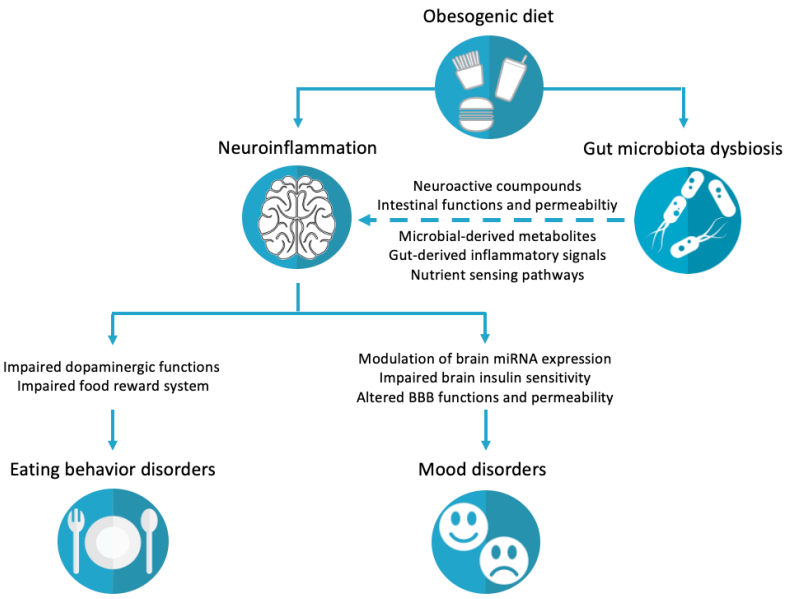


Figure 10. Potential factors modulated by the gut microbiota and implicated in mood disorders and eating behaviors

Eating behaviors are regulated through homeostatic and hedonic feeding circuits. The hypothalamus has been recognized for its implication in homeostasis regulation of food intake whereas the mesolimbic dopamine system is strongly associated with food seeking behaviors, characterized as the non-homeostasis regulation of feeding. The latter includes behaviors associated with motivation, reinforcement and hedonia [309]. This process is, among others, dependent on dopamine neurons in the ventral tegmental area and on the nucleus accumbens (NAcc). Therefore, disruption of dopaminergic functions could lead to food overconsumption and obesity [284, 310]. In animal models, an obesogenic diet has been associated with reduction of gene expression of dopamine transporter (DAT) [311] and dopamine receptor 2 (DR2) in the brain [284, 311]. Using a reversal approach, mice switched from an HFHS diet to a chow diet supplemented with fructo-oligoasaccharides completely reversed the decreased expression level of DAT and DR2 [311]. Moreover, the supplementation of normal weight mice with the glycoside Rebaudioside A (Reb A), a molecule strongly contributing to the sweet taste of stevia, was associated with reduced mRNA expression levels of DAT and tyrosine hydroxylase in the NAcc. An alteration of the gut microbiota composition was also observed in mice supplemented with Reb A [312]. These results strengthen the concept that the gut microbiota is regulating central dopaminergic functions which is of high relevance in the context of obesity.

An alteration of the food reward system might also contribute to the increased energy intake leading to obesity by increasing preferential consumption of palatable energy-dense foods to reach the anticipated satisfaction [313]. In obese patients, a food reward assessment based on a smart phone application showed less pleasure associated with the consumption of food, supporting the notion of a reward-deficiency [314]. Using a long-term (12h) two-bottle preference test, HFD-induced obese mice displayed lower intake of the 1% sucrose solution due to their low ability to detect low concentration of sweet solutions as compared to control mice fed a chow diet [313]. The supplementation of these mice with inulin-type fructans reversed the low intake of the sucrose solution observed to a level similar to that seen in chow-fed mice suggesting that modulation of the gut microbiota can improve the sensory deficit and thereby the satisfaction associated with the consumption of palatable foods [313]. In humans, Rezzi et al. showed that microbial derived metabolites in the urine of people characterized as having «chocolate desiring preferences» were clustering differently than humans being indifferent to these preferences [315]. Hence, an altered microbiome in obese people could promote choice for highly palatable foods to compensate for the sensory deficit and reach the desired satisfaction [313].

Other approaches that can strongly influence the composition of the gut microbiota such as bariatric surgery have also been associated with the reduction of hedonic eating. Postoperative gut microbiota profile of obese women that underwent laparoscopic sleeve gastrectomy was linked with beneficial change in hedonic behaviors [316]. Indeed, participants showed lower preferences for high-caloric food one-month post-surgery. Interestingly, the bacterial genus *Akkermansia* which has been strongly associated with a healthy metabolic profile [261] was inversely correlated with the postoperative desires for sweet [316]. Considering that the bacterial population is mostly influenced by the nutrients consumed by the host, bacteria are thus also dependent on human behaviors. It has been suggested that decreased bacterial diversity would allow some bacteria to spend more resources on modulating eating behaviors by generating cravings to promote their own fitness [305].

Potential treatment of obesity-associated cognitive impairment targeting the gut microbiota

Considering the importance attributed to the composition of the gut microbiota in obese patients suffering from behavioral issues, targeting the gut microbiota using prebiotics, probiotics, symbiotics or postbiotics could represent an important strategy to prevent or treat food and/or emotion-related behaviors. Prebiotics have been shown to have anxiolytic and antidepressive-like effects in a normal weight animal model exposed to chronic social stress [317]. In humans, evidence is less consistent. Obese women suffering from MDD submitted to a 25% calorie-restricted diet and supplemented with 10g/day of inulin showed no effect of the prebiotic supplementation on MDD-associated behaviors. Weight loss achieved by caloric restriction was the main predictor of improved behaviors [318]. As for probiotics, it has first been proposed that they could be used as a complementary therapy in patients suffering from MDD [319], a concept that has evolved with time and led to the creation of a new term: psychobiotic. The latest is defined as « a live organism that, when ingested in adequate amount, produces a health benefit in patients suffering from psychiatric illness» [320]. Probiotics, mainly *Bifidobacterium* and *Lactobacillus* species, have been shown to protect against depressive like-behaviors in a HF-fed rat model of depression [321]. In obese women undergoing a two-phase weight reduction program, supplementation with *Lactobacillus rhamnosus* CGMCC1.3724 resulted in higher body weight loss and was associated with increased satiety efficiency (represented by the efficiency to suppress desire to eat), decreased food craving and reduced depressive symptoms [254]. However, these beneficial effects were not observed in obese men undergoing the same weight loss program, suggesting a potential sex-specific response to the treatment [254].

Additionally, targeting specific microbial-derived metabolites could also represent a promising strategy to address behavioral issues associated with obesity. In an exploratory study, Osadchiy et al. demonstrated an association between indole metabolites (indoles, indoleacetic acid, skatole) derived from gut tryptophan and functional and anatomical connectivity of different brain regions implicated in the regulation of non-homeostatic control of feeding. These results strengthen the concept that gut-derived metabolites could

act through a gut-brain axis to modulate hedonic food intake and obesity [322]. In obese mouse models and patients, indole metabolites have been shown to protect against obesity associated disorders [323, 324]. The consumption of a HFD can induce a shift in tryptophan metabolism in favor of the kynurenine pathway through modulation of the activity of indoleamine-2-3 dioxygenase 1 (IDO1), an enzyme responsible for 90% of tryptophan catabolism [323]. In an obese mouse model, the deletion of IDO1 was associated with improved insulin sensitivity, reduced endotoxemia and blunted inflammatory markers [323]. In humans, incidence of type 2 diabetes is inversely correlated with serum concentration of indolepropionic acid [325]. It is still too early to conclude on the implication of gut-derived tryptophan metabolites in the control of non-homeostatic feeding in obese patients, but this certainly warrants further investigation. Overall, few studies have addressed the effect of gut microbiota modulators on obesity associated mood disorders and eating behaviors, but the perspectives offered by some investigations are sufficiently promising to justify further relevant research.

Finally, the therapeutical applications of the gut microbiota in obesity are not only restricted to the treatment of behavioral issues through a gut-brain axis but include a wide range of other well documented beneficial applications, such as supplementation with prebiotics and probiotics [227, 234, 259, 326], whereas the potential xenobiotic detoxifying capacity of the gut microbiota remains an untapped field with great potential [327].

Body detoxication of persistent organic pollutants (POPs)

POPs & obesity

Persistent organic pollutants (POPs) are environmental contaminants that can affect several organs and systems and that are considered dangerous for human health and wildlife. Some of them are complex molecules, that bioaccumulate over time, bio-magnify along the food chain and are liposoluble. Organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) & dioxins, such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD) are the most common categories of POPs and are

chemicals that were mostly used as pesticides, cooling and isolating fluids, flame retardant or that are bi-products of industrial processes.

Human exposition occurs mostly through consumption of contaminated food mainly from animal origin such as fish, dairy products, eggs and meat [328]. These compounds accumulate in adipose tissues [2, 329] and can stay in the organism for decades since they are highly resistant to degradation [2, 330]. POPs are known endocrine disruptors, meaning they can interfere with hormonal response, which explains why they can affect reproduction, have been associated to certain types of hormone-dependent cancers, such as breast and prostate cancer [331, 332], and can disrupt metabolic processes [333]. It should also be kept in mind that some populations are more at risk of exposure, such as northern communities, since they consumed higher quantities of potentially contaminated foods, such as fish, which could play a role in the higher prevalence of metabolic diseases in these communities [334].

The liposolubility of POPs is an important issue in obesity since excess body fat allows more POPs to accumulate in the body. Many studies including narrative reviews have reported the contribution of POPs to the development of obesity and impaired glucose metabolism [2, 49, 335, 336]. In case of weight loss or insulin resistance, associated with increased lipolysis, pollutants stored in adipose tissue are released into the circulation [49]. Many studies showed the increase in circulating POPs after weight loss, whether it is through dietary intervention or bariatric surgery [337], which can trigger levels exceeding health based guidelines [338]. Although beneficial effects of weight loss on cardiometabolic health are well established, harmful effects of POPs released at the same time are also possible [339, 340]. Some of these undesirable effects of weight loss such as decrease in oxidative capacity of skeletal muscle [341], total triiodothyronince (T_3) concentration [342], and resting (RMR) [342] and sleeping (SMR) metabolic rate [343] could mitigate the positive impacts of weight loss on cardiometabolic health and promote weight regain [49, 340]. To lower total body burden, lipectomy could be an alternative in obese patients, since the POPs stored in the removed adipose tissue would not be able to be released in circulation anymore. Although reducing total body burden, lipectomy would

not change concentration of POPs in adipose tissues and could increase risks related to future POPs exposure as it would lower storage capacity.

Another important aspect of POPs on health is their capacity to impact reproduction as well as future generations. They can be transmitted through the placenta and breastfeeding [344] and can impact the epigenome signatures of different types of cells, including germinal cells [345, 346], which can modify gene expression in future generations. It has been shown that breastmilk from obese women contains higher concentrations of POPs than breastmilk from lean women [347], which can have detrimental effects on the infant. It can modify gut microbiota composition and function at an early developmental stage [348], which can have unknown future repercussions. In mice, offspring exposed to PBDE-47 *in utero* and during lactation had a higher chance of developing obesity when fed a HFD, which was due to impaired lipid metabolism and gut microbiota dysbiosis [349]. These findings underlie the importance of considering POPs in an obesity context, due to unfavorable metabolic effects and negative impact on reproduction.

Gut microbiota & POPs

The community of microorganisms in the human gut can be affected by numerous factors including diet and environment. Changes in the gut microbiota by POPs could be one of the mechanisms by which these contaminants can affect cardiometabolic health, as studies in animal models have shown that PBDEs, PCBs and DDE can directly impact gut microbiota composition [350-352] and thus could change the production of microbial-derived metabolites. As fermentation is one of the key functions of gut microbes, which is characterized by a production of SCFAs, it has been hypothesised that pollutants could affect cardiometabolic health by interfering with the fermentation capability. Hoffman et al. showed that PCB-126 interfered with microbial production of propionate and succinate [353]. They also found that these effects were abolished by an increase in dietary fiber consumption. In a similar way, fecal SCFAs were changed in mice supplemented in DDE, a metabolite of DDT [352]. Another way POPs could disturb host-microbe homeostasis and metabolic health could be through the Aryl Hydrocarbon Receptor (AhR) [255]. A decrease in AhR activity has been associated to metabolic diseases and could be reversed

by supplementation in AhR agonists or by *Lactobacillus reuteri,* that naturally produces AhR ligands, as it has been observed in mice [354].

Hypothesis of microbiome implication on body detoxication

As persistent organic pollutants are mostly absorbed through consumption of certain foods, it has been hypothesized that nutritional or food-based interventions, for example by increased consumption of dietary polyphenols or polyphenol-rich supplements, could be a way to lower POPs-associated toxicity [355]. Other nutritional strategies have been postulated, such as consuming a vegan diet or lowering absorption through the consumption of olestra, a non-absorbable fat substitute that have also been shown to be lower POPs absorption [356]. Although there are currently not enough studies to support the concept of using prebiotic or probiotic to stimulate a body detoxification mechanism from organic pollutants, a few hypotheses are herein proposed, such as change in pollutants bioavailability, their bioremediation and in their enterohepatic circulation, as developed below and illustrated in Figure 11.

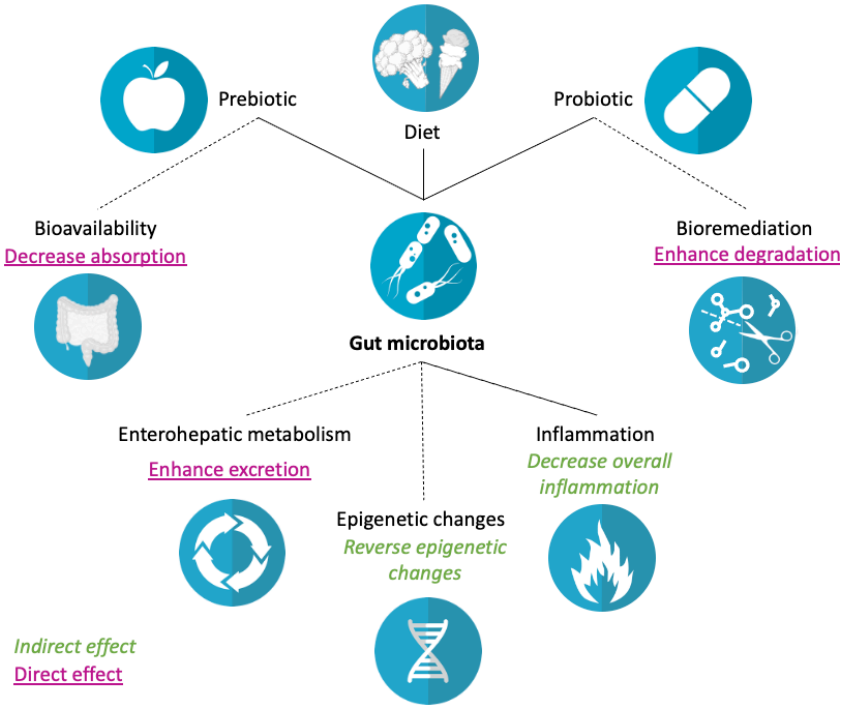


Figure 11. Potential mechanisms by which changes in the gut microbiota can impact the (re)absorption and toxicity of POPs

A. Bioavailability

In terms of chemicals and pollutants, an important factor to consider is their bioavailability. If the amount of contaminants cannot be changed by limiting their intake through dietary interventions, then lowering their bioavailability may be an alternative to decrease toxicity. For example, ingesting foods with a low digestibility may bind to POPs and thus reduce their absorption and exposure to key organs. This process has been shown with other pollutants such as methylmercury (MeHg), where polyphenols from green tea, black tea and coffee reduced bioaccessibility of MeHg from fish [229]. Prebiotics such as fibers and polyphenols could also have a binding capacity to POPs and prevent their absorption in the small intestine. Indeed, lignin has been shown to bind pesticide under simulated gastrointestinal conditions [357], rice bran fiber to bind polychlorinated biphenyls (PCBs) [358] and nori, an algae rich in fiber, to increase fecal excretion of dioxin in rats [359]. These data reinforce the binding potential of prebiotics to POPs to lower their bioavailability and reduce their absorption.

B. Bioremediation

Changing the microbial communities in the gut could enhance the number of microorganisms producing enzymes that could accelerate the degradation of POPs. In the environmental field, contamination by POPs is also problematic and one of the most efficient solution to remove them is through bioremediation, e.g. the degradation of pollutants by microorganisms [360, 361]. It is conceivable that some of our gut microorganisms have the enzymatic capacity to promote the degradation of POPs and thus reduce their absorption. An *in vitro* study showed that hepatocytes were able to absorb PCB-77 and PCB-153, but could not produce any metabolites, meaning they do not contain the enzymes necessary for the first step of degradation, i.e. removal of the chlorine atoms [362]. Interestingly, this study showed that it was not the same case for a mix of *Clostridium* spp. bacteria found in the commensal human gut microbiota as they could produce metabolites within a few hours, which suggests that microbial activity is essential for the first step of PCB degradation. Methanogenic archaea are also possible microorganisms that could perform bioremediation in the human gut [363], as they are known to biodegrade petroleum, and some POPs are petroleum-based, and can be found in

the commensal human gut microbiota. Although these studies reveal great potential, more preclinical and clinical studies are needed to confirm if microorganisms can be supplemented safely to help biodegrade pollutants in the human gastrointestinal tract.

C. Enterohepatic metabolism

The main function of bile acids is to enhance lipid absorption; thus, liposoluble molecules such as POPs are also absorbed through bile acids. In case of weight loss, POPs stored are released in small amounts from adipose tissue to circulation, pass through liver, bind with bile salts and transported into the intestine where they can either be excreted or reabsorbed [49]. Enhancing the excretion of bile acids by altering enterohepatic turnover thus represents a key potential mechanism to promote body detoxification [364]. Many factors can influence enterohepatic circulation, such as POPs themselves [365], olestra [49] fibers [358] as well as the gut microbiota [366]. Thus, some of these factors could be potential targets to increase POPs excretion through the enterohepatic circulation. Interestingly, specific strains of probiotics, such as *Lactobacillus plantarum KCTC3928* and probiotic mixture, such as VSL#3 have been shown to increase fecal bile acid deconjugation and excretion [237, 367], which would suggest that a supplementation in probiotics could be a therapeutic avenue for obese patients ongoing weight loss, as the probiotics could lower reabsorption of POPs released by adipose tissue loss by enhancing their elimination through bile acid excretion. These findings point towards the key implication of the gut-liver axis to modulate the impact of POP on health.

D. Indirect impacts of targeting gut microbiota on POPs toxicity

The use of prebiotics, probiotics or diet intervention targeting gut microbiota can also help lower impact of POPs indirectly, by lowering inflammation and reversing epigenetic changes. Low-grade inflammation is a comorbidity associated to obesity, and the link between changes in gut microbiota and increased inflammation in the host, through metabolic endotoxemia is well documented [167]. Many interventions targeting the gut microbiota, such as prebiotic and probiotic supplementation, have shown a reduction in circulating LPS and inflammatory cytokines [167, 220]. As POPs can increase low-grade inflammation [49], these interventions could indirectly lower POPs toxicity. Epigenetic

signatures can be affected by endocrine disruptors [345, 346], which can affect gene expression of the exposed individual and also predispose future generations to metabolic and reproductive dysfunction [368]. Diet and microbiota are known to be able to modify these epigenetic signatures [369] and could be therapeutic avenues to reverse epigenetic changes made by POPs as it has been observed by probiotics in different conditions [248].

Concluding remarks

As worldwide obesity is a major public health issue, it is critical to develop better therapeutic avenues to limit its development and lower associated comorbidities. As the gut microbiota can be modulated to impact on numerous pathologies including metabolic diseases, we propose that targeting this new "organ" is a promising course of action to complement other preventive or therapeutic strategies. We propose that supplementation with probiotics, prebiotics, synbiotics and postbiotics along with the promotion of healthier eating habits should be considered. Personalised nutrition and personalised treatments, according to genetic background and gut microbiota composition, could be even more effective. Another interesting therapeutic avenue is FMT in obese patients, that could allow to lower dysbiosis and blunt inflammation and improve insulin sensitivity, but this will need to be more tightly regulated to avoid safety issues. Targeting the gut microbiome could be specialised even further in context of obesity to modify specific organs, such as the brain to help change eating behaviors and lower depression. Interventions on the microbiome might also help body detoxification of lipophilic pollutants such as POPs by lowering their absorption or re-absorption as well as enhancing their degradation and excretion. In an environment where pollution is everywhere, finding simple and safe ways to help detoxication should be prioritised in health research and the approval of new chemicals should be done more carefully. Although the processes that could directly or indirectly affect POPs toxicity discussed in this article are promising, more studies are needed to determine their efficacy and potential in terms of interventions at a population level. Whether it is in the context of obesity, depression, cancer or other diseases, there are a few key points that need to be considered when applying treatment through the gut microbiota. Indeed, the gut microbiome is complex, unique for each individual and can change rapidly depending of different factors, such as sexual dimorphism, age, diet, health

status, environment, including pollutant exposure and medication that can be easily overlooked. The possibilities of treatment development for different diseases through the gut microbiome are extensive, as the gut is connected to many organs and systems. The more we will learn from the host-microbe interaction, the more we will be able to target specific reactions of the gut, such as production of metabolites, to have a beneficial impact on the host.

5. Objectifs de la thèse

À la suite de la revue de littérature précédente, plusieurs constats peuvent être faits. Tout d'abord, la prévalence de l'obésité et des désordres métaboliques et psychologiques ne cesse d'augmenter. De plus, le traitement et la prévention de ces troubles de santé doivent se faire par une multitude de changements au niveau individuel ainsi qu'au niveau populationnel. Ensuite, l'une des stratégies individuelles pouvant être adoptée est la thérapie nutritionnelle médicale, soit une modification des habitudes alimentaires. Finalement, le microbiote intestinal (sa composition et sa fonction) semble jouer un rôle important sur la santé de l'hôte, particulièrement en contexte d'obésité et pourrait être utilisé comme cible thérapeutique.

De ce fait, **l'objectif principal** de cette thèse était d'étudier certaines interventions alimentaires ou supplémentations en contexte de développement de l'obésité ou dans un contexte de perte de poids. **L'objectif secondaire** était de mieux comprendre quel est le rôle du microbiote intestinal dans l'impact de ces interventions et par le fait même, son potentiel thérapeutique.

Pour ce faire, cette thèse se sépare en quatre chapitres, trois chapitres portant sur des démonstrations chez des modèles animaux et un portant sur une étude clinique randomisée contrôlée.

Chapitre 1 : Ce chapitre s'intéressait à deux concepts, le premier étant la pollution endogène due à une libération des polluants organiques persistants (POP) accumulés au

sein du tissu adipeux par une perte de poids. Le deuxième étant le potentiel d'un extrait de canneberge riche en polyphénol sur l'excrétion de ces polluants et l'amélioration de la santé métabolique. Des souris ont été soumises à une diète obésogène avec ou sans ajout de POP et à la suite de leur phase de gain de poids, à une diète faible en gras et en sucre combiné à une supplémentation en extrait de canneberge ou d'un véhicule.

Chapitre 2 : Ce deuxième chapitre visait à montrer l'importance de l'environnement sur la colonisation du microbiote intestinal ainsi que sur le développement de désordres métaboliques associés à la consommation d'une diète obésogène. Pour ce faire, des souris ont été traitées avec une fraction de canneberge riche en proanthocyanidines ou à un véhicule et les fèces de ces animaux ont été utilisées pour coloniser des souris axéniques dans deux types d'hébergement différents. Le but était d'étudier l'impact de l'environnement sur la colonisation du microbiote intestinal ainsi que le phénotype métabolique des animaux receveurs d'un nouveau microbiote intestinal.

Chapitre 3 : L'impact différentiel de certaines sources uniques de protéines sur le développement de désordres métaboliques, notamment la résistance à l'insuline a déjà été démontré. Cependant, ce troisième chapitre s'intéressait à comparer la source la plus utilisée dans la littérature, soit la caséine, à un mélange représentatif de l'alimentation nord-américaine composé de 10 sources, animales et végétales, en contexte obésogène. De plus, l'observation des changements drastiques au niveau métabolique, mais également au niveau du microbiote intestinal, nous a poussés à étudier des métabolites microbiens (les BCFA) qui pourraient jouer un rôle causal sur le développement de la résistance à l'insuline hépatique.

Chapitre 4 : Ce dernier chapitre visait à étudier l'impact d'un probiotique sur la santé métabolique et psychologique de sujets avec un surpoids soumis à un protocole de restriction calorique modéré. Le but étant de voir si le probiotique potentialise les effets bénéfiques de la perte de poids sur la santé métabolique ainsi que sur les comportements alimentaires et l'humeur, dont l'anxiété, le stress et la dépression.

Chapitre 1 : A polyphenol-rich cranberry extract protects against endogenous exposure to persistent organic pollutants during weight loss in mice

Béatrice So-Yun Choi[a,b], Thibault Vincent Varin[a,b] Philippe St-Pierre[a,b], Geneviève Pilon[a,b], Angelo Tremblay[a,b,c] and André Marette[a,b,d,*]

[a] Quebec Heart & Lung Institute, Université Laval, 2725 Ch Ste-Foy, Québec, QC, Canada, G1V 4G5
[b] Institute of Nutrition and Functional Foods, Université Laval, 2440 Boulevard Hochelaga Suite 1710, Québec, QC, Canada, G1V 0A6
[c] Department of Kinesiology, Faculty of Medicine, Université Laval, 2300 Rue de la Terrasse, Québec, QC, Canada, G1V 0A6
[d] Department of Medicine, Faculty of Medicine, Université Laval, 1050 Avenue de la Médecine, Québec, QC, Canada, G1V 0A6
*Corresponding author: andre.marette@criucpq.ulaval.ca

Adapté pour des raisons de format de l'article suivant [370] :

Choi BS, Varin TV, 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
https://doi.org/10.1016/j.fct.2020.111832

1.1 Résumé

L'augmentation dramatique de l'obésité et des maladies qui y sont associées requiert de nouvelles stratégies pour favoriser la perte de poids. Malgré ses effets bénéfiques bien connus, la perte rapide de masse grasse peut également conduire à la libération endogène de molécules liposolubles aux effets potentiellement nocifs, comme les polluants

organiques persistants (POP). L'objectif de cette étude était d'évaluer l'impact d'un extrait de canneberge (CE) riche en polyphénols sur la libération de POP pendant la perte de poids. Des souris C57BL/6J ont été nourries avec un régime obésogène avec ou sans un mélange de POP, puis ont été soumises à une diète faible en gras. Les souris exposées aux POP ont ensuite été séparées en deux groupes pendant la perte de poids, recevant soit le CE soit un véhicule. Malgré la perte de graisse plus importante chez les souris traitées à l'extrait de canneberge, leurs taux circulants de POP n'ont pas augmenté et leur homéostasie du glucose s'est améliorée suite à la perte de poids par rapport aux souris traitées avec le véhicule. L'extrait a également induit des changements dans le microbiote intestinal, y compris la prolifération de *Parvibacter*, un membre de la famille des *Coriobacteriaceae* qui a un rôle potentiel dans le métabolisme des xénobiotiques. Nos données suggèrent donc que le microbiote intestinal peut être ciblé par des extraits riches en polyphénols pour se protéger d'une exposition accrue aux POP et de leurs effets métaboliques néfastes lors d'une perte de poids rapide.

1.2 Abstract

The dramatic rise in occurrence of obesity and associated diseases calls for new strategies to promote weight loss. While its beneficial effects are well known, rapid loss of fat mass can also lead to the endogenous release of liposoluble molecules with potential harmful effects, such as persistent organic pollutants (POP). The aim of this study was to evaluate the impact of a polyphenol-rich cranberry extract (CE) on POP release during weight loss. C57BL/6J mice were fed an obesogenic diet with or without a mixture of POP and then changed to a low-fat diet. The POP-exposed mice were then separated in two groups during weight loss, receiving either CE or vehicle. Despite the higher fat loss in the CE-treated mice, their circulating levels of POP were not enhanced, and their glucose homeostasis was further improved during weight loss compared to vehicle-treated mice. CE extract also induced changes in the gut microbiota, including blooming of *Parvibacter*, a member of the *Coriobacteriaceae* family which has been predicted to play a role in xenobiotic metabolism. Our data thus suggests that the gut microbiota can be targeted by polyphenol-

rich extracts to protect from increased POP exposure and their detrimental metabolic effects during rapid weight loss.

1.3 Introduction

Persistent organic pollutants (POP) are a category of environmental contaminants that can biomagnify i.e. increase in concentration along the food chain and bioaccumulate over time in adipose tissue[1]. The main exogenous exposure is through consumption of contaminated foods, such as fish, while the main endogenous exposure is through weight loss, since the liposoluble pollutants get liberated from the adipose depots into the bloodstream. These contaminants have been shown to increase in the circulation of obese subjects after weight loss, whether it is through lifestyle changes[2,3] or bariatric surgery[4,5].

Throughout the years, pollutants classified as POP have been used in a variety of products, such as insecticides, flame-retardants, coolant fluids and occasionally released as by-products by the industry. While POP are either banned or highly regulated since the adoption of the Stockholm Convention on Persistent Organic Pollutants in 2001, they are still widely present in the environment due to their persistent nature[6]. Their high solubility in lipids gives them the ability to accumulate in adipose tissue and, given their long half-life that can be up to 10-15 years[7], they can remain stored for decades once they are absorbed. Hence, biomagnification of these pollutants along the food chain makes humans prone to a high lifetime exposure to POP. As they are known to be endocrine disruptors, they can contribute not only to cardiometabolic diseases[8] but also to the development of certain types of cancers[9] and the impairment of reproductive functions[10]. Given that the main exposition is through ingestion of contaminated food, some populations are more at risk of exposure, such as communities living in the Arctic[11-13], which could contribute to the high prevalence of obesity and type 2 diabetes in these populations[14]. Recently, POP have also been shown to have a disruptive effect on the intestinal microbiota[15], which could also explain their implication in the development of cardiometabolic diseases[8]. Additionally, POP are known to induce epigenetic changes that can be transmitted through several generations and can predispose offspring to metabolic problems[16,17]. Even if the global exposure has gone down recently, the fact that the detrimental effects of POP were

observed in humans displaying lower levels than those observed at the time of POP banishment[3] reinforces the relevance to investigate their impact on metabolic regulation.

Dietary interventions such as use of prebiotics or probiotics as positive modulators on gut microbiome and gut health is a strategy gaining in popularity. Since POP could impact health through modification of the gut microbiome[18-20] the use of nutrients and prebiotics such as lignin[21,22] or inulin[23] has been proposed as a way to help detoxify from POP[24,25]. The definition of prebiotics has evolved and recently broaden to include polyphenols as prebiotic phytonutrients[26], also known for their benefits on cardiometabolic health through their effects on the gut microbiota[27]. Indeed, our group has previously reported beneficial impact of a polyphenol-rich cranberry extract on prevention[28] and treatment[29] of obesity and related metabolic impairments although not in a context of POP exposure during weight loss. Polyphenols could exert an effect to lower absorption or reabsorption of contaminants through the gut as they were previously shown to be effective at lowering the bioavailability of another environment pollutant, methylmercury[30]. Therefore, in this study we have tested the hypothesis that cranberry polyphenols have a positive effect on gut microbiota which could lower the negative impacts of endogenous POP exposure on metabolic health in a context of weight loss.

1.4 Material & Methods

Animals & diets

Single-caged male C57BL/6J aged 6 weeks were acclimatized to the animal facility for 12 days prior to start of the experiment on a 12h light/dark cycle and were kept on regular (low-fat) chow diet. The experimental design of the study is shown in Figure 1. For the first 12 weeks of the treatment, mice were fed a high-fat diet (HFD) from Research diet (D12451) with added corn oil containing the mixture of POP (described below). Mice receiving the same amount of oil but without POP were used as a non-exposed control group. To induce weight loss, mice were switched to a low-fat diet (LFD), from Research diet - D12450H) for four additional weeks. During the last six weeks of the protocol, mice received a polyphenol-rich cranberry extract (CE) or an equal volume of vehicle (water).

This extract was provided by Nutra Canada the same commercial source providing the extrat that has been previously characterized[28]. The total polyphenolic content is 30,2% of dry weight, with 8,6% of dry weight from proanthocyanidins. It was given daily at 200 mg/kg based on previous studies[28,29]. To minimize stress, gavage was started at week 10 i.e. 2 weeks prior to switching to the LF diet to induce weight loss. To avoid any behavioral impact of drastic diet change, mice were gradually changed from HFD to LFD over 5 days (Day 1 80% HFD – 20% LFD, day 2 60% HFD – 40 LFD, until 100% LFD on day 5). Body composition (lean mass, fat mass and fluid) was measured at weeks 0, 12 and 16 by qNMR (Bruker Minispec LF90). Fresh feces were collected during acclimatization and at week 10 and 16 for 16S sequencing. At weeks 6 and 14, mice were fasted 4 hours prior to glycemia measurement and blood collection through the saphenous vein. At weeks 9 and 16, mice were fasted 6 hours prior to an oral glucose tolerance test (oGTT) where they were gavaged with 50% dextrose (2µl/g body weight) and blood was collected 0, 15, 30, 60, 90 and 120 minutes after gavage. At the end of the protocol, animals were anesthetized with isoflurane, plasma was collected through cardiac puncture and then mice were euthanized by cervical dislocation. Tissues were collected and weighted. All manipulations were approved by the *Comité de protecton des animaux de l'Université Laval* (CPAUL).

POP mixture design & quantification

The POP mixture incorporated to the diet was composed of 18 different chemicals from four different categories: DDT and metabolites, PCB, PBDE and dioxin (see Table 1) and were obtained from Sigma Aldrich or Caledon Tech. The types and quantities of pollutants were based on levels measured in salmon to correspond to human consumption according to measures made at IMR (Institute of Marine Research, Norway). The total quantity included in the diet is calculated to be representative of a human eating a portion of 200g of fish 5 times per week, over a period of 20 years. Quantification of POP in the diet was made through the Direction of the expertise and food analysis laboratory (DLEAA) at the Ministry of Agriculture, Fisheries and Food of Quebec. Briefly, 3g of food were used for extraction with ethyl acetate, followed by salting out and centrifugation. Purification was made by gel permeation chromatography (GPC) and on silica gravimetric column. Extracts

were then concentrated and measured by gas Chromatography coupled with tandem mass spectrometry GC-MS/MS.

Plasma analysis

Insulin was measured through ELISA with the Mouse Ultrasensitive Insulin kit (Alpco), according to manufacturer's instructions. Plasma levels of cholesterol and triglycerides were measured by the biochemical analysis platform of the Quebec Heart and Lung Institute by colorimetry (Roche Modular P.) and detection limits were 0.08 mmol/L for total cholesterol and 0.05 mmol/L for triglycerides. Circulating POP were measured by the Public health expertise and reference center (INSPQ) in Quebec City, Canada. Briefly, 200 µl of plasma was used for liquid-liquid extraction and eluted in two fractions, mixture of dichloromethane:hexane and a solvent mixture of acetone:dichloromethane. Both fractions were evaporated and taken up in hexane for analysis by gas chromatography coupled to mass detection (GC-MS). If POP levels in circulation were lower than their detection level, half the value of the minimally detectable value was used. Circulating POP results were normalized for adipose tissue loss by dividing the individual values of circulating contaminants in g/L of plasma by adipose tissue loss between weeks 12 and 16, in g. Raw values can be found in Supplemental Table 1.

Liver triglycerides

To measure hepatic triglyceride levels, 50 mg of liver were used for a standard chloroform-methanol Folch lipid extraction as previously described[31] and triglycerides were measured by commercial kit (Randox Laboratories, Crumlin, UK).

16S rRNA amplicon sequencing and analysis

For fecal microbiota analysis, bacterial genomic material was extracted from fresh feces (stored at -80 °C between the collection time and the extraction) with the ZymoBIOMICS DNA Miniprep kit (Zymo Research) according to manufacturer's instructions and then sent to the sequencing platform at *Institut de biologie intégrative et des systèmes* (IBIS, Quebec City) for PCR amplification of the V3-V4 hypervariable region of the bacterial 16S rRNA gene as previously described[7]. Cutadapt (v1.14[32]) was used to trim forward and reverse

primers from reads. Sequences were processed with DADA2 (v1.10.1[33]) in R environment (http://www.R-project.org). Taxonomic assignment of amplicon sequence variants (ASVs) was performed using the RDP classifier algorithm (v2,2[34]) trained against the Silva database (version 132)[35]. Taxa that appeared less than three times in the entire dataset were removed. The resulting ASVs table was rarefied to the smallest sample size (10589 sequences) in order to take into account differences in sampling depth.

Bacterial alpha-diversity was assessed with Shannon and Simpson's reciprocal indexes. A stacked bar plot at order level was generated to show overall bacterial community composition. The linear discriminant analysis (LDA) effect size (LefSe) method was performed to detect differentially abundant bacteria between two distinct biological conditionse[36]. A p-value of <.05 and a LDA score ≥ 2.5 will be considered statistically significant. All raw sequences were deposited in the public European Nucleotide Archive server under accession number PRJEB37127.

Statistical analysis

Data are expressed as mean±SEM. During the weight gain phase, statistically significant was assessed by comparing the mice in the POP exposed group to the mice in the control group by a T-test for parametric data sets and Mann-Whitney for non-parametric data sets. To assess statistically significant differences after cranberry treatment started, one-way analysis of variance (ANOVA) with Bonferroni post hoc test were performed for parametric data sets and Kruskal-Wallis with Dunn's multiple comparison test for non-parametric data sets. For repeated measures (such as weight curves and oGTT) two-way ANOVA with tukey post-hoc test was used. To determine whether data sets would be treated as parametric or non-parametric, a Shapiro-Wilk normality test was performed. For all statistical analysis, $p<0.05$ was considered significant and all analysis were performed with GraphPad Prism 8.

1.5 Results

Short term-exposure to a diverse mixture of POP was not sufficient to aggravate metabolic health in a weight-gain context

During the weight gain phase, the addition of a relevant POP-mixture to a HFD did not affect metabolic parameters. Mice from both groups gained an average of 10g of body weight (Fig. 2a) over 10 weeks, that was mainly explained by an increase of fat mass by over 600% (Fig. 2b). Fasting glycemia and insulinemia at week 6 (Fig. 2c, d) as well as glucose tolerance and glucose-stimulated insulin secretion did not vary either between the groups (Fig. 2e-g). These results were consistent with findings from *Baker et al.,* even if the POP administration was different as we used a mixture of POP incorporated in the diet at lower levels than the gavage of PCB-77 used in their study[37]. As stress can affect weight change in rodents, daily gavage started 2 weeks prior to the gradual diet switch from HFD to LFD. Therefore, to remove possible bias, these weeks (10 to 12) were excluded from the weight gain phase.

Polyphenol-rich cranberry extract favors LF diet-induced weight loss and further improves glucose homeostasis

As expected, all mice switched to the LFD lost weight between weeks 12 and 16. The anticipated effects of weight loss on metabolic health were clearly observed when comparing weight as well as fasting glucose and insulin values, as well as oral glucose tolerance measured before (Fig. 2c-f) and after (Fig 3c-g) weight loss for all groups. However, obese mice switched to LFD diet and treated daily with the CE showed greater weight loss as compared to the POP-vehicle group (Fig. 3a). This enhanced weight loss was mainly explained by a significant greater reduction of adipose tissue mass (-39.7 ± 3.6%) in the POP-cranberry group as compared to the POP-vehicle group (-29.1 ± 1.2%). The tendency for a greater loss of adipose tissue in the control group of -34.7 ± 1.7% (P=0.063) as compared to the POP-vehicle group further suggests that POP exposure per se may lower LFD-induced weight loss (Fig. 3b).

Fasting glycemia was ameliorated in CE-treated POP-exposed mice compared to vehicle POP-exposed animals, but no changes in insulinemia were observed (Fig. 3c, d). In

agreement with the fasting glucose result at week 14, the OGTT performed at week 16 showed improved glucose tolerance in CE-treated POP-exposed mice which was significant at times 30-, 60- and 120 min post-glucose challenge (Fig. 3e) although no significant differences were observed between the areas under the curves (AUC) (Fig. 3f). No changes were observed on glucose-stimulated insulin responses between the groups (Fig. 3g). Despite that the finding of greater weight and adipose tissue loss in CE-treated POP-exposed mice as compared to the vehicle-treated group, we found no changes in either the blood lipid profile (Fig. 3g-h) or hepatic steatosis (Fig. 3i, j).

Greater loss of fat mass by CE extract in LFD-treated obese mice did not result in higher POP in circulation

As POP are liposoluble and accumulate in adipose depots, it is known that after a rapid weight loss that they can be released and accumulate in circulation proportionally to the amount of fat loss[4]. Out of 18 supplemented POP given in the diet, 15 were measurable in circulation (6 PCB, 7 PBDE, DDE and DDT). To take into account the changes of fat mass, POP levels were expressed by the amount of adipose tissue loss to correlate the amount of POP released to that of total adipose tissue loss[38]. All of the detectable contaminants were significantly enhanced in the POP-exposed groups as compared to the control group (Fig. 4a-c). It is interesting to note that some of the chemicals were actually detectable in the control group even if their diet was not purposely contaminated. We found that some contaminants were significantly decreased (PCB-101 and PBDE-154) or strongly tend to decrease (DDE, $p=0.0606$) in CE-treated POP-exposed mice, indicating that cranberry polyphenols can lower circulating levels of pollutants released from adipose tissue after weight loss (Fig. 4a-c).

Weight loss in POP-exposed mice and CE treatment induces selective changes in the gut microbiota

Fresh feces were collected at baseline, at the end of the weight gain phase (week 10) and at the end of the weight loss phase (week 16) to assess gut microbiota composition. As anticipated, comparing the relative abundance at the order level (Fig. 5a) revealed that the main changes on overall gut microbiota composition was related to the dietary treatment.

At baseline (time 0), before any treatment, mice were kept on the chow diet and therefore presented similar gut microbiota composition. After 10 weeks on HFD, however, a clear shift in the gut microbiota composition was observed, irrespectively of POP exposure. Similarly, between week 10 and week 16, we can observe that switching animals to the LFD to induce weight loss was associated with major changes the overall gut microbiota composition (Fig. 5a).

The effect of POP supplementation during the 10-week period of HFD-induced weight gain, and before any CE supplementation, was mild and limited to the overrepresentation of two genera in the control group, *Candidatus_Soleaferrea* and *Ruminiclostridium,* whereas POP exposure was associated with upregulation of *Ruminococcaceae_UCG_009* (Fig. 5b). All these genera are part of the Ruminococcaceae family in the Firmicutes phylum. Shannon and Simpson's reciprocal indexes were not impacted by the diet, meaning the alpha diversity was not affected by the POP mixture during the HFD-induced obesity phase (Fig. 5c, d). On the other hand, when POP-exposed mice were switch to the LFD to induce weight loss we found that two genera i.e. *Alistipes* and *Parvibacter* were overrepresented in the POP group and that *Ruminiclostridium_6* was conversely overrepresented in the control group (Fig. 5e). Interestingly, as compared to the POP-exposed vehicle group, mice exposed to POP but also treated with CE showed an underrepresentation of many genera in their fecal microbiome. Remarkably, only one genus, *Parvibacter*, a member of the *Coriobacteriaceae* family, was found to be increased in the POP-exposed CE-treated group as compared to vehicle-treated counterparts (Figure 5f). These differences were also accompanied by a reduced alpha diversity in the in the CE-treated POP-exposed fecal microbiota (Figure 5g, h).

1.6 Discussion

As obesity and associated cardiometabolic diseases are complex and multifactorial disorders, it is important to consider all possible contributors to their development. The importance of environmental pollution has not been studied as much as many other factors and more work is critically needed to demonstrate and better understand how these

chemicals can affect weight regulation and metabolic outcomes. In this study, we focused on the effect of POP release during weight loss and the potential of a polyphenol-rich cranberry extract as a proof of concept that targeting the gut microbiota could help to lower pollutant exposure and their deleterious effects on glucose homeostasis.

During weight gain no changes in metabolic parameters and only small changes in gut microbiota composition were observed in POP-contaminated mice as compared to the control group. The POP mixture did not statistically worsen metabolic phenotype in HFD-fed mice even if a small trend is observed. This might be related to the amount and time of the exposure to the pollutants, but may also be explained by a protective role of increase fat mass storage capacity, sparing from POP toxicity and metabolic disruption, as previously proposed by Lee *et al.*[39] Indeed, it has been hypothesized that protection against the endocrine-disrupting effects of POP can be related to their storage in adipose tissue, leading to the concept that an enlarged fat tissue compartment could protect against the deleterious effects of POP exposure. Thus, we believe that in a LFD context, the negative impact of POP exposure might have been more clearly observable. While the impact of POP exposure on the gut microbiota was relatively modes, this could be explained by the fact that lipids are primarily absorbed in the small intestine whereas analyses of fecal samples is mostly representative of colonic microbial communities. Indeed, the majority of the chemical pollutants probably did not reach the colon as it would be expected they would be absorbed with dietary lipids during the weight gain phase. Indeed, POP are dissolved in micelles made of hydrolyzed dietary lipids and bile acids[40], which can then be absorbed in the small intestine. POP could also directly impact host and microbiota bile acid metabolism[41], which could affect their theoretical excretion rate.

A key finding of this study is that CE treatment lowered fasting glycemia and improved glucose tolerance during weight loss, and more than in animals that were also subjected to the weight reducing LFD protocol but treated with vehicle (water) only. This beneficial effect of CE on glucose homeostasis may be related to higher adipose tissue loss in CE-treated animals. Our data confirmed the hypothesis that CE may impede the anticipated greater release of POP upon rapid weight loss, as demonstrated by the relatively lower

levels of POP in the plasma of CE-treated mice, despite greater fat loss in these animals. POP endogenous exposure alone during the weight-loss phase did not have a major impact in our model, as observed when comparing the control and POP-vehicle groups, but there was an interesting trend for lower adipose tissue loss in the POP-vehicle group. This finding suggests either a direct impact of POP on weight loss prevention or an indirect effect where the body is trying to protect itself from POP by maintaining adiposity.

We also hypothesized that CE may reduce the impact of released pollutants during weight loss owing to their documented effects on the gut microbiota. We found that CE treatment of POP-exposed mice decreased the alpha diversity of the fecal microbiome, suggesting that in this group a significant amount of POP was able to reach the colon and disturb the gut microbiota, as reduced diversity upon exposure to POP has been observed by others[23]. Three potential mechanisms may underlie the beneficial effect of cranberry polyphenols in the context of rapid weight loss. They may exert their effects through 1) reducing POP bioavailability, 2) modifying enterohepatic metabolism and/or 3) favoring a niche for microbial communities implicated in POP detoxification.

The first mechanism is based on the concept that the majority of polyphenols cannot be degraded by digestive processes and thus they reach the colon mostly intact, where they can be metabolized by bacteria[27]. It is possible that polyphenols bind to POP, like they bind other pollutants[30] and thus prevent their absorption in the upper intestine and then reach the colon where they may be excreted rather than reabsorbed. Second, as mentioned above, POP depend of bile acids to be absorbed or reabsorbed in the intestine, thus changing the enterohepatic metabolism could affect their absorption[39]. As polyphenols have been shown to increase bile acid excretion[42], it could also contribute to the lower circulating levels of POP observed in CE-treated POP-exposed animals.

Perhaps the most plausible or effective mechanism is that CE promotes degradation of POP by bioremediation[43], the degradation of chemicals by microorganisms. Indeed, cranberry polyphenols may provide a favorable niche to specific bacterial species through their well-documented prebiotic activity. This concept is supported by both animal[44] and human[18]

studies. Indeed, De *et al.* reported that hepatic cells were not able to carry the first step of biodegradation of a PCB, while bacteria could[45], pointing to their importance in body detoxication. In the present study, the relative abundance of *Parvibacte*r, a member of the *Coriobacteriaceae* family[46], was enhanced in POP-exposed HFD fed mice compared to mice kept on a control HFD but was also enhanced in CE-treated POP-exposed mice compared vehicle-treated POP-exposed mice. Little is currently known about the role *Parvibacter* in the context of obesity and metabolic homeostasis, but it has been suggested that the *Coriobacteriaceae* family is implicated in the metabolism of xenobiotics[47], suggesting an important role of this family and possibly *Parvibacter* in detoxication. We postulate that *Parvibacter* was increased in the gut microbiota as a protective response of the body to POP exposure and that treatment with cranberry polyphenols further mobilized this detoxification pathway, thus reduced POP levels in the circulation of these mice and despite greater weight loss and body fat reduction.

It is also noteworthy that mice consuming the control HFD (i.e. not POP supplemented) still had detectable amounts of all 15 pollutants measured in circulation. This could be explained by the fact that these chemicals are omnipresent in the environment and likely contaminated the animal fat source (lard) of the HFD. From an ecological standpoint, it is disconcerting to note that POP are sufficiently persistent to even penetrate the standardized production of laboratory animal feed. While this could be conceived as a limitation of the study, it should be noted that the levels of circulating contaminants detected in non-POP exposed animals still remained very low as compared to POP-exposed mice, and thus did not confound our ability to determine significant differences between the vehicle and CE-treated mouse groups for both POP levels, and metabolic endpoints.

Conclusion

This study shows that polyphenols can prevent the increase in POP concentrations that is normally observed in response to body fat loss. As a proof of concept, a polyphenol-rich CE was used to show that prebiotics could lower body load of POP during their release from adipose tissue in a weight loss context. Other types of polyphenols such as lignin[21,22] or inulin[23] should also be investigated for their ability to reduce POP exposure in a weight

loss context. More work will be required to optimize the use of polyphenols to help lower body contamination of POP through body detoxication mechanisms and to protect against their deleterious impact on human health.

1.7 Acknowledgements

We thank Valérie Dumais, Christine Dion, Christine Dallaire, Jacinthe Julien and Joanie Dupont-Morissette for their expert help with animal experimentation, Annette Bernhard and Lise Madsen for their help with POP mixture design as well as Nutra Canada for providing the polyphenol-rich cranberry extract and Stéphanie Dudonné and Yves Desjardins for its phenolic characterization. We would also like to thank the Public health expertise and reference center (INSPQ) and the Ministry of Agriculture, Fisheries and Food of Quebec (MAPAQ) for POP measurements. This work was funded by a grant from the *Fondation de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ)* to AT, the Canadian Institutes for Health Reseach and the Sentinel North program to AM, the latter funded by the Canada First Research Excellence Fund. AM was the recipient of a CIHR/Pfizer research Chair in the pathogenesis of insulin resistance and cardiovascular diseases. BSYC is funded by doctoral scholarships from Sentinel North and from a NSERC-funded CREATE-SMAART program.

CRediT authorship contribution statement

Béatrice So-Yun Choi: Methodology, Investigation, Formal analysis, Writing - Original Draft, Visualization. Thibault Vincent Varin: Software, Formal analysis, Writing - Review & Editing, Visualization. Philippe St-Pierre: Methodology, Project administration, Writing - Review & Editing. Geneviève Pilon: Conceptualization, Methodology, Writing - Review & Editing. Angelo Tremblay: Conceptualization, Writing - Review & Editing, Funding acquisition. André Marette: Conceptualization, Writing - Review & Editing, Supervision, Funding acquisition.

1.8 References

1. Petriello, M.C., Newsome, B. & Hennig, B. Influence of nutrition in PCB-induced vascular inflammation. *Environ Sci Pollut Res Int* **21**, 6410-6418 (2014).
2. Imbeault, P., Tremblay, A., Simoneau, J.A. & Joanisse, D.R. Weight loss-induced rise in plasma pollutant is associated with reduced skeletal muscle oxidative capacity. *American journal of physiology. Endocrinology and metabolism* **282**, E574-579 (2002).
3. Pelletier, C., Doucet, E., Imbeault, P. & Tremblay, A. Associations between Weight Loss-Induced Changes in Plasma Organochlorine Concentrations, Serum T3 Concentration, and Resting Metabolic Rate. *Toxicological Sciences* **67**, 46-51 (2002).
4. Hue, O.*, et al.* Increased plasma levels of toxic pollutants accompanying weight loss induced by hypocaloric diet or by bariatric surgery. *Obesity surgery* **16**, 1145-1154 (2006).
5. Kim, M.J.*, et al.* Fate and complex pathogenic effects of dioxins and polychlorinated biphenyls in obese subjects before and after drastic weight loss. *Environ Health Perspect* **119**, 377-383 (2011).
6. Magulova, K. & Priceputu, A. Global monitoring plan for persistent organic pollutants (POPs) under the Stockholm Convention: Triggering, streamlining and catalyzing global POPs monitoring. *Environ Pollut* **217**, 82-84 (2016).
7. Ritter, R.*, et al.* Intrinsic human elimination half-lives of polychlorinated biphenyls derived from the temporal evolution of cross-sectional biomonitoring data from the United Kingdom. *Environ Health Perspect* **119**, 225-231 (2011).
8. Lee, D.H., Porta, M., Jacobs, D.R., Jr. & Vandenberg, L.N. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. *Endocr Rev* **35**, 557-601 (2014).
9. Lim, J.E., Park, S.H., Jee, S.H. & Park, H. Body concentrations of persistent organic pollutants and prostate cancer: a meta-analysis. *Environ Sci Pollut Res Int* **22**, 11275-11284 (2015).
10. Anas, M.K.*, et al.* In utero and lactational exposure to an environmentally relevant organochlorine mixture disrupts reproductive development and function in male rats. *Biol Reprod* **73**, 414-426 (2005).
11. Schaebel, L.K., Bonefeld-Jorgensen, E.C., Vestergaard, H. & Andersen, S. The influence of persistent organic pollutants in the traditional Inuit diet on markers of inflammation. *PloS one* **12**, e0177781 (2017).
12. Hjermitslev, M.H., Long, M., Wielsoe, M. & Bonefeld-Jorgensen, E.C. Persistent organic pollutants in Greenlandic pregnant women and indices of foetal growth: The ACCEPT study. *Sci Total Environ* **698**, 134118 (2020).
13. Binnington, M.J.*, et al.* Mechanistic polychlorinated biphenyl exposure modeling of mothers in the Canadian Arctic: the challenge of reliably establishing dietary composition. *Environ Int* **92-93**, 256-268 (2016).
14. Zuk, A.M., Tsuji, L.J.S., Nieboer, E., Martin, I.D. & Liberda, E.N. Examining environmental contaminant mixtures among adults with type 2 diabetes in the Cree First Nation communities of Eeyou Istchee, Canada. *Scientific reports* **9**, 15909 (2019).

15. Petriello, M.C., Hoffman, J.B., Vsevolozhskaya, O., Morris, A.J. & Hennig, B. Dioxin-like PCB 126 increases intestinal inflammation and disrupts gut microbiota and metabolic homeostasis. *Environ Pollut* **242**, 1022-1032 (2018).
16. Skinner, M.K.*, et al.* Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *BMC medicine* **11**, 228 (2013).
17. Navarro, P.*, et al.* Maternal folic acid supplementation does not counteract the deleterious impact of prenatal exposure to environmental pollutants on lipid homeostasis in male rat descendants. *J Dev Orig Health Dis*, 1-11 (2019).
18. Velmurugan, G.*, et al.* Gut microbial degradation of organophosphate insecticides-induces glucose intolerance via gluconeogenesis. *Genome Biol* **18**, 8 (2017).
19. Cheng, S.L.*, et al.* Gut Microbiota Modulates Interactions Between Polychlorinated Biphenyls and Bile Acid Homeostasis. *Toxicol Sci* **166**, 269-287 (2018).
20. Zhang, L.*, et al.* Persistent Organic Pollutants Modify Gut Microbiota-Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation. *Environ Health Perspect* **123**, 679-688 (2015).
21. Ta, C.A.*, et al.* Binding capacity of various fibre to pesticide residues under simulated gastrointestinal conditions. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* **37**, 1147-1151 (1999).
22. Sera, N.*, et al.* Binding effect of polychlorinated compounds and environmental carcinogens on rice bran fiber. *J Nutr Biochem* **16**, 50-58 (2005).
23. Hoffman, J.B., Flythe, M.D. & Hennig, B. Environmental pollutant-mediated disruption of gut microbial metabolism of the prebiotic inulin. *Anaerobe* **55**, 96-102 (2019).
24. Petriello, M.C.*, et al.* Modulation of persistent organic pollutant toxicity through nutritional intervention: emerging opportunities in biomedicine and environmental remediation. *Sci Total Environ* **491-492**, 11-16 (2014).
25. 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. *International journal of obesity (2005)* (2020).
26. Gibson, G.R.*, et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* **14**, 491-502 (2017).
27. Anhe, F.F., Choi, B.S.Y., Dyck, J.R.B., Schertzer, J.D. & Marette, A. Host-Microbe Interplay in the Cardiometabolic Benefits of Dietary Polyphenols. *Trends Endocrinol Metab* **30**, 384-395 (2019).
28. Anhe, F.F.*, et al.* A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut* **64**, 872-883 (2015).

29. Anhe, F.F., *et al.* A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss. *Mol Metab* **6**, 1563-1573 (2017).
30. Girard, C., Charette, T., Leclerc, M., Shapiro, B.J. & Amyot, M. Cooking and co-ingested polyphenols reduce in vitro methylmercury bioaccessibility from fish and may alter exposure in humans. *Sci Total Environ* **616-617**, 863-874 (2018).
31. Anhe, F.F., *et al.* Treatment with camu camu (Myrciaria dubia) prevents obesity by altering the gut microbiota and increasing energy expenditure in diet-induced obese mice. *Gut* (2018).
32. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *2011* **17**, 3 (2011).
33. Callahan, B.J., *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**, 581-583 (2016).
34. Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**, 5261-5267 (2007).
35. Quast, C., *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**, D590-596 (2013).
36. Segata, N., *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* **12**, R60 (2011).
37. Baker, N.A., *et al.* Coplanar polychlorinated biphenyls impair glucose homeostasis in lean C57BL/6 mice and mitigate beneficial effects of weight loss on glucose homeostasis in obese mice. *Environ Health Perspect* **121**, 105-110 (2013).
38. Jansen, A., Lyche, J.L., Polder, A., Aaseth, J. & Skaug, M.A. Increased blood levels of persistent organic pollutants (POP) in obese individuals after weight loss-A review. *J Toxicol Environ Health B Crit Rev* **20**, 22-37 (2017).
39. Lee, Y.M., Kim, K.S., Jacobs, D.R., Jr. & Lee, D.H. Persistent organic pollutants in adipose tissue should be considered in obesity research. *Obes Rev* **18**, 129-139 (2017).
40. Moser, G.A. & McLachlan, M.S. The influence of dietary concentration on the absorption and excretion of persistent lipophilic organic pollutants in the human intestinal tract. *Chemosphere* **45**, 201-211 (2001).
41. Fader, K.A., *et al.* 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-elicited effects on bile acid homeostasis: Alterations in biosynthesis, enterohepatic circulation, and microbial metabolism. *Scientific reports* **7**, 5921 (2017).
42. Chambers, K.F., Day, P.E., Aboufarrag, H.T. & Kroon, P.A. Polyphenol Effects on Cholesterol Metabolism via Bile Acid Biosynthesis, CYP7A1: A Review. *Nutrients* **11**(2019).
43. Sharma, J.K., *et al.* Advances and perspective in bioremediation of polychlorinated biphenyl-contaminated soils. *Environ Sci Pollut Res Int* **25**, 16355-16375 (2018).
44. Wolf, A.R., *et al.* Bioremediation of a Common Product of Food Processing by a Human Gut Bacterium. *Cell Host Microbe* **26**, 463-477 e468 (2019).

45. De, S., Ghosh, S. & Dutta, S.K. Congener specific polychlorinated biphenyl metabolism by human intestinal microbe Clostridium species: Comparison with human liver cell line-HepG2. *Indian journal of microbiology* **46**, 199-207 (2006).
46. Clavel, T., Charrier, C., Wenning, M. & Haller, D. Parvibacter caecicola gen. nov., sp. nov., a bacterium of the family Coriobacteriaceae isolated from the caecum of a mouse. *Int J Syst Evol Microbiol* **63**, 2642-2648 (2013).
47. Claus, S.P.*, et al.* Colonization-induced host-gut microbial metabolic interaction. *mBio* **2**, e00271-00210 (2011).

1.9 Tables

Table 1. Composition of POP mixture in the diet. Quantities added to the diet and measured of the 18 POPs included in the mixture**.**

POP	Quantity added (ug/kg of diet)	Quantity measured (ug/kg of diet)	Group
pp-DDD	304	206	DDT and metabolites
pp-DDT	137	91.6	
pp-DDE	729	569	
PCB-52	94.2	83.0	PCB (Polychlorobiphenyl)
PCB-101	182	166	
PCB-28	38.0	44.0	
PCB-138	273	268	
PCB-153	289	349	
PCB-180	68.3	57.0	
PCB-114	146	133	
PBDE-47	114	127	PBDE (Polybromodiphenylethers)
PBDE-100	21.3	28.2	
PBDE-154	12.2	12.7	
PBDE-209	30.4	Not available	
PBDE-28	6.76	9.44	
PBDE-99	19.7	17.8	
PBDE-153	3.49	4.11	
TCDD	0.05	Not available	Dioxin and furans

1.10 Figure Legends

Figure 1. Experimental design.

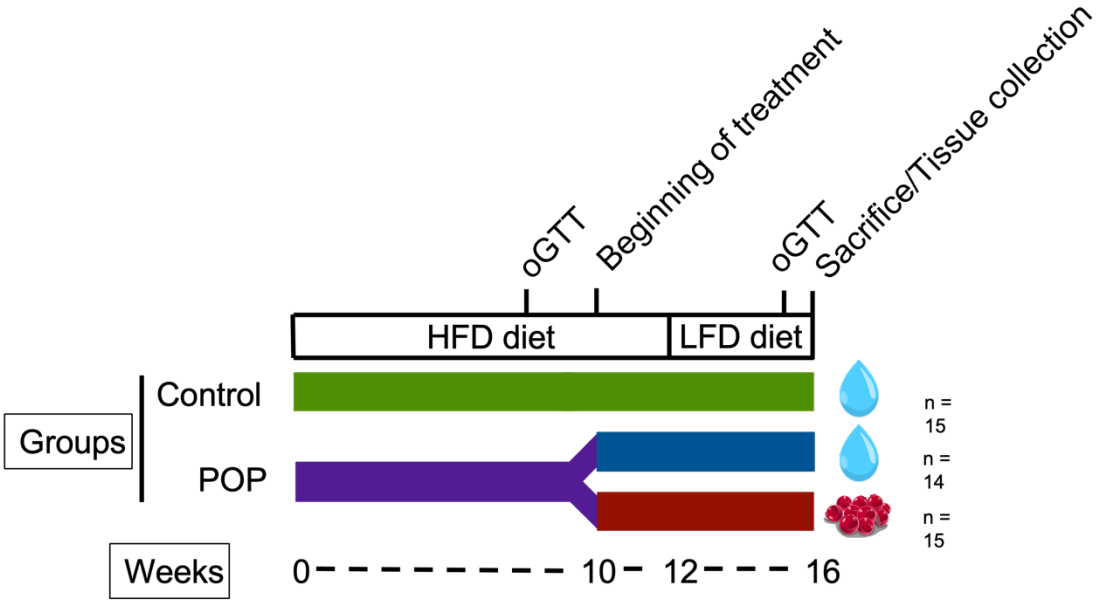
Figure 2. Impact of high-fat high-sucrose (HFHS) diet and POP. Weight gain (a), adipose tissue gain (b), fasting glycemia (c) and insulinemia at week 6 (d) (blood drawn from saphenous vein after 4h of fasting). Glycemia (e), area under the curve (f) and insulinemia (g) during the oral glucose tolerance test (OGTT), (glucose administration (1g/kg) through gavage, blood drawn from saphenous vein after 6h fasting). n=15-29. Data are presented as the mean +/- SEM.

Figure 3. Impact of cranberry extract treatment and weight loss. Weight loss (a), adipose tissue loss (b), fasting glycemia (c) and insulinemia at week 6 (d) (blood drawn from saphenous vein after 4h of fasting). Glycemia (e), area under the curve (f) and insulinemia (g) during the oral glucose tolerance test (OGTT), (glucose administration (1g/kg) through gavage, blood drawn from saphenous vein after 6h fasting). Total plasmatic cholesterol (g), plasmatic triglycerides (h) liver weight at sacrifice(i) and hepatic triglycerides (j) n=14- 15. Data are presented as the mean +/- SEM. Data are significantly different ($p < 0.05$) according to a two-way ANOVA. *indicates a significant difference between POP and POP-Cranberry.

Figure 4. Circulating POP corrected for adipose tissue loss. PCBs (polychlorobiphenyl) (a), PBDEs (Polybromodiphenylether) (b) DDE & DDT (dichlorodiphenyldichloroethylene & dichlorodiphenyltrichloroethane (c) n=3 for control group and n=14-15 for POP treated groups Data are presented as the mean +/- SEM. Data are significantly different ($p < 0.05$) according to a one-way ANOVA.

1.11 Figures

Figure 1



Weeks 0 to 10: Induction of obesity (HFD) and POP accumulation.
Week 10: Beginning of treatment (cranberry extract) or vehicle (water).
Weel 12: Diet change, HFD → LFD (without POP) to induce weight loss and POP liberation in circulation.

Figure 2

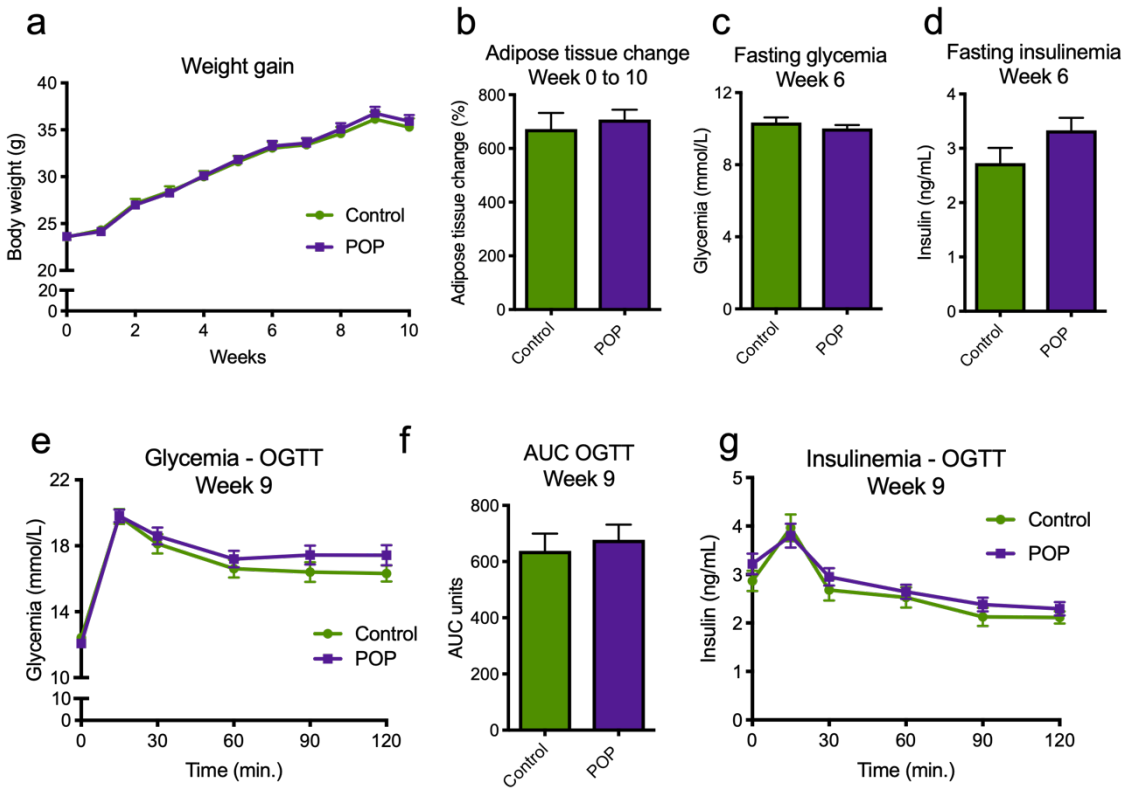

a
Weight gain
Body weight (g)
Weeks
Control
POP
b
Adipose tissue change
Week 0 to 10
Adipose tissue change (%)
c
Fasting glycemia
Week 6
Glycemia (mmol/L)
d
Fasting insulinemia
Week 6
Insulin (ng/mL)
e
Glycemia - OGTT
Week 9
Glycemia (mmol/L)
Time (min.)
f
AUC OGTT
Week 9
AUC units
g
Insulinemia - OGTT
Week 9
Insulin (ng/mL)
Time (min.)

Figure 3

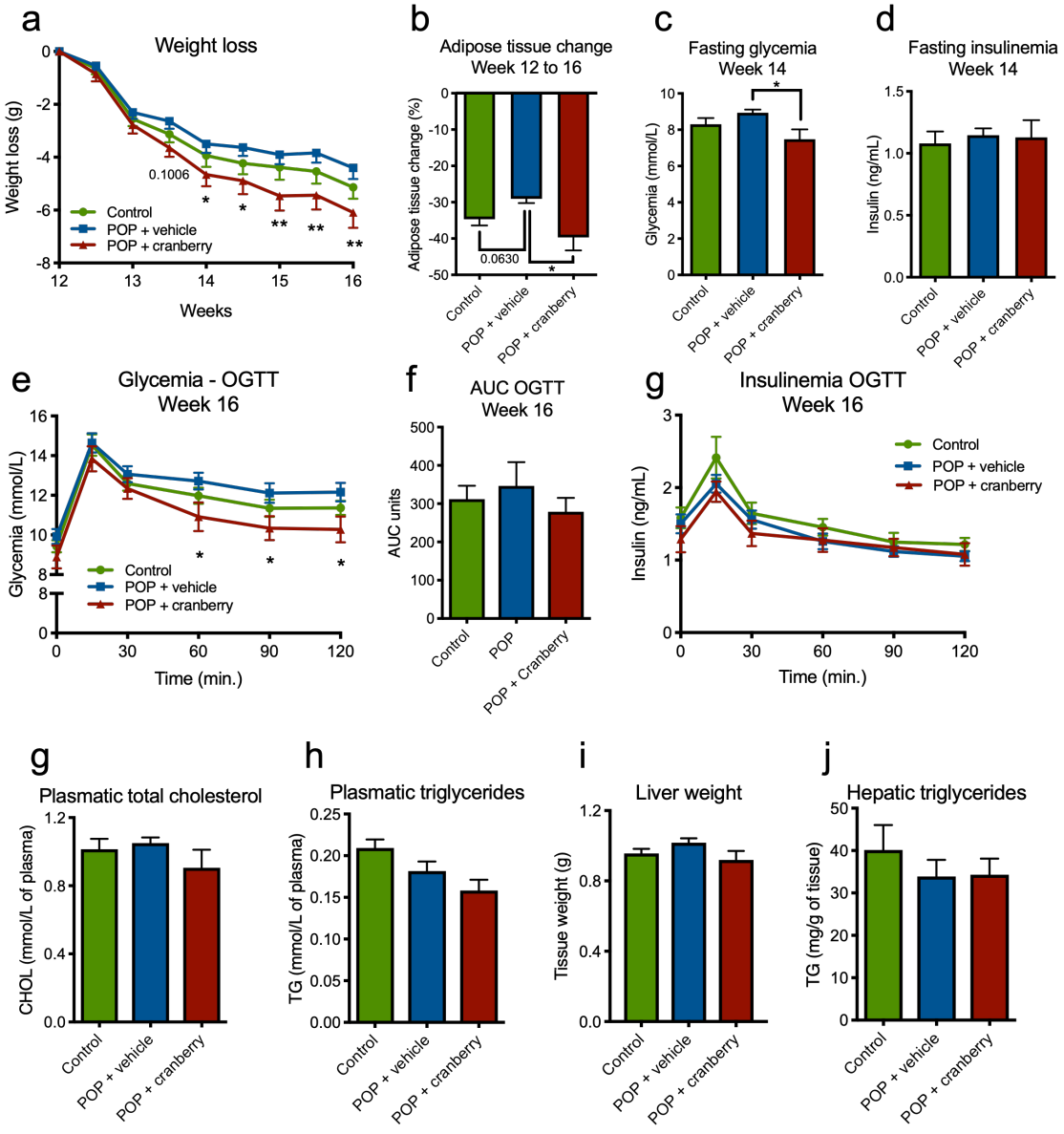
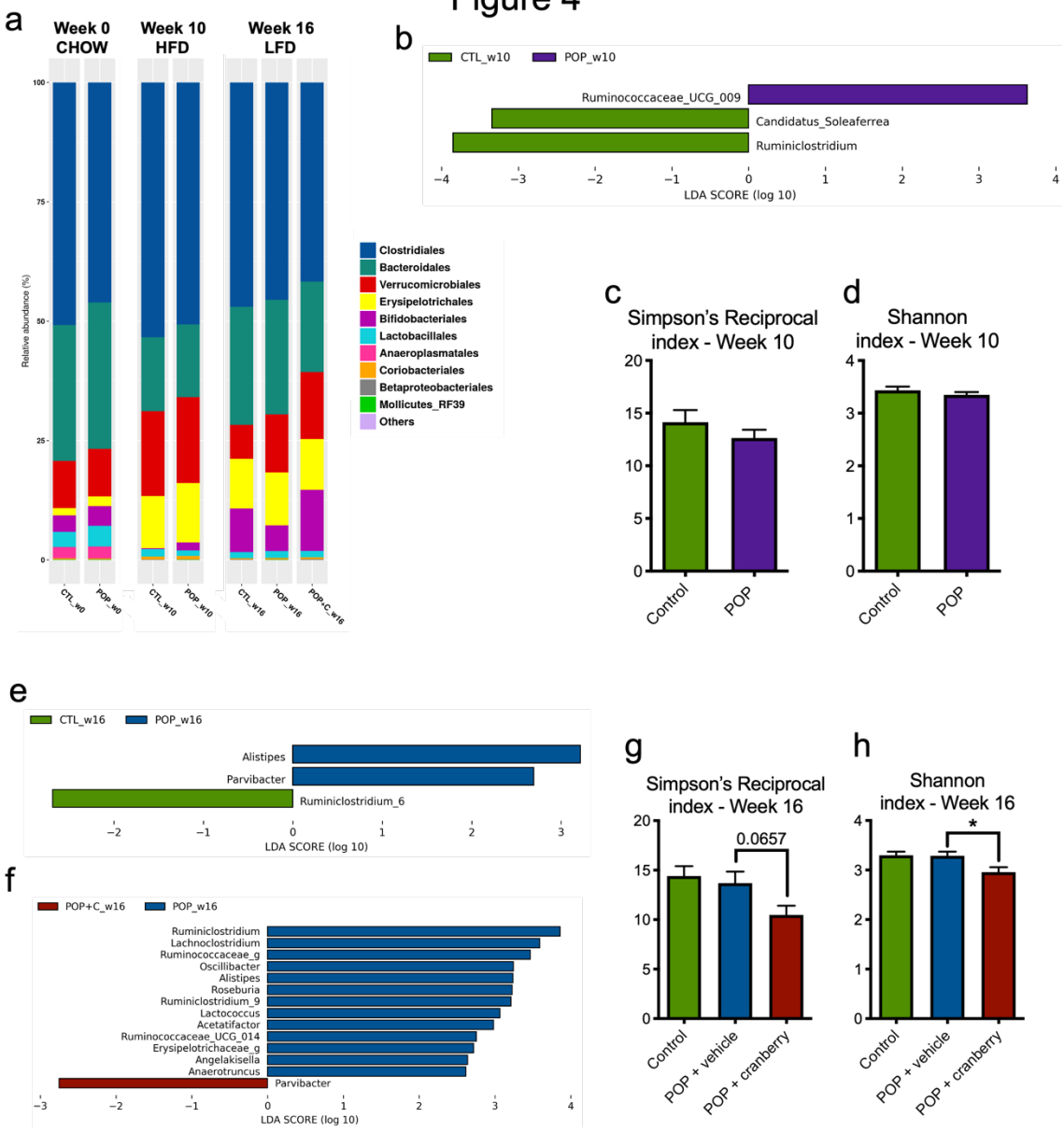

a
Weight loss
Weight loss (g)
Weeks
0.1006
Control
POP + vehicle
POP + cranberry
b
Adipose tissue change
Week 12 to 16
Adipose tissue change (%)
0.0630
Control
POP + vehicle
POP + cranberry
c
Fasting glycemia
Week 14
Glycemia (mmol/L)
d
Fasting insulinemia
Week 14
Insulin (ng/mL)
e
Glycemia - OGTT
Week 16
Glycemia (mmol/L)
Time (min.)
f
AUC OGTT
Week 16
AUC units
Control
POP
POP + Cranberry
g
Insulinemia OGTT
Week 16
Insulin (ng/mL)
Time (min.)
g
Plasmatic total cholesterol
CHOL (mmol/L of plasma)
h
Plasmatic triglycerides
TG (mmol/L of plasma)
i
Liver weight
Tissue weight (g)
j
Hepatic triglycerides
TG (mg/g of tissue)

Figure 4


a
Week 0 CHOW
Week 10 HFD
Week 16 LFD
Relative abundance (%)
CTL_w0
POP_w0
CTL_w10
POP_w10
CTL_w16
POP_w16
POP+C_w16
Clostridiales
Bacteroidales
Verrucomicrobiales
Erysipelotrichales
Bifidobacteriales
Lactobacillales
Anaeroplasmatales
Coriobacteriales
Betaproteobacteriales
Mollicutes_RF39
Others
b
CTL_w10
POP_w10
Ruminococcaceae_UCG_009
Candidatus_Soleaferrea
Ruminiclostridium
LDA SCORE (log 10)
c
Simpson's Reciprocal index - Week 10
Control
POP
d
Shannon index - Week 10
Control
POP
e
CTL_w16
POP_w16
Alistipes
Parvibacter
Ruminiclostridium_6
LDA SCORE (log 10)
f
POP+C_w16
POP_w16
Ruminiclostridium
Lachnoclostridium
Ruminococcaceae_g
Oscillibacter
Alistipes
Roseburia
Ruminiclostridium_9
Lactococcus
Acetatifactor
Ruminococcaceae_UCG_014
Erysipelotrichaceae_g
Angelakisella
Anaerotruncus
Parvibacter
LDA SCORE (log 10)
g
Simpson's Reciprocal index - Week 16
0.0657
Control
POP + vehicle
POP + cranberry
h
Shannon index - Week 16
*
Control
POP + vehicle
POP + cranberry

1.12 Supplementary Material

Supplemental table 1. Circulating POP at week 16, in µg/L of plasma.

		Control		POP + vehicle		POP-cranberry	
Group	POP	Average	SEM	Average	SEM	Average	SEM
DDT and metabolites	p,p'-DDE	0.010	0.000	5.056	0.204	5.352	0.344
	p,p'-DDT	0.025	0.000	1.586	0.048	1.506	0.098
PCB	PCB-52	0.150	0.000	1.302	0.188	1.524	0.144
	PCB-101	0.005	0.000	1.407	0.050	1.424	0.096
	PCB-28	0.025	0.000	0.745	0.029	0.799	0.060
	PCB-138	0.012	0.004	15.01	0.658	14.17	0.974
	PCB-153	0.018	0.004	15.66	0.692	14.91	0.902
	PCB-180	0.007	0.002	2.695	0.121	2.509	0.178
PBDE	PBDE-47	0.075	0.012	0.792	0.045	0.963	0.083
	PBDE-100	0.005	0.000	0.707	0.049	0.805	0.056
	PBDE-154	0.005	0.000	0.267	0.008	0.251	0.015
	PBDE-209	0.021	0.011	0.079	0.014	0.062	0.007
	PBDE-28	0.005	0.000	0.107	0.005	0.121	0.009
	PBDE-99	0.087	0.013	0.677	0.023	0.665	0.039
	PBDE-153	0.011	0.003	0.179	0.008	0.163	0.013

Chapitre 2 : Gnotobiotic mice housing conditions critically influence the phenotype associated with the transfer of fecal microbiota in a context of obesity

Laurence Daoust[1,2,#], Béatrice S-Y Choi[1,2,#], Anne-Laure Agrinier[1,2], Thibault V. Varin[2], Adia Ouellette[1,2], Geneviève Pilon[1,2], Emile Levy[2,3], Yves Desjardins[2], Fernando F. Anhê[4], Vanessa P. Houde[1,2] & André Marette[1,2*]

[1] Quebec Heart & Lung Institute, Université Laval, Québec City, Québec, Canada.

[2] Institute of Nutrition and Functional Foods, Université Laval, Québec City, Québec, Canada.

[3] Research Centre, CHU Ste-Justine, Nutrition, Université de Montréal, Montréal, Québec, Canada.

[4] Department of Biochemistry and Biomedical Sciences, Farncombe Family Digestive Health Research Institute and Centre for Metabolism, Obesity, and Diabetes Research McMaster University, Hamilton, Ontario, Canada.

[#] These authors contributed equally.

* Corresponding author: andre.marette@criucpq.ulaval.ca

Keywords

Housing conditions, gut microbiota, obesity, NAFLD, fecal transplant, untargeted metabolomic

2.1 Résumé

Le microbiote intestinal est une cible potentielle pour la prévention ou le traitement des comorbidités associées à l'obésité, comme le diabète de type 2, les maladies cardiométaboliques et la stéatose hépatique non alcoolique. La transplantation de microbiote fécal (FMT) dans un modèle de souris axénique (GF) est une procédure courante pour étudier le rôle causal du microbiote intestinal dans la physiopathologie de l'obésité. Cependant, le manque de standardisation de cette technique peut contribuer à certaines divergences observées entre les études dans la littérature. L'objectif de ce projet était de déterminer l'impact de l'hébergement de souris GF colonisées avec le microbiote intestinal de souris traitées à l'extrait de canneberge enrichi en proanthocyanidines (PAC) ou de leurs homologues traitées avec un véhicule dans deux environnements distincts, sur le développement d'un phénotype métabolique associé à l'obésité et sur la colonisation

intestinale bactérienne. Pour ce faire, des souris GF nourries avec un régime riche en gras et en sucre (HFHS) ont été colonisées et logées dans des cages individuelles stériles et ventilées dans des conditions d'hébergement rigoureuses, soit dans le secteur GF, soit dans le secteur exempt d'organismes pathogènes spécifiques (SPF) de la même animalerie. De manière inattendue, après 8 semaines, nous avons observé un phénotype hépatique opposé dépendant de l'environnement chez les souris colonisées. Les souris hébergées dans le secteur GF recevant le microbiote fécal PAC ont montré une diminution significative du poids du foie et de l'accumulation des triglycérides et du cholestérol hépatiques par rapport à leur groupe contrôle respectif. Le phénotype inverse, c'est-à-dire une accumulation plus importante de lipides dans le foie, a été observé chez les souris logées dans le secteur SPF recevant le microbiote fécal PAC. De plus, les conditions de logement ont eu un impact sélectif sur la colonisation des taxons microbiens ainsi que sur le profil des métabolites fécaux. Ces résultats suggèrent que l'environnement dans lequel les souris gnotobiotiques sont logées influence fortement la composition et la fonction du microbiote intestinal et peut conduire à des phénotypes distincts après la colonisation. Une meilleure standardisation de l'utilisation des souris GF est nécessaire et des conditions d'hébergement strictes devraient être suivies.

2.2 Abstract

The gut microbiota is a potential target for prevention or treatment of obesity-associated comorbidities such as type 2 diabetes, cardiometabolic and non-alcoholic fatty liver diseases. Fecal microbiota transplant (FMT) in a germ-free (GF) mouse model is a common procedure to study the causal role of the gut microbiome in the pathophysiology of obesity. However, the lack of standardization of this technique may contribute to some discrepancies observed between studies in the literature. The objective of this project was to determine the impact of housing GF mice colonized with the gut microbiota of either proanthocyanidins-enriched cranberry extract (PAC) treated mice or their vehicle-treated counterparts in two distinct environments on the development of an obesity-associated metabolic phenotype and bacterial intestinal colonization. To do so, GF mice fed a high-fat high-sucrose (HFHS) diet were colonized and housed in sterile individual ventilated cages under rigorous housing conditions either in the GF sector or in the specific pathogen

free (SPF) sector of the same animal facility. Unexpectedly, after 8 weeks, we observed opposite liver phenotype dependent on the housing environment in colonized mice. Mice housed in the GF sector receiving the PAC fecal microbiota showed a significant decrease in liver weight and both hepatic triglycerides and cholesterol accumulation compared to their respective control group. The inverse phenotype i.e. a higher lipid accumulation in the liver, was observed in mice housed in the SPF sector receiving the PAC fecal microbiota. In addition, housing conditions had a selective impact on the colonization of microbial taxa as well as fecal metabolites profile. These results suggest that the environment in which gnotobiotic mice are housed strongly influences gut microbiota composition and function and can lead to distinctive phenotypes post colonization. Better standardisation of the use of GF mice is needed and strict housing conditions should be followed.

2.3 Introduction

The development of culture-independent methods has enabled the identification of previously unknown taxa and contribute to better understanding the role of the intestinal microbiota in human health. The implication of the gut microbiota has emerged as a potential target for the prevention or the treatment of obesity-associated comorbidities, such as type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD) and cardiometabolic diseases (1-3). Nowadays, publications assessing the role of the microbiome is these pathologies are numbered in thousands. Different methods have been used to establish causality, not just association, between the effect of the microbiome and metabolic disorders associated with obesity (4), and fecal microbial transplantation (FMT) to germ-free (GF) mice is the most common approach. The latter aims to demonstrate the causal role of the gut microbiota in specific pathophysiological traits by colonizing GF mice with fecal samples from donors and thereafter assessing phenotype-transmissibility in GF recipient mice (i.e., gnotobiotic mice). Using this technique, it is possible to determine the contribution of the gut microbiota to a given pathology or condition as the composition and the functionality of the transplanted microbiome sample is known (5). This technique has also allowed researchers to better mimic human physiology by transplanting human fecal samples to GF mice (6-8).

While FMT techniques have undoubtedly contributed to a better understanding of the role of gut microbiome in a growing number of conditions and diseases, some concerns remain regarding the reproducibility of this approach when replicated in different facilities. The lack of standardization of FMT protocols may contribute to some of the discrepancies observed between studies in the literature (9) thereby limiting reproducibility and translatability of microbiome data in the field. Gnotobiotic isolators are considered the gold standard for housing gnotobiotic animals, but require expensive maintenance costs and are not designed for individual mice housing (10). For short-term housing, studies have shown the efficacy of the individually ventilated cages (IVC), which are suitable for multiple groups testing and allow procedures to be less time-consuming (11, 12). However, the main issue is that this equipment is not always present in animal facilities. Hence, many researchers rely on using more accessible and/or less costly alternatives, such as administering antibiotics to conventional mice or housing/handling colonized GF mice in a specific pathogen free (SPF) environment designed to maintain rodents in an environment free of certain pathogens (13, 14). However, it is documented that the surrounding environment in which mice are housed significantly impacts the composition and functionality of the gut microbiome (15). Likewise, in humans, delivery by caesarian section rather than through birth canal dictates the type/source of microbes that pioneer newborn colonization, and has been associated with increased risk to develop metabolic diseases later in life, as newborns are first exposed to the hospital's bacterial environment as opposed to those naturally born and thus first exposed to the vaginal microbiota (16, 17). Furthermore, as FMT is increasingly considered as a potential strategy for the treatment of obesity and related metabolic diseases (18, 19), a better understanding of the impact of the environment on successful microbiota transfer is needed to ensure clinical translatability and efficacy.

In the present study, we aimed to determine the impact of distinct housing conditions on the metabolic outcomes of GF mice colonized with the gut microbiota of donor mice treated with a known gut microbiota modulator (cranberry proanthocyanidins (PAC)). PAC are a class of polymeric polyphenols and are well-known to improve metabolic and liver health

in obese animals through modulating gut microbiota composition (20-23). We compared the impact of maintaining high-fat high-sucrose (HFHS)-fed mice colonized with fecal materials from PAC or vehicle treated HFHS-fed donor mice in either the GF or the SPF sector of our animal facility. Unexpectedly, we observed housing-specific divergent liver phenotypes after colonizing GF mice with the same fecal material. Our results call for caution in extrapolating the causal implication of the gut microbiome in FMT-driven metabolic phenotyping when housing conditions are not strictly controlled for, and further highlight the need to standardize such experiments to ensure reproducibility and translatability of FMT results in the field.

2.4 Material & Methods

Production of PAC fraction from polyphenol-rich cranberry extract

PAC were extracted from a frozen cranberry ethanolic extract produced by Diana Food (SD002240009/CN18000880) containing 32.9% of polyphenols and 10.5% of PAC. The final enriched fraction was produced by the method described by Gu et al. (24) contained 88.1% of polyphenols, 2.2% of PAC with a DP (degree of polymerization) 1-4 and 23% of PAC with a DP >5.

Animals

C57BL/6J donor male mice (The Jackson Laboratory, Bar Harbor, ME) arrived at 6 weeks of age and were acclimatized for 12 days. Mice were thereafter single-housed in positive flow ventilated cages in a 12h dark-light cycle SPF facility designed to maintain rodents in an environment free of certain pathogens with water and food ad libitum. After acclimatization, mice were fed either a standard chow diet (Teklad, Envigo, IN, USA) or a HFHS diet (65% lipids; D17051002 (Research Diet, NJ, USA)). Chow- and HFHS-fed mice received water daily by gavage, whereas an additional group of HFHS-fed mice was orally treated with 83.3mg/kg/day of a cranberry PAC extract. This dose is representative of the amount of PAC in 200 mg/kg of cranberry extract, as previously published by our research team (20, 25). Food intake was measured 3 times per week and mice were weighted weekly. Body composition was measured by quantitative nuclear magnetic resonance (qNMR) with a Bruker Minispec LF90II (Bruker Optics, Germany). At week 8,

mice were place in clean cages without the bedding for 24 hours and feces were collected for assessment of fecal energy excretion. Fresh feces were collected for 16S rRNA gene-based analysis of gut microbial profile at the end of the treatment. Additional fresh feces were also collected at the end of the treatment for preparation of fecal slurries used for fecal microbial transplants (FMT). At the end of the study, mice were fasted for 6h and gavaged with their daily treatment 2h before euthanasia by cardiac punction under anesthesia with isoflurane. Organs and plasma were collected for further analysis.

Fecal microbiota transplantation (FMT) studies

FMT solutions from vehicle-treated HFHS-fed (HFHS) and cranberry PAC-treated HFHS-fed (HFHS+PAC) donor mice were prepared as follows: Fifty mg (+/- 5 mg) of feces were collected for each of the 12 donor mice within each group at week 8 and kept at -80°C until further use. Fecal samples from each group were thereafter combined and diluted in cold sterile PBS (20µl/mg of feces), vortexed for 15 minutes at maximum speed, and centrifuged for 5 minutes (4C°, 300 rpm). The fecal slurry was then aliquoted and stored at -80°C. Each aliquot was thawed once, when used for gavaging the animals. Seven- to nine-week-old GF recipient mice (n=40) were purchased from the International Microbiome Centre (University of Calgary, Alberta, Canada). Upon arrival, recipient mice were randomly housed in the axenic-gnotobiotic facility (n=20) or in the SPF facility (n=20) in individually positive flow ventilated sterile cages (ISO cage P and GM500 from Techniplast, respectively) and orally administered with 200µl of fecal slurries from HFHS or HFHS+PAC donor mice twice over the study period (i.e., upon arrival and 4 weeks later). Recipient mice received ad libitum double-irradiated HFHS diet (D17051002-1.5V, Research Diet); diet was changed 3 times per week, and sterile water changed weekly for the duration of the study. Body weight was taken weekly, and fresh feces were collected for 16S rRNA gene-based gut microbial analysis and untargeted metabolomics. In the SPF facility, cages were only opened under a biosafety cabinet with HEPA filters, handled with sterile gloves disinfected between each mouse and changed between groups. Upon 8 weeks of colonization, mice were euthanasia by cardiac punction under anesthesia with isoflurane. Organs and plasma were collected for further analysis.

Polyphenol measurements

Dihydroxyphenyl-γ-valerolactone sulfate were extracted from plasma according to a previously described method (26). A volume of 110µL of plasma, mixed 1:1 with phosphoric acid 4% were loaded through a preconditioned Oasis HLB microelution plate (Waters, Milford, MA, USA) with 250µL methanol and 250 µL acetic acid 0.1%. Loaded plates were washed with 200µL water and 200µL acetic acid 0.2%, then eluted with 75µL 70:29.5:0.5 acetone/water/acetic acid containing 1 µg/mL 4-hydroxybenzoic-2,3,5,6-d4 acid. Extracts were directly analyzed by UHPLC-ESI-Q-TOF (Acquity ultra-performance liquid chromatography I-Class system combined to an electrospray ionization source and quadrupole time-of-flight Synapt G2-Si Mass Spectrometer, Waters, Milford, MA, USA). Dihydroxyphenyl-□-valerolactone sulfate were identified with exact mass of [M-H]- (287.0225 m/z) and presence of specific fragmented ion products (207.0586 m/z) in the pool of all samples and quantified as 3,4-dihydroxyphenyl-□-valerolactone equivalent (27).

Fecal energy excretion

Fecal pellets collected during a 24h period after 8 weeks of treatment were dried and energy density was determined using direct calorimetry (Adibactic bomb calorimetry, Parr instruments, Moline, IL, USA).

Biochemical analysis

Liver lipids were extracted using the chloroform-methanol Folch lipid extraction as previously described (20). Triglycerides and cholesterol were quantified using commercial kits (Infinity Triglycerides Reagent, Thermo Fisher Scientific) according to the manufacturer's instructions. Circulating triglycerides were quantified in the plasma collected at chemiluminescence (Dimension Vista 1500, Siemens, Germany) at the biochemical analysis platform of the Quebec Heart Lung Institute. Proteins from approximatively 80 mg of liver tissue were extracted in PBS buffer (containing Igepal 1% and protease inhibitors; MilliporeSigma) for IL-6 quantification. After centrifugation, proteins were dosed in supernatants (bicinchoninic acid dosage; Thermo Fisher Scientific) to standardize the loading of the amount of total proteins for the ELISA. Determination of

mouse IL-6 was done by ELISA, according to the manufacturer's instructions (R&D Systems, MN, USA).

Hematoxylin & Eosin staining

Liver tissues were fixed in paraformaldehyde, embedded in paraffin and stained with hematoxylin and eosin at the Institut de biologie intégrative et des systèmes (IBIS) at Laval University.

Liver antioxidant activity

For the assessment of the endogenous antioxidant defense, tissue samples were homogenized in a buffer (50 mM Tris-HCl and 0.1 mM EDTA-Na2 pH 7.8), centrifuged at 10 000 g for 5 min at 4°C and the supernatants were collected. The activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) were determined as previously describe (28). For GPx activity, hepatic tissue homogenates were added to a phosphate buffered saline buffer (pH 7.0) containing 10 mM GSH, 0.1 U of G-Red and 2 mM NADPH with 1.5% H2O2 to initiate the reaction. Absorbance was monitored every 30 seconds at 340 nm for 5 min.

16S rRNA high-throughput sequencing & analysis of fecal sequencing data

Fresh feces in SPF and GF mice were harvested after 4 and 8 weeks of treatment. Feces were immediately snap frozen at -80°C. Fecal DNA was extracted using the NucleoSpin 96 Soil, 96-well kit for DNA from soil from Macherey-Nagel according to manufacturer's instructions. Samples were sent to Génome Québec for 16S amplification and sequencing. For each DNA sample, amplification of the V3-V4 region was performed using the primers 341F (5-CCTACGGGNGGCWGCAG-3) and 805R (5-GACTACHVGGGTATCTAATCC-3) (Illumina, CA, USA). Libraries were purified using AxyPrep magnetic beads (Axygen Biosciences, CA, USA) and libraries were assessed using DNA 7500 chips (Agilent Technologies, CA, USA) and picogreen (Life Technologies, CA, USA). High-throughput sequencing (2 × 300 bp paired-end) was performed on a MiSeq platform (Illumina, CA, USA) at Génome Québec. Sequences were processed using the DADA2 package (v1.10.1) (29) in the R environment (http://www.R-

project.org) and associations to bacterial taxa was obtained using the RDP classifier algorithm (v2.2) (30) trained against the Silva database 132 (31). To quantify bacterial alpha-diversity, Shannon and Simpson's reciprocal indexes were calculated using the phyloseq package (32). The functional profile of KEGG Orthology (KO) for each sample was predicted from 16S rRNA amplicon data with Tax4Fun (33). In order to normalize sampling effort, samples were rarefied to an even sampling depth of 14198 and 10212 sequences for fecal and mucus sequencing data, respectively.

Untargeted metabolomics

Samples were randomized and extracted from protocol adapted from Cheng et al., 2020 (34). Briefly, 15-60 mg of feces was homogenized with grade-HLPC methanol in ratio of 1:5 (w:v). After vortexing for 6 minutes (RT, 1400 rpm), samples were ultra-sonicated for 30 minutes at room temperature, then vortexed again. Samples were centrifuged at 17 000 g for 20 minutes at 4°C and the supernatants were collected and stored at -80 °C until LC-MS analysis. The peaklist obtained after preprocessing (features defined by their mass/charge value and retention time) were analyzed with MetaboAnalystR package in R (35). Data were auto-scaled (mean-centering and division by the square root of standard deviation of each variable) to enforce Gaussian distribution enabling relative comparison. Univariate and multivariate analysis were performed with t-tests, fold-change analyses and principal component analyses (PCA) to detect significant features and to visually separate trends between groups. A fold-change and a p-value threshold of, respectively, 2 and 0.05 were used to discriminate significant features. The putative identification of the metabolites was carried out from the databases: Human Metabolite Data Base (36), KEGG(37). Identifications with corresponding fragmentations were retained to determine the class of compounds.

Statistical analysis

A student-t test (for parametric data sets) or Mann-Whitney test (for non-parametric data sets) were used to assign significance between two groups (Graph Pad Prism, USA). Shapiro-Wilk test was used to assess the normal distribution of the data ($p>0.05$). A two-way repeated measures ANOVA with a Bonferroni post hoc test was used when assessing

the significance of the effect at different time points. We applied the linear discriminant analysis effect size (LEfSe) method for differential abundance analysis for fecal sequencing data (38). A linear discriminant analysis (LDA) score ≥ 2.5 was considered statistically significant. According to the distribution of data, Spearman's or Pearson's rank correlation were used to assess the degree of association between metabolic parameters and bacterial genera/metabolites. Bacterial genera present in at least 50% of all samples and significantly correlated ($P<0.1$) were considered and visually represented. Metabolites significantly different between the two sectors after performing an ANOVA ($P<0.05$), controlled for FDR and significantly correlated ($P<0.05$; FDR-corrected) were considered and visually represented.

2.5 Results

Administration of a cranberry PAC extract in HFHS-fed donor mice reduce body weight gain without changes in energy intake and excretion

Donor mice were fed a HFHS diet and supplemented with a cranberry PAC extract or the vehicle (water) by daily gavage for 8 weeks (Fig 1A). Chow-fed mice were used as a healthy reference group to assess efficacy of the HFHS diet to promote obesity. We observed decreased body weight gain throughout the treatment in HFHS + PAC mice compared to HFHS control mice (Suppl. Fig. 1A), which reached significance when assessing total body weight gain (Fig. 1B). This decrease in total body weight gain was mainly associated with a decreased fat mass (Fig. 1C) as lean mass was similar between groups (Figure 1D). Cumulative food intake throughout the 8 weeks was similar between both HFHS-fed groups (Suppl. Fig. 1B) as was 24h fecal energy excretion (Suppl Fig. 1C). Liver weight and triglycerides were similar between HFHS-fed groups (Suppl. Fig. 1D, E).

PAC extract induces specific changes in the gut microbiota of HFHS-fed donor mice and is associated with changes in fecal metabolites

We analyzed the composition of the gut microbiota after 8 weeks of treatment using 16S rRNA gene sequencing. As previously reported, feeding mice a HFHS diet in mice strongly shifted gut microbial composition as compared to standard chow-fed mice (Fig. 1E).

Conversely, while changes to gut bacterial communities were more subtle between vehicle- and PAC-treated HFHS-fed mice (Fig. 1E), intra-community variations were similar between these groups as captured by two alpha-diversity indices (Fig. 1F-G). We therefore applied the LeFSe method to filter the taxa that better discriminate between groups and to calculate their effect size. This analysis disclosed the genera *Blautia* and *Intestinimonas* as key discriminative features in the gut microbiota of HFHS+PAC mice, whereas the genus *Anaerotruncus* was strongly discriminative of gut microbial communities from vehicle-treated HFHS mice (Fig. 1H). We next applied untargeted metabolomics as an exploratory approach to better understand the role of gut microbiome in shaping the metabolic phenotype. This analysis revealed five metabolites that differed significantly in the feces of HFHS compared to HFHS-PAC treated-mice (Fig.1I). Lastly, we assessed microbial metabolism of PAC after gavage by measuring plasma levels of dihydroxyphenyl-γ-valerolactone sulfate, one of the main microbial metabolite resulting from PAC degradation (39), and confirmed it was detectable in all mice receiving the PAC extract and not in the vehicle-treated mice (Fig. 1J).

The composition of the fecal slurry reflects changes in the donors' gut microbiota

We used amplicon-based sequencing to analyze and validate the composition of the fecal slurries derived from feces of PAC- or vehicle-treated HFHS mice (Fig. 1K) against the fecal microbial profile found in these mice (Fig. 1F). We validated higher relative abundance of *Intestinimonas* and, to a lesser extent, *Blautia* in fecal slurries of HFHS + PAC as opposed to the vehicle-treated HFHS mice. However, at odds with the gut microbial profile of vehicle-treated HFHS mice, *Anaerotruncus* was not different in fecal slurries of these mice as compared to HFHS + PAC mice.

Housing conditions strongly influence the liver phenotype in HFHS-fed germ-free mice receiving the cranberry PAC FMT without changing weight gain

We next performed FMT using fecal slurries from HFHS-vehicle or HFHS-PAC donor mice to GF mice maintained in either GF or SPF housing conditions (Fig. 2A). The gnotobiotic mice were single housed for 8 weeks, and we implemented additional measures that aimed to replicate as closely as possible the axenic-gnotobiotic settings in the SPF

facility. These included, but was not limited to, sterilization of cages, water and food, handling of animals under a biosafety cabinet, and use of sterile gloves (see Table 1 for details). Colonization with fecal slurries from HFHS + PAC mice did not impact body weight gain or food intake, regardless of housing condition, as compared to counterparts that received fecal material from HFHS + vehicle donor mice (Suppl. Fig. 2A-C & G-I). As the intestine is physiologically and anatomically connected to the liver, we sought to investigate the effect of gut microbes transferred by FMT on that organ. When housed in the GF sector, we observed a significant decrease in liver weight and hepatic triglycerides and cholesterol accumulation in GF mice that received FMT from HFHS + PAC mice as compared to those colonized with feces from vehicle-treated HFHS mice (Fig. 2B-D), Plasma triglycerides were not different between groups (Fig. 2E). These changes were also associated with a trend towards lower IL-6 levels in the liver of GF mice that received FMT from HFHS + PAC mice, suggesting reduced inflammatory tone along with decreased hepatic steatosis in these mice (Fig. 2F). The latter was further corroborated by H&E staining of liver sections showing increased accumulation of lipid droplets in FMT HFHS mice housed in the GF sector as opposed to their counterparts (Fig. 2G). Remarkably, when assessing the liver phenotype of GF recipient mice housed in SPF conditions, we observed opposing effects. In contrast to GF mice kept in the GF facility, FMT HFHS + PAC mice kept in SPF conditions showed a tendency for increased liver weight and significantly greater liver triglycerides and cholesterol levels as compared to their FMT HFHS counterparts (Fig. 2H-J). Plasma triglycerides and liver IL-6 were not different between groups (Fig. 2K-L), whereas H&E staining of liver sections corroborated the more pronounced liver deposition in FMT HFHS + PAC mice as compared to FMT HFHS (Fig. 2M).

The activity of hepatic antioxidant enzymes was also assessed as it is tightly related to oxidative/antioxidative balance in the liver, which can be disturbed in a context of obesity. In mice housed in the GF sector and bearing the HFHS + PAC microbiota, we observed decreased activity of superoxide dismutase (SOD), no change in the activity of catalase (CAT), and an increase of glutathione peroxidase activity (GPx) (Suppl. Fig. 2D-F). On the other hand, mice bearing the HFHS + PAC microbiota in the SPF environment had a

decrease in both SOD and CAT activity, while no change was observed in GPx, suggesting a reduced redox function during SPF housing (Suppl. Fig. 2J-L). These results suggest that although mice were colonized with the same fecal slurries, their housing conditions strongly influenced the development of hepatic metabolism and redox function, potentially linked to a different environmental pressure on the microbial colonization of the gut.

Housing environment of gnotobiotic mice exert a strong pressure on the colonization of the gut microbiota

We next assessed the composition and predicted function of the gut microbiota in FMT HFHS and FMT HFHS + PAC mice housed in both the GF and SPF sector (Fig. 3A-G & Suppl. Fig. 3 A-D). Consistent with donor fecal samples and fecal slurry composition, we observed an overrepresentation of *Intestinimonas* in both groups bearing gut microbiota from the HFHS+PAC independently of housing condition and time. An overrepresentation of *Enterohabdus* in both groups bearing the gut microbiota from the HFHS was also observed at both 4 weeks and 8 weeks post-FMT (Fig 3 A-B, Suppl. Fig. 3C-D). Several changes in microbiota composition that were dependent on housing conditions were also consistent over time, such as increase in relative abundance of *NK4A214* in the FMT HFHS-PAC group and of *Clostridia_o_f_g* in the FMT HFHS in the GF sector (Fig. 3A, Fig. Suppl. 3C) and an overrepresentation of *Alistipes* in FMT HFHS-PAC and of *Monoglobus* in the FMT HFHS group in the SPF sector (Fig. 3B, Fig. Suppl. 3D). Overall alpha-diversity measured by Shannon and Simpson's reciprocal indexes was similar between groups in their respective sectors (Fig. 3C-F).

Interestingly, the use of KEGG Orthology to predict the functional alterations in the gut microbiota revealed changes in gnotobiotic mice housed in the GF sector only. FMT HFHS + PAC mice in the GF sector showed an increased in functional pathways associated with membrane transport (ABC transporters and phosphotransferase system) and carbohydrate metabolism (starch and sucrose metabolism) whereas FMT HFHS mice showed increased functional pathways associated with bacterial infectious diseases (*Staphylococcus aureus* infection) and signal transduction (two component system) (Fig 3G). The absence of significant changes in the SPF sector suggests the compositional changes of the gut

microbiota in the SPF sector were not robust enough to induce functional alterations in the gut microbiota.

We next investigated the impact of housing conditions on fecal metabolites using untargeted metabolomics as an exploratory approach and found a similar trend in that less changes were present between colonization groups in the SPF sector compared to the GF sector. Although animals grouped similarly in both sectors on principal component analysis (Fig. 3H-I), four metabolites were found to be significantly different when comparing both mouse groups housed in the GF sector (Fig. 3J) whereas no significant changes were observed between mouse group housed in the SPF sector.

Liver phenotype correlates with distinctive bacteria and metabolites in the GF and SPF sector

To identify specific factors that could explain the influence of housing conditions on liver health after gut colonization, correlations were made between 16S data and the 3 mostly affected liver parameters, namely weight, triglycerides and cholesterol. In both experimental group of mice housed in the GF environment, we observed a negative correlation between the liver weight and the following bacterial genera: Colidextribacter, Lachnoclostridium, Lachnospireaceae, Lactoccocus, Oscillospireaceae, Ruminoccocaceae, Tuzzerella and an unknow genus, GCA-900066575. Liver triglycerides negatively correlated with Lactococcus and Oscillospireaceae. In addition, liver cholesterol positively correlated with Blautia and negatively with Lactococcus (Fig. 4A). In mice housed in the SPF environment, liver weight negatively correlated with Akkermansia and positively correlated with Blautia, Lachnospiraceae, Roseburia and an unknow genus. As for liver triglycerides, this parameter negatively correlates with Bacteroides and positively correlates with Butyricicoccus, Lachospireaceae_NK4A136_group, Roseburia and Ruminococcaecaeae_g. Finally, liver cholesterol negatively correlates with Akkermansia and Bacteroides and positively correlates with Blautia, Roseburia, Ruminococcaceae_g and an unknow genus (Fig. 4A).

We then used a similar approach to identify potential metabolites influenced by housing conditions that correlated with metabolic parameters (Fig 4B). Remarkably, we observed significant correlations, positive or negative, for all liver parameters but only for both experimental group of mice housed in the GF sector as opposed to the SPF sector. Interestingly, two identified metabolites i.e., monocarboxylic acid / Steroids and steroid derivatives and monoradylglycerols negatively correlated with the liver weight and all 3 parameters, respectively.

2.6 Discussion

The purpose of this study was to determine the influence of housing GF mice colonized with the fecal microbiota of PAC-treated donor mice in two distinct environments i.e., GF or SPF sector, on the development of an obesity-associated metabolic phenotype and bacterial intestinal colonization. No changes on weight gain or food intake were observed over the 8 weeks of HFHS diet between mice housed in either sector. Surprisingly, we observed opposite liver phenotypes influenced by FMT of gnotobiotic mice that were dependent on the housing environment. On one hand, GF mice treated with the FMT HFHS + PAC showed decreased liver lipid accumulation whereas the administration of the same fecal slurry to GF mice housed in the SPF sector exacerbated the development of a fatty liver associated with the consumption of a HFHS diet. Even though experimental protocols in both sectors happened simultaneously and determinant factors were identical, such as age and origin of the animals, water, food and fecal colonization solutions, the different housing environments favored the colonization of different bacterial taxa and function which led to divergent liver phenotypes. To the best of our knowledge, this study is the first to compare simultaneous colonization of GF mice in an axenic/gnotobiotic setting vs sterile cages in a classic SPF sector. Most published studies have compared the efficacy of standard gnotobiotic isolators *vs* the more recent IVC system to maintain the gnotobiotic status of the rodents (11, 12).

One of the most important aspects when manipulating gnotobiotic animals is to strictly follow procedures to maintain mice in a sterile environment and avoid contaminations with external microorganisms. These could arise from human skin and hair or from the

procedure room (10). Previous work by Lebeuf *et al.* (40) suggest that the strong bacterial environmental pressure in the procedure room of the SPF facility could have contributed to the complete opposite liver phenotypes we observed in gnotobiotic mice colonized with the FMT HFHS + PAC. By comparing CFU present in housing room of both the SPF and GF sector in the animal facility of our research institute, they observed a bacterial load 1000 times higher in the room of the SPF sector as opposed to the GF sector. The lack of strict access restriction and the request to wear only standard protection equipment for animal facility personnel in rooms of the SPF sector, which are recognized standards for SPF staff, certainly contributes to highest bacterial load as opposed to the GF sector where only trained animal technicians wearing full body protection are allowed. The increased risk of contamination when manipulation mice in the SPF sector could promote the colonization of environmental bacteria in gnotobiotic mice and later affect the development of specific metabolic phenotypes. For instances, *Acinetobacter* and *Aquabacterium*, which were not detected in the fecal slurry, were found to be present in 3 mice housed in the SPF sector at T8 whereas none of those were detected in mice housed in the GF sector. *Acinetobacter* is an aerobic environmental bacterium that can resist to disinfectant and often triggers nosocomial infections in hospitalised patients (41). On the other hand, *Aquabacterium* is an aerobic environmental bacterium mostly found in water and hot springs (42). Interestingly, a disrupted state of anaerobiosis in the colon is associated with inflammatory bowel diseases (43) which could help explain the presence of aerobic microbes in the gut of HFHS-fed mice, which also have disrupted gut integrity (44, 45). Our results also suggest that the use of the IVC system in the GF sector allows to expose more subtle changes associated with the different FMT. When assessing functionality of the gut microbiome using KEGG orthology, we observed no significant change between the treated groups in the SPF sector as opposed to mice housed in the GF sector. We also performed untargeted metabolomics analysis using LC-MS on fecal samples and observed the same pattern. Significantly different metabolites between both groups were only found in the GF and not the SPF sector. This was further confirmed when looking at correlations between liver phenotype and fecal metabolites. Indeed, many significant correlations were observed in the GF sector whereas none were found this the SPF sector.

One of the important markers of the FMT is the overrepresentation of *Intestinimonas* in fecal samples of FMT HFHS + PAC treated mice of both sectors. This bacterium was first found to be overrepresented in HFHS-donor mice treated with the CE PAC-enriched extract. Interestingly, the reduced abundance of this bacterium has previously been associated with obesity in a metabolic disease cohort (46). *Intestinimonas* was also detected in the fecal slurry of FMT HFHS + PAC and subsequently overrepresented in GF mice housed in both sectors that received this FMT at both time points sampled (T4 and T8). As liver phenotypes were opposite, these results suggest that the presence of *Intestinimonas* alone is unlikely to be the cause of the observed phenotype. This also strengthens the hypothesis that in many cases, a single bacterial species is not sufficient to account for a metabolic phenotype, but rather a bacterial consortium and/or an ecological niche are necessary. Thus, even if some bacteria were overrepresented in mice receiving the same FMT independently of their housing sector such as *Monoglobus*, *Enterorhabdus* and *Intestinimonas*, the rest of the consortium could have influenced their functionality.

To our surprise, bacteria correlating with liver phenotypes in both sectors were very different. Indeed, only *Blautia* correlated positively with liver parameters in both housing sectors and interestingly, this genus was enhanced in fecal samples from donor mice receiving the daily PAC treatment. Recently, Liu *et al.* (47) have highlighted the potential probiotic effect of this bacterium through its ability to metabolize different compounds that would normally be non-digestible for the host and its production of bacteriocins. Furthermore, *Lactococcus* in the GF sector and *Roseburia* in the SPF sector were respectively negatively and positively correlated with all three measured liver parameters, making them the most robust associations to accumulation of lipids in the liver. In accordance with our results, lactic acid bacteria such as *Lactococcus* have been frequently reported for their hepatoprotective activity (48-50). As for *Roseburia*, Aron-Wisnewsky *et al*. (51) reported discordant results regarding its microbial signature in the progression of NAFLD in humans. Moreover, *Ruminococaceeae_g*, which were not found to be overexpressed in any treated group when looking at LDA scores, had opposite correlations with liver phenotype in both sectors. Indeed, it negatively correlated with liver weight in mice housed in the GF sector and positively with liver triglycerides and cholesterol in mice

housed in the SPF sector. Although this correlation does not imply causality, this finding leads to the notion that the same microorganism can exert differential function depending on their ecological niche, available substrates, etc. A distinctive example in the literature is the case of *Prevotella copri*. This bacteria has been associated with improved postprandial blood responses in a large cohort of 1098 individuals (52) whereas others have suggested *Prevotella Copri* to be one of the main drivers of insulin resistance through microbial branched-chain amino acids biosynthesis (53). Some microorganisms can even have deleterious effect on the host when in specific environmental conditions, defined as a pathobiont (54), such as *Escherichia coli* (*E. coli*) (55). Indeed, the co-administration of *E. coli* mpk and *Bacteroides vulgatus* protects gnotobiotic IL2 $^{-/-}$ mice against colitis. On the other hand, IL2 $^{-/-}$ mice monocolonized with *E. coli* mpk develop colitis (55). These studies show the importance of preclinical models such as gnotobiotic mice to assess causality links of specific bacterium, which might in turn be environment-dependent.

Conclusion

In summary, our study shows for the first time that a simultaneous colonization of GF mice in two distinct environments (SPF vs GF) of the same animal facility can lead to differential metabolic phenotypes, that is, improved or exacerbated liver physiology associated with an obesogenic diet. These results emphasize the need for standardization of procedures surrounding FMT in rodent models to improve reproducibility and translatability. It is believed the findings of the present study further highlight the impact of environmental pressure, not only on success of colonization of the gastrointestinal tract by specific bacterial taxa, but also on key functional changes at the community level.

2.7 Acknowledgements

We thank Christine Dallaire, Jacinthe Julien, Joanie Dupont-Morissette, Cynthia Faubert and Alexandre Pleau for the expert help with animal care as well as Perrine Feutry for the polyphenol measurements.

Funding

This work was funded by the Weston Garfield Foundation and by a Foundation grant (FDN-143247) from the Canadian Institutes for Health Research (CIHR). AM is the recipient of a CIHR/Pfizer research Chair in the pathogenesis of insulin resistance and cardiovascular diseases and J.A.-DeSève foundation. LD is funded by the Jean-Paul-Houle fund from Laval University. BSYC is funded by doctoral scholarships from the Sentinel North program at Laval University (Canada First Research Excellence Fund) and from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Author contribution

LD, BSYC, GP and AM conceived the study and wrote the manuscript with inputs from all co-authors. LD and BSYC conducted animal studies and related measurements with the help from AO. TVV performed 16S sequencing analyses. ALA performed the untargeted metabolomic analyses. YD prepared the PAC extract. EL performed antioxidant enzymes analysis. FFA gave feedback and suggestions over the duration of the study and the manuscript preparation. GP, VH and AM supervised the study. LD, BSYC, GP and AM are responsible for the integrity of the work as a whole. All authors reviewed the manuscript.

Disclosure statement

No author declared a conflict of interest**.**

2.8 References

1. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004;101(44):15718-23.
2. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457(7228):480-4.
3. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.
4. Koh A, Backhed F. From Association to Causality: the Role of the Gut Microbiota and Its Functional Products on Host Metabolism. Mol Cell. 2020;78(4):584-96.
5. Ericsson AC, Franklin CL. Manipulating the Gut Microbiota: Methods and Challenges. ILAR journal. 2015;56(2):205-17.
6. Wrzosek L, Ciocan D, Borentain P, Spatz M, Puchois V, Hugot C, et al. Transplantation of human microbiota into conventional mice durably reshapes the gut microbiota. Scientific reports. 2018;8(1):6854.
7. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. Nature. 2016;529(7585):212-5.
8. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science translational medicine. 2009;1(6):6ra14.
9. Gheorghe CE, Ritz NL, Martin JA, Wardill HR, Cryan JF, Clarke G. Investigating causality with fecal microbiota transplantation in rodents: applications, recommendations and pitfalls. Gut Microbes. 2021;13(1):1941711.
10. Basic M, Bleich A. Gnotobiotics: Past, present and future. Lab Anim. 2019;53(3):232-43.
11. Hecht G, Bar-Nathan C, Milite G, Alon I, Moshe Y, Greenfeld L, et al. A simple cage-autonomous method for the maintenance of the barrier status of germ-free mice during experimentation. Lab Anim. 2014;48(4):292-7.
12. Lundberg R, Bahl MI, Licht TR, Toft MF, Hansen AK. Microbiota composition of simultaneously colonized mice housed under either a gnotobiotic isolator or individually ventilated cage regime. Scientific reports. 2017;7:42245.
13. Lundberg R, Toft MF, August B, Hansen AK, Hansen CH. Antibiotic-treated versus germ-free rodents for microbiota transplantation studies. Gut Microbes. 2016;7(1):68-74.
14. Le Roy T, Debedat J, Marquet F, Da-Cunha C, Ichou F, Guerre-Millo M, et al. Comparative Evaluation of Microbiota Engraftment Following Fecal Microbiota Transfer

in Mice Models: Age, Kinetic and Microbial Status Matter. Front Microbiol. 2018;9:3289.
15. Unger AL, Eckstrom K, Jetton TL, Kraft J. Facility-dependent metabolic phenotype and gut bacterial composition in CD-1 mice from a single vendor: A brief report. PLoS One. 2020;15(9):e0238893.
16. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature. 2019;574(7776):117-21.
17. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell Host Microbe. 2015;17(5):690-703.
18. Allegretti JR, Mullish BH, Kelly C, Fischer M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. The Lancet. 2019;394(10196):420-31.
19. Kootte RS, Levin E, Salojarvi J, Smits LP, Hartstra AV, Udayappan SD, et al. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. Cell Metab. 2017;26(4):611-9 e6.
20. Anhe FF, Roy D, Pilon G, Dudonne S, Matamoros S, Varin TV, et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. Gut. 2015;64(6):872-83.
21. Anhe FF, Nachbar RT, Varin TV, Vilela V, Dudonne S, Pilon G, et al. A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss. Mol Metab. 2017;6(12):1563-73.
22. Neto CC, Mortzfeld BM, Turbitt JR, Bhattarai SK, Yeliseyev V, DiBenedetto N, et al. Proanthocyanidin-enriched cranberry extract induces resilient bacterial community dynamics in a gnotobiotic mouse model. Microb Cell. 2021;8(6):131-42.
23. Ntemiri A, Ghosh TS, Gheller ME, Tran TTT, Blum JE, Pellanda P, et al. Whole Blueberry and Isolated Polyphenol-Rich Fractions Modulate Specific Gut Microbes in an In Vitro Colon Model and in a Pilot Study in Human Consumers. Nutrients. 2020;12(9).
24. Gu L, Kelm M, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S, et al. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. J Agric Food Chem. 2002;50(17):4852-60.
25. Anhe FF, Nachbar RT, Varin TV, Vilela V, Dudonne S, Pilon G, et al. A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss. Molecular metabolism. 2017.
26. Dudonné S, Dubé P, Pilon G, Marette A, Jacques H, Weisnagel J, et al. Modulation of Strawberry/Cranberry Phenolic Compounds Glucuronidation by Co-Supplementation with Onion: Characterization of Phenolic Metabolites in Rat Plasma

Using an Optimized μSPE-UHPLC-MS/MS Method. J Agric Food Chem. 2014;62(14):3244-56.
27. Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, et al. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). Metabolomics. 2007;3(3):211-21.
28. Veilleux A, Grenier E, Marceau P, Carpentier AC, Richard D, Levy E. Intestinal lipid handling: evidence and implication of insulin signaling abnormalities in human obese subjects. Arterioscler Thromb Vasc Biol. 2014;34(3):644-53.
29. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581-3.
30. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7.
31. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590-6.
32. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8(4):e61217.
33. Asshauer KP, Wemheuer B, Daniel R, Meinicke P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics (Oxford, England). 2015;31(17):2882-4.
34. Cheng K, Brunius C, Fristedt R, Landberg R. An LC-QToF MS based method for untargeted metabolomics of human fecal samples. Metabolomics. 2020;16(4):46.
35. Pang Z, Chong J, Li S, Xia J. MetaboAnalystR 3.0: Toward an Optimized Workflow for Global Metabolomics. Metabolites. 2020;10(5).
36. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, et al. HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res. 2018;46(D1):D608-d17.
37. Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 2021;49(D1):D545-d51.
38. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
39. Mena P, Bresciani L, Brindani N, Ludwig IA, Pereira-Caro G, Angelino D, et al. Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity. Nat Prod Rep. 2019;36(5):714-52.

40. Lebeuf M, Turgeon N, Faubert C, Robillard J, Paradis E, Duchaine C. Managing the Bacterial Contamination Risk in an Axenic Mice Animal Facility. Can J Microbiol. 2021.
41. Doughari HJ, Ndakidemi PA, Human IS, Benade S. The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes Environ. 2011;26(2):101-12.
42. Khan IU, Habib N, Asem MD, Salam N, Xiao M, Zhou EM, et al. Aquabacterium tepidiphilum sp. nov., a moderately thermophilic bacterium isolated from a hot spring. Int J Syst Evol Microbiol. 2019;69(2):337-42.
43. Henson MA, Phalak P. Microbiota dysbiosis in inflammatory bowel diseases: in silico investigation of the oxygen hypothesis. BMC Syst Biol. 2017;11(1):145.
44. Daniel N, Rossi Perazza L, Varin TV, Trottier J, Marcotte B, St-Pierre P, et al. Dietary fat and low fiber in purified diets differently impact the gut-liver axis to promote obesity-linked metabolic impairments. Am J Physiol Gastrointest Liver Physiol. 2021;320(6):G1014-G33.
45. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761-72.
46. Thingholm LB, Rühlemann MC, Koch M, Fuqua B, Laucke G, Boehm R, et al. Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial Functional Capacity and Composition. Cell Host Microbe. 2019;26(2):252-64.e10.
47. Liu X, Mao B, Gu J, Wu J, Cui S, Wang G, et al. Blautia-a new functional genus with potential probiotic properties? Gut Microbes. 2021;13(1):1-21.
48. Naudin CR, Maner-Smith K, Owens JA, Wynn GM, Robinson BS, Matthews JD, et al. Lactococcus lactis Subspecies cremoris Elicits Protection Against Metabolic Changes Induced by a Western-Style Diet. Gastroenterology. 2020;159(2):639-51.e5.
49. Nam Y, Kim JH, Konkit M, Kim W. Hepatoprotective effects of Lactococcus chungangensis CAU 1447 in alcoholic liver disease. J Dairy Sci. 2019;102(12):10737-47.
50. Lee NY, Yoon SJ, Han DH, Gupta H, Youn GS, Shin MJ, et al. Lactobacillus and Pediococcus ameliorate progression of non-alcoholic fatty liver disease through modulation of the gut microbiome. Gut Microbes. 2020;11(4):882-99.
51. Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. Nature reviews Gastroenterology & hepatology. 2020;17(5):279-97.
52. Asnicar F, Berry SE, Valdes AM, Nguyen LH, Piccinno G, Drew DA, et al. Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals. Nat Med. 2021;27(2):321-32.
53. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376-81.
54. Jochum L, Stecher B. Label or Concept - What Is a Pathobiont? Trends Microbiol. 2020;28(10):789-92.

55. Waidmann M, Bechtold O, Frick JS, Lehr HA, Schubert S, Dobrindt U, et al. Bacteroides vulgatus protects against Escherichia coli-induced colitis in gnotobiotic interleukin-2-deficient mice. Gastroenterology. 2003;125(1):162-77.

2.9 Tables

Table 1. Characteristics of housing conditions in the SPF sector and GF sector.

Characteristics		GF sector	SPF sector
General	Mice	♂ C57BL/6 aged 7-9 weeks	
	Mouse provider	IMC : University of Calcary	
	Diet	Double irradiated (20-40 kGy) - Research Diet	
	Water	Autoclaved (121°C for 40min)	
	Housing	ISOcage P - Techniplast	GM500 - Techniplast
Opening of cages	Disinfectant	Submerged in MB10	Spayed with oxivir
	Biosafety cabinets	IBS (ISOcage biosafety) - Techniplast	BS60 - Techniplast
	Room bacteria load [1]	94 CFU	95209 CFU
Handlers	Protection equipment	Tyvek coverall and powered air respirator by Bullard.	N95 mask, gown and sterile gloves
	Sex	$♀_1$ and $♂_1$	$♀_2$ and $♀_3$

CFU: Colony Forming Unit; GF: Germ-free; IMC: International Microbiome Center; SPF: Specific-pathogen free.

2.10 Figure Legends

Figure 1. Administration of a PAC extract in donor HFHS-fed mice lowers body weight gain and changes gut microbiota composition. (A) Donor mice were fed a chow diet and daily gavage with water or fed a HFHS diet and daily gavaged with water or 83.3mg/kg of a PAC extract over 8 weeks. (B) Total body weight gain, (C) fat mass, (D) lean mass were measured. Gut microbiota representation of 16S sequencing are (E) heatmap showing the abundance of bacterial genera (log10 transformed) detected in the fecal samples of chow, HFHS and HFHS + PAC fed mice; (F) simpson reciprocal and (G) shannon alpha-diversity indexes and (H) LEfSe, used to assess the genera that more strongly discriminates the composition of the gut microbiota of HFHS and HFHS + PAC fed mice at T8. (I) Fecal metabolites that significantly distinguish HFHS and HFHS + PAC fed mice at T8 are shown in a heatmap. (J) plasma level of dihydroxyphenyl-γ-valerolactone sulfate. (K) The abundance of genera (log10 transformed) detected in fecal slurries from HFHS and HFHS + PAC fed mice is shown in a heatmap. Values are expressed as the mean ± SEM (chow n=8, HFHS n=12, HFHS + PAC n=12). A student t-test was performed between HFHS and HFHS-PAC groups when respecting statistical postulates, otherwise a Mann-Whitney was performed. * $P<0.05$; ** $P<0.01$; PAC: proanthocyanidins.

Figure 2. Housing condition impacts the liver phenotype of GF mice receiving FMT from PAC-treated donor mice or control donor mice. (A) Feces from each HFHS and HFHS + PAC fed donor mice were collected at T8 and pooled according to their respective group to produce the fecal colonization solutions. GF mice were housed either in the GF sector or in the SPF sector and were colonized with their respective FMT solution (FMT HFHS or FMT HFHS + PAC) at arrival and 4 weeks into the study; *Mice housed in the GF Sector*: (B) Liver weight; (C) Liver triglycerides; (D) Liver cholesterol; (E) Plasmatic triglycerides; (F) Liver IL-6; (G) H&E staining of liver section; *Mice housed in the SPF sector*: (H) Liver weight; (I) Liver triglycerides; (J) Liver cholesterol; (K) Plasmatic triglycerides; (L) Liver IL-6; (M) H&E staining; of liver section. Values are expressed as the mean ± SEM (n=10/group). A student t-test was performed between FMT HFHS and

FMT HFHS-PAC groups from respective environment when statistical postulates were respected, otherwise a Mann-Whitney was performed. * $P<0.05$; GF: Germ-free; SPF: Specific pathogen-free.

Figure 3. Fecal composition and functionality of the gut microbiome in FMT treated HFHS-GF mice housed in both the GF and SPF sectors. LEfSe was calculated to assess the genera that more strongly discriminates the composition of the gut microbiota at T8 in FMT treated HFHS-fed GF mice housed in (A) the GF sector and (B) the SPF sector. Alpha-diversity indexes, (C, E) Simpson reciprocal and (D, F) Shannon alpha-diversity were assessed respectively in FMT HFHS and FMT HFHS + PAC. (G) LEfSe was calculated to assess the functions of the gut microbiome that more strongly discriminated FMT HFHS and FMT HFHS + PAC mice housed in the GF sector; (H) Principal Component Analysis (PCA) of fecal metabolites in FMT treated HFHS GF mice housed in both the GF sector; (I) PCA of fecal metabolites in FMT treated HFHS GF mice housed in both the SPF sector; (J) Heatmap represents fecal metabolites that significantly distinguish FMT treated GF mice housed in the GF sector; (n=10/group). GF: Germ-free; SPF: Specific pathogen-free.

Figure 4. Correlation between bacteria, metabolites and liver phenotype in FMT mice differ between both housing conditions. (A) According to the distribution of data, Spearman's or Pearson's rank correlation was used to assess the degree of association between liver parameters and bacterial genera that were present in more than 50% of all samples. Only significant associations ($P<0,1$) are represented by the colored dots; (B) Spearman's or Pearson's rank correlation was used to assess the degree of association between liver parameters and fecal metabolites that were significantly different the two sectors after performing an ANOVA ($P<0.05$), controlled for FDR and significantly correlated ($P<0.05$; FDR-corrected) are represented by the colored dots; (n=10/group).

2.11 Figures

Figure 1

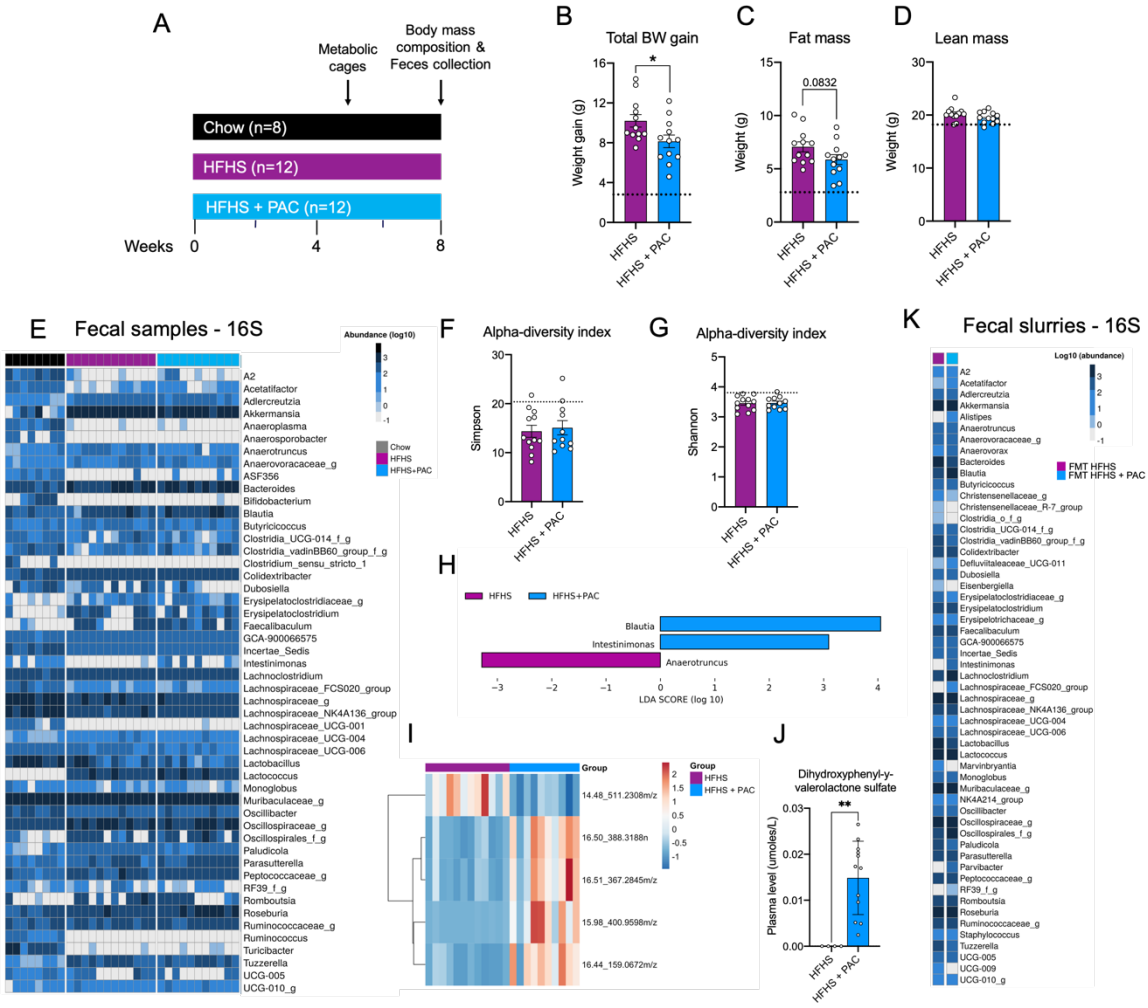


Figure 2

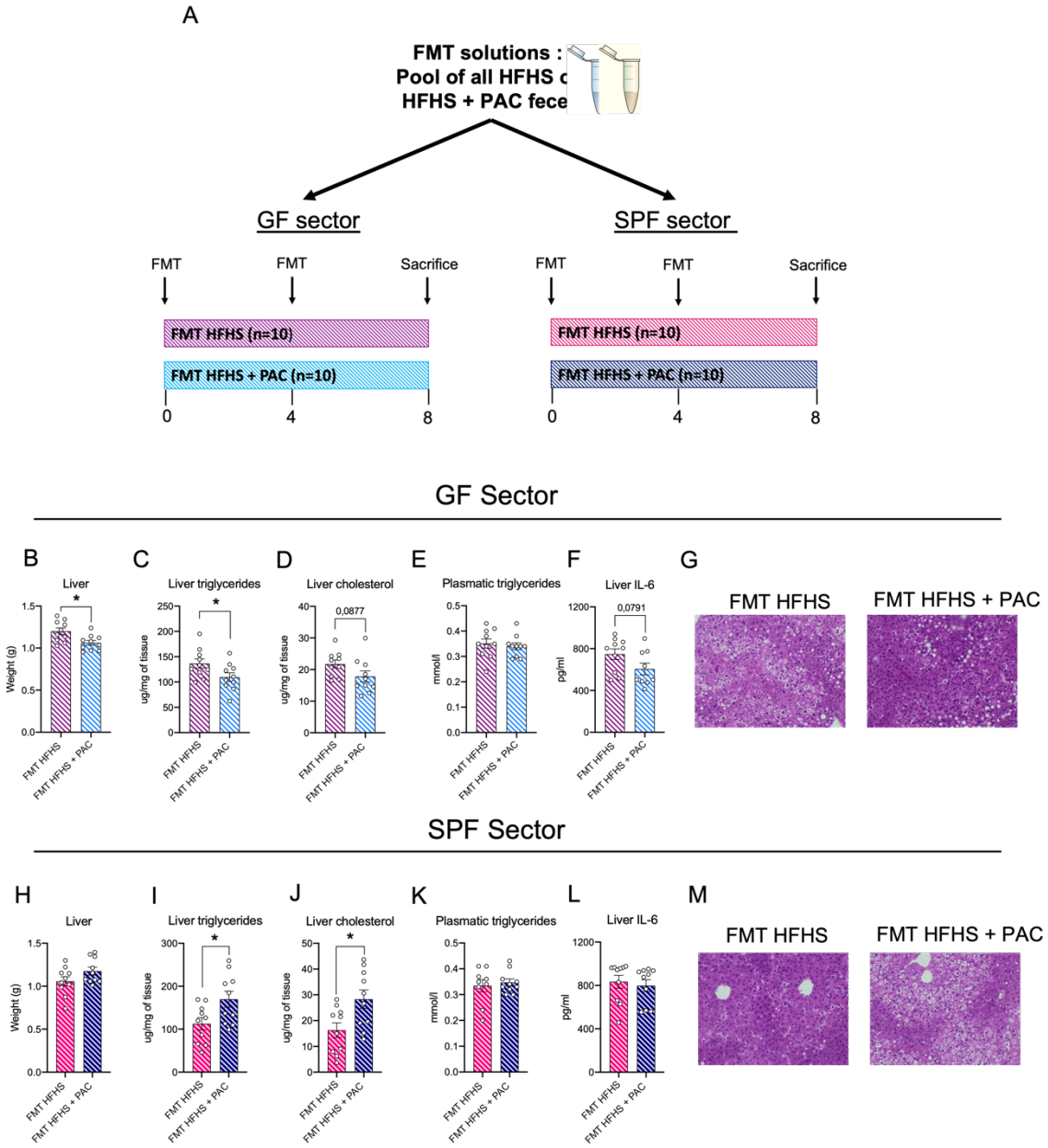

A
FMT solutions :
Pool of all HFHS c
HFHS + PAC fece
GF sector
SPF sector
FMT
FMT
Sacrifice
FMT HFHS (n=10)
FMT HFHS + PAC (n=10)
0
4
8
GF Sector
B
Liver
Weight (g)
C
Liver triglycerides
ug/mg of tissue
D
Liver cholesterol
0,0877
E
Plasmatic triglycerides
mmol/l
F
Liver IL-6
0,0791
pg/ml
G
FMT HFHS
FMT HFHS + PAC
SPF Sector
H
I
J
K
L
M

Figure 3

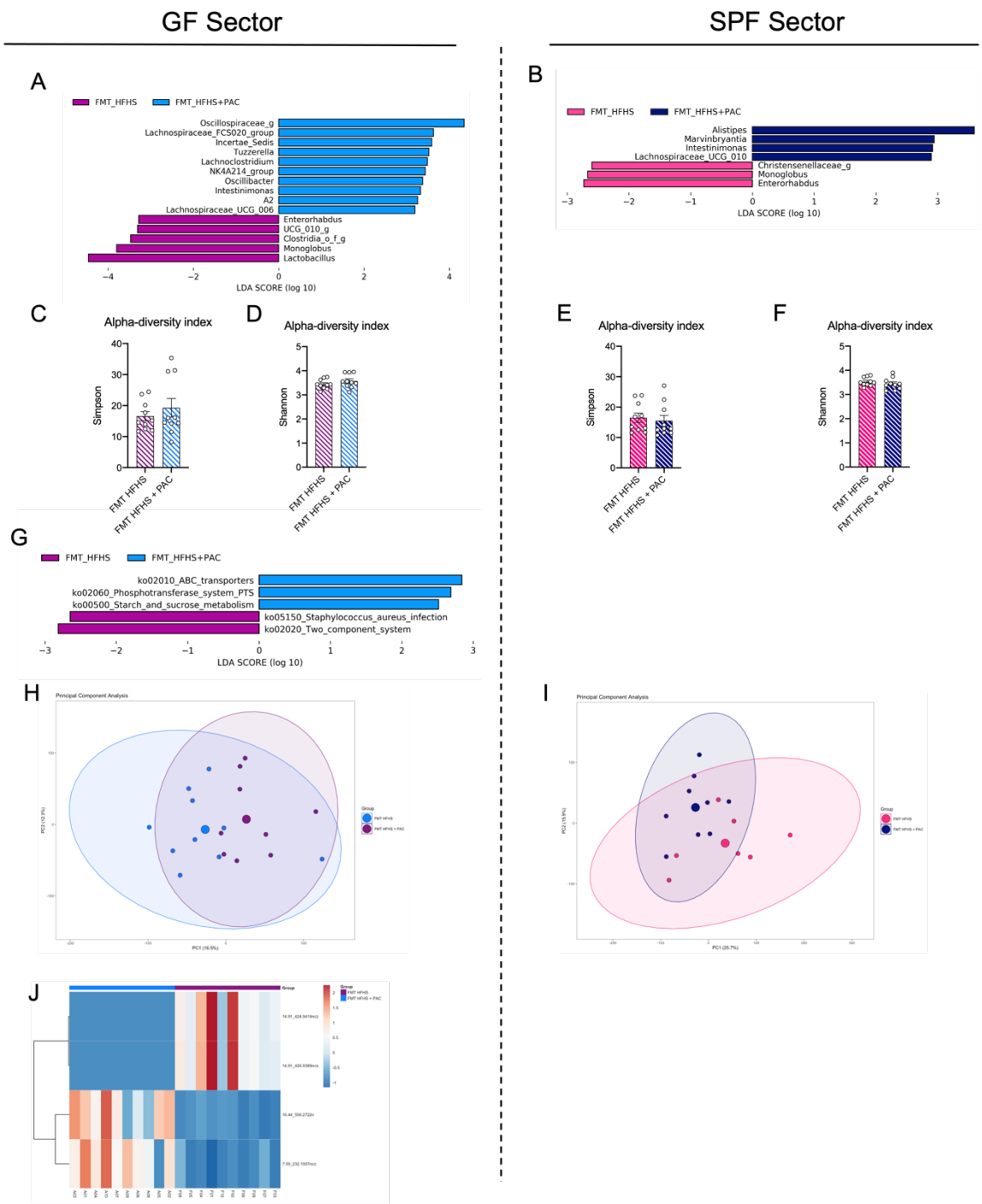
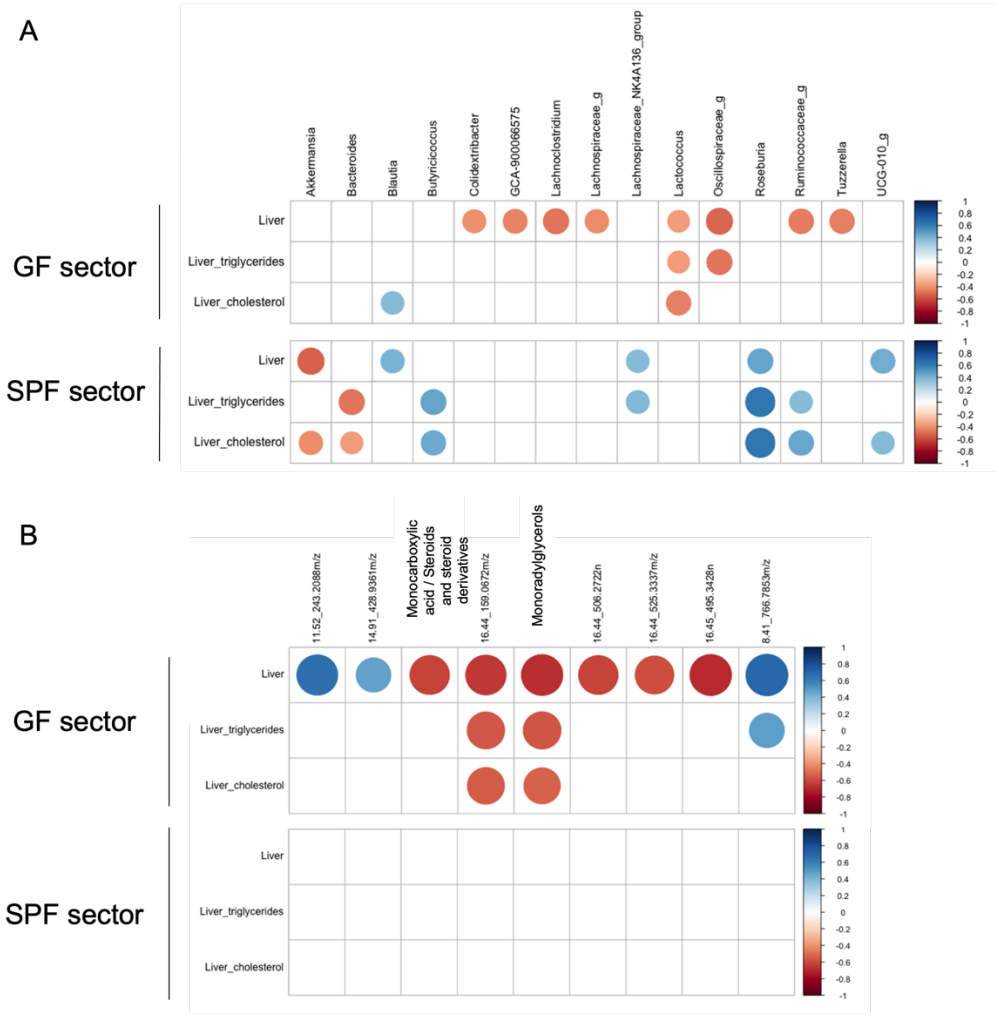
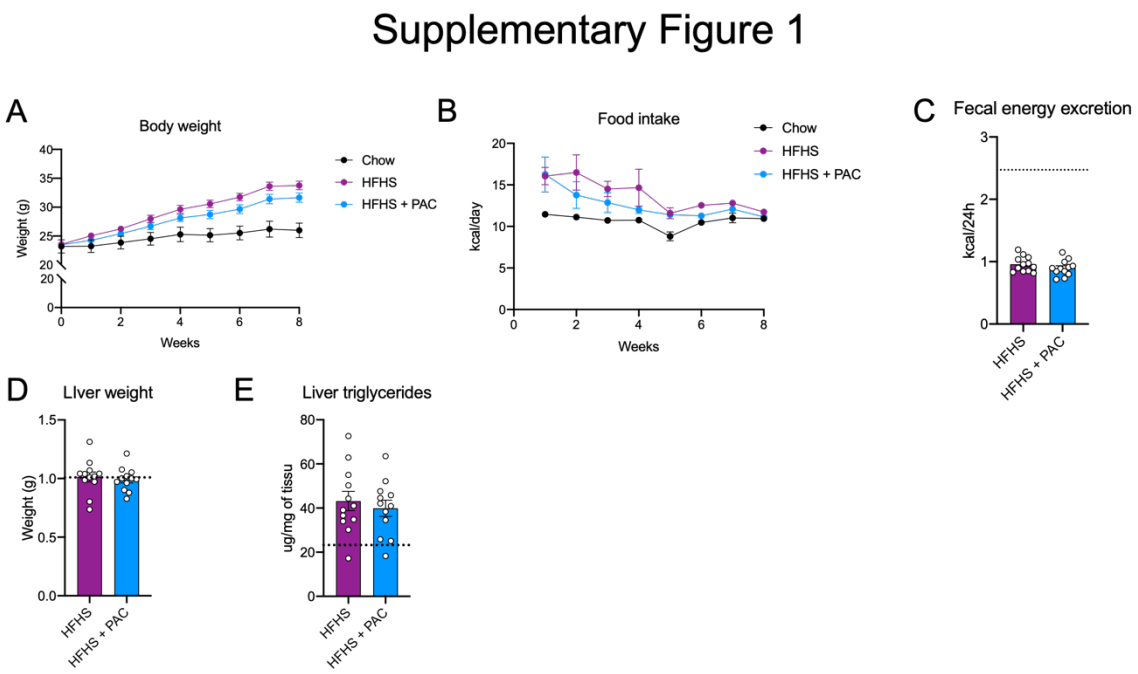

GF Sector
SPF Sector
A
FMT_HFHS
FMT_HFHS+PAC
Oscillospiraceae_g
Lachnospiraceae_FCS020_group
Incertae_Sedis
Tuzzerella
Lachnoclostridium
NK4A214_group
Oscillibacter
Intestinimonas
A2
Lachnospiraceae_UCG_006
Enterorhabdus
UCG_010_g
Clostridia_o_f_g
Monoglobus
Lactobacillus
LDA SCORE (log 10)
B
FMT_HFHS
FMT_HFHS+PAC
Alistipes
Marvinbryantia
Intestinimonas
Lachnospiraceae_UCG_010
Christensenellaceae_g
Monoglobus
Enterorhabdus
LDA SCORE (log 10)
C
Alpha-diversity index
Simpson
FMT HFHS
FMT HFHS + PAC
D
Alpha-diversity index
Shannon
FMT HFHS
FMT HFHS + PAC
E
Alpha-diversity index
Simpson
FMT HFHS
FMT HFHS + PAC
F
Alpha-diversity index
Shannon
FMT HFHS
FMT HFHS + PAC
G
FMT_HFHS
FMT_HFHS+PAC
ko02010_ABC_transporters
ko02060_Phosphotransferase_system_PTS
ko00500_Starch_and_sucrose_metabolism
ko05150_Staphylococcus_aureus_infection
ko02020_Two_component_system
LDA SCORE (log 10)
H
Principal Component Analysis
I
Principal Component Analysis
J

Figure 4

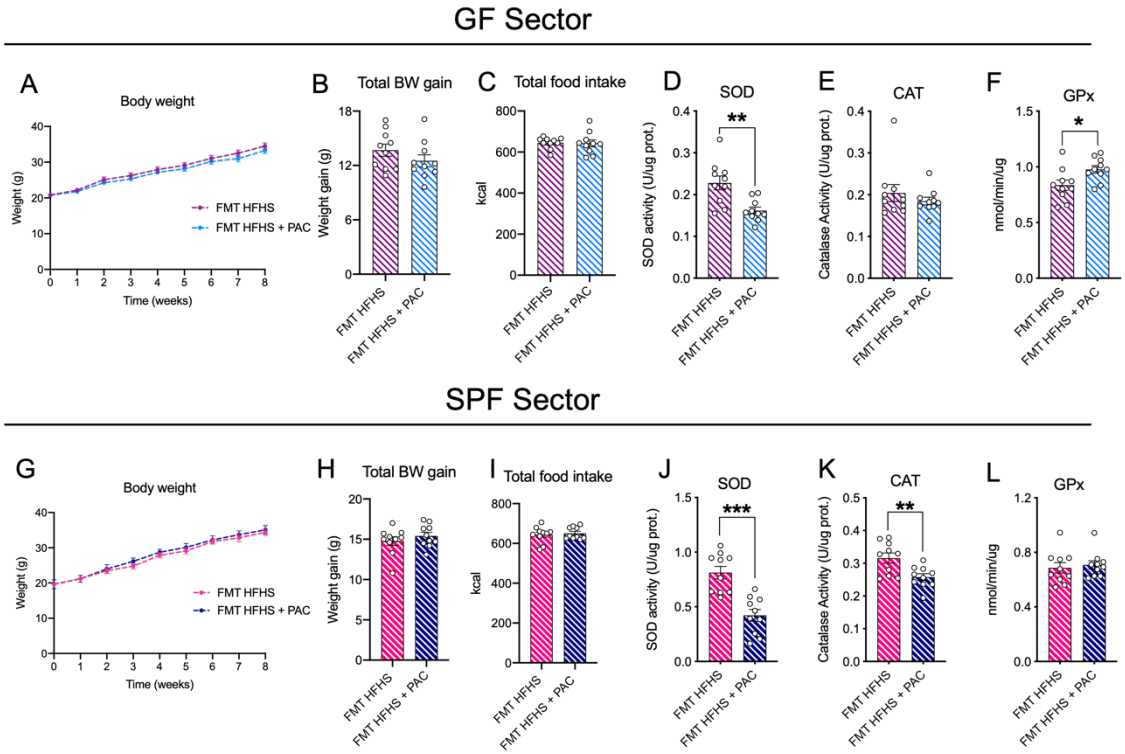

A
GF sector
SPF sector
Akkermansia
Bacteroides
Blautia
Butyricicoccus
Colidextribacter
GCA-900066575
Lachnoclostridium
Lachnospiraceae_g
Lachnospiraceae_NK4A136_group
Lactococcus
Oscillospiraceae_g
Roseburia
Ruminococcaceae_g
Tuzzerella
UCG-010_g
Liver
Liver_triglycerides
Liver_cholesterol
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
B
GF sector
SPF sector
11.52_243.2098m/z
14.91_428.9361m/z
Monocarboxylic acid / Steroids and steroid derivatives
16.44_159.0672m/z
Monoradylglycerols
16.44_506.2722n
16.44_525.3337m/z
16.45_495.3428n
8.41_766.7853m/z
Liver
Liver_triglycerides
Liver_cholesterol

2.12 Supplementary Material



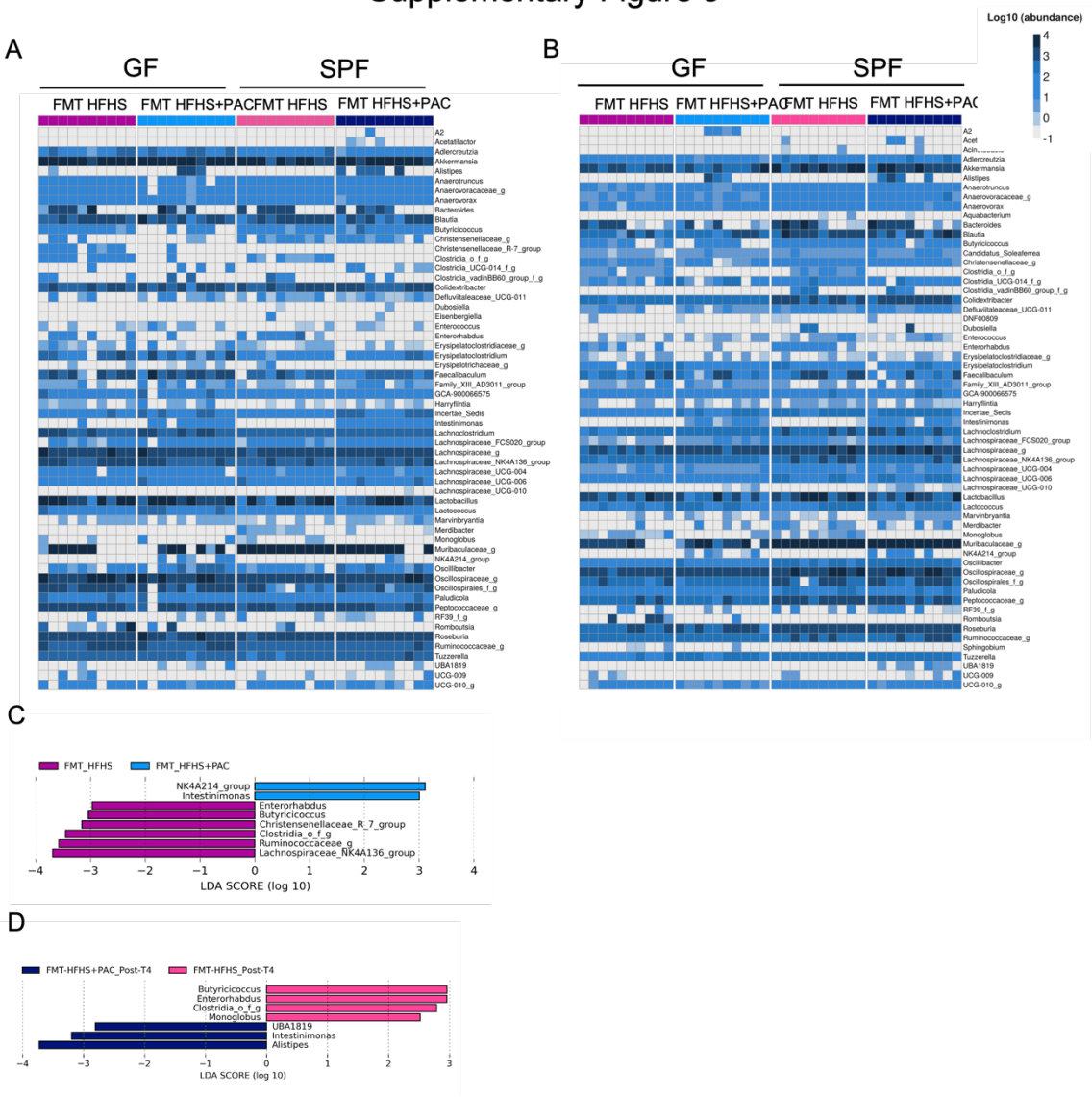
Supplementary Figure 1. Additional metabolic parameters measured in HFHS-fed donor mice. Additional parameters evaluating energy metabolism were measured such as (A) body weight and (B) food intake in kcal over 8 weeks of treatment, (C) fecal energy excretion in kcal and (D) horizontal activity measured over 24 hours, (E) liver weight and (F) liver triglycerides. Values are expressed as the mean ± SEM (chow n=8, HFHS n=12, HFHS + PAC n=12). A student t-test was performed between HFHS and HFHS-PAC groups when respecting statistical postulates, otherwise a Mann-Whitney was performed. PAC: proanthocyanidins.

Supplementary Figure 2



Supplementary Figure 2. Body weight gain, food intake and liver antioxidant activity in FMT treated HFHS-GF mice housed in both the GF and SPF sectors. *Mice housed in the GF Sector*: (A) body weight over 8 weeks, (B) total body weight gain, (C) total food intake in kcal, (D) liver SOD activity, (E) liver CAT activity (F) liver GPx. *Mice housed in the SPF sector*: (G) body weight over 8 weeks, (H) total body weight gain, (I) total food intake in kcal, (J) liver SOD activity (K) liver CAT activity and (L) liver GPx. Values are expressed as the mean ± SEM (n=10/group). A student t-test was performed between HFHS and HFHS-PAC groups when respecting statistical postulates, otherwise a Mann-Whitney was performed. **P*<0,05; ***P*<0,01; CAT: Catalase; GF: germ-free; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; SPF: Specific pathogen-free.

Supplementary Figure 3



Supplementary Figure 3. Gut microbiota composition in FMT treated HFHS-GF mice housed in both the GF and SPF sectors. Heatmaps representing the abundance of genera (log10 transformed) detected in the gut microbiota of FMT treated HFHS-GF mice housed in both the GF and SPF sectors at (A) T4 i.e. preceding the second colonization and (B) T8. LEfSe was calculated to assess the genera that more strongly discriminates the composition of the gut microbiota at T4 in FMT treated HFHS-fed GF mice housed in (C) the GF sector and (D) the SPF sector. (n=10/group); GF: Germ-free; SPF: Specific pathogen-free.

Chapitre 3 : Feeding diversified protein sources exacerbates hepatic insulin resistance via increased gut microbial branched-chain fatty acids and mTORC1 signaling in obese mice

Béatrice S.-Y. Choi[1,2,#], Noëmie Daniel[1,2,#], Vanessa P. Houde[1,2], Adia Ouellette[1,2], Bruno Marcotte[1,2], Thibault V. Varin[2], Cécile Vors[2], Perrine Feutry[2], Olga Ilkayeva[3], Marcus Ståhlman[4], Philippe St-Pierre[1,2], Fredrik Bäckhed[4,5], Angelo Tremblay[1,2], Phillip J. White[3,6] and André Marette[1,2,*]

[1]Quebec Heart and Lung Institute (IUCPQ), Laval University, Canada
[2]Institute of Nutraceuticals and Functional Foods (INAF), Laval University, Canada
[3]Duke Molecular Physiology Institute and Department of Medicine, Duke University, USA
[4]Wallenberg Laboratory, University of Gothenburg, Sweden
[5]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark
[6]Department of Pharmacology & Cancer Biology, Duke University, USA
# equal contributions
*corresponding author (andre.marette@criucpq.ulaval.ca)

Adapté pour des raisons de format de l'article suivant [371] :

Choi BS, Daniel N, Houde VP, Ouellette A, Marcotte B, Varin TV, Vors C, Feutry P, Ilkayeva O, Ståhlman M, St-Pierre P, Bäckhed F, Tremblay A, White PJ, Marette A. 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(1):3377. https://doi.org/10.1038/s41467-021-23782-w

3.1 Résumé

Les modèles animaux de pathologies humaines sont classiquement nourris avec des diètes purifiés contenant de la caséine comme unique source de protéines. Nous démontrons que l'apport d'une source de protéine mixte reflétant celle du régime occidental exacerbe l'obésité et la résistance à l'insuline induites par la diète, en potentialisant la signalisation hépatique mTORC1/S6K1 par rapport à la caséine seule. Ces effets impliquent des altérations du microbiote intestinal, comme le montrent les études de transplantation du microbiote fécal. L'impact négatif de la source de protéine mixte est également lié à des changements rapides dans la production microbienne d'acides gras à chaîne ramifiés (BCFA) et à une augmentation des acylcarnitines plasmatiques et hépatiques, indiquant une oxydation mitochondriale aberrante des acides gras. Nous montrons également que les BCFA, l'acide isobutyrique et l'acide isovalérique, augmentent la production de glucose et activent la voie mTORC1/S6K1 dans les hépatocytes. Nos résultats démontrent que l'altération de la source de protéines alimentaire exerce un impact rapide et important sur le microbiote intestinal et la production de BCFA, avec des conséquences significatives sur le développement de l'obésité et de la résistance à l'insuline.

3.2 Abstract

Animal models of human diseases are classically fed purified diets that contain casein as the unique protein source. We show that provision of a mixed protein source mirroring that found in the western diet exacerbates diet-induced obesity and insulin resistance by potentiating hepatic mTORC1/S6K1 signaling as compared to casein alone. These effects involve alterations in gut microbiota as shown by fecal microbiota transplantation studies. The detrimental impact of the mixed protein source is also linked with early changes in microbial production of branched chain fatty acids (BCFA) and elevated plasma and hepatic acylcarnitines, indicative of aberrant mitochondrial fatty acid oxidation. We further show that the BCFA, isobutyric and isovaleric acid, increase glucose production and activate mTORC1/S6K1 in hepatocytes. Our findings demonstrate that alteration of dietary protein

source exerts a rapid and robust impact on gut microbiota and BCFA with significant consequences for the development of obesity and insulin resistance.

3.3 Introduction

Obesity is a complex disease associated with numerous comorbidities, including metabolic syndrome and cardiovascular diseases[1]. These pathologies are commonly studied via animal models fed an obesogenic diet that is rich in fat and sucrose to mimic the western diet. In these diets much attention is given to fat and carbohydrate sources as the main culprit for the development of these diseases; however, very little consideration is given to the protein source. Indeed, the vast majority of commercially purified obesogenic diets use casein from bovine milk protein as the sole source of dietary protein. Moreover, all the Nutrient Requirement guidelines[2] for laboratory animals which serve as a frame of reference for rodent diet producers are based on casein only. This is problematic for two important reasons: 1) casein does not have the same composition as the plant and animal sources of proteins that are abundant in most human diets, and 2) casein may reduce body weight gain as compared to other protein sources, especially in its hydrolyzed form[3,4]. Furthermore, the molecular mechanisms underlying the metabolic effects of different dietary protein types remains elusive[4-8].

Here we show that a mixture more representative of the complex composition of dietary protein consumed by humans in western societies promotes distinct metabolic perturbations, such as increase weight gain and insulin resistance, compared to a diet containing only casein and we explore the potential role of the gut microbiota in these effects. More specifically, we show the impact of consuming mixed dietary proteins compared to casein on liver metabolism through an incomplete mitochondrial oxidation of fatty acids as well as the activation of the mTOR/S6K1 signaling pathway. These findings highlight the importance of considering protein source in the diet of animal models of diet-induced obesity.

3.4 Material & Methods

Diet

For this study, we have elaborated low-fat low sucrose (LFLS: 75% kcal carbohydrates, 10% kcal sucrose, 10% kcal fat) and high-fat high-sucrose (HFHS: 35% kcal carbohydrates, 30% kcal sucrose, 50% kcal fat) homemade diets containing a protein mix (PM) designed to represent the human consumption according to the US Department of Agriculture data[51]. The corresponding LFLS and HFHS diets containing casein (C) as the only protein source were used as controls. The protein sources consisted of cooked meat (chicken/pork/beef, Happy Yak, Canada), fish (cod, Seagarden, Norway), plant (soy, Teklad Envigo, USA and pea/rice, Canadian Protein, Canada), egg (egg white, Teklad Envigo, Canada) and dairy (whey, Canadian Protein, Canada and casein, MP Biomedicals, USA). All of the protein sources were provided in a lyophilized form, and details of their process are available on the respective websites of the providers, with the exception of beef, pork and chicken (Happy Yak, Canada), which are lyophilized lean cuts of meat without any additional processing. Composition of each protein source used in the PM have been analyzed (Environex) and the information is available in Supplementary Table 3. The resulted PM consisted of 81% protein, 11% fat, 3% carbohydrates, 1% fiber and negligeable amount of ash and humidity. Diets were then matched for protein, fat, carbohydrate, fiber. All diets were isonitrogenous (15% kcal) and lard and corn oil content were adjusted such that the saturated: polyunsaturated fatty acid ratio (SAT: PUFA) in the four experimental diets represented the daily American dietary intake[9] (Supplementary Tables 1 and 2).

Animals

Animals were single-housed, except for the GF study, where mice were housed 3 per cage, all animals were housed in ventilated cages at a humidity of 40-50% and a temperature of 22 °C on a 12-hour dark-light cycle with ad libitum food. 6-week-old male C57BL/6J mice for the 12-week protocol,10-week-old male C57BL/6J mice for the 2-week protocol and 11-week-old male C57BL/6J mice for the 2-week antibiotic protocol (The Jackson Laboratory, USA) were acclimatized for 2 weeks on LFLS-C diet prior the beginning of their respective treatment. Animals were then distributed into treatment groups accordingly to their body weight and fed with their respective diets for 12 weeks (LFLS-C, LFLS-PM, HFHS-C or

HFHS-PM) or 2 weeks (HFHS-C or HFHS-PM). Food intake was measured three times a week and body weight weekly. Body composition was measured by nuclear magnetic resonance with a Bruker Minispec (LF90) apparatus. Fresh feces were collected to analyse gut microbiota composition as well as short-chain and branched-chain fatty acids content.

For the 12-week protocol, a fasting blood sample was collected at week 5 and an oral glucose tolerance test, with a dose of 1 mg of glucose/g of body weight, was performed at week 11, both after a 6-hour fast. Sacrifice was done at week 12 after a 6-hour fast and animals were injected intravenously with saline or insulin (3.8 U/kg) 5 minutes prior to sacrifice for analysis of insulin signaling in the tissues. For the 2-week antibiotic protocol, half the mice were put on antibiotics (1g/L of Ampicillin and 0.5g/L of Neomycin), in drinking water changed 3 times per week in bottles wrapped in aluminum foil, one week prior to the start or the HFHS diets and for the whole duration of dietary intervention. For both 2-week protocols, a meal-test was conducted at the end of the protocol, where 0.5 g of their respective diet was given to the animals after a 12-hour fast. The mice had 15 minutes to eat the food and were euthanized 2h after the end of the meal test.

For the fecal microbiota transplantation (FMT) study, 8-9-week-old male C57BL/6J germ-free mice (International Microbiome Center, University of Calgary) were ordered and colonized upon arrival in the axenic-gnotobiotic sector of our animal facility. For fecal microbiota transplantation solutions, fecal samples from each of the 15 mice from groups HFHS-C and HFHS-PM of the first 12-week protocol were respectively pooled and diluted in sterile cold PBS at 2ml/100mg of feces, vortexed 3 minutes and centrifuged 3 minutes at 800g at 4C. Stool suspension was then aliquoted and thawed only once, when used for fecal microbiome transplant, where each mouse received 200ul of solution by gavage. Upon arrival, mice were separated in two groups according to BW (FMT-HFHS-C of FMT-HFHS-PM) and received the first colonization. Every 2 weeks for the whole duration of the study mice received another FMT. Mice were group-housed 3 by 3 on a 12-hour dark-light cycle with ad libitum food. Food intake was measured three times per week, while body weight was assessed weekly. Animals were thoroughly observed for any signs of fighting within each cage, and none were noted. All mice were fed HFHS-C diet.

All animals were euthanatized by cardiac puncture (under isoflurane anaesthesia) and cervical dislocation before tissue collection for analysis. All manipulations were approved (#2017-156-1) by the *Comité de protection des animaux de l'Université Laval* (CPAUL) and complied with the Canadian Council on Animal Care (CCAC) guidelines.

Biochemical analyses

ELISA assays were used to measure insulin (Alpco Mouse Ultrasensitive Insulin kit), c-peptide (Crystal Chem Mouse C-Peptide kit) and lipopolysaccharides (LPS) (MyBioSource Mouse Lipopolysaccharides kit) in circulation according to manufacturer's instructions. Imidazole propionate and urocanate were measured in plasma according to the method previously described[18]. Briefly, plasma samples were extracted with 10 volumes of acetonitrile containing deuterated internal standards. The supernatant was dried and the imidazole propionate and urocanate were derivatized into butyl esters using butanol: HCL (conc) [95:5]. The samples were separated on a BEH C18 column (Waters, Milford, USA) using a gradient consisting of water with 0.1% formic acid (A-phase) and acetonitrile with 0.1% formic acid (B-phase). Mass spectrometric analysis was performed using an TQ-XS mass spectrometer (Waters, Milford, USA). The imidazole propionate and urocanate were detected by multiple reaction monitoring using the transitions 197/81 and 195/93, respectively. Quantification was made using external standard curves. Amino acids, acyl-carnitines and organic acids were measured using stable isotope dilution techniques. Amino acids and acyl-carnitine species were measured using flow injection tandem mass spectrometry, organic acids profile was determined by capillary gas chromatography-mass spectrometry (TRACE ISQ instrument, Thermo Election Corporation). Samples were equilibrated with a cocktail of internal standards, de-proteinated by precipitation with methanol, aliquoted supernatants were dried, and then esterified with hot, acidic methanol (acyl-carnitines) or n-butanol (amino acids)[26].

Immunoblotting

Liver and gastrocnemius muscle were powdered with liquid nitrogen and then homogenized under rotation for 2-hours at 4°C in a 10-fold mass excess of ice-cold lysis buffer (50 mM

Hepes pH 7.5, 150 mM NaCl, 1 mM EGTA, 20 mM β-glycerophosphate, 1% NP-40, 10 mM NaF, 2 mM Na_3VO_4, 0.1 mM PMSF and protease inhibitors cocktail). Lysates were clarified by centrifugation at 16000g for 10 min at 4°C and the proteins were measured with a BCA assay (ThermoFisher Scientific, Burlington, Canada). Tissue lysates (5-30 μg) were denatured in SDS sample buffer and submitted to SDS-PAGE followed by transfer on nitrocellulose membranes (Pall Corporation, Mississauga, Canada). Membranes were blocked for 1-hour at room temperature and then probed with the primary antibody overnight at 4°C. After washing in TBST (50 mM Tris-HCl pH 7.5, 0.15 mM NaCl and 0.1% Tween-20), the membranes were incubated with HRP-conjugated secondary antibodies for 1-hour at room temperature. Cells were washed with PBS and lysed in ice-cold lysis buffer (50 mM Hepes pH 7.5, 150 mM NaCl, 1 mM EGTA, 20 mM β-glycerophosphate, 1% NP-40, 10 mM NaF, 2 mM Na_3VO_4, 0.1 mM PMSF and protease inhibitors cocktail). Cell lysates were clarified by centrifugation at 16000g for 10 min at 4°C and the proteins were measured with a BCA assay (ThermoFisher Scientific, Burlington, Canada). Lysates in SDS sample buffer were then subjected to immunoblotting. The detection was performed with an ECL reagent (Millipore, Etobicoke, Canada). See Supplementary Method Table 1 for the full details on the antibodies used and the immunoblotting conditions. Immunoblots were analyzed using ImageJ software (NIH, Bethesda, USA). Uncropped and unprocessed scans of blots from representative gels are found in the Data Source file.

Quantitative PCR analysis

RNA was extracted from freeze-powdered brown adipose tissue with the Direct-zol RNA Miniprep Plus kit accordingly to the manufacturer's instructions (Zymo Research, Irvine, USA). Gene expression was assessed by the ΔΔCt method and *Actin* and *Hprt* were used as the reference genes. *Akkermansia muciniphila* bacterial quantification was performed from extracted fecal bacterial DNA. Primer sequences are available in Supplementary Method Table 2.

16S rRNA amplicon sequencing

Fecal DNA was extracted from fresh feces with the ZymoBIOMICS DNA Miniprep kit (Zymo Research) according to manufacturer's instructions and then sent to the sequencing

platform at *Institut de biologie intégrative et des systèmes* (IBIS) for PCR amplification of the V3-V4 region and 16S rRNA sequencing analysis. Forward and reverse primers were removed from 16S rRNA gene amplicons using Cutadapt (v3.1[52]). Sequence reads were analyzed using the DADA2 package (v1.145.0[53]) in R (v3.6.0; http://www.R-project.org). Forward and reverse reads were first trimmed at 270 bp and 205 bp respectively, to remove low quality regions. Sequences with an expected error threshold > 2 and > 4 for the forward and reverse reads respectively, with ambiguous bases, and with quality score less than 3 or equal to 2 were discarded. Dereplication and denoising of filtered sequences were carried out using DADA2 default parameters. Denoised forward and reverse reads were merged (all reads with any mismatches were removed) and searched for chimeras. Taxonomic assignment of amplicon sequence variants (ASVs) was performed using the RDP classifier algorithm (v2.2[54]) trained against the Silva database 132[55] (https://zenodo.org/record/1172783#.YGEfARKQikA). In order to normalize sampling effort, samples were rarefied to an even sampling depth of 10459 sequences. ASVs with a number of sequences <0.05% of total number of sequences and present in less than 3 samples were discarded at this step. A phylogenetic tree was built in R using the DECIPHER (v2.14.0[56]) and phangorn packages (v2.5.5[57]). Data visualization and analyses were performed in R with the phyloseq (v1.28[58]) package. Overall bacterial community composition was visualized in heatmaps at the genus level. Presence of "f" or "g" at the end of taxon denotes unclassified family or genus, respectively. To quantify bacterial alpha-diversity, Shannon and Simpson's reciprocal indexes were calculated. Principal coordinates analysis (PCoA) was performed on unweighted UniFrac distance matrix in order to measure beta-diversity. The statistical significance of differentially abundant bacteria between the two distinct biological conditions were measured with LEfSe[59]. A p-value of <.05 and a linear discriminant analysis (LDA) score ≥ 2.5 will be considered statistically significant. The raw sequence data generated in this work were deposited into the European Nucleotide Archive (ENA) under accession PRJEB37442.

Short chain fatty acids (SCFA)

SCFA were measured in the feces of all the animals at week 11 for the 12-week study and at week 2 for the 2-week studies. SCFA were extracted according to a protocol previously

published by García Villalba et al.[60] with some modifications. Right after collection, faeces were weighed and 1 mL of 0.5% phosphoric acid were added per 100 mg of material. Faecal suspensions were homogenized 2 min with a Bead Ruptor 12 (Omni International, Kennesaw, GA, USA), then centrifuged at 18000 g for 10 min at 4°C. Supernatant were collected and an equal volume of ethyl acetate, spiked with internal standard 4-methylvaleric acid were added. To extract SCFA, samples were mixed 2 min at 2400 rpm using a VWR VX-2500 Multitube Vortexer (VWR, Radnor, PA, USA), then centrifuged at 18000 g for 5 min at 4°C. The organic phase was transferred to an autosampler vial for gas chromatography analysis. A 5-points calibration curve were prepared with a mix of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and internal standard 4-methyl valeric acid. SCFA quantification were performed on a Shimadzu GC 2010 Plus equipped with a Nukol Supelco capillary GC column (30 m x 0.25 mm id, 0.25 µm) and a FID detector.

Cell Culture and stimulation

FAO rat hepatocytes cells were cultured in RPMI 1640 containing 10% FBS. L6 rat myoblasts (kind gift of Dr Amira Klip, Hospital for Sick Children, Toronto, ON, Canada) were grown in α-MEM with 10% FBS and differentiated into myotubes in α-MEM with 2% FBS. For immunoblotting, FAO cells were serum-deprived for 24 hours and treated with or without isobutyric acid (1 mM) (MilliporeSigma Canada, Oakville, ON, Canada) or isovaleric acid (1 mM) (MilliporeSigma Canada) for the last 1-24 hours of deprivation.

Glucose production

FAO cells were incubated 16-18 hours in serum-free medium, with or without insulin (1 nM), isobutyric acid (1-1000 µM) (MilliporeSigma Canada, Oakville, ON, Canada) or isovaleric acid (1-1000 µM) (MilliporeSigma Canada). The cells were washed three times with PBS and then incubated with phenol- and glucose-free DMEM medium supplemented with 20 mM sodium L-lactate and 2 mM sodium pyruvate for 5 hours with or without the indicated concentration of insulin (1 nM), isobutyric acid (1-1000 µM) or isovaleric acid (1-1000 µM). Cell supernatants were collected, and glucose concentration was measured with the Amplex-Red Glucose assay kit (Invitrogen, Burlington, ON, Canada) accordingly to the

manufacturer's instructions. Cells were lysed with 50 mM NaOH and protein concentration was determined using a BCA protein assay kit to normalize glucose production.

Measurement of 2-Deoxyglucose Uptake

Fully differentiated L6 myotubes were serum-deprived for 5 hours in α-MEM and treated with isobutyric acid (0.1-1 000 μM) or isovaleric acid (0.1-1 000 μM) for the last two hours of deprivation. The cells were also stimulated with or without insulin (100 nM) during the last 30 min of deprivation. Glucose uptake was measured in cells incubated for 8 min in HEPES-buffered saline containing 10 μmol/l unlabeled 2-deoxyglucose and 0,33 μCi/ml 2-[1,2-3H(N)]-deoxy-D-glucose (Perkin Elmer). The reaction was terminated by washing three times with ice-cold 0.9% NaCl. Cell-associated radioactivity was determined by lysing the cells with 0.05 N NaOH, followed by liquid scintillation counting and normalization to protein concentration.

Statistical analysis

Data are expressed as means ± s.e.m. For the 12-week and the antibiotic protocols, a two-way or a three-way analysis of variance (ANOVA) was performed to isolate the main effects of diet (D), protein (P) insulin condition (I), or antibiotic condition (A) factors as well as the corresponding interaction effects. A Tukey post-hoc test with a type I error set at .05 was then applied (SigmaPlot® v12.0, San Jose, CA, USA) when the $p<.10$ threshold of main factors was achieved.The exact p-values for main effects are indicated under the title of each graph and subsequent significant differences established by post-hoc test were recorded as follows: *$p<.05$, **$p<.01$, ***$p<.001$. Trends ($.05 \leq$ p-value $< .10$) were also reported in graphs for additional indication. Data violating ANOVA normality and homoscedasticity postulates were log-transformed before the test was applied. Differences in parameters involving repeated measures were assessed using a mixed linear model (proc MIXED procedure) in SAS (SAS Studio, SAS® University Edition USA), with treatment, time (T) and treatment*time interaction as fixed effects and an autoagressive (1) covariance matrix to account for within-subject correlations. The skewness in the distribution of model residuals was considered and data were log-transformed when required. For the 2-week and the germ-free protocols a two-tailed Student's *t* test or its non-parametric equivalent Mann-Whitney

test when data normality and homoscedasticity were not respected was performed. and significant differences were recorded as follows: *p<.05, **p<.01, ***p<.001 For BCFA measurement *in vitro*, a Kruskal-Wallis one-way ANOVA followed by a two-tailed Dunn's post-hoc test versus basal or insulin was performed in each basal or insulin condition. P-values of general ANOVA are indicated under the title of each graph. Features were considered statistically different at p<.05 and significant differences were recorded as follow: *p<.05, **p<.01, ***p<.001 Trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Statistics regarding metataxonomic analysis are described in the corresponding subsection (see above). Microsoft Excel (v16.47.1) and Graphpad Prism (v9.1.0) software were used to compile data and make graphs and figures.

3.5 Results

Protein source influences the obesogenic effect of high-fat high-sucrose feeding

We designed a protein mix (PM) that better reflects the protein composition of diets consumed by humans. This PM was incorporated into either a low-fat low-sucrose (LFLS) diet or a high-fat high-sucrose (HFHS) diet. We then compared the respective LFLS-PM and HFHS-PM diets to classical control diets which contained casein (C) as the single protein source. PM and C diets were matched for total carbohydrates, lipids and protein amount (Fig. 1 and Supplementary Table 1). Our PM was formulated according to the USDA database[9] recording the protein intake of the global North American population and therefore can be considered representative of the protein mixture found in a western diet. The PM included ten different protein sources: plants (rice, 21.1%; soy, 6.1%; pea; 6.1%), red (beef, 13.4%) and white (chicken, 13.8% and pork 13.4%) meats, dairy products (casein, 15.4% and whey, 3.8%), egg (3.7%) and fish (cod, 3.3%) (Fig. 1 and Supplementary Table 2). Compared to casein, the PM diet contained more than double the glycine, almost twice as much alanine, arginine and cysteine, 50% more aspartic acid and 50% less proline (Fig. 1b, Supplementary Table 3). The PM diet also slightly differed from casein (10-20% of variation) in the content of serine, glutamic acid, tyrosine and lysine, which were more abundant (+11-17%), and threonine and phenylalanine (-12-16%) which were less abundant in PM compared to casein

(Fig. 1b). Importantly, the diets were isonitrogenous and amino acid proportions were matched between LFLS diets and HFHS diets (Supplementary Table 1 and 3).

The protein source did not influence food intake or weight gain in mice fed the LFLS diet. However, PM amplified the obesogenic effects of HFHS feeding on body weight gain over a 12-week period (Fig. 2a). This occurred without changes in energy intake and protein consumption (Supplementary Fig 1a, b). The greater weight gain observed in HFHS-PM-fed mice compared to their HFHS-C-fed counterparts (Fig. 2b) was due to an increase in both fat mass and lean mass (Supplementary Fig. 1c, d) as evidenced by body composition analysis, as well as increased mass of visceral adipose tissue, brown adipose tissue, and gastrocnemius muscle (Supplementary Fig. 1e-h).

Further examination of brown adipose tissue (BAT) revealed that PM downregulated the expression of several thermogenic genes in obese mice. Notably, uncoupling protein 1 (*Ucp1*), Cell death activator CIDE-A (*Cidea*) and Type II iodothyronine deiodinase (*Dio2*) mRNA levels were markedly lower in BAT of mice fed the HFHS-PM diet compared to HFHS-C fed mice (Supplementary Fig. 1i-k). In contrast, PPAR-γ-co-activator 1α (*Pgc1α*) gene expression was mainly affected by dietary fat and sucrose rather than protein source (Supplementary Fig. 1l). This suggests that lower BAT thermogenic activity may have contributed to the higher weight gain in mice fed the HFHS-PM diet.

The PM diet also exacerbated the detrimental effect of HFHS feeding on glucose homeostasis. Mice fed the HFHS-PM diet displayed similar fasting glycemia but higher fasting insulinemia and larger glucose and insulin excursions during an oral glucose tolerance test compared to HFHS-C-fed mice (Fig. 2c-g). The increased insulin response likely reflects higher glucose-stimulated insulin secretion and not lower clearance of the hormone given that C-peptide levels were also increased during the OGTT (Fig. 2h) and that this beta-cell product has negligible extraction by the liver. In the LFLS fed mice the PM had no effect on glucose homeostasis which is in line with the lack of effect on body weight gain.

Given that dietary protein and amino acids can impair insulin signaling through mechanistic target of rapamycin/S6 kinase 1 (mTOR/S6K1) dependent phosphorylation of insulin receptor substrate 1 (IRS1) on multiple inhibitory serine residues[10-14], we next tested whether our PM diet impacts metabolic health via this nutrient sensing mechanism. Consistent with higher mTOR/S6K1 signaling in liver of HFHS-PM fed mice, we observed higher inhibitory phosphorylation of IRS1 on serine 1101 concomitant with increased phosphorylation of S6 on Ser240-244 compared to HFHS-C mice (Fig. 2i-k). In addition to higher S6 phosphorylation, PKC theta, an alternative IRS-1 S1101 kinase activated by diacylglycerol (DAG) and associated with type 2 diabetes[15,16], was also increased in the liver of HFHS-PM-fed mice as compared to HFHS-C-fed mice (Fig. 2i, l). Importantly, the net result of these effects in HFHS-PM fed mice was significantly lower insulin-mediated phosphorylation of Akt on serine 473 compared to their HFHS-C fed counterparts (Fig. 2m, n). IRS1 and IRS2 protein expression were not modulated by the protein source (Fig. 2i, Supplementary Fig. 2a-c). While the mixed protein source clearly potentiated HFHS mediated inhibitory phosphorylation of IRS1 in liver, addition of PM to the HFHS diet had no impact on insulin signaling in skeletal muscle (Supplementary Fig. 2d-j), suggesting that the mixed protein primarily caused hepatic rather than peripheral insulin resistance, thus PM likely exerts its effect on glucose tolerance by promoting hepatic glucose production rather than lowering glucose uptake in peripheral tissues.

Protein source modulates gut microbiota independent of dietary fat and carbohydrate

Diet is a key environmental factor shaping the gut microbiota[17]. To evaluate the extent to which different protein sources influence bacterial populations, 16S rRNA gene sequencing was performed on DNA extracted from fresh fecal samples collected after 11 weeks of dietary treatment. Examination of the overall bacterial composition notably revealed a drastic reduction of Verrucomicrobiales by PM and a blooming of Lactobacillales by HFHS and PM interaction (Supplementary Fig. 3a). Among the 30 genera identified by taxonomic assignment recorded in the heatmap, some were only present in the HFHS context like *Romboutsia* or seemed to be particularly enhanced by the PM like *Intestinimonas* (Supplementary Fig. 3b). PCoA analysis showed a clear diet specific clustering of microbiota from LFLS and HFHS mice, as well as a tendency for the two HFHS groups to cluster

separately (Supplementary Fig. 3c). As expected, the HFHS diet modulated relative abundance of several bacterial genera and promoted *Romboutsia*, *Adlercreutzia* and *Tyzzerella* while reducing *Bifidobacterium*, *Facecalibacterium* and a *Muribaculaceae* genus (Supplementary Fig. 4a-d).

Importantly, the PM induced a significant shift in gut bacteria in both LFLS-fed and HFHS-fed mice that was independent from the effect of dietary fat and carbohydrate. Indeed, *Akkermansia muciniphila* was decreased by PM in both diets (Fig. 3a-e) and bacterial alpha-diversity displayed by Shannon and Simpson's reciprocal indexes (Fig. 3f, g) were influenced most by protein sources. Accordingly, we also observed greater metabolic endotoxemia in PM-fed mice compared to their lean control, as revealed by the HFHS diet-induced increase in plasma LPS, which was significantly increased ($p<0.001$) in HFHS-PM compared their LFLS-PM fed counterparts while only a tendency ($p=0.06$) was observed between the HFHS-C and LFLS-C fed mice (Supplementary Fig. 4e). While histidine levels did not differ between HFHS-fed animals consuming casein *versus* PM, we felt it was still important to determine the circulating level of imidazole propionate (ImP), a microbially produced metabolite derived from histidine, since it was recently shown to impair insulin signaling via activation of mTOR[18]. Neither plasma ImP nor its precursor urocanate were found to be affected by the protein source (Supplementary Fig. 4f, g). Irrespective of LFLS or HFHS consumption, relative abundance of *Adlercreutzia*, *Tyzzerella* and *Intestinimonas* genera were enriched while *Bacteroides* and the *Akkermansia* genera were depleted by the PM diet (Fig. 3a-d). Notably, *Tyzzerella*, *Adlercreutzia*, *Acetatifactor*, *Lachnospiraceae-UCG_006* and *Ruminiclostrium_g* genera stood out as they were more abundant in both the HFHS context and in PM fed animals (Fig. 3b, d and Supplementary Fig. 4a-d). This could suggest a potential association between the fecal enrichment of these bacteria and the exacerbation of the deleterious effects due to the HFHS and PM interaction.

Propionate, acetate and butyrate are the main short-chain fatty acids (SCFA) produced in the gut, and are mostly associated with a positive metabolic phenotype[19,20] although this has been the subject of some debate[21]. SCFA are primarily derived from bacterial fermentation of fibers but can also be produced by protein fermentation[22]. Branched-chain fatty acids (BCFA)

(e.g. isobutyric acid and isovaleric acid) are a class of SCFA produced in the gut upon proteolytic fermentation of branched-chain amino acids (BCAA). In the 12-week study, the fecal content of the major SCFA (acetic, butyric and propionic acid) were all increased by the PM source in mice fed the LFLS diet. However, this protein effect was blunted in HFHS-fed animals (Fig. 3h-j). On the other hand, the minor SCFA (valeric, isobutyric and isovaleric acid) were clearly increased by the PM source, and this effect was most pronounced in HFHS-fed animals (Fig. 3k-m).

Early effects of the PM on gut microbiota and BCFA production and hepatic metabolism

To better understand the chronology of metabolic events and the mechanism linking our diet with diversified protein mix with worsening metabolic features, a short-term two-week study was next performed in mice fed either the HFHS-C or the HFHS-PM diet. At this early time-point there was no effect of the diet on body weight, food intake or tissue composition (Fig. 4a and Supplementary Fig. 5a-h). Postprandial blood samples were collected to assess circulating glucose, insulin and C-peptide levels, which were also found to be similar between the two groups (Fig. 4b, c and Supplementary Fig. 5i). Immunoblotting also showed no changes in phosphorylation levels of S6, Akt or IRS1 in the liver or skeletal muscle (Supplementary Fig. 4j-m).

However, at this early timepoint there was clear differences in the microbiota from HFHS-C and HFHS-PM-fed mice (Fig. 4d). Of the 30 genera found in the 12-week protocol and the 40 genera found in the 2-week protocol, 27 were commonly detected, demonstrating reproducibility between both studies despite the change in treatment duration (Supplementary Fig. 3b, 6a). Thus, only two weeks of PM treatment was sufficient to induce gut microbiota changes similar to that observed after 12 weeks, such as enhanced alpha-diversity, modulated beta-diversity and blooming of *Ruminiclostridium*, *Adlercreutzia* and *Tyzzerella* bacteria with a decrease of *Akkermansia* relative abundance (trend with LEfSe analysis -p=.05- confirmed by qPCR analysis) (Fig. 4d-g). This indicates that the changes in gut microbiota composition precede the metabolic impact of the mixed protein sources.

Importantly, we also observed that the fecal levels of SCFA and especially BCFA were already increased after only 2 weeks of the HFHS-PM diet, consistent with the 12-week study, demonstrating that the protein effect on these microbial metabolites took place prior to the development of obesity and insulin resistance (Fig. 4h-m).

Whereas this short-term diet intervention was not sufficient to perturb body weight gain, glucose homeostasis and insulin action, changes in circulating and tissue metabolites were already present. In line with the higher glycine and lower proline content of the PM diet (Fig. 1b and Supplementary Table 3), we observed clear differences in the abundances of glycine across plasma, liver and skeletal muscle pools and lower levels of proline in plasma and skeletal muscle (Supplementary Fig. 7a-c). In the liver, we also observed an increase in glutamate/glutamine, aspartate/asparagine, and the urea cycle intermediate ornithine, a trend for higher levels of the tricarboxylic acid (TCA) cycle intermediates fumarate, malate, and alpha-ketoglutarate (αKG) as well as a significant increase in succinate (Fig. 4n and Supplementary Fig. 7b). These effects are likely due to the higher content of alanine and aspartate in the PM diet (Fig. 1b and Supplementary Table 3). Indeed, alanine and aspartate are both metabolized in the liver by the alanine aminotransferase (ALT) and aspartate transaminase (AST) enzymes, respectively, to yield glutamate which can feed both the urea and TCA cycles[23].

In addition to the anticipated changes in amino acids we observed a clear increase in the accumulation of even chain acylcarnitines in both plasma and liver but not skeletal muscle of PM-fed mice (Fig. 4o and Supplementary Tables 4-6). Accumulation of even chain acylcarnitines results from mitochondrial overload and incomplete oxidation of fatty acids and is a hallmark of metabolic dysfunction[24]. Thus, our data demonstrate that the PM diet exerts an early effect on hepatic fatty acid metabolism prior to the onset of obesity or changes in insulin sensitivity.

Transplantation of the fecal microbiota from HFHS-PM fed donor mice recapitulates some of the metabolic phenotypes in germ-free recipient mice

The interaction between diet, host and gut microbiome is complex and thus we next evaluated the contribution of the gut microbiota to the metabolic phenotypes induced by the HFHS-PM diet by performing a fecal microbiota transplantation (FMT) study. Germ-free (GF) mice were colonized with fecal slurry from either HFHS-C or the HFHS-PM donor mice, which were then fed HFHS-C diet and maintained in our axenic-gnotobiotic animal facility to avoid interference from confounding environmental factors. While no significant effect was observed on body weight gain of the recipient GF mice, we found that the microbiota from the HFHS-PM donor animals increased the weight of adipose tissues (significant in inguinal and brown fat depots) as compared to GF mice colonized with the HFHS-C microbiota (Fig. 5a-g). Furthermore, whereas both groups exhibited similar fasting glucose and insulin, and glucose excursions during a GTT were comparable (Fig. 5h-j), mice bearing the microbiota from HFHS-PM donors showed an exacerbated glucose-stimulated insulin response (Fig. 5k) as compared to their counterpart mice bearing the fecal microbiota from HFHS-C donors, which was highly significant (post-hoc analysis : $p=0.006$) at the 15 min peak of insulin secretion), and further illustrated by calculation of the GSIS during the peak of insulin response (Fig. 5l). 16S rRNA gene sequencing of the gut microbiota composition revealed that FMT-HFHS-PM mice had greater alpha diversity than FMT-HFHS-C mice, represented by Shannon and Simpson's reciprocal indices, consistent with data from donor mice (Fig. 3f, g and 5m-n). The recipient mice also exhibited similar changes in their bacterial taxa composition as seen in their donor counterparts, as reflected by an overrepresentation of *Akkermansia muciniphila* in mice transplanted with the HFHS-C fecal samples and an overrepresentation of a genus from the *Lachnospiraceae*_NK4A136_group, *A2* and *Intestinimonas* in the mice transplanted with HFHS-PM fecal samples (Fig. 3b, d and 5o). Thus, major taxonomic changes in donor mice were successfully transferred by FMT even if all recipient mice were fed the same diet. These data demonstrate that changes in the composition of the gut microbiota are involved in at least part of the phenotypic effects of the HFHS-PM diet, notably contributing to the greater body fat accretion and insulin resistance as demonstrated by the higher insulin secretion required to achieve glucose control.

An intact gut microbiota is required for BCFA production in HFHS-PM fed mice

To gain further insight into the role of the gut microbiota in the metabolic consequences of PM feeding, we carried out a second 2-week study with additional groups of mice receiving broad-spectrum antibiotics to disrupt the microbiota. As expected from previous work[25], antibiotic treatment alone also had some impact on postprandial glycemia and insulinemia (Supplementary Fig. 8a, b), as well as body and tissue weights, (Supplementary Fig 8c-i), which did not seem to be related to energy intake (Supplementary Fig. 8j). Nonetheless, antibiotic-mediated disruption of the microbiota markedly blunted the HFHS-PM-induced fecal production of SCFA and BCFA (Fig 6a-f) confirming that the microbiota was responsible for their raised levels upon dietary treatments. We also fully reproduced the early and diet-dependent changes in hepatic acylcarnitines (AC) from the previous 2-week study (Fig. 6g-h, and Supplementary Table 7). Interestingly, whereas antibiotic treatment completely abolished the effect of PM-feeding on SCFA and BCFA, only a small fraction of the PM-diet effects on hepatic AC were altered by antibiotic treatment. Notable, among these 3-hydroxyisovaleryl (C5-OH) and 3-hydroxyisobutyryl (C4-OH) which were raised by PM-feeding but blunted with antibiotic treatment, are produced from mitochondrial oxidation of the BCFA, isovaleric and isobutyric acid respectively[11,26]. These results highlight the selective role of the gut microbiota in the regulation of these key metabolites and demonstrate that not all metabolic effects of PM-feeding are driven by the changes in the gut microbiota.

BCFA elevate glucose production and activate mTOR/S6K1 signaling in hepatocytes

In the animal studies, changes in isobutyric and isovaleric acid were the most striking as their content was not only driven by HFHS diet but also by the PM and by their interaction (Fig. 3l-m). Moreover, the production of these BCFA and that of their hepatic oxidation intermediates was completely blunted with antibiotic treatment in HFHS-PM diet fed mice. While they have been reported to be increased in obesity[27] and in non-alcoholic fatty liver disease (NAFLD)[28] the impact of these BCFA on host metabolism has not been directly studied. Here we evaluated their impact on glucose metabolism in hepatic and muscle cells. We found that both isobutyric acid and isovaleric acid dose-dependently increased hepatic glucose production (HGP) in FAO hepatoma cells (Fig. 7a-b). Isovaleric acid was more potent as its glucose producing action was detectable at 250 µM whereas the effect of

isobutyric acid was only observed at 1 mM. BCFA increased glucose production even in the presence of insulin, but isovaleric acid was again more potent than isobutyric acid to overcome the suppressive effect of insulin. This impact on hepatic glucose metabolism was associated with a robust but transient activation of the mTOR/S6K1 pathway in these cells as revealed by increased S6 phosphorylation (Fig. 7c-d). On the other hand, L6 myotubes treated with BCFA displayed no change in glucose uptake (Supplementary Fig. 9a, b). These *in vitro* findings point towards the liver as the main target of BCFA, which aligns with the mTOR/S6K1 and insulin signaling data from our *in vivo* observations.

3.6 Discussion

The role of dietary lipids and carbohydrates in the development of obesity and associated cardiometabolic diseases has been a major focus over the last 50 years. However, the potential role of dietary proteins in these pathologies remains poorly studied. We show here that inclusion of a diversified mix of proteins mirroring the western diet in a HFHS regimen causes early broad changes in the gut microbiota, selectively increases the production of BCFA, and induces early alterations in hepatic lipid oxidation which promote hepatic mTOR/S6K1 activation, liver insulin resistance, and elevated gluconeogenesis. Importantly, these changes in systemic insulin levels and hepatic lipid oxidation were observed upon altering the source of dietary protein without modifying total protein intake, and were coupled with impaired BAT thermogenesis, leading to higher lipid deposition in adipose tissues and exacerbate HFHS-induced obesity.

Central to this concept are the early and robust effects of the PM diet on the gut microbial populations. Remarkably, the PM significantly reshaped the gut microbial communities within two weeks of commencement of the PM diet. Since the shift in gut microbiota occurred prior to any change in systemic insulin sensitivity or body weight gain we interpret this to mean that the microbial changes underlie at least part of the obesogenic and insulin desensitizing effects of PM inclusion in the HFHS diet. This is further supported by the FMT studies in GF mice where we have shown the direct contribution of the gut microbiota by showing that fecal slurry from the HFHS-PM fed donor mice could reproduce some key metabolic phenotypes such as adipose tissue accretion and insulin resistance as shown by the

exacerbated insulin responses during the OGTT. Further studies will be needed to disentangle the individual *versus* combined role of the microbiome versus the diet *per se* in recapitulating the effect of HFHS-PM treatment on other key phenotypes such as increased body weight and aberrant liver function.

Indeed, the gut ecosystem has emerged as a central hub for mammalian energy intake regulation by processing nutrients into absorbable bioactive compounds[29]. Bacterial populations are modulated by diet and, in return, can produce metabolites that can either be used as fuel for microbial cross-feeding or pass into the host circulation and exert beneficial or harmful effects[30]. The fermentable fibers constitute the major energy source for bacteria, but their availability declines along the intestine[31]. Thus, peptides and proteins are degraded in the distal colonic populations, leading to production of a broad spectrum of metabolites[30] such as BCFA like isobutyric and isovaleric acid which are derived from valine and leucine, respectively[32-34].

Unlike the well-studied major SCFA, (acetic, butyric and propionic acid) data on the metabolic effects of BCFA is sparse. Yet, metabolomic studies in fecal human samples show that isovaleric acid is increased in obese patients compared to lean ones[27], that isobutyric acid is higher in NAFLD patients compared to healthy controls[28] and that both isobutyric acid and isovaleric acid are increased in patients with hypercholesterolemia[35]. These data suggest that BCFA might play a pathologic role in cardiometabolic disease. In our study, we showed that fecal excretion of BCFA increased with consumption of HFHS diet compared to LFLS diet, and this effect was greatest with PM consumption compared to casein. Our *in vitro* studies further revealed the gluconeogenic potential of isobutyric and isovaleric acids and their ability to activate the mTOR/S6K1 pathway, highlighting a novel gut-liver cross talk that appears to underlie the deleterious interaction of HFHS diet and PM. These results are consistent with Hu *et al.* who identified 3-hydroxyisobutyrate (3-HIB), which is derived from valine and isobutyric acid[36], as a substrate for gluconeogenesis[37]. It was also shown that 3-HIB can stimulate endothelial fatty acid uptake in muscle and is associated with T2D[38]. Another *in vitro* study demonstrated that isobutyric and isovaleric acid ability to block lipolysis in rat and human adipocytes[39]. The authors of this study suggested that this effect

of BCFA might promote insulin sensitivity, however an alternate outcome could be greater adipose tissue expansion as was observed with HFHS-PM-fed mice in our study.

Among the many effects of PM on the gut microbiota we found that PM particularly depleted *Akkermansia muciniphila* which occurred after only 2 weeks of dietary exposure and preceded any changes in body weight gain or glucose homeostasis. This effect was also found in colonized GF mice after FMT from HFHS-PM fed mice as compared to GF mice after FMT from HFHS-C fed mice. High abundance of this bacterium in HFHS-fed mice has been associated with protection against obesity and an increase insulin sensitivity[40]. Moreover, its administration as a probiotic notably improved metabolic health in both obese mice and humans[41-43]. Our data indicates that casein promotes *Akkermansia muciniphila* and thus could have a protective effect on the development of obesity when used in obesogenic murine diets. On the contrary, the PM favoured a more diversified gut microbiota composition, while disturbing the ecological niche of *Akkermansia muciniphila*. In addition, the HFHS-PM interaction also promoted enrichment of *Tyzzerella* that was previously correlated with lifetime risk of cardiovascular diseases[44] and a low dietary quality[45].

Other than early changes in microbial populations and microbe derived SCFA, we also observed robust acylcarnitine accumulation in the liver and plasma of HFHS-PM-fed mice. Compared to casein, PM induced an overall increase of short, medium and long chain acylcarnitines in the plasma. Elevated circulating even chain acylcarnitines (particularly short and long chain) are a hallmark of metabolic disease, present in T2D patients[46] and may be a predictive marker of prediabetes[47]. However, it is unclear if they promote insulin resistance or simply represent biomarkers of dysregulated metabolism[48]. The acylcarnitine profile in HFHS-PM-fed animals and the increase in multiple TCA intermediates observed in liver is consistent with a state of mitochondria overload and suggest the combination of PM with a HFHS diet interferes with hepatic lipid oxidation. It is interesting that abrogation of gut microbiota by antibiotic treatment did little to blunt these broad changes in specific hepatic acylcarnitines, suggesting that not all the metabolic effects of PM-feeding can be attributed to changes in the microbiota. These effects are reminiscent of those observed with modulation of dietary BCAA supply[26,49] and suggest that altered amino acid supply might

account for this effect of the PM diet. Thus, additional studies are warranted to determine which component of the PM diet is responsible for the rapid shift in hepatic acylcarnitine metabolism.

The role of the mTOR signaling pathway in obesity and energy metabolism has been thoroughly investigated in the last two decades. This nutrient sensing pathway allows cells to react to environmental factors in insulin target tissues, such as muscle, liver, and fat[50] and can be activated by different intracellular and extracellular signals. It has been shown that mTOR can be activated by amino acids, particularly by BCAA[11]. However, we did not detect any difference in BCAA levels in circulation nor in tissues in PM-fed mice, rather our studies suggest that BCFA are responsible for the increased mTOR/S6K1 activation observed in liver of mice fed the protein mix. Koh *et al.* recently demonstrated that microbially-produced ImP is a key metabolite increased in type 2 diabetic patients that can activate the mTOR pathway[18]. However, we have measured ImP in this study and found no differences between mice fed casein and the PM sources indicating that this microbial product is not contributing to the effect of the mixed dietary proteins on hepatic mTOR/S6K1 activation and insulin resistance. Another potential mechanism of PM-induced hepatic insulin resistance is the incomplete oxidation of lipids by the TCA cycle which can lead to the accumulation of other lipid intermediates such as DAG which activate stress-sensitive kinases, such as PKC that can negatively phosphorylate IRS to promote insulin resistance. This potential mechanism is supported by our observations of increased levels of acylcarnitines and protein levels of PKC theta, a well-known IRS-1 S1101 kinase, that was induced by the combination of HFHS and PM.

Our findings are relevant to the use of pre-clinical models in metabolic investigations. We demonstrate that altering the source of dietary protein without modifying total protein intake has a major impact on the microbial and metabolic effects of obesogenic diets and should be considered when designing dietary studies in animal models of obesity and diabetes. Notably, we found that inclusion of a protein mixture that mirrors the protein composition of the western diet accentuates the effects of HFHS feeding on weight gain, hepatic insulin sensitivity, and gut microbiota composition compared to the classical casein-based diet that is still widely used. In this regard, while we tried our best to match the HFHS-C and HFHS-

PM diets as much as possible (including matching for SAT:PUFA ratio), we cannot completely exclude the potential contribution of other minor nutritional factors we could not completely adjusted for. This includes monounsaturated fatty acids, some fibers that we could not discriminate according to the soluble/insoluble classification, as well as potential advanced glycation end-products issued from meat cooking. However, since these non-adjusted components represent less than 5% of the energy intake variation between the diets used, their impact is most likely negligible as compared to that conferred by the major differences in protein composition.

In summary, we have demonstrated that dietary protein sources have a major impact on obesity and insulin resistance that is mechanistically link to direct and early changes in gut microbiota and BCFA production, leading to activation of the mTOR/S6K1 pathway and hepatic insulin resistance. Cellular studies further provided direct evidence that BCFA can exert cell-autonomous effects on mTOR/S6K1 activation and stimulating hepatic gluconeogenesis. Our work thus reveals the importance of considering the dietary protein source in designing nutritional interventions aimed at improving weight management, and that modifying the composition of dietary proteins may offer therapeutic benefits for insulin resistance and T2D.

3.7 Acknowledgements

This work was funded by a Foundation scheme grant (FDN-143247) from the Canadian Institutes of Health Research (CIHR) to AM, which is also the recipient of a CIHR/Pfizer research Chair in the pathogenesis of insulin resistance and cardiovascular diseases. PJW is supported by a Pathways Award from the American Diabetes Association #1-16-INI-17. BSYC is funded by doctoral scholarships from the Sentinel North program at Laval University (Canada First Research Excellence Fund) and from the Natural Sciences and Engineering Research Council of Canada (NSERC). We thank Christine Dallaire, Christine Dion, Jacinthe Julien, Jenny Rancourt-Mercier, Joanie Dupont-Morissette, Geneviève Guèvremont and Alexandre Pleau for the expert help with animal care, Roxanne Nolet for the SCFA and BCFA measurements as well as Tabitha George for metabolite analyses performed at the DMPI metabolomics core laboratory.

Author contribution

BSYC, ND, PJW and AM conceived the study and wrote the manuscript with inputs from all co-authors. BSYC and ND conducted animal studies and related analysis. BSYC, VPH, AO and BM performed cell culture experiments. VPH realized qPCR and western blotting. TVV performed 16S sequencing analyses. OI and PJW performed the plasma and tissues metabolomic analyses. MS and FB performed ImP and urocanate analyses. PF performed SCFA and BCFA analyses. ND and CV conducted statistical analyses. PSTP, PJW, AT and AM supervised the study. BSYC, ND and AM are responsible for the integrity of the work as a whole. All authors reviewed the manuscript.

Data availability

Source data are provided with this paper. The raw sequence data generated in this work were deposited into the European Nucleotide Archive (ENA) under accession identification PRJEB37442.

Code availability

No custom code was used in the present work.

Declaration of interests

The authors declare no conflict of interest.

3.8 References

1. Despres, J.P. & Lemieux, I. Abdominal obesity and metabolic syndrome. *Nature* **444**, 881-887 (2006).
2. National Research Council, *Nutrient Requirements of Laboratory Animals: Fourth Revised Edition,* 1995. 192 (The National Academies Press, Washington, DC, 1995).
3. Clausen, M.R.*, et al.* Intake of hydrolyzed casein is associated with reduced body fat accretion and enhanced phase II metabolism in obesity prone C57BL/6J mice. *PLoS One* **10**, e0118895 (2015).
4. Liisberg, U.*, et al.* The protein source determines the potential of high protein diets to attenuate obesity development in C57BL/6J mice. *Adipocyte* **5**, 196-211 (2016).
5. Comerford, K.B. & Pasin, G. Emerging Evidence for the Importance of Dietary Protein Source on Glucoregulatory Markers and Type 2 Diabetes: Different Effects of Dairy, Meat, Fish, Egg, and Plant Protein Foods. *Nutrients* **8**(2016).
6. Ijaz, M.U.*, et al.* Beef, Casein, and Soy Proteins Differentially Affect Lipid Metabolism, Triglycerides Accumulation and Gut Microbiota of High-Fat Diet-Fed C57BL/6J Mice. *Front Microbiol* **9**, 2200 (2018).
7. Fan, M.*, et al.* Dietary Protein Consumption and the Risk of Type 2 Diabetes: ADose-Response Meta-Analysis of Prospective Studies. *Nutrients* **11**(2019).
8. Hill, A.M., Harris Jackson, K.A., Roussell, M.A., West, S.G. & Kris-Etherton, P.M. Type and amount of dietary protein in the treatment of metabolic syndrome: a randomized controlled trial. *Am J Clin Nutr* **102**, 757-770 (2015).
9. US Department of Agriculture, Center for Nutrition Policy and Promotion (2014) Nutrient Content of the US Food Supply, 1909–2010. http://www.cnpp.usda.gov/ USFoodSupply-1909-2010 (accessed January 2018).
10. Chevrier, G.*, et al.* Low-Molecular-Weight Peptides from Salmon Protein Prevent Obesity-Linked Glucose Intolerance, Inflammation, and Dyslipidemia in LDLR-/-/ApoB100/100 Mice. *J Nutr* **145**, 1415-1422 (2015).
11. Newgard, C.B.*, et al.* A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* **9**, 311-326 (2009).
12. Tome, D. 90th Anniversary Commentary: The mTORC1 Complex-A Central Player in the Control and Regulation of Amino Acid Sufficiency. *J Nutr* **148**, 1678-1682 (2018).
13. Tremblay, F.*, et al.* Overactivation of S6 kinase 1 as a cause of human insulin resistance during increased amino acid availability. *Diabetes* **54**, 2674-2684 (2005).
14. Tremblay, F.*, et al.* Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. *Proc Natl Acad Sci U S A* **104**, 14056-14061 (2007).
15. Li, Y.*, et al.* Protein kinase C Theta inhibits insulin signaling by phosphorylating IRS1 at Ser(1101). *J Biol Chem* **279**, 45304-45307 (2004).
16. Szendroedi, J.*, et al.* Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. *Proc Natl Acad Sci U S A* **111**, 9597-9602 (2014).

17. Rothschild, D.*, et al.* Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210-215 (2018).
18. Koh, A.*, et al.* Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1. *Cell* **175**, 947-961 e917 (2018).
19. Chambers, E.S., Preston, T., Frost, G. & Morrison, D.J. Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. *Curr Nutr Rep* **7**, 198-206 (2018).
20. Gill, P.A., van Zelm, M.C., Muir, J.G. & Gibson, P.R. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment Pharmacol Ther* **48**, 15-34 (2018).
21. den Besten, G.*, et al.* The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **54**, 2325-2340 (2013).
22. Diether, N.E. & Willing, B.P. Microbial Fermentation of Dietary Protein: An Important Factor in Diet(-)Microbe(-)Host Interaction. *Microorganisms* **7**(2019).
23. White, P.J.*, et al.* Muscle-Liver Trafficking of BCAA-Derived Nitrogen Underlies Obesity-Related Glycine Depletion. *Cell Rep.* **33**, 108375 (2020).
24. Koves, T.R.*, et al.* Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab* **7**, 45-56 (2008).
25. Zarrinpar, A.*, et al.* Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. *Nat Commun* **9**, 2872 (2018).
26. White, P.J.*, et al.* Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export. *Mol Metab* **5**, 538-551 (2016).
27. Tiihonen, K., Ouwehand, A.C. & Rautonen, N. Effect of overweight on gastrointestinal microbiology and immunology: correlation with blood biomarkers. *Br J Nutr* **103**, 1070-1078 (2010).
28. Da Silva, H.E.*, et al.* Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep* **8**, 1466 (2018).
29. Rowland, I.*, et al.* Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* **57**, 1-24 (2018).
30. Canfora, E.E., Meex, R.C.R., Venema, K. & Blaak, E.E. Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat Rev Endocrinol* **15**, 261-273 (2019).
31. Windey, K., De Preter, V. & Verbeke, K. Relevance of protein fermentation to gut health. *Mol Nutr Food Res* **56**, 184-196 (2012).
32. Zarling, E.J. & Ruchim, M.A. Protein origin of the volatile fatty acids isobutyrate and isovalerate in human stool. *The Journal of laboratory and clinical medicine* **109**, 566-570 (1987).
33. Bai, W., Geng, W., Wang, S. & Zhang, F. Biosynthesis, regulation, and engineering of microbially produced branched biofuels. *Biotechnol Biofuels* **12**, 84 (2019).
34. Oliphant, K. & Allen-Vercoe, E. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome* **7**, 91 (2019).
35. Granado-Serrano, A.B.*, et al.* Faecal bacterial and short-chain fatty acids signature in hypercholesterolemia. *Sci Rep* **9**, 1772 (2019).

36. Jaskiewicz, J.*, et al.* Catabolism of isobutyrate by colonocytes. *Archives of biochemistry and biophysics* **327**, 265-270 (1996).
37. Hu, H., Jaskiewicz, J.A. & Harris, R.A. Ethanol and oleate inhibition of alpha-ketoisovalerate and 3-hydroxyisobutyrate metabolism by isolated hepatocytes. *Archives of biochemistry and biophysics* **299**, 57-62 (1992).
38. Jang, C.*, et al.* A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. *Nat Med* **22**, 421-426 (2016).
39. Heimann, E., Nyman, M., Palbrink, A.K., Lindkvist-Petersson, K. & Degerman, E. Branched short-chain fatty acids modulate glucose and lipid metabolism in primary adipocytes. *Adipocyte* **5**, 359-368 (2016).
40. Anhe, F.F.*, et al.* A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut* **64**, 872-883 (2015).
41. Everard, A.*, et al.* Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 9066-9071 (2013).
42. Plovier, H.*, et al.* A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat. Med.* **23**, 107-113 (2017).
43. Depommier, C.*, et al.* Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med* **25**, 1096-1103 (2019).
44. Kelly, T.N.*, et al.* Gut Microbiome Associates With Lifetime Cardiovascular Disease Risk Profile Among Bogalusa Heart Study Participants. *Circ Res* **119**, 956-964 (2016).
45. Liu, Y.*, et al.* Dietary quality and the colonic mucosa-associated gut microbiome in humans. *Am J Clin Nutr* (2019).
46. Mihalik, S.J.*, et al.* Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. *Obesity (Silver Spring)* **18**, 1695-1700 (2010).
47. Sun, L.*, et al.* Early Prediction of Developing Type 2 Diabetes by Plasma Acylcarnitines: A Population-Based Study. *Diabetes care* **39**, 1563-1570 (2016).
48. Schooneman, M.G., Vaz, F.M., Houten, S.M. & Soeters, M.R. Acylcarnitines: reflecting or inflicting insulin resistance? *Diabetes* **62**, 1-8 (2013).
49. White, P.J. & Newgard, C.B. Branched-chain amino acids in disease. *Science (New York, N.Y.)* **363**, 582-583 (2019).
50. Laplante, M. & Sabatini, D.M. mTOR signaling in growth control and disease. *Cell* **149**, 274-293 (2012).
51. What We Eat in America, NHANES 2011-2012, individuals 2 years and over (excluding breast-fed children), day 1. Available: www.ars.usda.gov/nea/bhnrc/fsrg.
52. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *2011* **17**, 3 (2011).
53. Callahan, B.J.*, et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**, 581-583 (2016).

54. Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**, 5261-5267 (2007).
55. Quast, C*., et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**, D590-596 (2013).
56. Wright, E.S. DECIPHER: harnessing local sequence context to improve protein multiple sequence alignment. *BMC Bioinformatics* **16**, 322 (2015).
57. Schliep, K.P. phangorn: phylogenetic analysis in R. *Bioinformatics* **27**, 592-593 (2011).
58. McMurdie, P.J. & Holmes, S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**, e61217 (2013).
59. Segata, N*., et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* **12**, R60 (2011).
60. Garcia-Villalba, R*., et al.* Alternative method for gas chromatography-mass spectrometry analysis of short-chain fatty acids in faecal samples. *J Sep Sci* **35**, 1906-1913 (2012).

3.9 Figure Legends

Figure 1: A new protein mix was formulated to oppose casein use in rodent diets. (**a**) Macronutrient composition of LFLS (15% kcal proteins, 10% kcal fat, 75% kcal carbohydrates) and HFHS (15% kcal proteins, 50% kcal fat, 35% kcal carbohydrates) diets. (**b**) Description of the protein sources used to formulate Casein (100% casein) and Protein Mix (13.4% beef, 13.4% pork, 13.8% chicken, 15.4% casein, 3.9% whey, 3.3% cod, 6.1% soy, 6.1% pea, 21.1% rice, 3.7% egg) portions in diets and resulting amino acid composition, in percentage of protein source. See also Supplementary Tables 1-3.

Figure 2: Protein mix compared to casein potentiates diet-induced obesity and glucose intolerance through mTOR pathway activation in the liver. Mice were fed with a LFLS-C (green), a LFLS-PM (green, hatched), a HFHS (blue) or a HFHS-PM (blue, hatched) diet. (**a**) Body weight curve and (**b**) total body weight gain over the 12 weeks of dietary treatment. (**c-h**) At week 11, mice were fasted for 6 hours and challenged with an oral glucose load (1 mg/g body weight): (**c**) glycemia and (**d**) insulinemia before the ingestion of glucose, (**e)** glycemic response and (**f**) area under the curve as well as (**g**) insulinemic response following the glucose load. (n=14 for LFLS-C group and n=15 biologically independent mice for the three other groups except for (d) where n=14 for HFHS-PM group). (**h**) C-peptide response before and 15 minutes after the ingestion of the bolus of glucose (n=4 for LFLS-PM group, n=6 for LFLS-C and HFHS-PM groups and n=7 biologically independent mice for HFHS-C group). (**i-n**) Hepatic insulin signaling of mice fasted for 6 hours and injected intravenously with saline ("-", n=5 for all groups) or insulin ("+", n=9 for LFLS-C and HFHS-PM groups and n=10 independent experiments for LFLS-PM and HFHS-C groups) for 5 minutes before euthanasia: immunoblots and quantification of densitometry analyses for (**i-k**) pIRS1 Ser1101, total IRS1, pS6 S240-244 and total S6 (**i, l**) PKC theta (n=14 for LFLS-C and HFHS-PM groups and n=15 biologically independent mice for LFLS-PM and HFHS-C groups) and **(m, n)** pAkt Ser473 and total Akt. Actin and eEF2 have been used as loading control. Representative images are from different gels and separated by a dash line. As twice as many mice were injected with insulin compared to saline, the number of representative animals (2:1) is pictured on the gels. Arb. units, Arbitrary Units. Data are means±s.e.m. Statistical analyses were performed using a two-way ANOVA, a three-way ANOVA or a

mixed model for repeated measures, followed by a Tukey post-hoc test. P-values of general effect for diet (D), protein (P), time (T) and insulin condition (I) factors are recorded under the title of each graph, followed by the p-values of the corresponding factor interaction effects. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file. See also Supplementary Fig. 1 and 2.

Figure 3: Protein mix shifts gut microbiota composition independently of LFLS or HFHS diet after 12 weeks of dietary treatment. (**a**, **b**) Cladograms showing differentially abundant bacteria between (**a**) LFLS-C (pink) and LFLS-PM (yellow) groups and (**b**) HFHS-C (green) and HFHS-PM (blue) groups, represented as the taxonomic levels from phylum to order, and abbreviations for family and genus levels. The central point denotes the root of the tree of bacteria. The size of each node represents the relative abundance of taxa. (**c**, **d**) Histograms of LDA scores identifying genera differentially represented between (**a**) LFLS-C and LFLS-PM and (**b**) HFHS-C and HFHS-PM groups, with a cut-off value of 2.5 for LDA score. Presence of 'g' at the end of taxon denotes unclassified genus. **(e)** Relative quantity of fecal *Akkermansia muciniphila* evaluated by qPCR. Alpha diversity represented by **(f)** Shannon and (**g**) Simpson's reciprocal indexes. (n=14 for LFLS-C group and n=15 biologically independent mice for the three other groups). Fecal levels of major SCFA (**h**) acetic, (**i**) butyric and (**j**) propionic acids and minor SCFA (**k**) valeric, (**l**) isobutyric and (**m**) isovaleric acids after 12 weeks of dietary intervention (n=14 for LFLS-C and HFHS-C groups and n=15 biologically independent mice for LFLS-PM and HFHS-PM groups). For panels e-m: LFLS-C: green; LFLS-PM: green, hatched; HFHS-C: blue; HFHS-PM: blue, hatched. Data are means±s.e.m. Statistical analyses were performed using a two-way ANOVA followed by a Tukey post-hoc test. P-values of general effect for diet (D) and protein (P) factors and diet x protein (DxP) interaction are recorded under the title of each graph. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file. See also Supplementary Fig. 3 and 4.

Figure 4: Two weeks of protein mix is not enough to affect weight gain and development of insulin resistance in HFHS-fed mice but induces changes in microbial composition, SCFA production and postprandial metabolite profile. (**a**) Total body weight gain of mice fed with HFHS-C (purple) or HFHS-PM (purple, hatched) diet during a 2-week study. Mice were food-deprived for 12 hours after 2 weeks of dietary treatment and food was given back to animals during 15 min. Blood was collected 30 min later by saphenous vein for postprandial (**b**) glycemia and (**c**) insulinemia assessment. (n=12 biologically independent mice for both groups). Gut microbiota differences are represented by (**d) a** histogram plotting differentially abundant bacteria detected by LEfSe using a threshold of LDA score >2.5 between HFHS-C (green) and HFHS-PM (blue), **(e)** quantification of fecal *Akkermansia muciniphila* evaluated by gene expression through qPCR, and **(f)** Shannon and **(g)** Simpson's reciprocal diversity indexes. n=6 biologically independent mice for both groups. Mice selected for gut microbiota composition analysis were the closest to median on main physiological parameters (body weight, glycemia and insulinemia). Fecal levels of major SCFA (**h**) acetic, (**i**) butyric and (**j**) propionic acid and minor SCFA (**k**) valeric, (**l**) isobutyric and (**m**) isovaleric acid after 2 weeks of dietary intervention. Two hours following refeeding after the food deprivation, submandibular blood was collected for plasma metabolite profiles determination. Mice were euthanized and muscle and liver were harvested for metabolites profile. Hepatic (**n**) organic acid profile and (**o**) acylcarnitines (AC) profiles. n=12 biologically independent mice for both groups. Data are means±s.e.m for panels a-g and median, interquartile range, minimum and maximum for panels h-o. Statistical analyses were performed using a two-tailed Student's *t* test or its nonparametric equivalent Mann-Whitney test. Detailed significant differences are recorded as follows: *p<.05, **p<.01. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file. See also Supplementary Fig. 5-7 and Supplementary Tables 4-6. Short-chain AC: C2 to C4; Medium-chain AC: C5 to C10; Long-chain AC: C12 to C22.

Figure 5: FMT is sufficient to induce some phenotypical changes caused by PM in a HFHS context. Germ-free mice were colonized by fecal slurry from the either HFHS-C-fed

mice (light blue) or HFHS-PM-fed mice (light blue, hatched). (**a**) Body weight curve, (**b**) total body weight gain and **(c)** total energy intake of mice all fed HFHS-C and housed in an axenic-gnotobiotic facility. (**d-g**) Tissue weights of (**d**) inguinal white adipose tissue (iWAT) (**e**) intrascapular brown adipose tissue (BAT), (**f**) visceral adipose tissue (VAT), and (**g**) gastrocnemius muscle after 11 weeks. At week 10, mice were fasted for 6 hours and challenged with an oral glucose load (1 mg/g body weight). (**h**) Glycemia and (**i**) insulinemia before the ingestion of glucose. **(j)** Glycemic response, (**k**) insulinemic response and (**l**) area under the curve (AUC) for GSIS during the peak of insulin response (baseline T0-T30), following the glucose load. Fecal microbiota alpha diversity represented by **(m)** Shannon and (**n**) Simpson's reciprocal indexes. (**o**) Histograms of LDA scores identifying genera differentially represented between FMT HFHS-C (light blue) and FMT HFHS-PM (blue) groups, with a cut-off value of 2.5 for LDA score. n=12 biologically independent mice for both groups except for panels h-l where n=11 for FMT HFHS-PM group, and panel C where n=4 . Data are means±s.e.m. Statistical analyses were performed using a two-tailed Student's *t* test or its nonparametric equivalent Mann-Whitney test, or a two-way repeated measure ANOVA followed by a Tukey post-hoc test. P-values of general effect for microbiota (M) and time (T) factors and microbiota x time (MxT) interaction are recorded under the title of each graph. Detailed significant differences are recorded as follows: *p<.05, ***p<.001. Source data are provided as a Source Data file.

Figure 6: Antibiotics (Abx) prevent SCFA and BCFA production while mitigating diet impact on hepatic acylcarnitines. Mice were fed with a HFHS-C (purple) or a HFHS-PM (purple, hatched) and administered with an antibiotic cocktail (orange, and orange, hatched respectively). Fecal levels of major SCFA (**a**) acetic, (**b**) propionic and (**c)** butyric acid and minor SCFA (**d**) valeric, (**e**) isobutyric and (**f**) isovaleric acid after 2 weeks of dietary intervention. Hepatic **(g)** acylcarnitine profile and specific **(h)** acylcarnitine changes attributed to antibiotic treatment, protein source or both. Short-chain AC: C2 to C4; Medium-chain AC: C5 to C10; Long-chain AC: C12 to C22. Data are means±s.e.m. Metabolite concentrations were log10 transformed and Pareto scaled before generating a heatmap. Statistical analyses were performed using a two-way ANOVA followed by a Tukey post-hoc test. n=12 biologically independent mice for both groups. P-values of general effect for

protein (P) and antibiotic (A) factors and protein x antibiotic (PxA) interaction are recorded under the title of each graph. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file. See also Supplementary Fig. 7 and 8.

Figure 7: BCFA increase hepatic glucose production and activate the mTOR/pS6K1 pathway *in vitro*. Branched chain fatty acids (BCFA) effect on hepatic glucose production in hepatocytes (FAO) in basal or insulin (1nM) condition with **(a)** isovaleric (isoV) or **(b)** isobutyric (IsoB) acid (1-1 000µM); data corrected for total protein. For IsoV treatment in basal condition (dark red): n=6 independent experiments for 1µM, 500µM and 750µM, n=7 for 10µM and 250µM, n=11 for 1mM, and n=13 for 100nM. For IsoV treatment in insulin condition (light purple): n=5 independent experiments for 750µM, n=6 for 1µM, n=7 for 10µM, n=8 for 250µM and 500µM and n=12 for 100µM and 1mM. For IsoB treatment in basal condition (blue-green): n=6 independent experiments for 250µM, n=7 for 1µM, 10µM, 500µM and 750µM, n=13 for 100µM, n=15 for 1mM. For IsoB treatment in insulin condition (kaki green): n=6 independent experiments for 750µM, n=7 for 1µM, 10µM and 250µM, n=9 for 500µM and n=14 for 100µM and 1mM. Immunoblots and quantification of densitometry analyses for pS6 S240-244 and total S6 in FAO cells exposed to 1mM of **(c, d)** isovaleric (dark red) and **(e, f)** isobutyric (blue-green) acid. eEF2 has been used as loading control and n=4 independent experiments. Data are means±s.e.m. A Kruskal-Wallis test followed by a two-tailed Dunn's post-hoc test versus Vehicule/ Vehicule+Insulin, was performed in each condition and general p-value is recorded under the title of each graph. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Source data are provided as a Source Data file. See also Supplementary Fig. 9.

3.10 Figures

FIGURE 1

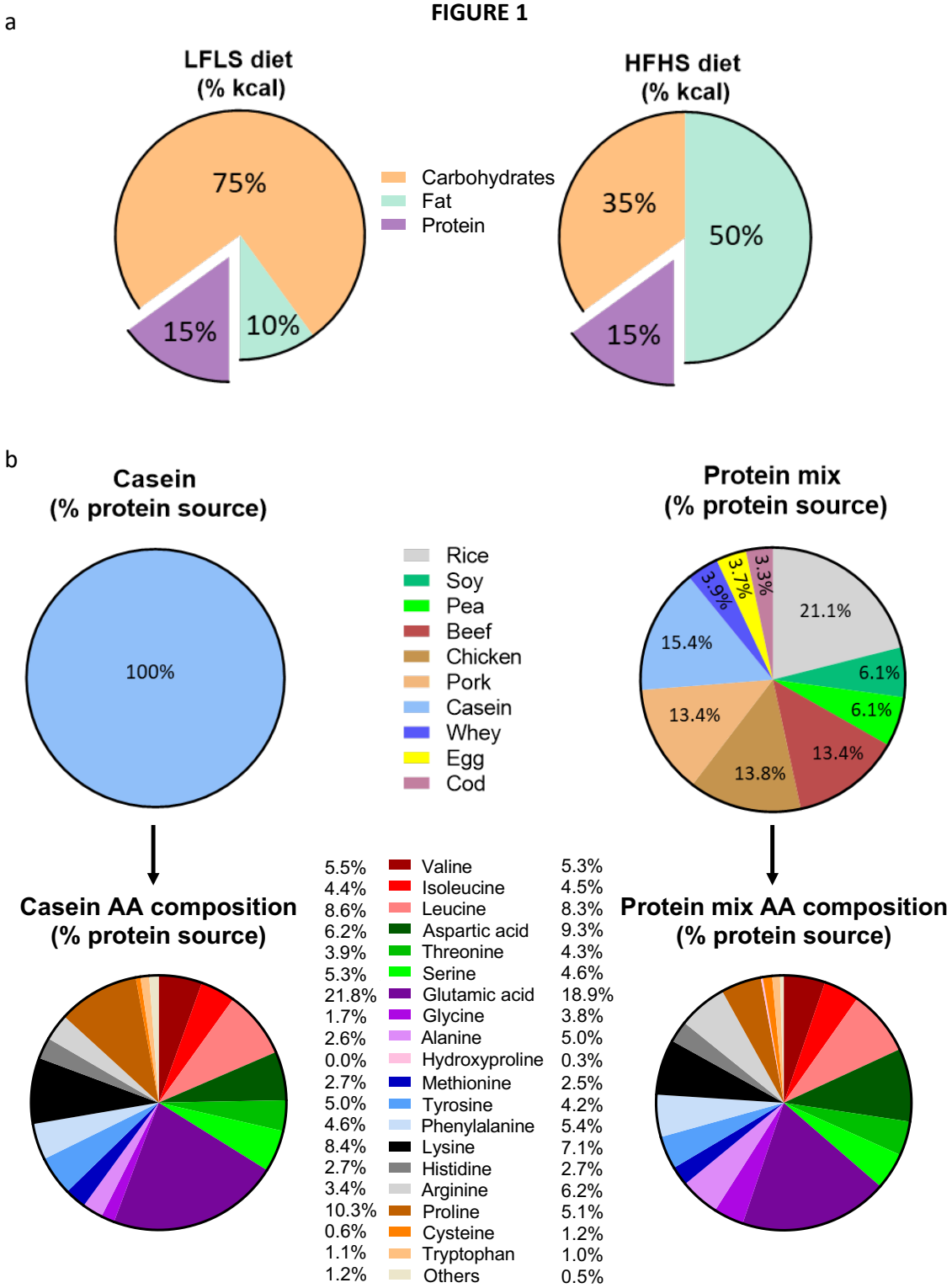


FIGURE 2

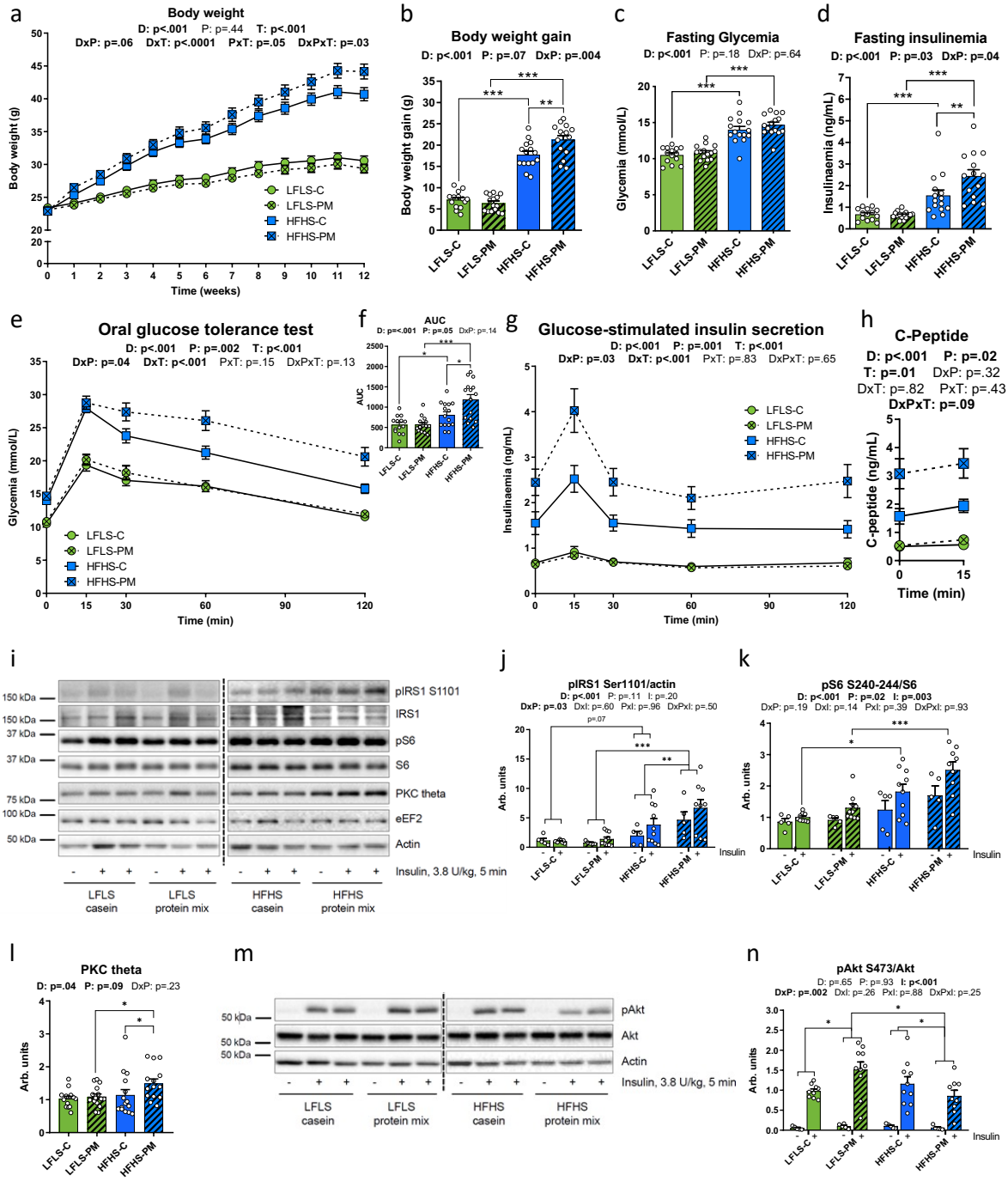

a
Body weight
D: p<.001 P: p=.44 T: p<.001
DxP: p=.06 DxT: p<.0001 PxT: p=.05 DxPxT: p=.03
Body weight (g)
Time (weeks)
LFLS-C
LFLS-PM
HFHS-C
HFHS-PM
b
Body weight gain
D: p<.001 P: p=.07 DxP: p=.004
Body weight gain (g)
c
Fasting Glycemia
D: p<.001 P: p=.18 DxP: p=.64
Glycemia (mmol/L)
d
Fasting insulinemia
D: p<.001 P: p=.03 DxP: p=.04
Insulinaemia (ng/mL)
e
Oral glucose tolerance test
D: p<.001 P: p=.002 T: p<.001
DxP: p=.04 DxT: p<.001 PxT: p=.15 DxPxT: p=.13
Glycemia (mmol/L)
Time (min)
f
AUC
D: p=<.001 P: p=.05 DxP: p=.14
AUC
g
Glucose-stimulated insulin secretion
D: p<.001 P: p=.001 T: p<.001
DxP: p=.03 DxT: p<.001 PxT: p=.83 DxPxT: p=.65
Insulinaemia (ng/mL)
Time (min)
h
C-Peptide
D: p<.001 P: p=.02
T: p=.01 DxP: p=.32
DxT: p=.82 PxT: p=.43
DxPxT: p=.09
C-peptide (ng/mL)
Time (min)
i
150 kDa
150 kDa
37 kDa
37 kDa
75 kDa
100 kDa
50 kDa
pIRS1 S1101
IRS1
pS6
S6
PKC theta
eEF2
Actin
Insulin, 3.8 U/kg, 5 min
LFLS casein
LFLS protein mix
HFHS casein
HFHS protein mix
j
pIRS1 Ser1101/actin
D: p<.001 P: p=.11 I: p=.20
DxP: p=.03 DxI: p=.60 PxI: p=.96 DxPxI: p=.50
Arb. units
Insulin
k
pS6 S240-244/S6
D: p<.001 P: p=.02 I: p=.003
DxP: p=.19 DxI: p=.14 PxI: p=.39 DxPxI: p=.93
Arb. units
Insulin
l
PKC theta
D: p=.04 P: p=.09 DxP: p=.23
Arb. units
m
50 kDa
50 kDa
50 kDa
pAkt
Akt
Actin
Insulin, 3.8 U/kg, 5 min
LFLS casein
LFLS protein mix
HFHS casein
HFHS protein mix
n
pAkt S473/Akt
D: p=.65 P: p=.93 I: p<.001
DxP: p=.002 DxI: p=.26 PxI: p=.88 DxPxI: p=.25
Arb. units
Insulin

FIGURE 3

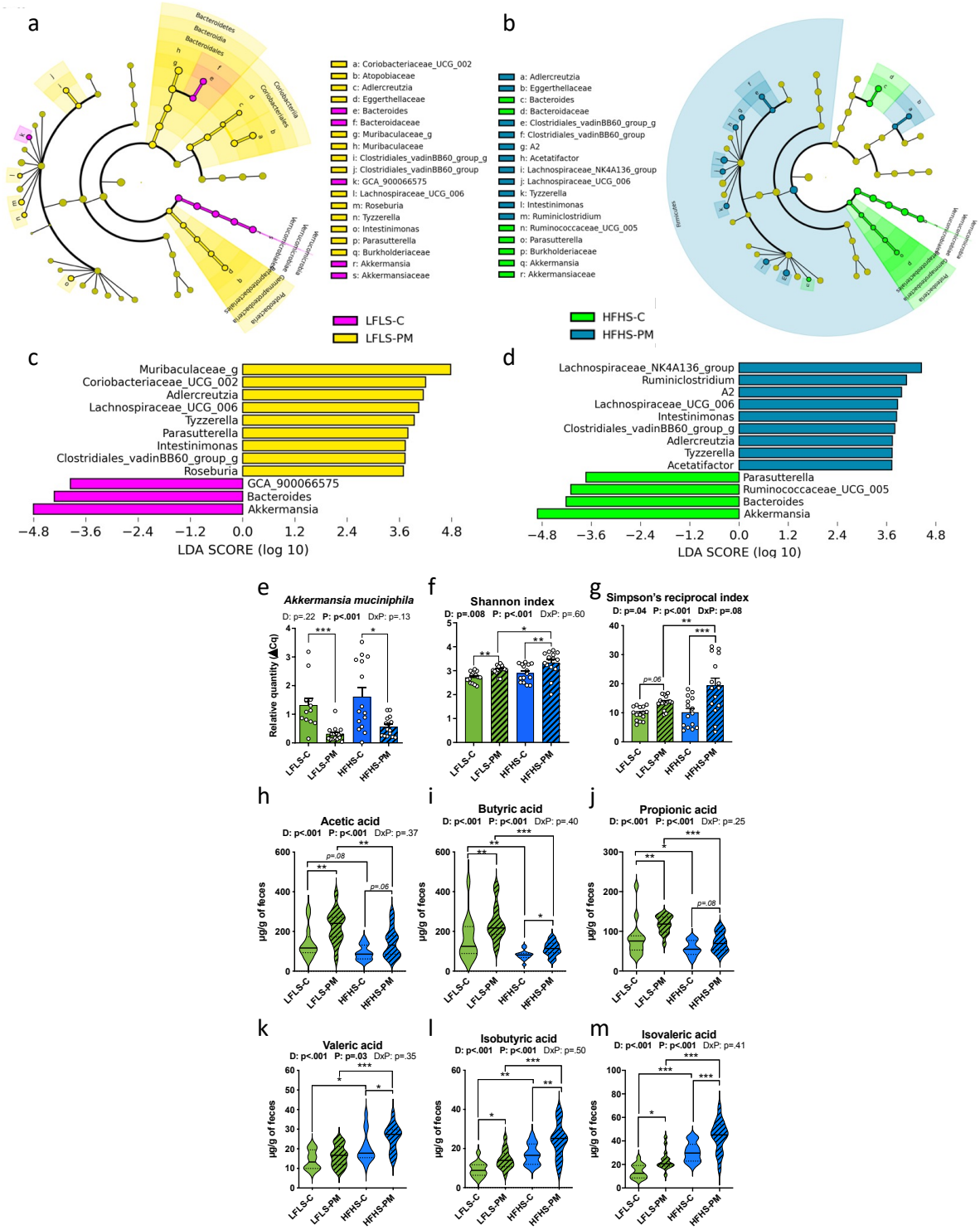

a
b
c
d
e
f
g
h
i
j
k
l
m
LFLS-C
LFLS-PM
HFHS-C
HFHS-PM
LDA SCORE (log 10)
Akkermansia muciniphila
Shannon index
Simpson's reciprocal index
Acetic acid
Butyric acid
Propionic acid
Valeric acid
Isobutyric acid
Isovaleric acid
Relative quantity (ΔCq)
µg/g of feces

FIGURE 4

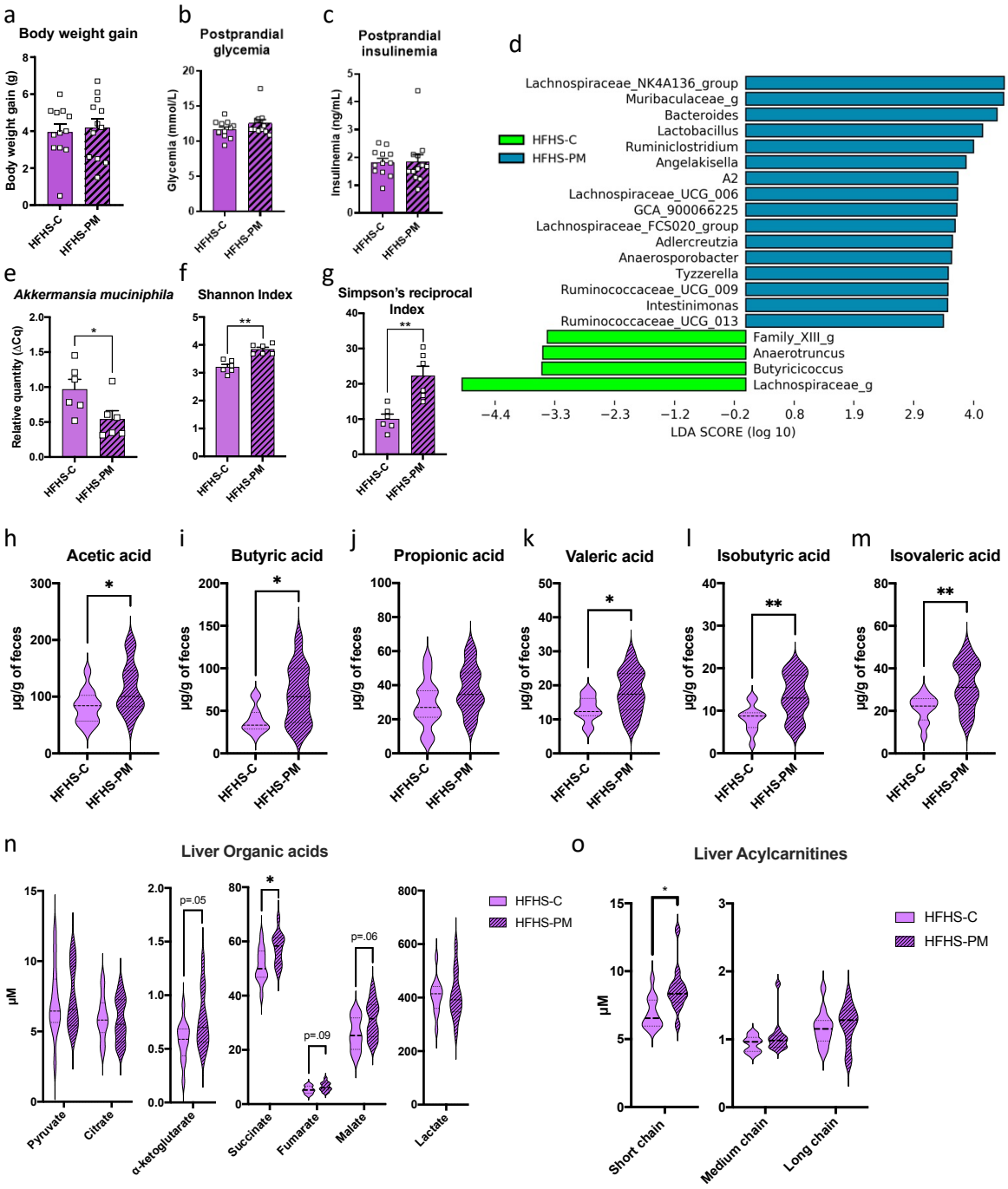

a
Body weight gain
Body weight gain (g)
HFHS-C
HFHS-PM
b
Postprandial glycemia
Glycemia (mmol/L)
c
Postprandial insulinemia
Insulinemia (ng/mL)
d
Lachnospiraceae_NK4A136_group
Muribaculaceae_g
Bacteroides
Lactobacillus
Ruminiclostridium
Angelakisella
A2
Lachnospiraceae_UCG_006
GCA_900066225
Lachnospiraceae_FCS020_group
Adlercreutzia
Anaerosporobacter
Tyzzerella
Ruminococcaceae_UCG_009
Intestinimonas
Ruminococcaceae_UCG_013
Family_XIII_g
Anaerotruncus
Butyricicoccus
Lachnospiraceae_g
HFHS-C
HFHS-PM
−4.4 −3.3 −2.3 −1.2 −0.2 0.8 1.9 2.9 4.0
LDA SCORE (log 10)
e
Akkermansia muciniphila
Relative quantity (ΔCq)
f
Shannon Index
g
Simpson's reciprocal Index
h
Acetic acid
i
Butyric acid
j
Propionic acid
k
Valeric acid
l
Isobutyric acid
m
Isovaleric acid
µg/g of feces
n
Liver Organic acids
µM
Pyruvate
Citrate
α-ketoglutarate
Succinate
Fumarate
Malate
Lactate
p=.05
p=.09
p=.06
o
Liver Acylcarnitines
Short chain
Medium chain
Long chain

FIGURE 5

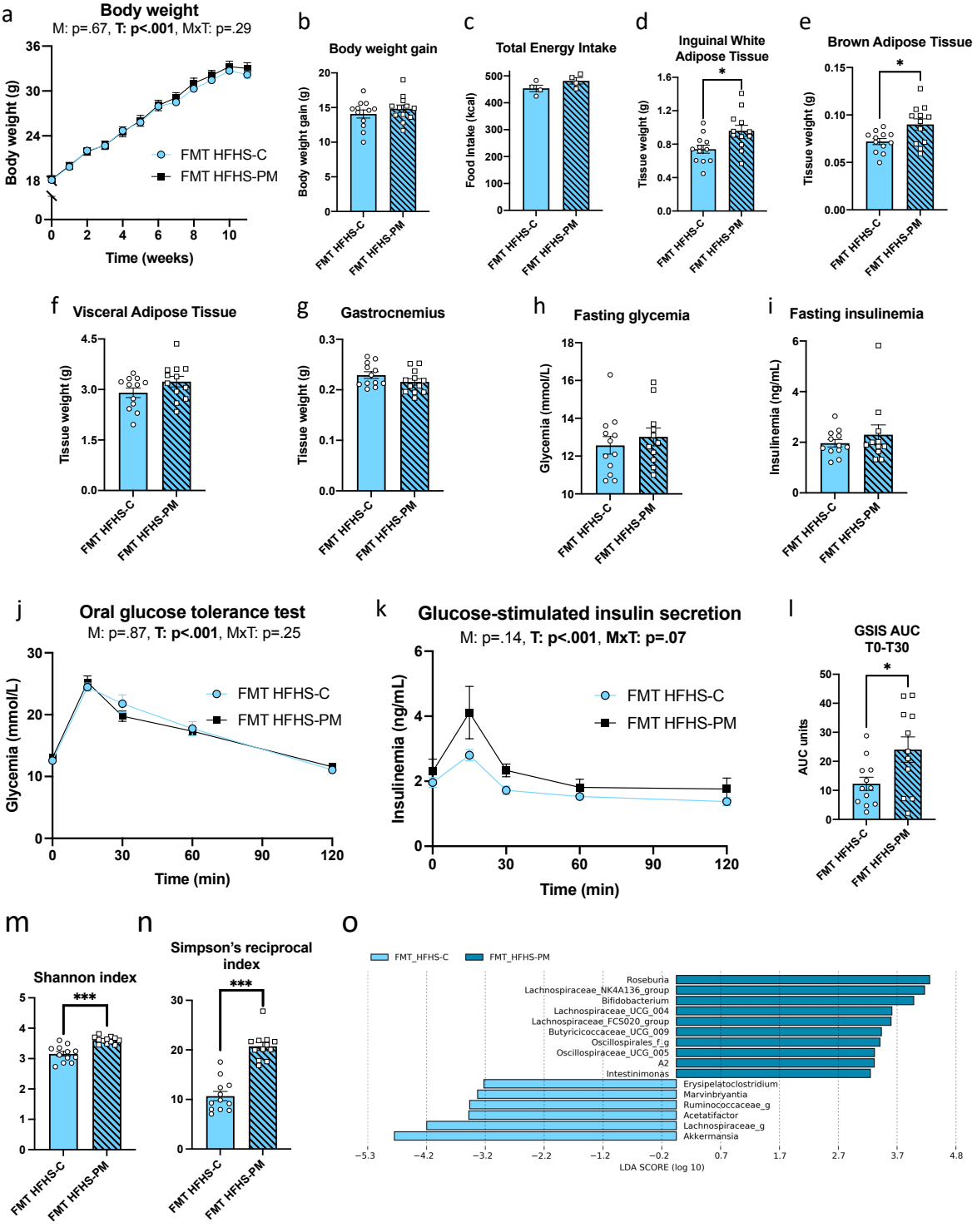

a
Body weight
M: p=.67, T: p<.001, MxT: p=.29
Body weight (g)
Time (weeks)
FMT HFHS-C
FMT HFHS-PM
b
Body weight gain
Body weight gain (g)
c
Total Energy Intake
Food intake (kcal)
d
Inguinal White Adipose Tissue
Tissue weight (g)
e
Brown Adipose Tissue
Tissue weight (g)
f
Visceral Adipose Tissue
Tissue weight (g)
g
Gastrocnemius
Tissue weight (g)
h
Fasting glycemia
Glycemia (mmol/L)
i
Fasting insulinemia
Insulinemia (ng/mL)
j
Oral glucose tolerance test
M: p=.87, T: p<.001, MxT: p=.25
Glycemia (mmol/L)
Time (min)
k
Glucose-stimulated insulin secretion
M: p=.14, T: p<.001, MxT: p=.07
Insulinemia (ng/mL)
Time (min)
l
GSIS AUC T0-T30
AUC units
m
Shannon index
n
Simpson's reciprocal index
o
FMT_HFHS-C
FMT_HFHS-PM
Roseburia
Lachnospiraceae_NK4A136_group
Bifidobacterium
Lachnospiraceae_UCG_004
Lachnospiraceae_FCS020_group
Butyricicoccaceae_UCG_009
Oscillospirales_f.g
Oscillospiraceae_UCG_005
A2
Intestinimonas
Erysipelatoclostridium
Marvinbryantia
Ruminococcaceae_g
Acetatifactor
Lachnospiraceae_g
Akkermansia
LDA SCORE (log 10)

FIGURE 6

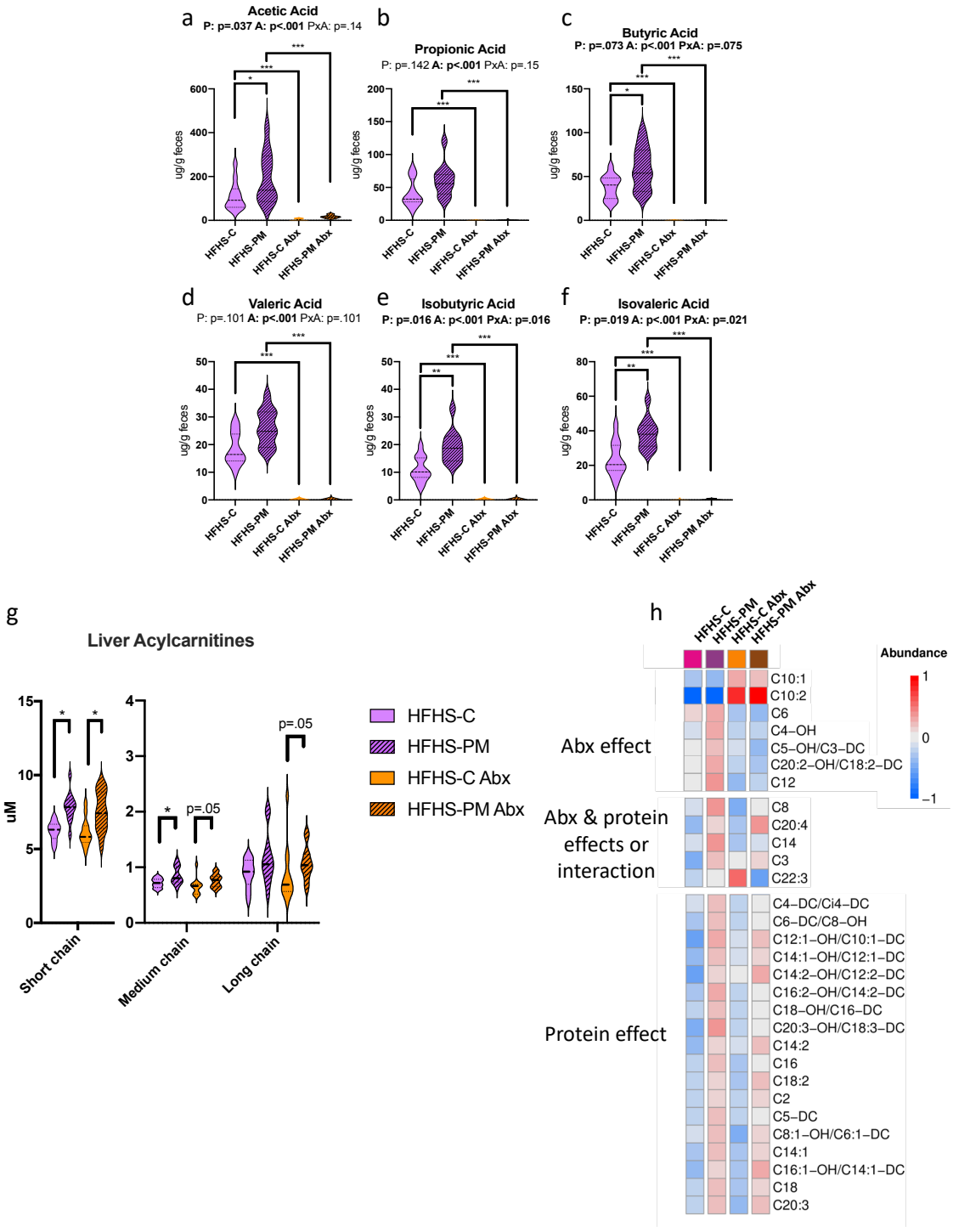
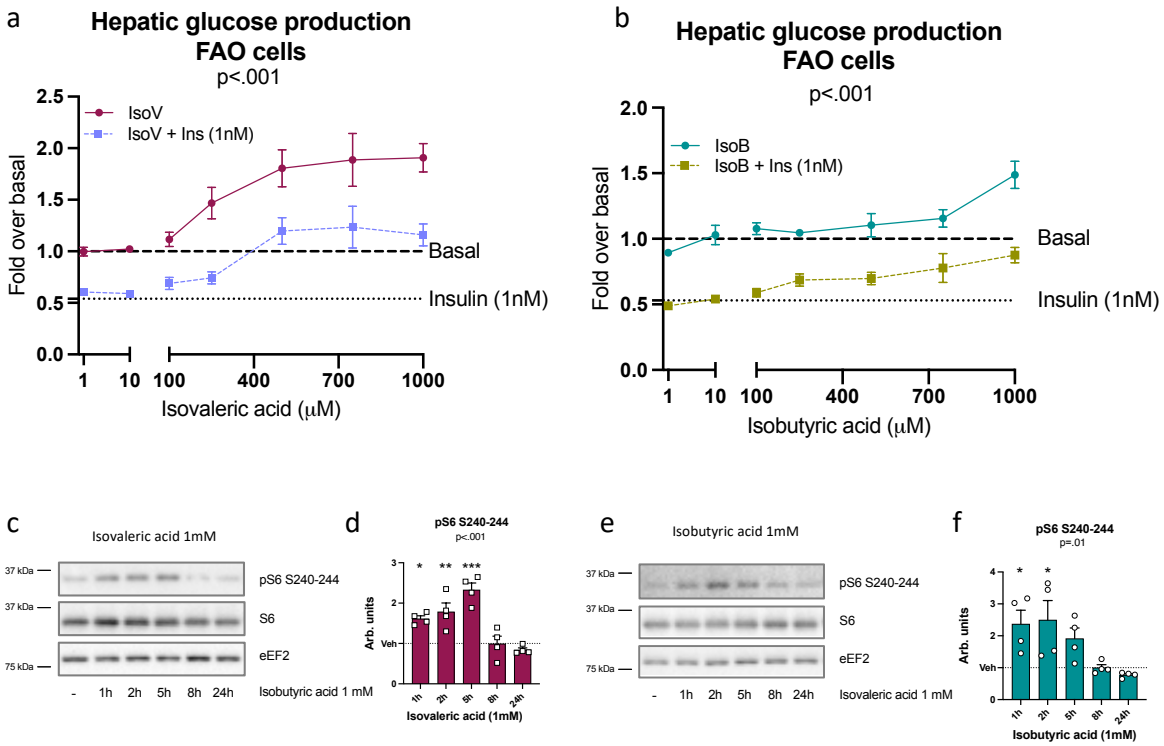
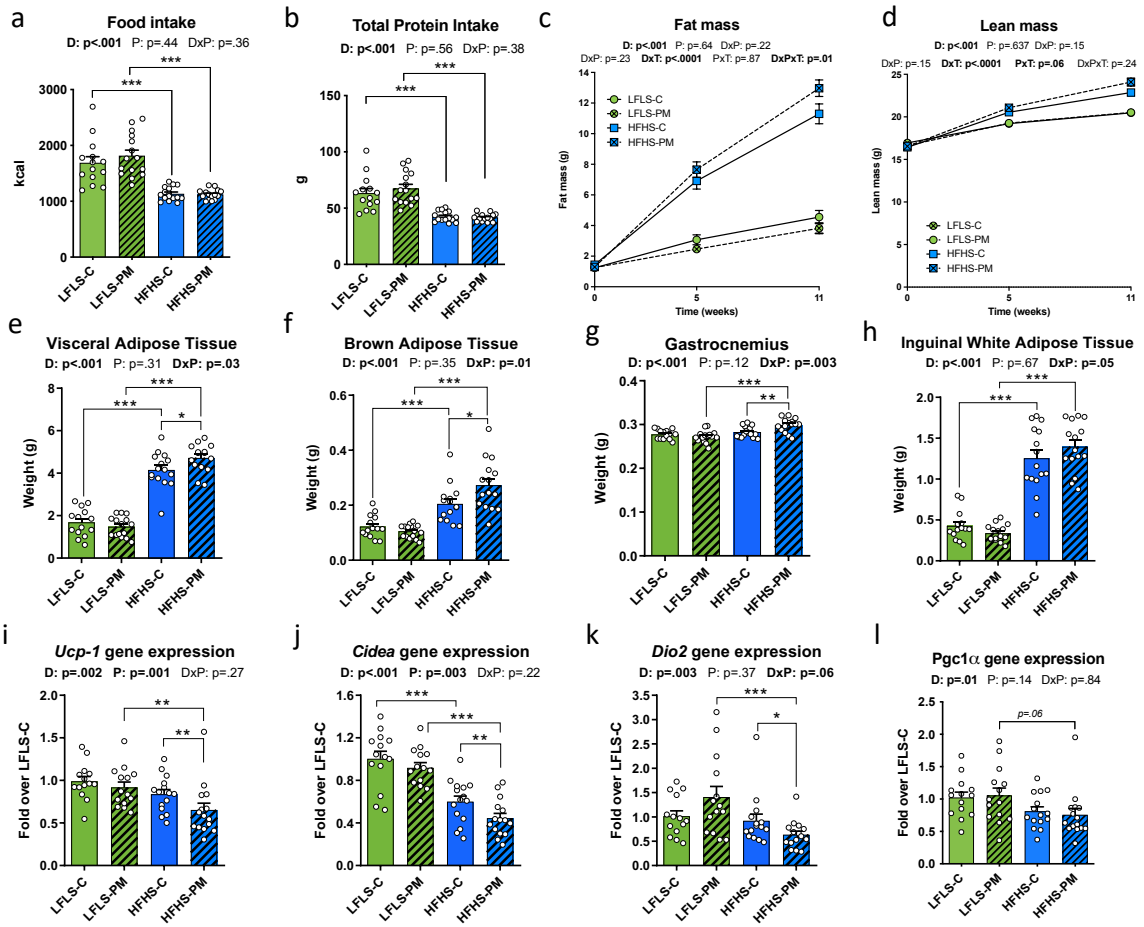

a
Acetic Acid
P: p=.037 A: p<.001 PxA: p=.14
b
Propionic Acid
P: p=.142 A: p<.001 PxA: p=.15
c
Butyric Acid
P: p=.073 A: p<.001 PxA: p=.075
d
Valeric Acid
P: p=.101 A: p<.001 PxA: p=.101
e
Isobutyric Acid
P: p=.016 A: p<.001 PxA: p=.016
f
Isovaleric Acid
P: p=.019 A: p<.001 PxA: p=.021
ug/g feces
HFHS-C
HFHS-PM
HFHS-C Abx
HFHS-PM Abx
g
Liver Acylcarnitines
uM
p=.05
Short chain
Medium chain
Long chain
HFHS-C
HFHS-PM
HFHS-C Abx
HFHS-PM Abx
h
Abundance
Abx effect
C10:1
C10:2
C6
C4–OH
C5–OH/C3–DC
C20:2–OH/C18:2–DC
C12
Abx & protein effects or interaction
C8
C20:4
C14
C3
C22:3
Protein effect
C4–DC/Ci4–DC
C6–DC/C8–OH
C12:1–OH/C10:1–DC
C14:1–OH/C12:1–DC
C14:2–OH/C12:2–DC
C16:2–OH/C14:2–DC
C18–OH/C16–DC
C20:3–OH/C18:3–DC
C14:2
C16
C18:2
C2
C5–DC
C8:1–OH/C6:1–DC
C14:1
C16:1–OH/C14:1–DC
C18
C20:3

FIGURE 7


a
Hepatic glucose production
FAO cells
p<.001
IsoV
IsoV + Ins (1nM)
Fold over basal
Basal
Insulin (1nM)
Isovaleric acid (μM)
b
Hepatic glucose production
FAO cells
p<.001
IsoB
IsoB + Ins (1nM)
Fold over basal
Basal
Insulin (1nM)
Isobutyric acid (μM)
c
Isovaleric acid 1mM
pS6 S240-244
S6
eEF2
Isobutyric acid 1 mM
d
pS6 S240-244
p<.001
Arb. units
Isovaleric acid (1mM)
e
Isobutyric acid 1mM
pS6 S240-244
S6
eEF2
Isovaleric acid 1 mM
f
pS6 S240-244
p=.01
Arb. units
Isobutyric acid (1mM)

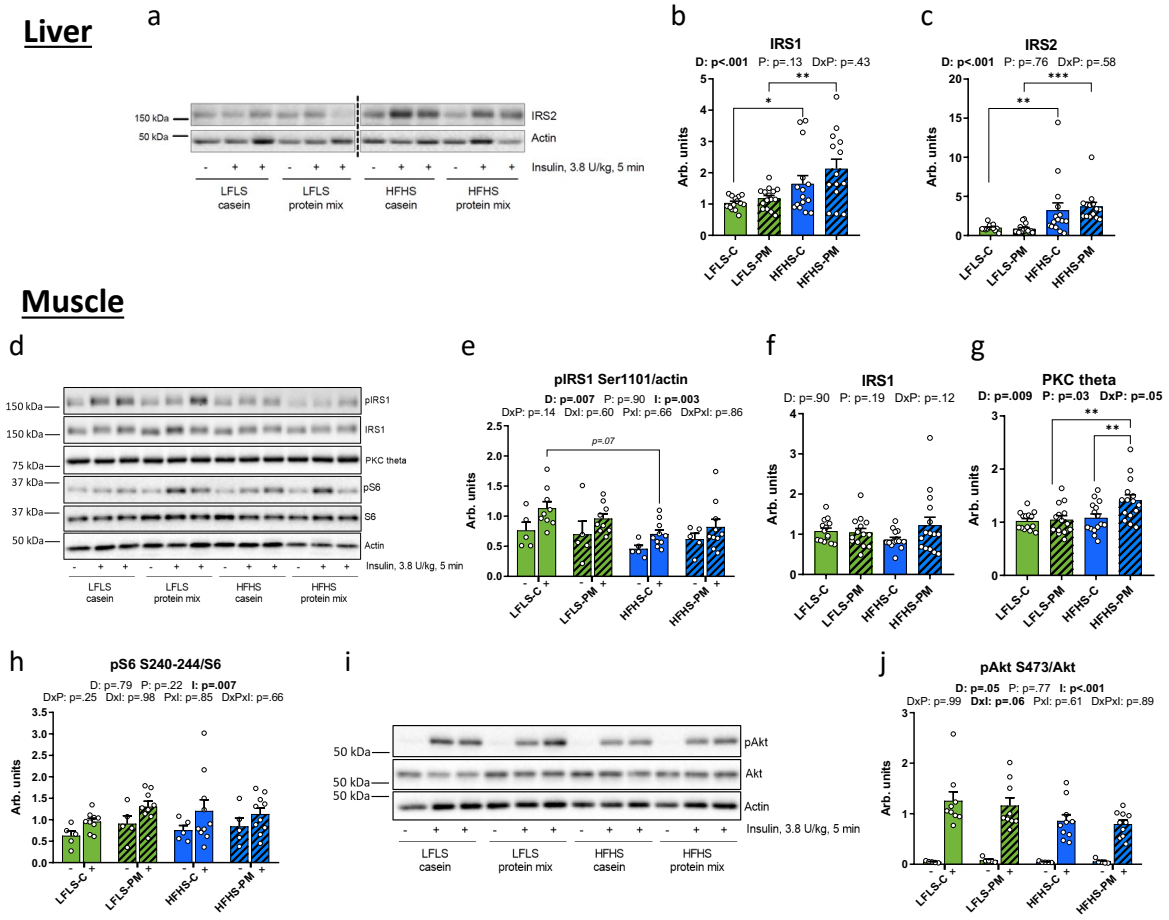
3.11 Supplementary Material

Supplementary Figure 1

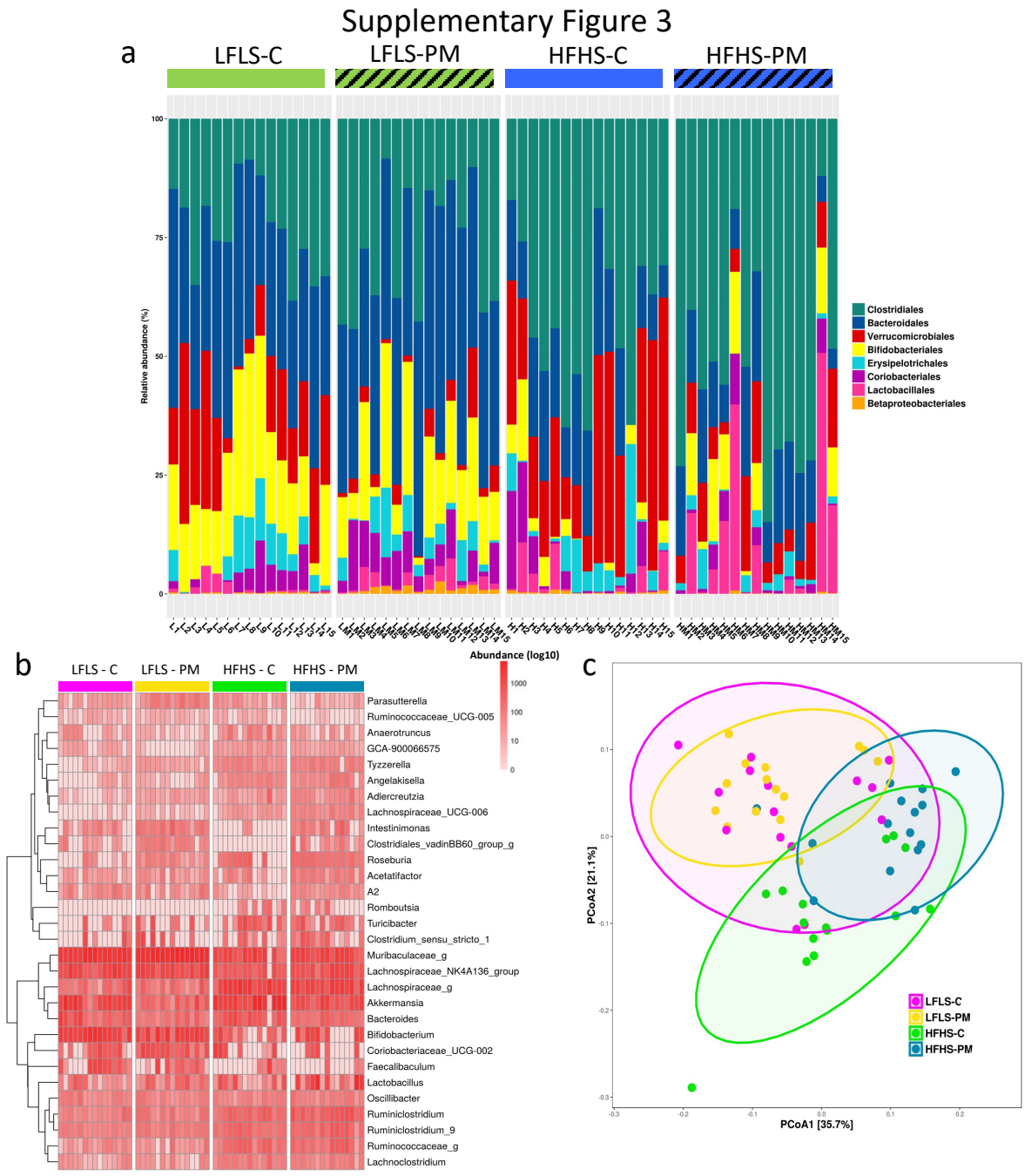


Supplementary Figure 1. Protein mix magnifies obesity-linked deleterious effect on phenotypic parameters and alters energy management. Mice were fed with a LFLS-C (green), a LFLS-PM (green, hatched), a HFHS (blue) or a HFHS-PM (blue, hatched) diet. **(a)** Total food intake and **(b)** protein intake recorded during the 12-week protocol. **(c)** Fat and **(d)** lean masses measured at weeks 0, 5 and 11 by quantitative NMR spectroscopy. **(e-h)** Tissue weights at 12 weeks of dietary treatment: **(e)** visceral adipose tissue, **(f)** intrascapular brown adipose tissue (BAT), **(f)** gastrocnemius muscle and **(h)** inguinal white adipose tissue. (**i**) *Ucp-1*, (**j**) *Cidea*, **(k)** *Dio2* and **(l)** *Pgc1α* mRNA relative gene expression quantified by RT-qPCR. Data are means±s.e.m. Statistical analyses were performed using a two-way ANOVA or a mixed model for repeated measures, followed by a Tukey post-hoc test. n=14 for LFLS-C group and n=15 biologically independent mice for the three other groups except for panels i-l where n=14 for LFLS-PM. P-values of general effect for diet (D), protein (P) and time (T) factors are recorded under the title of each graph, followed by the p-values of the corresponding factor interaction effects. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file.

Supplementary Figure 2

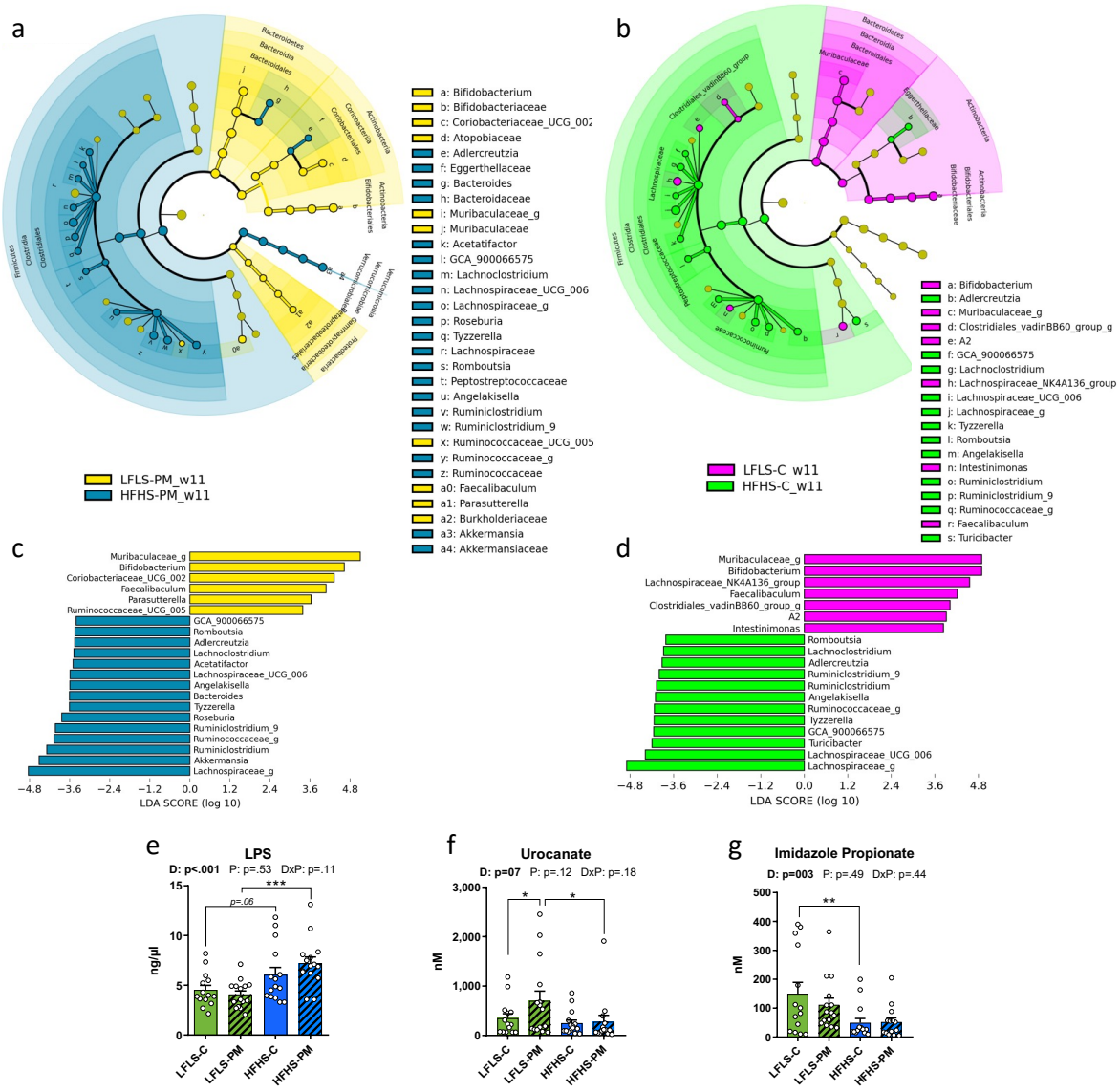


Supplementary Figure 2. Protein source does not affect total hepatic IRS1 and IRS2 and insulin signaling in the muscle. Mice were fed with a LFLS-C (green), a LFLS-PM (green, hatched), a HFHS (blue) or a HFHS-PM (blue, hatched) diet. At week 12, mice were fasted for 6 hours, injected with either saline (-) or insulin (+) and euthanized 5 minutes later. **(a-c)** Representative gels and quantification of densitometry analyses for total **(b)** IRS1 (see Figure 2 for gel) and **(a, c)** IRS2 in the liver. **(d-j)** Insulin signaling in the gastrocnemius muscle. **(d)** Representative gels and quantification of densitometry analyses for **(e)** pIRS1 Ser1101 and (**f**) total IRS1, **(g)** total PKC theta, and **(h)** pS6 S240-244. **(i)** Representative gels and quantification of densitometry analyses for **(j)** pAkt Ser473. Actin has been used as loading control. Arb. units, Arbitrary Units. Data are means±s.e.m. For total proteins (two-way ANOVA analysis), n=14-15 biologically independent mice; for insulin signaling (three-way ANOVA analysis), n=5 for all groups of independent mice injected with saline, and n=9 for LFLS-C and HFHS-PM groups and n=10 independent mice for LFLS-PM and HFHS-C groups injected with insulin. P-values of general effect for diet (D), protein (P), time (T) and insulin condition (C) factors are recorded under the title of each graph, followed by the p-values of the corresponding factor interaction effects. Detailed significant differences detected by Tukey post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file.



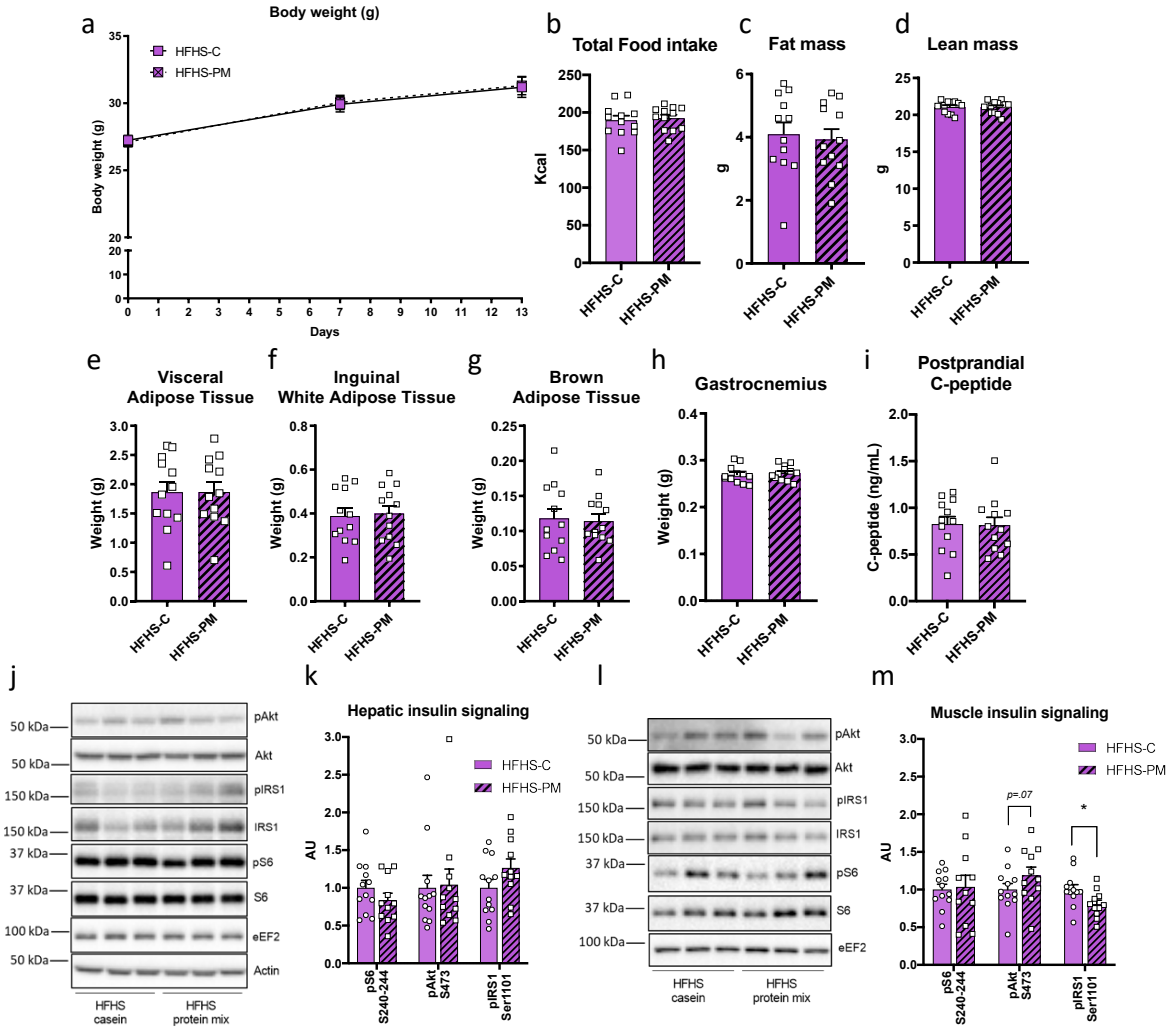
Supplementary Figure 3. Protein source and diet modulate fecal bacterial populations. (a) Stacked bar plotting the relative abundance of operational taxonomic units at order level. **(b)** Heatmap representing the overall bacterial community composition at genus level based on 16S-rRNA-encoding sequences in mice feces collected after 11 weeks of treatment. **(c)** Principal coordinates analysis (PCoA) based on unweighted Unifrac metric. The x and y axes accounted for 35.7% and 21.1% of the variance, respectively. n=14 for LFLS-C group and n=15 biologically independent mice for the three other groups. For panel a: LFLS-C (green); LFLS-PM (green, hatched); HFHS (blue);HFHS-PM (blue, hatched). **For panels b and c:** LFLS-C (pink); LFLS-PM (yellow)); HFHS (green);HFHS-PM (blue).

Supplementary Figure 4



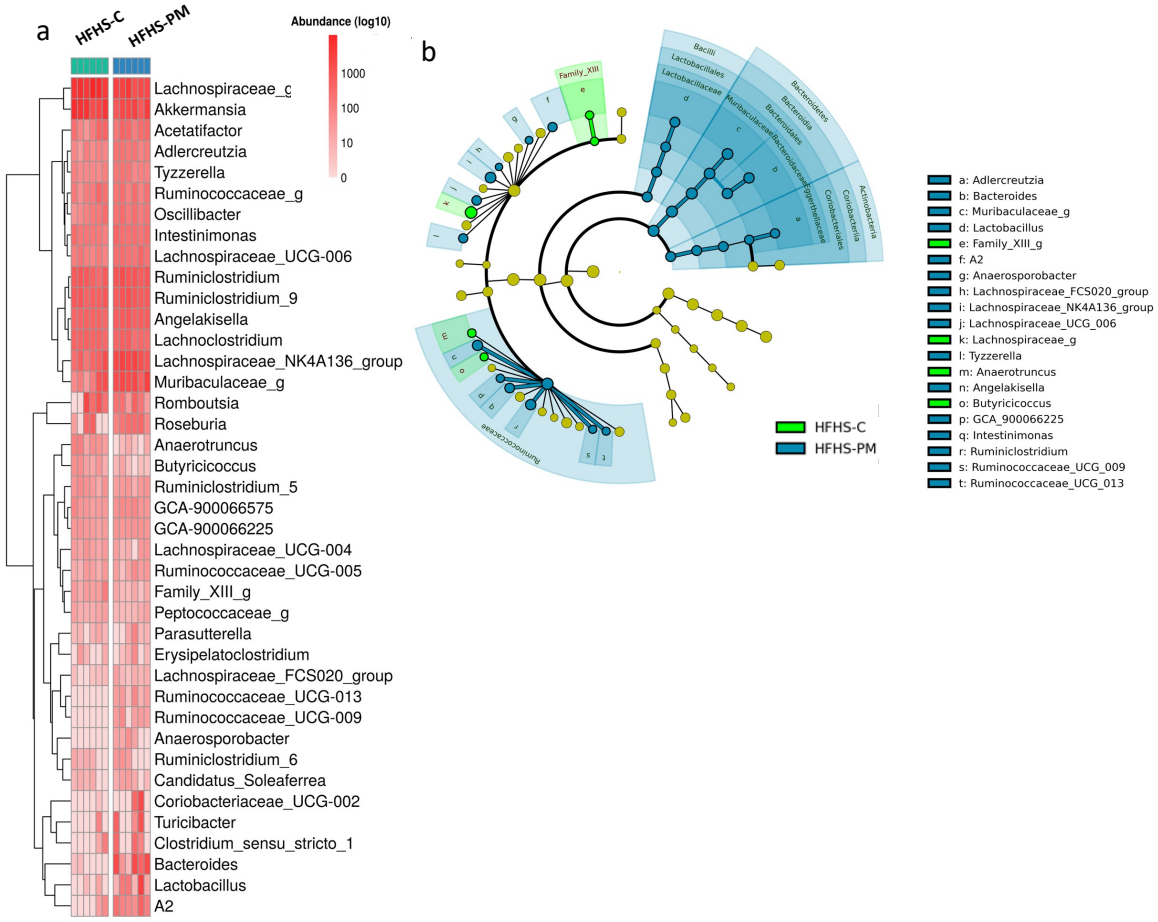
Supplementary Figure 4. HFHS diet modulates fecal bacterial populations either on casein or protein mix condition. Cladogram representations showing differentially abundant bacteria between **(a)** LFLS-PM and HFHS-PM groups and **(b)** LFLS-C and HFHS-C groups. LEfSe analysis identifying taxonomic differences between **(c)** LFLS-PM and HFHS-PM groups and **(d)** LFLS-C and HFHS-C fecal microbiotas. Histograms of LDA scores (cut-off value of 2.5) at the genus level. Plasma microbial metabolites: **(e)** Lipopolysaccharide (LPS), **(f)** urocanate and **(g)** imidazole propionate. Data are means±s.e.m. Statistical analyses were performed using a two-way ANOVA followed by a Tukey post-hoc test. n=13-15 biologically independent mice. P-values of general effect for diet (D) and protein (P) factors and diet x protein (DxP) interaction are recorded under the title of each graph. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01, ***p<.001. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. For panel a: LFLS-C (green); LFLS-PM (green, hatched); HFHS (blue);HFHS-PM (blue, hatched). **For panels b and c:** LFLS-C (pink); LFLS-PM (yellow); HFHS (green);HFHS-PM (blue). Source data are provided as a Source Data file.

Supplementary Figure 5



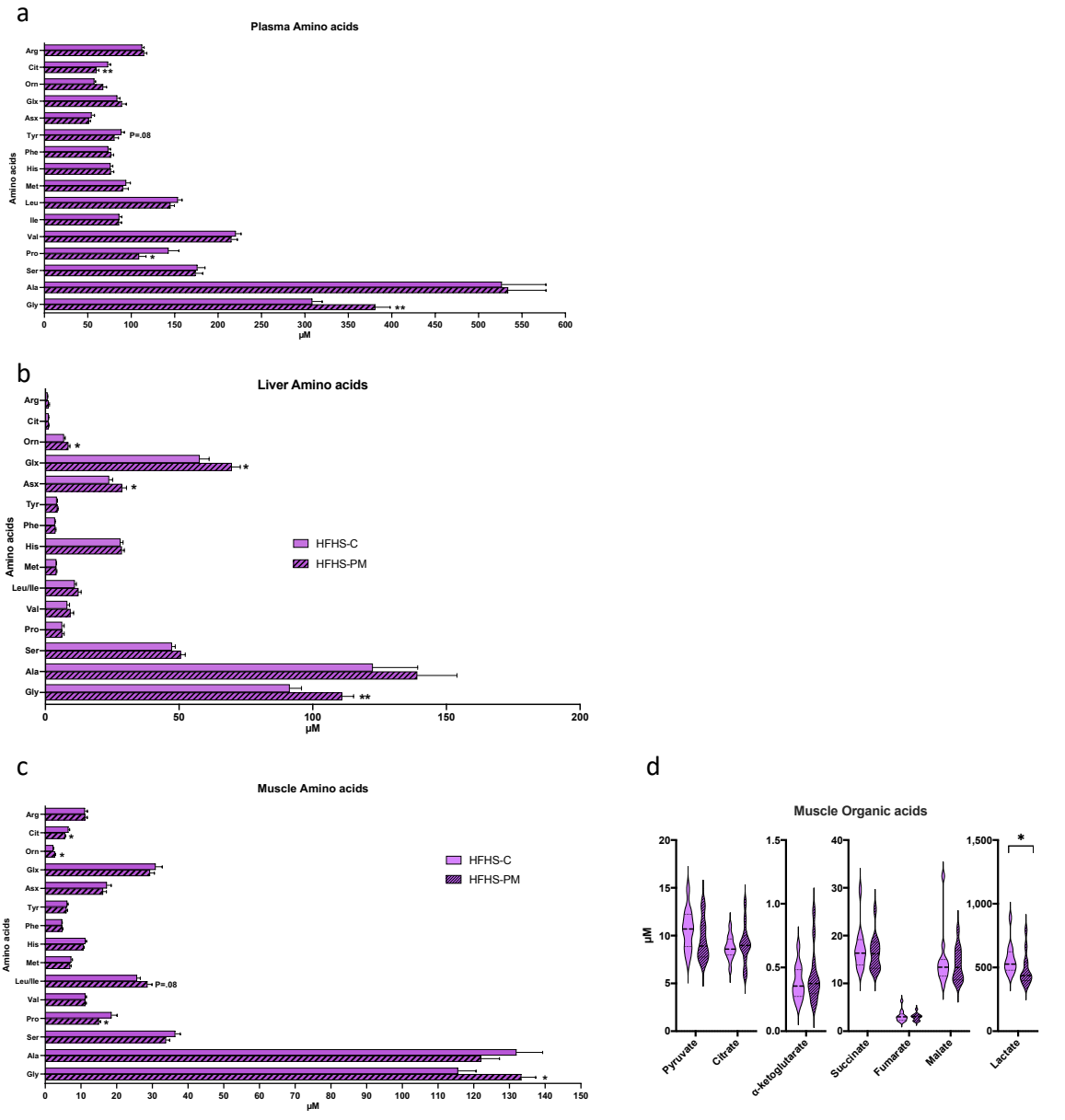
Supplementary Figure 5. Two weeks of casein replacement by protein mix is not long enough to induce metabolic or muscle insulin signaling impairment. (a) Body weight curve **(b)** total food intake, **(c)** fat mass and **(d)** lean mass measured by qNMR after 2 weeks of dietary intervention. **(e-h)** Tissue weights, (**e**) visceral adipose tissue, (**f**) inguinal white adipose tissue **(g)** intrascapular brown adipose tissue and (**h)** gastrocnemius muscle. **(i)** C-peptide in circulation in post-prandial state. n=12 independent mice for both HFHS-C (purple) and HFHS-PM (purple, hatched) groups. **(j-m)** Insulin signaling: hepatic (**k**) and muscle **(m)** quantification of densitometry analyses for pS6 S240-244 on total S6, pAkt S473 on total Akt, pIRS1 Ser1101 on total IRS1 and (**l, n**) corresponding representative immunoblots. Actin and eEF2 have been used as loading control. n=12 for HFHS-C and n=11 for HFHS-PM groups. Data are means±s.e.m. Statistical analyses were performed using a two-tailed Student's t test or its nonparametric equivalent Mann-Whitney test. Detailed significant differences are recorded as follows: *p<.05. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file.

Supplementary Figure 6



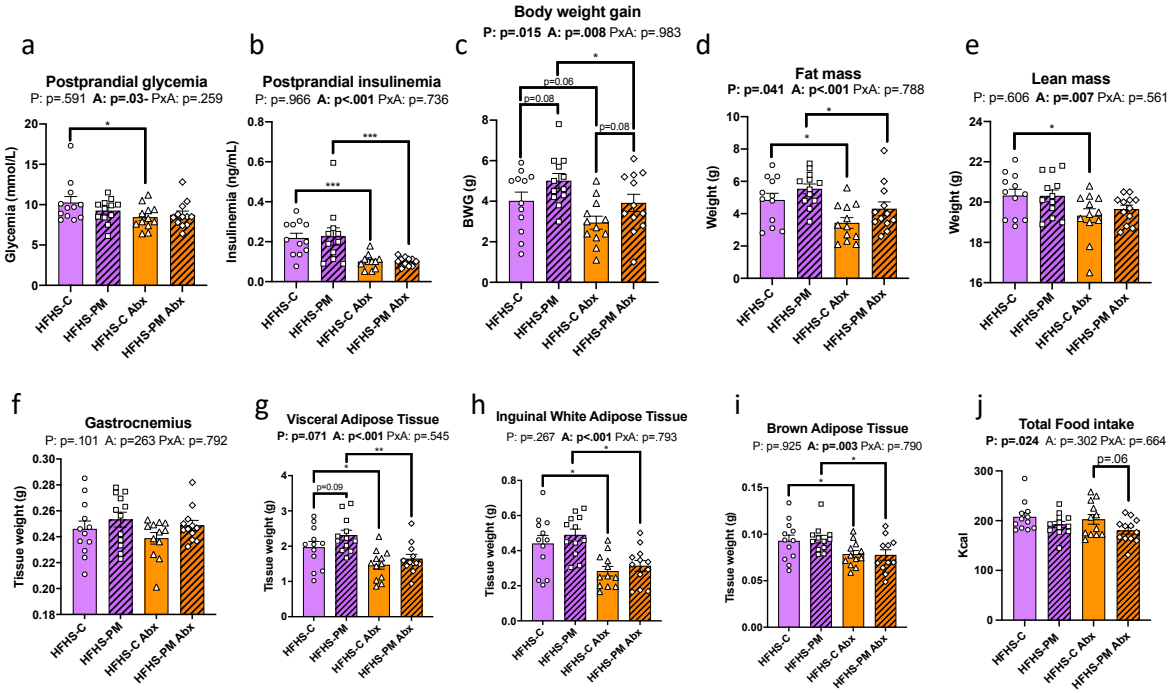
Supplementary Figure 6. Protein source modulates fecal bacteria after 2 weeks of treatment. Microbiota 16S-RNA sequencing of feces collected after 2 weeks of HFHS-C (green) or HFHS-PM (blue) dietary treatment . **(a)** Heatmap recording overall bacterial composition at the genus level. **(b)** Cladogram (the taxonomic levels from phylum to family are labelled, while genera are abbreviated). n=6 biologically independent mice for both groups. Presence of 'g' at the end of taxon denotes unclassified genus.

Supplementary Figure 7



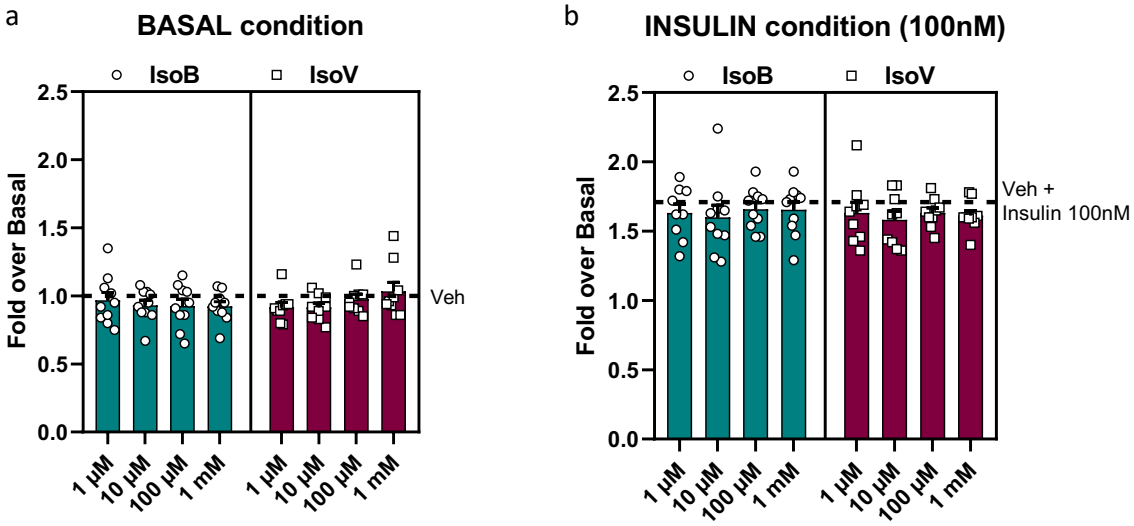
Supplementary Figure 7. Protein mix induces targeted changes in amino acids profiles in the plasma, liver and muscle of HFHS-fed mice. (a) Plasma, **(b)** liver and **(c)** muscle amino acids profiles in post-prandial state after two weeks of HFHS-C (purple) or HFHS-PM (purple, hatched) dietary challenge. **(d)** Muscle organic acids profile. n=12 independent mice for both groups. Data are means±s.e.m. Statistical analyses were performed using a two-tailed Student's t test or its nonparametric equivalent Mann-Whitney test. Detailed significant differences are recorded as follows: *p<.05, **p<.01. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file. Arg, arginine; Cit, citrulline; Orn, ornitine; Glx, Glutamine and/or glutamate; Asx, aspartate and/or asparagine; Tyr, tyrosine; Phe, phenylalanine; Hist, histidine; Met, Methionine; Leu, leucine; Ile, Isoleucine; Val, valine; Pro, proline; Ser, serine; Ala, alanine; Gly, glycine.

Supplementary Figure 8



Supplementary Figure 8. Antibiotic treatment affects food intake and body composition. Mice were fed with a HFHS-C (purple) or a HFHS-PM (purple, hatched) and administered with an antibiotic cocktail (orange, and orange, hatched respectively). Physiological parameters: postprandial **(a)** glycemia **(b)** insulinemia**,** and **(c)** total body weight gain **(d)** fat mass and **(e)** lean mass measured by qNMR after 2 weeks of dietary intervention. (**f-i**) Tissue weights of (**f**) gastrocnemius muscle **(g)** visceral adipose tissue, (**h**) inguinal white adipose tissue and **(i)** intrascapular brown adipose tissue. **(j)** Total energy intake. Data are means±s.e.m. Statistical analyses were performed using a two-way ANOVA followed by a Tukey post-hoc test. n=12 biologically independent mice for all groups, except for postprandial insulinemia where n=11 for HFHS-PM, HFHS-C Abx and HFHS-PM Abx groups. P-values of general effect for protein (P) and antibiotic (A) factors and protein x antibiotic (PxA) interaction are recorded under the title of each graph. Detailed significant differences detected by post-hoc test are recorded as follows: *p<.05, **p<.01. Exact p-values for trends (.05 ≤ p-value < .10) are recorded on graphs for additional indication. Source data are provided as a Source Data file.

Supplementary Figure 9



Supplementary Figure 9. BCFA do not impact glucose uptake *in vitro*. Branched chain fatty acids (BCFA) effect on skeletal muscle (L6) glucose uptake in **(a)** basal or **(b)** insulin condition (100 nM) with isobutyric (blue-green) or isovaleric acid (purple) (1-1 000µM); data corrected for total protein. Data are means±s.e.m. n=9-10 independent experiments. A Kruskal-Wallis test followed by a Dunn's post-hoc test versus Vehicule/Vehicule+Insulin, was performed in each condition. Source data are provided as a Source Data file.

SUPPLEMENTARY TABLE 1: Design of purified murine diets using a 10-source protein mix representative of human consumption.

	Dietary treatment			
Protein source	LFLS-C casein	LFLS-PM protein mix	HFHS-C casein	HFHS-PM protein mix
Macronutrient	**kcal %**	**kcal %**	**kcal %**	**kcal %**
Protein	**15**	**15**	**15**	**15**
Carbohydrate	**75**	**75**	**35**	**35**
sucrose	10	10	30	30
starch	65	65	5	5
Fat	**10**	**10**	**50**	**50**
saturated	3	3	18	18
MUFAs	3	3	15	15
PUFAs	3	3	15	14
SAT : PUFA	1	1	1	1
Fibers (g/100g)	**5**	**5**	**6**	**5**
Energy content (kcal/g)	**3.52**	**3.52**	**4.81**	**4.81**
Ingredient	**g**	**g**	**g**	**g**
Protein mix[1]	0	17.4	0	22
Protein	0	14	0	17.9
Carbohydrate	0	0.5	0	0.6
Fat	0	1.9	0	2.5
Total fibers	0	0.2	0	0.2
Casein	12.8	0	15.9	0
L-cystine	0.3	0.3	0.3	0.3
Corn starch[2]	62.4	68.7	5.9	6.4
Sucrose	6.6	7.3	30.3	34
Cellulose	5	5.2	5	5.3
Lard	2.7	1.1	16.4	16.2
Corn oil	1.2	1.2	7.6	8.3
Mineral mix[3]	6.7	6.7	6.7	6.7
Vitamin mix[4]	1.4	1.4	1.4	1.4
Choline bitartrate	0.3	0.3	0.3	0.3
BHT[5]	0.03	0.03	0.03	0.03
Total grams	99.4	109.6	89.8	100.9

[1] Home-made protein mix, see Table S1

[2] MP Biomedical corn starch : 90% starch, 10% humidity

[3] MP Biomedical mineral mixture 76 - 12% of sucrose

[4] Teklad Vitamin mix AIN-76A - 98% of sucrose

[5] Tert-butylhydroxytoluene

SUPPLEMENTARY TABLE 2: Profile of protein sources.

Protein source (Supplier, Country)		Chicken (Happy Yak, Canada)	Pork (Happy Yak, Canada)	Beef (Happy Yak, Canada)	Cod (Seagarden, Norway)	Soy (Teklad Envigo, USA)	Pea (Canadian Protein, Canada)	Rice (Canadian Protein, Canada)	Egg White (Teklad Envigo, USA)	Whey (Canadian Protein, Canada)	Casein (MP Biomedicals, USA)	Final Protein Mix (PM)
Corresponding USDA dietary		Poultry	Meat		Fish	Legumes, fruits, nuts, soy,		Grains	Egg	Dairy		
Energy (cal/100g)	cal/100g	428	451	549	389	385	399	420	322	382	363	432
Protein	%	**94.54**	**84.63**	**59.71**	**89.79**	**87.19**	**77.96**	**79.50**	**81.56**	**84.81**	**89.48**	**80.60**
Nitrogen	%	15.13	13.54	9.55	14.37	13.95	12.47	12.72	13.05	13.57	14.03	12.85
CHO	%	**0.00**	**0.00**	**0.68**	**0.00**	**2.85**	**3.56**	**7.93**	**5.72**	**4.62**	**0.23**	**2.63**
Total dietary fiber[1]	g/100g	NA	NA	NA	NA	1.70	4.80	3.20	NA	NA	NA	1.09
Total fat	g/100g	**5.51**	**12.51**	**34.19**	**3.26**	**2.81**	**8.12**	**7.80**	**0.44**	**1.35**	**0.49**	**11.06**
SAT	g/100g	1.78	4.44	15.10	0.77	0.79	1.61	3.00	0.15	0.30	0.31	4.40
MUFA	g/100g	1.84	5.36	15.39	0.61	0.42	2.13	1.96	0.17	0.27	0.10	4.33
PUFA	g/100g	1.64	2.11	1.12	1.74	1.45	4.00	2.40	0.09	0.72	0.03	1.61
ω3	g/100g	0.08	0.07	0.31	1.56	0.17	0.55	0.06	<0.01	<0.01	0.01	0.18
ω6	g/100g	1.54	1.97	0.78	0.15	1.28	3.44	2.33	0.08	0.71	0.01	1.40
TRANS fat	g/100g	0.02	0.04	1.05	0.01	0.02	0.02	0.09	<0,01	0.01	0.02	0.22
Cholesterol	mg/100g	227.60	264.70	214.70	478.30	<0.80	<0.80	<0.80	24.10	3.10	15.30	117.42
Ash	%	3.50	3.84	3.20	5.83	3.93	3.15	1.53	5.73	2.64	1.56	2.93
Humidity	%	2.13	2.32	2.22	3.64	3.21	7.17	3.24	6.51	3.59	8.22	3.85
Proportion in protein mix (g/100g of protein mix)		**11.9**	**12.8**	**18.2**	**3.0**	**5.7**	**6.3**	**21.5**	**3.7**	**3.7**	**13.2**	**100**
Protein proportion in protein mix[2] (g/100g of protein)		**13.8**	**13.4**	**13.4**	**3.3**	**6.1**	**6.1**	**21.1**	**3.7**	**3.8**	**15.4**	**100**

NA : Non Analyzed

[1] Since meats, dairy, fish, egg and casein source are not supposed to contain fibers, the total dietary fiber was calculated based on soy, pea and rice sources content.

[2] The proportion of each protein source is based on USDA data, Protein contribution from major food groups to the US food supply, 1909-2010

SUPPLEMENTARY TABLE 3: Amino acid profile of purified murine diets.

	Dietary treatment			
Protein source	LFLS-C casein	LFLS-PM protein mix	HFHS-C casein	HFHS-PM protein mix
Amino acid profile	g/100g of diet			
BCAA	2.4	2.3	3.3	3.2
Valine	0.7	0.7	1	0.9
Isoleucine	0.6	0.6	0.8	0.8
Leucine	1.1	1.1	1.5	1.5
Aspartic acid	0.8	1.2	1.1	1.6
Threonine	0.5	0.6	0.7	0.8
Serine	0.7	0.6	0.9	0.8
Glutamic acid	2.8	2.4	3.9	3.3
Glycine	0.2	0.5	0.3	0.7
Alanine	0.3	0.6	0.5	0.9
Methionine	0.3	0.3	0.5	0.4
Tyrosine	0.6	0.5	0.9	0.7
Phenylalanine	0.6	0.7	0.8	0.9
Lysine	1.1	0.9	1.5	1.2
Histidine	0.3	0.3	0.5	0.5
Arginine	0.4	0.8	0.6	1.1
Proline	1.3	0.7	1.8	0.9
Hydroxyproline	0	0	0	0
Cysteine	0.1	0.2	0.1	0.2
Tryptophan	0.1	0.1	0.2	0.2
Others	0.2	0.1	0.2	0.1
Total	12.9	12.8	17.7	17.6

SUPPLEMENTARY TABLE 4: Plasma acylcarnitine profile in postprandial state after 2 weeks of dietary treatment. Data are means±s.e.m. Statistical analyses were performed using a two-tailed Student's t test or its nonparametric equivalent Mann-Whitney test. An FDR correction (Two-stage step-up method of Benjamini, Krieger and Yekutieli, 5%) was applied on data to correct for multiple analysis. Significant differences are bolded. n=11-12.

Plasma Acylcarnitine (AC)	HFHS-C	HFHS-PM	Adjusted FDR	
	µM		p-value	q-value
C2	2.02E+01 ± 6.03E-01	3.09E+01 ± 2.89E+00	***0.006***	***0.011***
C3	2.76E-01 ± 2.82E-02	3.95E-01 ± 4.31E-02	***0.031***	*0.052*
C4/Ci4	3.62E-01 ± 3.26E-02	5.13E-01 ± 4.53E-02	***0.013***	***0.032***
C4-OH (C3-DC)	1.39E-01 ± 9.48E-03	2.16E-01 ± 3.03E-02	***0.017***	*0.046*
C4-DC/Ci4-DC	1.69E-02 ± 1.08E-03	2.20E-02 ± 2.09E-03	***0.041***	*0.061*
Total short chain AC	2.10E+01 ± 6.11E-01	3.20E+01 ± 2.97E+00	***0.005***	***0.011***
C5	1.14E-01 ± 8.42E-03	1.64E-01 ± 1.27E-02	***0.004***	***0.019***
C5:1	1.35E-01 ± 6.08E-03	1.35E-01 ± 7.00E-03	*0.996*	*0.726*
C5-OH/C3-DC(-M)	5.40E-02 ± 4.81E-03	5.94E-02 ± 7.92E-03	*0.908*	*0.474*
C5-DC (C6-OH)	1.86E-02 ± 2.10E-03	1.95E-02 ± 3.13E-03	*0.817*	*0.621*
C6 (C4:1-DC)	9.00E-02 ± 4.99E-03	1.46E-01 ± 1.23E-02	***<0.001***	***0.006***
C7-DC	2.10E-02 ± 3.70E-03	1.77E-02 ± 3.46E-03	*0.522*	*0.452*
C8	3.35E-02 ± 2.79E-03	4.46E-02 ± 3.77E-03	***0.027***	*0.049*
C8:1	5.12E-03 ± 1.43E-03	8.07E-03 ± 2.02E-03	*0.246*	*0.237*
C8:1-DC	6.22E-03 ± 8.48E-04	5.21E-03 ± 1.35E-03	*0.532*	*0.452*
C8:1-OH/C6:1-DC	2.08E-02 ± 2.50E-03	1.56E-02 ± 1.80E-03	*0.105*	*0.110*
C6-DC/C8-OH	3.21E-02 ± 1.40E-03	3.12E-02 ± 2.85E-03	*0.777*	*0.603*
C10	2.65E-02 ± 1.87E-03	4.08E-02 ± 5.40E-03	***0.004***	***0.042***
C10-OH/C8-DC	3.58E-02 ± 2.72E-03	4.26E-02 ± 4.12E-03	*0.182*	*0.181*
C10:1	2.19E-02 ± 1.31E-03	2.88E-02 ± 3.62E-03	***0.013***	*0.110*
C10:2	1.08E-03 ± 5.96E-04	1.73E-03 ± 6.89E-04	*0.449*	*0.431*
C10:3	1.08E-03 ± 6.11E-04	7.18E-04 ± 4.84E-04	*0.626*	*0.522*
Total medium chain AC	6.17E-01 ± 2.21E-02	7.60E-01 ± 4.47E-02	***0.009***	***0.024***
C12	4.55E-02 ± 3.43E-03	6.80E-02 ± 6.97E-03	***0.010***	***0.024***
C12-OH/C10-DC	1.39E-02 ± 1.15E-03	1.40E-02 ± 1.39E-03	*0.964*	*0.717*
C12:1	1.49E-02 ± 2.05E-03	2.47E-02 ± 4.12E-03	***0.045***	*0.061*
C14	7.90E-02 ± 3.25E-03	1.01E-01 ± 6.97E-03	***0.008***	***0.024***
C14-OH/C12-DC	5.99E-03 ± 1.00E-03	8.92E-03 ± 1.36E-03	*0.098*	*0.110*
C14:1-OH	8.17E-02 ± 5.37E-03	1.13E-01 ± 8.30E-03	***0.004***	***0.020***
C14:1-OH	1.62E-02 ± 1.45E-03	2.20E-02 ± 2.08E-03	***0.034***	*0.052*
C14:2	2.89E-02 ± 1.34E-03	3.66E-02 ± 2.64E-03	***0.016***	***0.035***
C16	2.54E-01 ± 1.07E-02	3.08E-01 ± 2.11E-02	***0.033***	*0.052*
C16-OH/C14-DC	1.03E-02 ± 9.20E-04	1.51E-02 ± 2.08E-03	***0.044***	*0.061*
C16:1	6.43E-02 ± 3.97E-03	7.24E-02 ± 6.54E-03	*0.303*	*0.279*
C16:1-OH/C14:1-DC	1.64E-02 ± 1.40E-03	2.00E-02 ± 1.58E-03	*0.102*	*0.110*
C16:2	2.38E-02 ± 1.72E-03	2.95E-02 ± 2.72E-03	*0.093*	*0.110*
C18	1.12E-01 ± 4.78E-03	1.40E-01 ± 3.35E-03	***0.001***	***0.004***
C18-OH/C16-DC	1.62E-02 ± 1.37E-03	2.28E-02 ± 1.38E-03	***0.003***	***0.016***
C18:1	2.98E-01 ± 1.09E-02	3.55E-01 ± 2.03E-02	***0.021***	***0.042***
C18:1-DC	8.67E-03 ± 9.18E-04	1.15E-02 ± 1.74E-03	*0.170*	*0.173*
C18:1-OH/C16:1-DC	1.49E-02 ± 1.07E-03	1.92E-02 ± 2.29E-03	*0.088*	*0.110*
C18:2	1.16E-01 ± 4.65E-03	1.43E-01 ± 8.86E-03	***0.013***	***0.032***
C18:2-OH	6.87E-03 ± 9.54E-04	1.06E-02 ± 8.64E-04	***0.008***	***0.024***
C20	7.86E-03 ± 1.14E-03	1.08E-02 ± 8.14E-04	***0.049***	*0.064*
C20-OH/C18-DC	1.81E-03 ± 6.19E-04	2.90E-03 ± 8.32E-04	*0.305*	*0.279*
C20:4	2.04E-02 ± 1.16E-03	2.50E-02 ± 2.37E-03	*0.099*	*0.110*
C22	5.63E-03 ± 7.26E-04	5.21E-03 ± 1.15E-03	*0.760*	*0.603*
Total long chain AC	1.26E+00 ± 4.70E-02	1.58E+00 ± 9.47E-02	***0.007***	***0.024***
Total AC	2.29E+01 ± 6.45E-01	3.44E+01 ± 3.09E+00	***0.004***	***0.011***

SUPPLEMENTARY TABLE 5 : Liver acylcarnitine profile in postprandial state after 2 weeks of dietary treatment.

Data are means±s.e.m. Statistical analyses were performed using a two-tailed Student's t test or its nonparametric equivalent Mann-Whitney test. An FDR correction (Two-stage step-up method of Benjamini, Krieger and Yekutieli, 5%) was applied on data to correct for multiple analysis. Significant differences are bolded. n=11-12.

Liver Acylcarnitine (AC)	HFHS-C µM	HFHS-PM µM	Adjusted FDR p-value	Adjusted FDR q-value
C2	6.36E+00 ± 3.28E-01	7.96E+00 ± 4.71E-01	***0.011***	*0.195*
C3	1.74E-01 ± 2.19E-02	2.39E-01 ± 2.47E-02	*0.061*	*0.314*
C4/Ci4	2.43E-01 ± 1.50E-02	2.67E-01 ± 2.49E-02	*0.417*	*0.719*
C4-OH (C3-DC)	1.30E-01 ± 9.87E-03	1.60E-01 ± 1.73E-02	*0.141*	*0.527*
C4-DC/Ci4-DC	2.52E-02 ± 1.13E-03	2.74E-02 ± 1.77E-03	*0.299*	*0.676*
Total short chain AC	6.94E+00 ± 3.35E-01	8.66E+00 ± 4.91E-01	***0.008***	*0.195*
C5	3.58E-02 ± 2.13E-03	4.46E-02 ± 3.29E-03	***0.036***	*0.261*
C5:1	2.19E-01 ± 1.59E-02	2.19E-01 ± 1.31E-02	*0.972*	>0.999
C5-OH/C3-DC(-M)	4.36E-02 ± 2.29E-03	4.40E-02 ± 3.07E-03	*0.913*	>0.999
C5-DC (C6-OH)	1.35E-01 ± 5.51E-03	1.70E-01 ± 1.00E-02	***0.001***	*0.195*
C6 (C4:1-DC)	2.43E-02 ± 1.95E-03	2.84E-02 ± 3.53E-03	*0.707*	*0.697*
C7-DC	1.27E-01 ± 1.81E-02	1.23E-01 ± 2.74E-02	*0.470*	>0.999
C8	4.95E-02 ± 3.33E-03	8.54E-02 ± 6.83E-03	***<0.001***	***0.007***
C8:1	2.41E-03 ± 4.55E-04	1.88E-03 ± 3.43E-04	*0.436*	*0.697*
C8:1-DC	1.41E-02 ± 1.12E-03	1.72E-02 ± 1.77E-03	*0.156*	*0.527*
C8:1-OH/C6:1-DC	1.18E-02 ± 8.00E-04	1.52E-02 ± 1.83E-03	***0.026***	*0.437*
C6-DC/C8-OH	2.28E-01 ± 1.12E-02	2.45E-01 ± 2.24E-02	*0.885*	*0.797*
C10	2.03E-02 ± 1.99E-03	2.30E-02 ± 3.02E-03	*0.470*	*0.752*
C10-OH/C8-DC	1.17E-02 ± 1.58E-03	1.62E-02 ± 4.60E-03	*0.312*	*0.697*
C10:1	8.56E-03 ± 5.44E-04	9.79E-03 ± 6.45E-04	*0.160*	*0.527*
C10:2	5.44E-04 ± 2.54E-04	5.44E-04 ± 2.56E-04	*1.000*	>0.999
C10:3	1.19E-03 ± 3.75E-04	1.21E-03 ± 2.86E-04	*0.974*	>0.999
Total medium chain AC	9.33E-01 ± 3.31E-02	1.04E+00 ± 7.43E-02	*0.214*	*0.536*
C12	2.02E-02 ± 7.99E-04	2.67E-02 ± 4.12E-03	***0.040***	*0.514*
C12-OH/C10-DC	5.04E-03 ± 3.65E-04	5.64E-03 ± 5.24E-04	*0.583*	*0.697*
C12:1	7.47E-03 ± 3.43E-04	8.59E-03 ± 7.71E-04	*0.199*	*0.550*
C12:1-OH/C10:1-DC	3.39E-03 ± 1.94E-04	4.79E-03 ± 5.10E-04	***0.018***	*0.211*
C12:2	9.74E-04 ± 2.34E-04	4.06E-04 ± 1.75E-04	*0.065*	*0.314*
C12:2-OH/C10:2-DC	6.85E-03 ± 3.16E-04	6.75E-03 ± 7.58E-04	*0.907*	>0.999
C14	5.71E-02 ± 2.36E-03	6.26E-02 ± 5.47E-03	*0.366*	*0.697*
C14-OH/C12-DC	6.64E-03 ± 4.05E-04	7.94E-03 ± 1.08E-03	*0.470*	*0.675*
C14:1	4.59E-02 ± 1.73E-03	4.89E-02 ± 4.32E-03	*0.523*	*0.805*
C14:1-OH/C12:1-DC	8.24E-03 ± 6.12E-04	1.05E-02 ± 9.00E-04	***0.048***	*0.313*
C14:2	1.22E-02 ± 9.21E-04	1.32E-02 ± 1.15E-03	*0.533*	*0.805*
C14:2-OH/C12:2-DC	2.72E-03 ± 2.05E-04	3.51E-03 ± 3.32E-04	*0.055*	*0.314*
C14:3	1.16E-03 ± 1.93E-04	1.04E-03 ± 2.34E-04	*0.718*	*0.929*
C14:3-OH/C12:3-DC	1.52E-03 ± 2.93E-04	1.10E-03 ± 3.87E-04	*0.138*	*0.719*
C16	1.94E-01 ± 1.16E-02	2.01E-01 ± 1.56E-02	*0.711*	*0.929*
C16-OH/C14-DC	1.47E-02 ± 1.33E-03	1.52E-02 ± 1.22E-03	*0.796*	*0.995*
C16:1	7.67E-02 ± 4.39E-03	7.81E-02 ± 7.19E-03	*0.871*	>0.999
C16:1-OH/C14:1-DC	1.26E-02 ± 7.31E-04	1.40E-02 ± 1.01E-03	*0.261*	*0.674*
C16:2	2.31E-02 ± 2.18E-03	2.64E-02 ± 2.19E-03	*0.289*	*0.675*
C16:2-OH/C14:2-DC	5.37E-03 ± 3.57E-04	6.35E-03 ± 5.96E-04	*0.172*	*0.536*
C16:3	2.50E-03 ± 2.67E-04	2.41E-03 ± 4.47E-04	*0.860*	>0.999
C16:3-OH/C14:3-DC	5.31E-04 ± 1.69E-04	3.68E-04 ± 1.21E-04	*0.440*	*0.745*
C18	8.74E-02 ± 7.02E-03	8.78E-02 ± 5.30E-03	*0.971*	>0.999
C18-OH/C16-DC	1.05E-02 ± 8.17E-04	1.12E-02 ± 9.29E-04	*0.628*	*0.885*
C18:1	2.67E-01 ± 2.25E-02	2.62E-01 ± 2.15E-02	*0.854*	>0.999
C18:1-OH/C16:1-DC	2.05E-02 ± 1.44E-03	1.99E-02 ± 1.44E-03	*0.768*	*0.977*
C18:2	1.33E-01 ± 1.32E-02	1.33E-01 ± 1.15E-02	*0.996*	>0.999
C18:2-OH/C16:2-DC	1.28E-02 ± 8.18E-04	1.35E-02 ± 1.18E-03	*0.634*	0.885
C18:3	1.12E-02 ± 1.33E-03	1.19E-02 ± 1.03E-03	*0.670*	0.914
C18:3-OH/C16:3-DC	1.49E-03 ± 1.93E-04	2.19E-03 ± 2.04E-04	***0.021***	0.220
C20	1.85E-02 ± 2.02E-03	2.16E-02 ± 1.57E-03	*0.237*	0.635
C20-OH/C18-DC/C22:6	2.16E-03 ± 2.82E-04	3.31E-03 ± 3.92E-04	***0.027***	0.240
C20:1	2.35E-02 ± 2.09E-03	2.61E-02 ± 2.21E-03	*0.413*	0.719
C20:1-OH/C18:1-DC	4.41E-03 ± 5.51E-04	4.83E-03 ± 5.18E-04	*0.413*	0.854
C20:2	1.79E-02 ± 1.66E-03	1.80E-02 ± 1.82E-03	*0.955*	>0.999
C20:2-OH/C18:2-DC	5.86E-03 ± 4.28E-04	6.34E-03 ± 2.45E-04	*0.346*	0.697
C20:3	9.30E-03 ± 6.20E-04	8.51E-03 ± 9.30E-04	*0.491*	0.790
C20:3-OH/C18:3-DC	2.17E-03 ± 2.39E-04	1.80E-03 ± 2.36E-04	*0.280*	0.675
C20:4	1.77E-02 ± 1.25E-03	2.26E-02 ± 2.60E-03	*0.109*	0.437
C22	3.45E-03 ± 3.77E-04	3.77E-03 ± 3.97E-04	*0.563*	0.832
C22:1	6.60E-03 ± 4.53E-04	7.58E-03 ± 3.61E-04	*0.106*	0.437
C22:2	8.92E-04 ± 1.08E-04	1.08E-03 ± 1.87E-04	*0.400*	0.719
C22:3	2.85E-04 ± 9.11E-05	7.20E-04 ± 1.67E-04	***0.032***	0.261
C22:4	1.59E-03 ± 2.60E-04	2.15E-03 ± 3.11E-04	*0.181*	0.536
C22:5	4.48E-04 ± 1.54E-04	9.66E-04 ± 2.15E-04	*0.063*	0.314
Total long chain AC	1.17E+00 ± 7.47E-02	1.22E+00 ± 8.75E-02	*0.681*	0.914
Total AC	9.04E+00 ± 4.02E-01	1.09E+01 ± 6.07E-01	***0.017***	0.211

SUPPLEMENTARY TABLE 6 : Muscle acylcarnitine profile in postprandial state after 2 weeks of dietary treatment.

Data are means±s.e.m. Statistical analyses were performed using a two-tailed Student's t test or its nonparametric equivalent Mann-Whitney test. An FDR correction (Two-stage step-up method of Benjamini, Krieger and Yekutieli, 5%) was applied on data to correct for multiple analysis. Significant differences are bolded. n=11-12.

Muscle Acylcarnitine (AC)	HFHS-C µM	HFHS-PM µM	p-value	Adjusted FDR q-value
C2	2.18E+00 ± 9.23E-02	2.46E+00 ± 1.87E-01	*0.194*	*0.718*
C3	3.58E-02 ± 4.89E-03	4.04E-02 ± 2.98E-03	*0.437*	*0.869*
C4/Ci4	6.21E-02 ± 5.46E-03	7.32E-02 ± 5.73E-03	*0.172*	*0.718*
C4-OH (C3-DC)	4.48E-02 ± 7.67E-03	7.20E-02 ± 9.18E-03	***0.033***	*0.718*
C4-DC/Ci4-DC	4.37E-02 ± 1.34E-03	4.31E-02 ± 2.02E-03	*0.813*	*>0.999*
Total short chain AC	2.37E+00 ± 9.83E-02	2.69E+00 ± 2.00E-01	*0.163*	*0.718*
C5	2.88E-02 ± 3.89E-03	3.02E-02 ± 4.10E-03	*0.804*	*>0.999*
C5:1	9.21E-02 ± 9.69E-03	1.12E-01 ± 5.71E-03	*0.089*	*0.718*
C5-OH/C3-DC(-M)	5.04E-02 ± 2.44E-03	4.85E-02 ± 1.54E-03	*0.521*	*0.911*
C5-DC (C6-OH)	4.21E-03 ± 1.20E-03	2.38E-03 ± 9.00E-04	*0.294*	*0.718*
C6 (C4:1-DC)	2.90E-02 ± 2.26E-03	3.08E-02 ± 3.72E-03	*0.689*	*>0.999*
C7-DC	1.93E-03 ± 6.92E-04	1.27E-03 ± 2.36E-04	*0.954*	*0.869*
C8	1.66E-02 ± 1.78E-03	1.57E-02 ± 1.65E-03	*0.700*	*>0.999*
C8:1	3.19E-03 ± 3.41E-04	2.39E-03 ± 4.61E-04	*0.180*	*0.718*
C8:1-DC	1.73E-03 ± 1.84E-04	2.27E-03 ± 5.79E-04	*0.544*	*0.869*
C8:1-OH/C6:1-DC	3.40E-03 ± 3.42E-04	3.44E-03 ± 4.04E-04	*0.938*	*>0.999*
C6-DC/C8-OH	8.23E-03 ± 7.70E-04	7.72E-03 ± 6.29E-04	*0.616*	*0.981*
C10	1.52E-02 ± 1.59E-03	1.70E-02 ± 1.51E-03	*0.414*	*0.869*
C10-OH/C8-DC	5.87E-03 ± 3.63E-04	6.22E-03 ± 6.05E-04	*0.631*	*0.981*
C10:1	3.85E-03 ± 4.82E-04	4.45E-03 ± 5.12E-04	*0.406*	*0.869*
C10:2	6.26E-04 ± 1.84E-04	1.18E-03 ± 2.83E-04	*0.118*	*0.718*
C10:3	4.46E-04 ± 1.34E-04	6.55E-04 ± 1.09E-04	*0.240*	*0.718*
Total medium chain AC	2.66E-01 ± 1.13E-02	2.86E-01 ± 1.13E-02	*0.141*	*0.718*
C12	3.27E-02 ± 3.23E-03	3.32E-02 ± 3.54E-03	*0.933*	*>0.999*
C12-OH/C10-DC	3.79E-03 ± 3.30E-04	4.74E-03 ± 4.99E-04	*0.128*	*0.718*
C12:1	8.83E-03 ± 9.58E-04	9.81E-03 ± 9.54E-04	*0.480*	*0.896*
C12:1-OH/C10:1-DC	3.75E-03 ± 5.65E-04	3.90E-03 ± 4.43E-04	*0.834*	*>0.999*
C12:2	1.36E-03 ± 2.28E-04	2.02E-03 ± 3.67E-04	*0.157*	*0.718*
C12:2-OH/C10:2-DC	5.30E-03 ± 5.18E-04	5.21E-03 ± 5.36E-04	*0.907*	*>0.999*
C14	1.14E-01 ± 1.21E-02	1.18E-01 ± 1.19E-02	*0.818*	*>0.999*
C14-OH/C12-DC	9.17E-03 ± 5.13E-04	1.05E-02 ± 1.33E-03	*0.100*	*0.869*
C14:1	4.69E-02 ± 5.42E-03	5.03E-02 ± 4.90E-03	*0.640*	*0.981*
C14:1-OH/C12:1-DC	1.25E-02 ± 7.42E-04	1.55E-02 ± 1.87E-03	*0.126*	*0.718*
C14:2	1.25E-02 ± 1.42E-03	1.30E-02 ± 1.19E-03	*0.777*	*>0.999*
C14:2-OH/C12:2-DC	3.69E-03 ± 4.10E-04	3.92E-03 ± 3.64E-04	*0.682*	*>0.999*
C14:3	8.57E-04 ± 1.97E-04	1.20E-03 ± 1.85E-04	*0.215*	*0.718*
C14:3-OH/C12:3-DC	9.19E-04 ± 2.37E-04	8.48E-04 ± 1.50E-04	*0.805*	*>0.999*
C16	6.95E-01 ± 8.29E-02	7.41E-01 ± 8.93E-02	*0.712*	*>0.999*
C16-OH/C14-DC	2.94E-02 ± 3.16E-03	3.36E-02 ± 4.67E-03	*0.462*	*0.893*
C16:1	1.08E-01 ± 9.65E-03	1.21E-01 ± 1.37E-02	*0.436*	*0.869*
C16:1-OH/C14:1-DC	1.78E-02 ± 1.36E-03	2.28E-02 ± 2.34E-03	*0.081*	*0.718*
C16:2	2.93E-02 ± 2.35E-03	3.15E-02 ± 4.01E-03	*0.636*	*0.981*
C16:2-OH/C14:2-DC	6.75E-03 ± 7.13E-04	8.90E-03 ± 1.11E-03	*0.118*	*0.718*
C16:3	2.83E-03 ± 4.09E-04	2.83E-03 ± 5.05E-04	*0.996*	*>0.999*
C16:3-OH/C14:3-DC	8.22E-04 ± 1.21E-04	8.34E-04 ± 1.66E-04	*0.955*	*>0.999*
C18	1.45E-01 ± 1.25E-02	1.64E-01 ± 1.95E-02	*0.417*	*0.869*
C18-OH/C16-DC	8.62E-03 ± 6.50E-04	1.09E-02 ± 1.13E-03	*0.095*	*0.718*
C18:1	4.09E-01 ± 3.78E-02	4.69E-01 ± 6.34E-02	*0.421*	*0.869*
C18:1-OH/C16:1-DC	2.37E-02 ± 1.79E-03	2.99E-02 ± 3.92E-03	*0.165*	*0.718*
C18:2	1.81E-01 ± 1.63E-02	2.16E-01 ± 2.93E-02	*0.308*	0.869
C18:2-OH/C16:2-DC	1.20E-02 ± 8.55E-04	1.63E-02 ± 2.18E-03	*0.079*	0.718
C18:3	9.86E-03 ± 9.16E-04	1.22E-02 ± 1.30E-03	*0.147*	0.718
C18:3-OH/C16:3-DC	1.21E-03 ± 1.50E-04	1.64E-03 ± 2.40E-04	*0.141*	0.718
C20	3.73E-03 ± 5.56E-04	4.86E-03 ± 4.40E-04	*0.126*	0.718
C20-OH/C18-DC/C22:6	6.13E-03 ± 1.05E-03	6.10E-03 ± 6.65E-04	*0.981*	*>0.999*
C20:1	1.96E-02 ± 1.50E-03	2.16E-02 ± 3.23E-03	*0.587*	0.980
C20:1-OH/C18:1-DC	2.09E-03 ± 2.10E-04	2.15E-03 ± 2.83E-04	*0.872*	*>0.999*
C20:2	1.16E-02 ± 1.47E-03	1.19E-02 ± 2.25E-03	*0.905*	*>0.999*
C20:2-OH/C18:2-DC	3.10E-03 ± 3.79E-04	3.12E-03 ± 2.63E-04	*0.961*	*>0.999*
C20:3	6.08E-03 ± 8.16E-04	5.03E-03 ± 7.53E-04	*0.357*	0.869
C20:3-OH/C18:3-DC	1.37E-03 ± 9.76E-05	1.12E-03 ± 1.81E-04	*0.238*	0.718
C20:4	1.13E-02 ± 2.15E-03	1.09E-02 ± 1.04E-03	*0.883*	*>0.999*
C22	6.47E-04 ± 1.20E-04	7.48E-04 ± 1.39E-04	*0.586*	0.980
C22:1	3.93E-03 ± 2.61E-04	4.29E-03 ± 1.40E-04	*0.235*	0.718
C22:2	1.01E-03 ± 1.56E-04	8.67E-04 ± 1.29E-04	*0.489*	0.896
C22:3	7.91E-04 ± 1.54E-04	6.05E-04 ± 1.36E-04	*0.377*	0.869
C22:4	2.76E-03 ± 4.31E-04	2.75E-03 ± 4.81E-04	*0.990*	*>0.999*
C22:5	3.75E-03 ± 6.33E-04	3.55E-03 ± 6.66E-04	*0.827*	*>0.999*
Total long chain AC	2.01E+00 ± 1.88E-01	2.23E+00 ± 2.60E-01	*0.500*	0.896
Total AC	4.65E+00 ± 2.18E-01	5.21E+00 ± 3.93E-01	*0.224*	0.718

SUPPLEMENTARY TABLE 7 : Liver acylcarnitine profile in postprandial state in the antibiotic study.
Data are means±s.e.m. Statistical analyses were performed using a two-way ANOVA followed by a Tukey post hoc test. Significant differences are bolded. n=12.

	µM				Main effects			Single effects			
Liver Acylcarnitines (AC)	HFHS-C	HFHS-PM	HFHS-C + Abx	HFHS-PM + Abx	P	A	PxA	HFHS-C vs HFHS-PM	HFHS-C+Abx vs HFHS-PM+Abx	HFHS-C vs HFHS-C+Abx	HFHS-PM vs HFHS-PM+Abx
C2	5.71E+00 ± 1.73E-01	7.17E+00 ± 2.65E-01	5.61E+00 ± 2.37E-01	6.96E+00 ± 3.61E-01	**<0.001**	0.573	0.85	**<0.001**	**0.001**	0.791	0.595
C3	1.20E-01 ± 9.13E-03	2.20E-01 ± 1.98E-02	1.71E-01 ± 2.12E-02	2.01E-01 ± 1.57E-02	**<0.001**	0.366	**0.047**	**<0.001**	0.230	**0.042**	0.428
C4/Ci4	2.21E-01 ± 1.68E-02	2.27E-01 ± 1.92E-02	1.97E-01 ± 1.84E-02	2.22E-01 ± 1.36E-02	0.364	0.407	0.579	0.801	0.302	0.329	0.845
C4-OH	1.50E-01 ± 1.05E-02	2.01E-01 ± 1.49E-02	1.37E-01 ± 2.40E-02	1.38E-01 ± 1.66E-02	0.126	**0.005**	0.197	0.049	0.861	0.256	**0.005**
C4-DC/Ci4-DC	2.50E-02 ± 2.04E-03	3.04E-02 ± 1.94E-03	2.39E-02 ± 1.74E-03	2.79E-02 ± 1.46E-03	**0.013**	0.32	0.695	**0.041**	0.128	0.668	0.328
Total SC AC	6.23E+00 ± 1.84E-01	7.85E+00 ± 2.83E-01	6.14E+00 ± 2.71E-01	7.55E+00 ± 3.83E-01	**<0.001**	0.513	0.725	**<0.001**	**0.001**	0.830	0.477
C5	5.13E-02 ± 4.49E-03	6.47E-02 ± 7.38E-03	5.56E-02 ± 3.65E-03	5.91E-02 ± 4.69E-03	0.153	0.931	0.301	0.084	0.775	0.427	0.500
C5:1	1.40E-01 ± 6.29E-03	1.31E-01 ± 6.95E-03	1.16E-01 ± 6.53E-03	1.33E-01 ± 1.09E-02	0.622	0.186	0.105	0.418	0.136	0.04	0.827
C5-OH/C3-DC	4.49E-02 ± 2.60E-03	4.87E-02 ± 3.75E-03	4.06E-02 ± 2.79E-03	3.49E-02 ± 2.01E-03	0.731	**0.003**	0.105	0.360	0.134	0.288	**0.002**
C5-DC	1.15E-01 ± 5.95E-03	1.46E-01 ± 8.04E-03	1.12E-01 ± 5.77E-03	1.36E-01 ± 8.02E-03	**<0.001**	0.367	0.596	**0.003**	**0.021**	0.791	0.312
C6	2.89E-02 ± 3.79E-03	3.25E-02 ± 6.74E-03	2.00E-02 ± 2.60E-03	1.88E-02 ± 1.35E-03	0.887	**0.003**	0.839	0.808	0.966	**0.042**	**0.022**
C7-DC	9.42E-02 ± 1.04E-02	8.77E-02 ± 6.00E-03	8.12E-02 ± 5.90E-03	8.90E-02 ± 1.19E-02	0.991	0.415	0.718	0.804	0.793	0.406	0.747
C8	6.60E-02 ± 5.97E-03	1.11E-01 ± 8.42E-03	5.05E-02 ± 3.94E-03	8.13E-02 ± 7.31E-03	**<0.001**	**0.001**	0.296	**<0.001**	**0.002**	0.104	**0.003**
C8:1	3.44E-03 ± 6.42E-04	4.14E-03 ± 1.58E-03	2.93E-03 ± 7.38E-04	3.61E-03 ± 6.40E-04	0.489	0.601	0.991	0.619	0.63	0.718	0.706
C8:1-DC	8.92E-03 ± 1.42E-03	1.15E-02 ± 2.68E-03	8.70E-03 ± 9.84E-04	1.03E-02 ± 8.55E-04	0.204	0.665	0.764	0.266	0.489	0.925	0.604
C8:1-OH/C6:1-DC	9.71E-03 ± 1.25E-03	1.31E-02 ± 1.12E-03	7.53E-03 ± 7.71E-04	1.22E-02 ± 1.00E-03	**<0.001**	0.146	0.557	**0.026**	**0.003**	0.150	0.534
C6-DC/C8-OH	1.24E-01 ± 9.18E-03	1.70E-01 ± 1.96E-02	1.33E-01 ± 2.17E-02	1.49E-01 ± 9.28E-03	**0.019**	0.65	0.621	**0.043**	0.175	0.977	0.503
C10	2.14E-02 ± 2.27E-03	2.40E-02 ± 5.56E-03	1.60E-02 ± 1.49E-03	2.06E-02 ± 2.05E-03	0.282	0.126	0.315	0.96	0.144	0.076	0.704
C10-OH/C8-DC	6.73E-03 ± 5.60E-04	8.53E-03 ± 1.11E-03	6.13E-03 ± 1.21E-03	8.14E-03 ± 9.55E-04	**0.061**	0.62	0.916	0.205	0.158	0.671	0.78
C10:1	2.43E-03 ± 6.33E-04	2.15E-03 ± 5.09E-04	4.09E-03 ± 7.22E-04	3.95E-03 ± 6.25E-04	0.741	**0.008**	0.912	0.755	0.876	0.067	**0.048**
C10:2	0.00E+00 ± 0.00E+00	0.00E+00 ± 0.00E+00	3.42E-04 ± 2.00E-04	6.66E-04 ± 3.12E-04	0.386	**0.009**	0.386	1.000	0.222	0.199	**0.015**
C10:3	2.09E-04 ± 1.53E-04	3.13E-04 ± 3.13E-04	2.55E-04 ± 2.03E-04	3.36E-04 ± 2.79E-04	0.707	0.888	0.963	0.765	0.816	0.894	0.947
Total MC AC	7.18E-01 ± 2.40E-02	8.55E-01 ± 4.64E-02	6.55E-01 ± 4.28E-02	7.61E-01 ± 3.37E-02	**0.002**	**0.044**	0.676	**0.013**	0.054	0.249	0.085
C12	2.05E-02 ± 2.97E-03	2.96E-02 ± 6.85E-03	1.49E-02 ± 3.38E-03	1.72E-02 ± 1.68E-03	0.139	**0.023**	0.881	0.343	0.248	0.083	0.125
C12-OH/C10-DC	3.22E-03 ± 5.12E-04	3.84E-03 ± 5.71E-04	3.65E-03 ± 6.78E-04	4.08E-03 ± 6.59E-04	0.395	0.589	0.876	0.477	0.623	0.623	0.786
C12:1	4.95E-03 ± 6.47E-04	6.17E-03 ± 1.13E-03	5.58E-03 ± 6.27E-04	7.14E-03 ± 7.01E-04	**0.092**	0.327	0.833	0.291	0.178	0.585	0.400
C12:1-OH/C10:1-DC	1.34E-03 ± 3.28E-04	3.28E-03 ± 6.03E-04	2.00E-03 ± 4.97E-04	2.90E-03 ± 5.29E-04	**0.007**	0.781	0.301	**0.009**	0.211	0.354	0.591
C12:2	4.79E-04 ± 2.20E-04	6.00E-04 ± 2.92E-04	0.00E+00 ± 0.00E+00	3.47E-04 ± 2.01E-04	0.268	**0.087**	0.591	0.684	0.246	0.112	0.397
C12:2-OH/C10:2-DC	2.85E-03 ± 3.92E-04	2.32E-03 ± 7.82E-04	2.80E-03 ± 4.79E-04	3.00E-03 ± 6.64E-04	0.788	0.609	0.543	0.535	0.809	0.945	0.429
C14	3.88E-02 ± 3.84E-03	5.73E-02 ± 8.70E-03	3.38E-02 ± 6.85E-03	4.00E-02 ± 3.57E-03	**0.023**	**0.047**	0.824	0.075	0.139	0.206	0.117
C14-OH/C12-DC	2.50E-03 ± 5.95E-04	3.72E-03 ± 7.98E-04	1.85E-03 ± 4.31E-04	3.21E-03 ± 6.28E-04	**0.046**	0.359	0.916	0.175	0.134	0.469	0.564
C14:1	2.28E-02 ± 2.56E-03	3.35E-02 ± 4.17E-03	2.17E-02 ± 3.00E-03	3.23E-02 ± 3.59E-03	**0.003**	0.735	0.995	**0.032**	**0.033**	0.814	0.807
C14:1-OH/C12:1-DC	3.94E-03 ± 4.08E-04	6.40E-03 ± 6.42E-04	5.02E-03 ± 7.67E-04	6.25E-03 ± 8.28E-04	**0.009**	0.499	0.375	**0.014**	0.206	0.271	0.879
C14:2	6.28E-03 ± 8.55E-04	9.78E-03 ± 1.81E-03	7.58E-03 ± 1.63E-03	1.02E-02 ± 1.02E-03	**0.027**	0.333	0.782	0.162	0.076	0.623	0.379
C14:2-OH/C12:2-DC	1.28E-03 ± 2.64E-04	2.75E-03 ± 4.53E-04	2.24E-03 ± 6.18E-04	3.31E-03 ± 6.33E-04	**0.017**	0.148	0.701	**0.049**	0.147	0.196	0.446
C14:3	1.91E-04 ± 1.91E-04	1.00E-03 ± 3.66E-04	8.24E-04 ± 5.15E-04	7.65E-04 ± 2.85E-04	0.303	0.584	0.234	0.119	0.909	0.22	0.646
C14:3-OH/C12:3-DC	1.62E-04 ± 9.59E-05	4.27E-04 ± 1.96E-04	2.94E-04 ± 2.26E-04	5.59E-04 ± 2.50E-04	0.194	0.513	1	0.357	0.357	0.644	0.644
C16	1.61E-01 ± 1.48E-02	2.09E-01 ± 2.99E-02	1.51E-01 ± 3.64E-02	1.83E-01 ± 1.70E-02	**0.038**	0.288	0.631	0.248	0.071	0.277	0.678
C16-OH/C14-DC	8.71E-03 ± 8.08E-04	1.15E-02 ± 1.66E-03	7.53E-03 ± 1.15E-03	9.76E-03 ± 1.31E-03	**0.06**	0.198	0.789	0.246	0.127	0.271	0.467
C16:1	5.22E-02 ± 6.24E-03	6.41E-02 ± 8.85E-03	4.62E-02 ± 8.75E-03	5.53E-02 ± 5.86E-03	**0.095**	0.305	0.842	0.294	0.185	0.386	0.557
C16:1-OH/C14:1-DC	6.12E-03 ± 8.13E-04	9.49E-03 ± 1.22E-03	6.53E-03 ± 9.33E-04	1.17E-02 ± 1.19E-03	**<0.001**	0.222	0.398	**0.029**	**0.001**	0.786	0.146
C16:2	1.47E-02 ± 2.06E-03	2.15E-02 ± 3.12E-03	1.47E-02 ± 2.67E-03	1.82E-02 ± 2.65E-03	**0.056**	0.549	0.75	0.114	0.252	0.842	0.517
C16:2-OH/C14:2-DC	3.56E-03 ± 3.96E-04	5.94E-03 ± 1.07E-03	3.84E-03 ± 6.90E-04	4.89E-03 ± 6.48E-04	**0.018**	0.617	0.666	**0.047**	0.161	0.961	0.510
C16:3	2.54E-03 ± 5.19E-04	3.12E-03 ± 5.30E-04	3.19E-03 ± 5.33E-04	3.18E-03 ± 5.50E-04	0.599	0.503	0.579	0.446	0.984	0.388	0.934
C16:3-OH/C14:3-DC	3.45E-04 ± 1.70E-04	6.89E-04 ± 2.36E-04	4.54E-04 ± 1.58E-04	6.42E-04 ± 2.72E-04	0.221	0.884	0.716	0.262	0.538	0.719	0.878
C18	8.64E-02 ± 6.60E-03	1.11E-01 ± 1.27E-02	8.18E-02 ± 1.13E-02	1.06E-01 ± 8.69E-03	**0.019**	0.637	0.985	0.089	0.094	0.749	0.728
C18-OH/C16-DC	7.68E-03 ± 6.02E-04	1.03E-02 ± 1.16E-03	7.98E-03 ± 8.62E-04	9.00E-03 ± 1.11E-03	**0.066**	0.616	0.419	0.063	0.455	0.827	0.356
C18:1	2.09E-01 ± 2.13E-02	2.56E-01 ± 3.28E-02	2.07E-01 ± 3.78E-02	2.50E-01 ± 2.61E-02	0.141	0.88	0.938	0.271	0.320	0.959	0.872
C18:1-OH/C16:1-DC	1.29E-02 ± 1.29E-03	1.54E-02 ± 2.07E-03	1.34E-02 ± 2.21E-03	1.62E-02 ± 2.10E-03	0.179	0.737	0.928	0.372	0.309	0.862	0.763
C18:2	9.88E-02 ± 1.10E-02	1.23E-01 ± 1.80E-02	9.52E-02 ± 2.00E-02	1.30E-01 ± 1.25E-02	**0.022**	0.989	0.396	0.287	**0.027**	0.554	0.542
C18:2-OH/C16:2-DC	1.26E-02 ± 1.37E-03	1.55E-02 ± 2.84E-03	1.22E-02 ± 1.47E-03	1.30E-02 ± 1.33E-03	0.436	0.544	0.781	0.455	0.722	0.815	0.532
C18:3	1.02E-02 ± 1.49E-03	1.25E-02 ± 2.87E-03	9.97E-03 ± 2.04E-03	1.25E-02 ± 2.28E-03	0.33	0.922	0.614	0.737	0.297	0.670	0.773
C18:3-OH/C16:3-DC	1.34E-03 ± 3.16E-04	1.13E-03 ± 3.33E-04	1.41E-03 ± 5.27E-04	1.92E-03 ± 5.04E-04	0.731	0.326	0.416	0.739	0.414	0.903	0.207
C20	1.46E-02 ± 1.43E-03	1.82E-02 ± 1.47E-03	1.61E-02 ± 2.21E-03	1.81E-02 ± 2.49E-03	0.156	0.73	0.687	0.198	0.467	0.597	0.968
C20-OH/C18-DC/C22:	1.82E-03 ± 3.81E-04	2.70E-03 ± 6.44E-04	2.51E-03 ± 3.86E-04	2.19E-03 ± 4.53E-04	0.562	0.847	0.22	0.203	0.642	0.314	0.462
C20:1	1.95E-02 ± 1.86E-03	2.27E-02 ± 2.52E-03	1.63E-02 ± 2.49E-03	1.98E-02 ± 1.85E-03	0.138	0.178	0.967	0.305	0.278	0.324	0.354
C20:1-OH/C18:1-DC	5.23E-03 ± 4.22E-04	5.86E-03 ± 5.22E-04	5.17E-03 ± 7.42E-04	6.68E-03 ± 8.09E-04	0.104	0.556	0.5	0.491	0.105	0.951	0.373
C20:2	1.20E-02 ± 1.27E-03	1.47E-02 ± 2.19E-03	1.33E-02 ± 2.07E-03	1.42E-02 ± 1.01E-03	0.294	0.791	0.6	0.267	0.708	0.577	0.854
C20:2-OH/C18:2-DC	6.47E-03 ± 3.55E-04	8.17E-03 ± 9.31E-04	5.36E-03 ± 6.18E-04	5.26E-03 ± 7.96E-04	0.265	**0.007**	0.214	0.098	0.927	0.272	**0.006**
C20:3	6.23E-03 ± 5.57E-04	8.76E-03 ± 1.16E-03	5.19E-03 ± 6.37E-04	8.59E-03 ± 8.52E-04	**<0.001**	0.473	0.604	**0.038**	**0.006**	0.383	0.888
C20:3-OH/C18:3-DC	1.36E-03 ± 4.00E-04	3.37E-03 ± 1.01E-03	1.79E-03 ± 4.64E-04	2.38E-03 ± 2.98E-04	**0.038**	0.649	0.251	**0.024**	0.493	0.621	0.258
C20:4	1.24E-02 ± 1.45E-03	2.00E-02 ± 2.66E-03	1.39E-02 ± 2.02E-03	2.63E-02 ± 2.53E-03	**<0.001**	**0.086**	0.287	**0.019**	**<0.001**	0.634	0.051
C22	2.99E-03 ± 3.10E-04	3.46E-03 ± 4.53E-04	3.70E-03 ± 4.86E-04	3.83E-03 ± 5.10E-04	0.509	0.234	0.71	0.466	0.838	0.269	0.559
C22:1	6.66E-03 ± 6.53E-04	8.05E-03 ± 1.02E-03	6.45E-03 ± 7.70E-04	6.47E-03 ± 4.99E-04	0.357	0.246	0.37	0.201	0.986	0.850	0.148
C22:2	1.15E-03 ± 2.68E-04	1.25E-03 ± 3.48E-04	7.25E-04 ± 3.69E-04	1.06E-03 ± 3.84E-04	0.538	0.378	0.728	0.850	0.496	0.385	0.705
C22:3	5.21E-04 ± 2.38E-04	6.14E-04 ± 3.74E-04	1.51E-03 ± 2.53E-04	3.16E-04 ± 1.95E-04	**0.051**	0.215	**0.023**	0.811	**0.004**	**0.014**	0.446
C22:4	1.84E-03 ± 5.08E-04	2.14E-03 ± 4.21E-04	1.58E-03 ± 4.93E-04	2.31E-03 ± 4.95E-04	0.293	0.923	0.658	0.664	0.291	0.703	0.807
C22:5	1.30E-03 ± 5.24E-04	1.15E-03 ± 3.50E-04	5.58E-04 ± 2.01E-04	1.23E-03 ± 4.24E-04	0.511	0.399	0.303	0.79	0.235	0.187	0.894
Total LC AC	8.92E-01 ± 7.38E-02	1.15E+00 ± 1.33E-01	8.58E-01 ± 1.47E-01	1.08E+00 ± 8.78E-02	**0.017**	0.507	0.745	0.135	0.054	0.485	0.811
Total AC	7.84E+00 ± 2.41E-01	9.85E+00 ± 4.07E-01	7.65E+00 ± 4.28E-01	9.39E+00 ± 4.56E-01	**<0.001**	0.412	0.722	**<0.001**	**0.003**	0.742	0.406

Supplementary Method Table 1: Antibodies and immunoblotting conditions

Antibody	Company	Catalogue number	Blocking reagent	Diluent (in TBST)	Dilution
Akt	Cell Signaling Technology	9272	BSA 5%	BSA 5%	1:2000
Phospho-Akt S473	Cell Signaling Technology	9271	BSA 5%	BSA 5%	1:2000
IRS1	Millipore	06-248	BSA 5%	BSA 5%	1:1000
IRS2	Millipore	06-506	BSA 5%	BSA 5%	1:1000
Phospho-IRS1 S1101	Cell Signaling Technology	2388	BSA 5%	BSA 5%	1:1000
Phospho-S6 S240-244	Cell Signaling Technology	5364	BSA 5%	BSA 5%	1:10000
S6	Cell Signaling Technology	2217	BSA 5%	BSA 5%	1:10000
PKC theta	Cell Signaling Technology	13643	BSA 5%	BSA 5%	1:1000
eEF2	Cell Signaling Technology	2332	BSA 5%	BSA 5%	1:1000
Actin	Millipore	MAB1501	Milk 5%	Milk 5%	1:20000
Anti-rabbit IgG-HRP	Jackson ImmunoResearch	111-035-144		Milk 5%	1:10000
Anti-mouse IgG-HRP	Jackson ImmunoResearch	115-035-146		Milk 5%	1:10000

Supplementary Method Table 2: Primer sequences

Gene	Forward	Reverse
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Cidea	TGCTCTTCTGTATCGCCCA	GCCGTGTTAAGGAATCTGCTG
PGC1α	TGGATGAAGACGGATTGC	TGGTTCTGAGTGCTAAGAC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Actb	CTCTAGACTTCGAGCAGGAG	AGAGTACTTGCGCTCAGGAG
Hprt	CCCCAAAATGGTTAAGGTTGC	AACAAAGTCTGGCCTGTATCC
Akkermansia muciniphila	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT

Chapitre 4 : *Lacticaseibacillus rhamnosus* HA-114 improves eating and mood-related behaviors in adults with overweight during weight loss : a randomized controlled trial

Béatrice S.-Y. Choi[1,2], Lucie Brunelle[3], Geneviève Pilon[1,2], Brunella Gonzalez Cautela[4], Thomas A. Tompkins[4], Vicky Drapeau[1,2,5], André Marette[1,2,6] & Angelo Tremblay[1,2,3*]

[1]Institute of Nutrition and Functional Foods (INAF), Laval University, Québec, QC, Canada
[2]Quebec Heart and Lung Institute (IUCPQ) Research Center, Laval University, Québec, QC, Canada
[3]Department of Kinesiology, Laval University, Québec, QC, Canada
[4] Rosell Institute for Microbiome and Probiotics, Montréal, QC, Canada
[5]Department of Physical Education, Faculty of Educational, Laval University, QC, Québec, Canada
[6]Department of Medicine, Faculty of Medicine, Laval University, Québec, QC, Canada
*****Corresponding author: angelo.tremblay@kin.ulaval.ca

4.1 Résumé

Le microbiote intestinal est un écosystème malléable influençant l'obésité et les maladies métaboliques chez l'humain. Des interventions ciblant cette communauté microbienne pourraient atténuer les comorbidités biologiques et psychologiques de l'excès de poids. Notre objectif était de déterminer si la supplémentation en *Lacticaseibacillus rhamnosus* HA-114 accentuait l'impact bénéfique de la perte de poids sur la santé métabolique et cognitive. Cet essai de 12 semaines, randomisé, en double aveugle et contrôlé par placebo, a évalué les marqueurs biologiques du métabolisme énergétique, les comportements alimentaires et l'humeur chez 152 adultes avec un surpoids recevant une supplémentation en *L. rhamnosus* HA-114 ou un placebo, qui suivaient également une intervention diététique induisant une perte de poids contrôlée. Bien que la supplémentation en probiotiques n'ait pas potentialisé la réduction du poids corporel ou de la masse grasse, une diminution significative de l'insuline plasmatique, de l'HOMA-IR, du LDL-cholestérol et des triglycérides a été observée dans le groupe supplémenté en probiotiques uniquement. En ce qui concerne les traits liés à l'alimentation et à l'humeur, les effets bénéfiques ont été observés uniquement dans le groupe recevant la supplémentation en probiotiques ou étaient significativement plus importants dans ce groupe, y compris la diminution des tendances à la l'hyperphagie, la désinhibition

et les envies de manger. Cette étude démontre la pertinence clinique de la supplémentation en probiotiques pour induire des résultats métaboliques et psychologiques bénéfiques chez les personnes en surpoids en cours de perte de poids.

4.2 Abstract

Gut microbiota has emerged as a modifiable ecosystem influencing obesity and metabolic diseases in humans. Interventions targeting this microbial community could attenuate the biological and psychological comorbidities of excess weight. Our aim was to determine if *Lacticaseibacillus rhamnosus* HA-114 supplementation accentuated beneficial impact of weight loss on metabolic and cognitive health. This 12-week randomized, double-blind, placebo-controlled trial assessed biological markers of energy metabolism, eating-behaviours and mood in 152 adults with overweight receiving *Lacticaseibacillus rhamnosus* HA-114 supplementation or placebo, that were also on a dietary intervention inducing a controlled weight loss. Although probiotic supplementation did not potentiate the reduction in body weight or fat mass, a significant decrease in plasma insulin, HOMA-IR, LDL-cholesterol and triglycerides was observed in the probiotic-supplemented group only. With respect to eating and mood-related traits, beneficial effects were either observed only in the group receiving probiotic supplementation or were significantly greater in this group, including decrease in binge eating tendencies, disinhibition and food-cravings. This study demonstrates the clinical relevance of probiotic supplementation to induce beneficial metabolic and psychological outcomes in individuals with overweight undergoing weight loss.

4.3 Introduction

The occurrence of gut microbiota dysbiosis has been implicated in a large spectrum of non-communicable diseases including obesity (1) and metabolic diseases (2). Over the past few decades, increased consumption of animal-derived fat and highly processed foods and decreased consumption of plant-derived fibres have been pointed out as potential causes for the increasing prevalence of obesity (3). In addition to directly affecting metabolism, these dietary changes can also significantly impact the gut microbiota composition and function (4) thus inducing dysbiosis.

Biological comorbidities of obesity are the main target of treatments due to their impact on cardiometabolic health and life expectancy, while psychological comorbidities are often neglected even if they can significantly affect health and quality of life. Indeed, there is a link

between obesity and mood disorders such as depression, stress, anxiety and low body self-esteem. Adults with obesity are 55% more likely to develop depression than non-obese individuals, while depressed adults are 58% more likely to become obese, demonstrating the reciprocity of these disorders (5). Furthermore, altered eating behaviors such as binge eating, are also common comorbidities of obesity; food addiction is present in 25-37% of individuals with obesity and can reach 60% among individuals with morbid obesity (6). The gut communicates with the brain in various ways, including the vagus nerve, microbiota-derived neuroactive compounds, enzymes or gut-derived inflammatory signals (7). Many studies on the association between obesity and psychological variables have been carried out and a potential role of the gut-brain axis has been hypothesized (8) as well as the concept of psychobiotics, "*live biotherapeutics or substances whose beneficial effects on the brain are bacterially mediated"* (9). Thus, we decided to examine a probiotic's potential to improve metabolic and cognitive health in adult with oberweight submitted to a dietary weight loss intervention.

4.4 Material and Methods

Participants

This is a randomized, double-blind, placebo-controlled clinical trial. Subjects were stratified based on sex and BMI and randomized in a 1:1 ratio to *L. rhamnosus* HA-114 or an appearance- and taste-matched placebo capsule provided by Lallemand Health Solutions (LHS; Montreal, Quebec, Canada). Randomization schedule was prepared by an associated statistician who was not involved in the study assessment and provided to the investigator. The manufacturer filled all the bottles dispensed to participants. Participants were recruited between January 2017 and March 2019 in Québec City, through advertisement on campus. To meet the inclusion criteria participants needed to be aged between 18 and 55 years old, have a BMI between 27.0 and 39.9 kg/m^2 and be sedentary to moderately active. Exclusion criteria were weight change of 10+ lbs in the last three months, taking antibiotics, supplements or any medication affecting energy metabolism, any serious health issues, drug or alcohol abuse and for women, being in menopause, pregnant or breastfeeding. Technical problems explain variations in subjects number for some variables, e.g. the collection of fasting blood samples. Primary outcome was change from baseline body weight and

secondary outcomes were change from baseline from a myriad of anthropometric, metabolic and cognitive measurements described below. All staff involved in product dispensing, visit assessments, conduct of the study, monitoring and analysis as well as participants remained blinded for the duration of the study. No premature unblinding occurred during the course of this study.

Intervention

The probiotic, *Lacticaseibacillus rhamnosus* HA-114, was given under the form of freeze-dried bacteria capsules (10 x 10^9 CFU per capsule), provided by LHS and stored refrigerated (2-8°C). Non-medicinal ingredients found in the probiotics and placebo capsules were potato starch, hydroxypropylmethylcellulose, titanium dioxide, and magnesium stearate. Participants were instructed to take the capsule daily, with a meal at the same time and to keep the product refrigerated during the study.

Dietary intervention consisted in a 12-week food plan, inducing a daily caloric restriction during the same period they consumed the probiotic formulation or the placebo. The energy content of the diet prescription was customized to each participant (resting metabolic rate X physical activity level – 500 kcal). To create a supportive and motivating environment and to assess whether dietary recommendations were still adequate and respected, participants came for follow-up visits every two weeks.

Measurements

At baseline and at the end of the intervention (12 weeks ± 1 week), participants came for 5-h visits starting in the morning after a minimum of 12h fasting, 24h without intense physical activity and 48h without alcohol consumption. During these visits, blood and stool was collected and anthropometric parameters, blood pressure, heart rate, resting metabolic rate by indirect calorimetry were measured, as well as appetite sensations by visual analog scales (VAS) before and after a standardised breakfast test meal as previously described (10, 11). Body composition was measured by dual energy absorptiometry with X-rays. Energy intake and diet quality was assessed by a three-day food record (12) and physical activity level by a three-day activity record on the same days (13).

Eating behaviors and mood-relates questionnaires

The Three Factor Eating Questionnaire (TFEQ) (14) was used to measure the main dimensions of human eating behaviors; cognitive restraint, disinhibition and susceptibility for hunger, with subscales for cognitive restraint, evaluating more precisely flexible and rigid control (15) strategic dieting behavior, attitude to self-regulation, avoidance of fattening foods (16). The Binge Scale (17) was used to assess binge eating tendencies and the Food Cravings Questionnaire (FCQ) (18) which differentiates State (S) and Trait (T) through different parameters such as lack of control, physiological state, the anticipation of positive reinforcement and relief from negative state to assess food cravings. Wellness and quality of life were assessed with the following questionnaires: the Body Esteem Scale (19) to measure distress-related body esteem, the Beck Depression Inventory (20) which evaluates depression symptoms, the State-Trait Anxiety Inventory (21), to measure anxiety symptoms, the Perceived Stress Scale (22) to evaluate the level of stress and the Gastrointestinal Symptom Rating Scale (GSRS) (23) to assess gastrointestinal symptoms including diarrhea, constipation, acid reflux, indigestion, and abdominal pain.

Strain detection

Stool samples were obtained before and after the protocol from a subgroup of participants (placebo, n=30 and probiotic, n=37), aliquoted in sterile tubes and frozen within 4 hours of collection. DNA was extracted from 150- 250 mg of homogenized stool samples using the ZymoBIOMICS 96 MagBead DNA kit (Zymo Research, USA, Ca.) with the addition of 0.5% v/v of β-mercaptoethanol to the MagBinding Buffer and the use of 900 µL ZymoBIOMICS™ MagWash 1. Absolute quantification of *L. rhamnosus* HA-114 was achieved using the CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, USA, Ca.) according to previously described methods (24) using specific forward (ACTCCAAAGAGCATTACCTCCG) and reverse (TGAATATGCCGGATCTAAGTCCA) primers.

Biochemical analysis

Blood samples were drawn from an antecubital vein through a venous catheter. The following fasted variables were measured: glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, ALT and AST, by a portable chemistry analyzer (Piccolo Xpress, Abaxis inc.), C-reactive protein, insulin and estradiol at the biochemical analysis platform of the Quebec Heart and Lung Institute and leptin and total ghrelin by ELISA (Human Leptin Instant ELISA Kit and Human Ghrelin ELISA Kit, Invitrogen, ThermoFisher Scientific).

Statistics

Power analysis was performed based on weight loss, using a t-test (assuming approximate normality) at a significance level of 5%, statistical power of 85%, and estimated a standard deviation of 2.7 kg for both groups (25, 26). The treatment effect was estimated using a linear mixed ANCOVA model adjusting for the effect of relevant covariates, sex and age, in the model. Appropriate non-parametric methods were applied if the data significantly deviated from the normality assumptions. For each endpoint, a single statistical model based on the experimental design and which includes effects for time and treatment were constructed. The fixed effects in the statistical model are time point (visit), treatment group (probiotic or placebo) and their interaction. No imputation was performed for missing observations, statistical models that account for unbalanced data were used instead. The analyses were conducted on the intention-to-treat population using JMP (v14.1.0).

4.5 Results

Participants' flow and pre-baseline characteristics

Overall, 152 healthy participants with an elevated BMI were included in the study (Supplementary Figure 1), which ended after recruitment was completed. Demographics and characteristics of randomized participants at pre-baseline (n=152) were similar between groups (Table 1). No serious adverse events were reported during the study.

Body weight and body composition

The dietary intervention, aiming at a decrease of 500 kcal/day in energy intake, reduced body weight, BMI, waist circumference, fat mass and percent body fat, whereas no significant

change in lean mass was induced by the protocol (Table 2). Although on average the placebo group lost 2.96 kg of fat mass compared to 3.24 kg for the probiotic group, there was no significant interaction with the probiotic supplementation ($p>0.05$).

Circulating markers, blood pressure and heart rate

Multiple blood markers related to metabolic and general health were measured in fasted blood samples (Table 2). Interestingly, fasting plasma insulin, LDL-cholesterol and triglycerides levels as well as HOMA-IR were decreased over time within the probiotic group only ($p<0.05$), although there was no significant group-by-time interaction. Leptin and ghrelin, which are hunger-related hormones produced mainly by the adipose tissue and gut, respectively, were measured in the fasted state. Although there was no significant group-by-time interaction, leptin decreased in both groups while ghrelin significantly increased in the probiotic group only ($p<0.05$). Other blood variables, blood pressure and resting heart rate remained within normal range in both groups and no changes were observed.

Energy expenditure and intake

As shown in Table 3, a small non-significant reduction in resting metabolic rate was observed in both groups. Physical activity levels increased slightly by the end of the intervention ($p<0.05$), but to an equivalent extent in the two groups, without a change in subjects' activity classification. As expected, due to dietary intervention, daily energy intake was decreased in the two groups and was attributable to a lower lipid consumption in the placebo group and a decrease in both lipid and carbohydrate intake in the probiotic group. Lipid intake was decreased in favor of protein in both groups.

Appetite sensation measurements

Appetite sensations were measured before and after the standardised breakfast. Four parameters were measured with visual analog scales (VAS), namely hunger, fullness, satiety and desire to eat. An analysis of the area under the curve (AUC) of all measurements for breakfast revealed no significant group-by-time interaction or within-group differences (Supplementary Figure 2A). Similarly, no significant difference in satiety quotients between the two groups was observed for all appetite sensations (Supplementary Figure 2B).

Eating behaviors

When evaluating binge eating behavior, a decrease was observed in the probiotic group only (Figure 1A), with a group-by-time interaction significance ($p<0.01$). The TFEQ revealed a decrease in disinhibition and hunger in the probiotic group only ($p<0.01$), and a group-by-time interaction effect was also observed for these parameters ($p=0.05$ and $p<0.01$, respectively) (Figure 1B). Cognitive restraint increased in both groups, but this effect was more pronounced in the probiotic group, with a significant group-by-time interaction ($p=0.05$). When evaluating the subscales for the restraint factor, we observerd a significant increase in both flexible and rigid restraint on the placebo and the probiotic groups and there was a tendency ($p=0.07$) for a group-by-time interaction on the rigid restraint, being further increased in the probiotic group compared to the placebo (Figure 1C). Furthermore, an increase in the component *Strategic Dieting Behavior* was present only in the probiotic group ($p=0.05$) and a significant group-by-time interaction effect was observed in the factors *Attitude to Self Regulation* and *Avoidance of Fattening Foods,* which were increased in the probiotic group compared to the placebo group ($p<0.05$). With respect to the State-Trait FCQ, we observed a higher number of dimensions changed in the probiotic group (7 out of 14) than in the placebo group (1 out of 14), suggesting a better control of food cravings in the probiotic group (Figure 1D-E). Interestingly, the dimension *lack of control* was lowered in the probiotic group both at the state and trait level with a significant group-by-time interaction ($p<0.05$ and $p<0.01$, respectively).

Mood-related parameters

Mood-related parameters assessed throughout this study included body esteem, anxiety, stress and depression, as well as gastrointestinal symptoms which can impact mood. An equivalent increase in body esteem was observed in the two groups (Figure 2A). In addition, there was a decrease over time in anxiety (trait), perceived stress and depression score in the probiotic group ($p<0.05$), although there was no group-by-time interaction significant effect (Figure 2B-D). Neither diet intervention nor probiotic supplementation affected overall gastrointestinal symptoms (Figure 2E).

Fecal L. rhamnosus HA-114 detection

L. rhamnosus HA-114 was not detected in baseline stool samples using strain-specific qPCR assays. At the end of the intervention, the strain was not detectable in any of the fecal samples provided by participants in the placebo group, while it was recovered and quantified in 35 of the 37 samples from the probiotic group (Figure 3).

4.6 Discussion

The aim of this study was to determine if a probiotic supplementation (*L. rhamnosus* HA-114) can improve the outcome of a weight-reducing program on body composition as well as behavioral variables related to the gut-brain axis. To this end, we tested the impact of a probiotic supplementation in combination with dietary supervision aiming at a decrease in energy intake. As expected, the program resulted in a significant decrease in body weight and fat in both groups, confirming the efficiency of the dietary intervention. However, there was no additional effect of the probiotic on body composition. In addition, the probiotic strain recovery from fecal samples in 95% of the participants allocated to receive the probiotic demonstrates good compliance to the present intervention.

Despite the similar weight loss in both groups, the probiotic intervention improved markers of metabolic health that were not observed in the placebo group. As indicated above, a significant reduction in fasting levels of insulin, LDL-cholesterol, triglycerides and HOMA-IR was observed only in the probiotic group. The favorable effects observed in this group suggest that probiotic supplementation can help prevent comorbidities related to metabolic syndrome such as insulin resistance and dyslipidemia which can ultimately lead to type 2 diabetes (T2D) (27).

There were no changes in appetite sensations in response to the standardized breakfast meal in either group, despite the observed changes in circulating hormones related to hunger. The lack of change in appetite sensations could be attributable to limitations of the VAS or a relatively moderate weight loss which may not have been sufficient to induce clear measurable changes. However, it is important to consider that the quantification of

circulating hormones and their influence on behavior and appetite control may not be representative of neurotransmitter signaling to the brain as homeostatic and hedonic signals in the control of food intake are very complex (28).

It is known that a weight loss due to diet restriction or surgery induces beneficial changes in eating and mood-related behaviours, such as an increase in body esteem and cognitive restraing combined with a decrease in disinhibition and hunger (29-31). This is concordant with our observation that body esteem improved in both groups, suggesting that this was modulated by weight loss. Beyond this effect, we demonstrated that at comparable body weight loss, *L. rhamnosus* HA-114 supplementation induced further improvements in behaviors compared to diet supervision with placebo. Our results suggest two categories of observations supporting this notion. First, we observed a significant decrease on several parameters for which no effect was observed in the control group; these include disinhibition and hunger measured by the TFEQ, several food-craving dimensions at the state level and trait level, Beck depression inventory score, binge eating tendencies, perceived stress and the anxiety at the trait level. Second, the existence of significant group-by-time interactions suggests a superiority of the probiotic intervention on some aspects, including decrease in binge eating tendencies, disinhibition, hunger, lack of control (both at the state and trait levels) and restraint behaviors. This increase was further characterized and results of the TFEQ-subscales suggest that the probiotic exacerbated control behaviors such as attitude to self regulation and avoidance of fattening food compared to the placebo, which could lead to a better long-term adherence to dietary recommandations and higher weight loss (32). Collectively, these results demonstrate a clear benefit of *L. rhamnosus* HA-114 supplementation on eating and mood-related behaviors during a weight loss program.

Many hypotheses could be explored further to characterize the underlying mechanism of action involved in the observed effects of *L. rhamnosus* HA-114. Some bacteria can modulate several processes via the gut-brain axis, by producing neurotransmitters such as GABA (33) and serotonin (34) or microbial metabolites, such as short-chain fatty acids (SCFA), which can cross the blood brain barrier and, in turn, influence brain function (35). Another hypothesis is the modulation of gut hormones production, which are known to

exert regulatory roles in key metabolic markers or processes such as glucose tolerance, insulin sensitivity, appetite control and fat storage (36). Improvement of gut barrier by modulators can also decrease metabolic endotoxemia and therefore reduce low-grade inflammation associated to obesity, metabolic (37) and non-metabolic disorders (38) as well as neuroinflammation (39). Furthermore, a strain of *L. rhamnosus* was shown to limit expansion of a pathobiont i.e. organisms that can cause harm under certain circumstances (40), thus reducing its negative impact on host's immunity and metabolism (41). Specifically, to *L. rhamnosus* HA-114, others have found that this strain can improve hippocampal dependent cognition deficits in a mouse model of Parkinson's disease (42) and reverse neurodegeneration in animal models of amyotrophic lateral sclerosis and Huntington's disease through modification of lipid metabolism (43), supporting the strong psychobiotic potential of this probiotic strain.

In conclusion, our results support the clinical relevance of the probiotic *L. rhamnosus* HA-114 supplementation during a weight loss dietary intervention in participants with overweight. Although further studies are needed to better understand the mechanisms underlying the effects of this probiotic strain and determine conditions under which a clinical outcome may be optimized, the results of this study reveal beneficial metabolic and psychological outcomes supporting the concept that probiotic supplementation could favor wellness and facilitate adherence during a diet-based weight reduction program.

4.7 Acknowledgements

The authors would like to thank Christian Couture for his help in statistical analyses, Annie Tremblay for providing valuable edits the manuscript and Amanda Piano for strain quantification by qPCR. The contribution of Catherine Lepage, Maya Purcell and Lola Bourgoin to the dietary supervision of participants is also gratefully acknowledged.

Funding

The work was supported by a grant from Lallemand Inc. Furthermore, AT is the recipient of a Canada Research Chair in Environment and Energy Balance. AM is the recipient of a CIHR/Pfizer research Chair in the pathogenesis of insulin resistance and cardiovascular

diseases. BSYC is funded by doctoral scholarships from the Sentinel North program at Laval University (Canada First Research Excellence Fund) and from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Author contribution

Conceptualization: AT, TAT, VD, Methodology: AT, VD, Investigation: BSYC, LB, Visualization: BSYC, Supervision: AT, AM, GP, VD, Project Administration: LB, AT, Funding Acquisition: AT, VD, Writing – Original Draft: BSYC and Writing – Review & Editing: AT, LB, GP, BGC, TAT, VD, AM.

Ethical Approval

This study was reviewed by the research ethics board (*Vice-rectorat à la recherche, à la création et à l'innovation - Comité universitaire d'éthique de la recherche, Université Laval*) and approved on April 7th, 2016, as well as the regulatory authority (Natural and Non-Prescription Health Products Directorate (NNHPD), Health Canada, Ottawa, Ontario), by which a Notice of Authorization letter was issued on September 13th, 2016. The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and its subsequent amendments and registered on clinicaltrial.gov under the identifier NCT02962583, URL: https://clinicaltrials.gov/ct2/show/NCT02962583?term=NCT02962583&draw=2&rank=1.

Competing Interests

The work was supported by a grant from Lallemand Inc. TAT and BGC are employed by the Rosell Institute for Microbiome and Probiotics, the research group of Lallemand Health Solutions Inc. The remaining authors declare no competing interests.

4.8 References

1. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.

2. Ha CW, Lam YY, Holmes AJ. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. World J Gastroenterol. 2014;20(44):16498-517.

3. Juul F, Martinez-Steele E, Parekh N, Monteiro CA, Chang VW. Ultra-processed food consumption and excess weight among US adults. Br J Nutr. 2018;120(1):90-100.

4. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021;19(1):55-71.

5. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry. 2010;67(3):220-9.

6. Gupta A, Osadchiy V, Mayer EA. Brain-gut-microbiome interactions in obesity and food addiction. Nat Rev Gastroenterol Hepatol. 2020;17(11):655-72.

7. van Son J, Koekkoek LL, La Fleur SE, Serlie MJ, Nieuwdorp M. The Role of the Gut Microbiota in the Gut-Brain Axis in Obesity: Mechanisms and Future Implications. Int J Mol Sci. 2021;22(6).

8. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota–gut–brain axis in obesity. The Lancet Gastroenterology & Hepatology. 2017;2(10):747-56.

9. Long-Smith C, O'Riordan KJ, Clarke G, Stanton C, Dinan TG, Cryan JF. Microbiota-Gut-Brain Axis: New Therapeutic Opportunities. Annu Rev Pharmacol Toxicol. 2020;60:477-502.

10. Sanchez M, Darimont C, Drapeau V, Emady-Azar S, Lepage M, Rezzonico E, et al. Effect of Lactobacillus rhamnosus CGMCC1.3724 supplementation on weight loss and maintenance in obese men and women. Br J Nutr. 2014;111(8):1507-19.

11. Sanchez M, Darimont C, Panahi S, Drapeau V, Marette A, Taylor VH, et al. Effects of a Diet-Based Weight-Reducing Program with Probiotic Supplementation on Satiety Efficiency, Eating Behaviour Traits, and Psychosocial Behaviours in Obese Individuals. Nutrients. 2017;9(3).

12. Tremblay A, Sévigny J, Leblanc C, Bouchard C. The reproducibility of a three-day dietary record. Nutr Res. 1983;3(6):819-30.
13. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. Am J Clin Nutr. 1983;37(3):461-7.
14. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985;29(1):71-83.
15. Westenhoefer J, Stunkard AJ, Pudel V. Validation of the flexible and rigid control dimensions of dietary restraint. Int J Eat Disord. 1999;26(1):53-64.
16. Bond MJ, McDowell AJ, Wilkinson JY. The measurement of dietary restraint, disinhibition and hunger: an examination of the factor structure of the Three Factor Eating Questionnaire (TFEQ). Int J Obes Relat Metab Disord. 2001;25(6):900-6.
17. Hawkins RC, 2nd, Clement PF. Development and construct validation of a self-report measure of binge eating tendencies. Addict Behav. 1980;5(3):219-26.
18. Nijs IM, Franken IH, Muris P. The modified Trait and State Food-Cravings Questionnaires: development and validation of a general index of food craving. Appetite. 2007;49(1):38-46.
19. Mendelson BK, Mendelson MJ, White DR. Body-esteem scale for adolescents and adults. J Pers Assess. 2001;76(1):90-106.
20. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71.
21. Spielberger C, Goruch R, Lushene R, Vagg P, Jacobs G. Manual for the state-trait inventory STAI (form Y). Mind Garden, Palo Alto, CA, USA. 1983.
22. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983;24(4):385-96.
23. Svedlund J, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci. 1988;33(2):129-34.
24. Ford AL, Nagulesapillai V, Piano A, Auger J, Girard SA, Christman M, et al. Microbiota Stability and Gastrointestinal Tolerance in Response to a High-Protein Diet with and without a Prebiotic, Probiotic, and Synbiotic: A Randomized, Double-Blind, Placebo-Controlled Trial in Older Women. J Acad Nutr Diet. 2020;120(4):500-16 e10.

25. Major GC, Alarie FP, Doré J, Tremblay A. Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control. Br J Nutr. 2009;101(5):659-63.
26. Major GC, Doucet E, Jacqmain M, St-Onge M, Bouchard C, Tremblay A. Multivitamin and dietary supplements, body weight and appetite: results from a cross-sectional and a randomised double-blind placebo-controlled study. Br J Nutr. 2008;99(5):1157-67.
27. Martin BC, Warram JH, Krolewski AS, Soeldner JS, Kahn CR, Martin BC, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. The Lancet. 1992;340(8825):925-9.
28. Woods SC. The control of food intake: behavioral versus molecular perspectives. Cell Metab. 2009;9(6):489-98.
29. Drapeau V, Jacob R, Panahi S, Tremblay A. Effect of Energy Restriction on Eating Behavior Traits and Psychobehavioral Factors in the Low Satiety Phenotype. Nutrients. 2019;11(2).
30. Pepino MY, Bradley D, Eagon JC, Sullivan S, Abumrad NA, Klein S. Changes in taste perception and eating behavior after bariatric surgery-induced weight loss in women. Obesity (Silver Spring). 2014;22(5):E13-20.
31. Dixon JB, Dixon ME, O'Brien PE. Depression in Association With Severe Obesity: Changes With Weight Loss. Arch Intern Med. 2003;163(17):2058-65.
32. Urbanek JK, Metzgar CJ, Hsiao PY, Piehowski KE, Nickols-Richardson SM. Increase in cognitive eating restraint predicts weight loss and change in other anthropometric measurements in overweight/obese premenopausal women. Appetite. 2015;87:244-50.
33. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A. 2011;108(38):16050-5.
34. Morais LH, Schreiber HLt, Mazmanian SK. The gut microbiota-brain axis in behaviour and brain disorders. Nat Rev Microbiol. 2021;19(4):241-55.

35. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol. 2019;16(8):461-78.
36. Martin AM, Sun EW, Rogers GB, Keating DJ. The Influence of the Gut Microbiome on Host Metabolism Through the Regulation of Gut Hormone Release. Front Physiol. 2019;10:428.
37. Scheithauer TPM, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, van Raalte DH, et al. Gut Microbiota as a Trigger for Metabolic Inflammation in Obesity and Type 2 Diabetes. Front Immunol. 2020;11:571731.
38. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017;13(4):851-63.
39. Guillemot-Legris O, Muccioli GG. Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. Trends Neurosci. 2017;40(4):237-53.
40. Jochum L, Stecher B. Label or Concept - What Is a Pathobiont? Trends Microbiol. 2020;28(10):789-92.
41. Natividad JM, Lamas B, Pham HP, Michel ML, Rainteau D, Bridonneau C, et al. Bilophila wadsworthia aggravates high fat diet induced metabolic dysfunctions in mice. Nat Commun. 2018;9(1):2802.
42. Xie C, Prasad AA. Probiotics Treatment Improves Hippocampal Dependent Cognition in a Rodent Model of Parkinson's Disease. Microorganisms. 2020;8(11).
43. Labarre A, Guitard E, Tossing G, Bareke E, Labrecque M, Tetreault M, et al. Probiotic Lacticaseibacillus rhamnosus HA-114 suppresses age-dependent neurodegeneration via mitochondrial beta-oxidation. Nature Portfolio - Preprint. 2020.

4.9 Tables

Table 1. Group characteristics at pre-baseline.

	Placebo (n = 74)	Probiotic (n = 78)
Sex		
Female	58	61
Male	16	17
Race		
Caucasian	61	65
African	6	6
Oriental asian	1	0
Arabic/Occidental asian	2	2
Latino-american	4	5
Age, Years (Mean ± SD)	32.7± 9.8	34.4 ± 8.6
Weight, kg (Mean ± SD)	93.9 ± 1.4	94.1 ± 1.3
Height, cm (Mean ± SD)	167.2 ± 9.2	166.2 ± 8.5
Body Mass Index, kg/m² (Mean ± SD)	31.8 ± 0.4	32.3 ± 0.4

Table 2. Anthropometric measurements, body composition changes, fasting blood markers, pressure and heart rate.

	Placebo		Probiotic	
	Baseline	3 months	Baseline	3 months
Body weight, kg	89.0 ± 13.3 (72)	84.8 ± 12.3** (63)	89.2 ± 12.1 (78)	84.2 ± 10.2** (69)
Body Mass Index, kg/m^2	31.7 ± 2.8 (72)	30.3 ± 2.7** (63)	32.2 ± 3.1 (78)	30.7 ± 3.2** (69)
Waist circumference, cm	96.6 ± 9.8 (72)	93.7 ± 9.3** (63)	96.3 ± 8.9 (78)	92.5 ± 8.3** (68)
Fat mass, kg	35.9 ± 7.3 (72)	32.7 ± 7.0** (63)	37.0± 7.1 (77)	33.5 ± 7.7** (68)
Lean mass, kg	48.8 ± 9.5 (72)	48.4 ± 9.7 (63)	47.8 ± 9.6 (77)	47.2 ± 9.0 (68)
Body fat, %	41.1 ± 6.4 (72)	39.1 ± 6.9** (63)	42.4 ± 6.7 (77)	40.1 ± 7.7** (68)
Glucose, mgl/dL	96.6 ± 13.7 (65)	96.9 ± 8.1 (53)	94.0 ± 7.5 (71)	93.8 ± 6.0 (60)
Insulin, pmol/L	76.1 ± 37.9 (62)	73.2 ± 44.9 (51)	67.1 ± 38.8 (72)	58.7 ± 35.6** (59)
HOMA-IR	2.7 ± 1.6 (62)	2.5 ± 1.5 (51)	2.3 ± 1.3 (71)	1.9 ± 1.2* (57)
LDL-Cholesterol, mg/dL	131.4 ± 35.6 (64)	128.9 ± 32.8 (53)	124.0 ± 34.0 (70)	118.2 ± 34.4* (58)
HDL-Cholesterol, mg/dL	53.9 ± 10.6 (64)	51.5 ± 10.4 (53)	57.3 ± 13.8 (70)	53.7 ± 12.6 (58)
Total Cholesterol, mg/dL	182.8 ± 38.6 (65)	180.4 ± 32.5 (53)	182.3 ± 38.1 (71)	172.1 ± 35.9 (60)
Triglycerides, mg/dL	122.0 ± 57.5 (64)	115.1 ± 64.6 (53)	107.6 ± 55.1 (71)	95.9 ± 44.4** (60)
Leptin, ng/mL	14.6 ± 9.5 (60)	10.5 ± 9.0** (50)	15.1 ± 10.5 (67)	11.1 ± 9.2* (54)
Total Ghrelin, ng/mL	4.5 ± 2.4 (61)	5.0 ± 2.1 (50)	4.8 ± 2.1 (69)	5.4 ± 2.5* (56)
Estradiol, pmol/L	224.2 ± 237.0 (62)	273.1 ± 261.9 (50)	228.7 ± 199.9 (65)	298.8 ± 308.8 (57)
Aspartate Aminotransferase, U/L	29.5 ± 12.0 (65)	27.3 ± 6.4 (53)	28.6 ± 24.8 (71)	24.7 ± 6.2 (60)
Alanine Aminotransferase, U/L	27.8 ± 18.4 (65)	23.2 ± 11.3 (53)	24.4 ± 16.0 (71)	22.2 ± 12.5 (61)
C-Reactive protein, mg/dL	3.7 ± 4.1 (60)	3.0 ± 2.9 (50)	3.2 ± 3.2 (70)	3.0 ± 3.5 (54)
Systolic Blood Pressure, mmHg	119.1 ± 11.8 (71)	120.1 ± 12.1 (61)	120.7 ± 11.2 (78)	117.6 ± 12.7 (69)
Diastolic Blood Pressure, mmHg	71.8 ± 9.2 (71)	72.0 ± 8.7 (61)	73.6 ± 9.1 (78)	71.3 ± 7.9 (69)
Resting Heart Rate, bpm	63.8 ± 7.8 (71)	62.8 ± 8.6 (61)	63.0 ± 11.0 (78)	62.6 ± 9.9 (69)

Data are represented as Mean ± SD (n), Within group significance (* = $p < 0.05$, ** = $p < 0.01$), HOMA-IR, Homeostatic model assessment of insulin resistance; HDL, High-density lipoprotein; LDL, Low-density lipoprotein.

Table 3. Energy metabolism (intake, expenditure and physical activity).

	Placebo		Probiotic	
	Baseline	3 months	Baseline	3 months
24h Energy expenditure, kcal/day	1692 ± 251 (62)	1633 ± 292 (52)	1676 ± 262 (68)	1634 ± 246 (58)
Physical Activity Level	1.46 ± 0.06 (72)	1.53 ± 0.13** (63)	1.48 ± 0.07 (78)	1.55 ± 0.15** (68)
Three-day food record				
Mean Total Daily Energy intake, kcal/day	2091 ± 529 (71)	1790 ± 383** (61)	2216 ± 548 (75)	1767 ± 343** (67)
Mean Total Daily Carbohydrates, g	245.4 ± 75.1 (71)	215.5 ± 50.4 (61)	261.5 ± 77.6 (75)	215.8 ± 48.4** (67)
Mean Total Daily Lipids, g	76.9 ± 23.9 (71)	59.2 ± 18.8** (61)	81.9 ± 26.1 (75)	56.5 ± 14.8** (67)
Mean Total Daily Proteins, g	93.3 ± 25.0 (71)	91.8 ± 19.6 (61)	93.2 ± 26.0 (75)	93.2 ± 23.2 (67)
Carbohydrates, %	46.8 ± 7.7 (71)	48.2 ± 4.9 (61)	47.4 ± 8.0 (75)	48.9 ± 5.6 (67)
Lipids, %	33.1 ± 5.9 (71)	29.5 ± 5.7* (61)	33.1 ± 6.5 (75)	28.8 ± 5.3** (67)
Proteins, %	18.2 ± 4.0 (71)	20.8 ± 3.2** (61)	17.1 ± 3.4 (75)	21.1 ± 3.4** (67)

Data are represented as Mean ± SD (n), Within group significance (* = $p < 0.05$, ** = $p < 0.01$)

4.10 Figure Legends

Figure 1. Eating behaviors. Scores for (A) the Binge Scale, n=72 at baseline and n=63 at 3-months for the placebo group and n=78 at baseline and n=69 at 3-months for the probiotic group, (B-C) the Three-Factor Eating Questionnaire, n=71-72 at baseline and n=62-63 at 3-months for the placebo group and n=78 at baseline and n=69 at 3-months for the probiotic group and (D-E) the State and Trait Food-Craving Questionnaire, n=72 at baseline and n=63 at 3-months for the placebo group and n=78 at baseline and n=69 at 3-months for the probiotic group. Desire = Intense desire to eat, Anticipation of positive = Anticipation of positive reinforcement, Relief from negative = Anticipation of relief from negative states, Physio. state = Physiological state, Consume = Intention and planning to consume food, Thoughts = Thoughts or preoccupation and Cues = Cues (signal/environment). Data are represented as Mean ± SD, Within group significance (* = $p \leq 0.05$, ** = $p<0.01$), group-by-time interaction significance ($^{\#}$ = $p \leq 0.05$, $^{\#\#}$ = $p<0.01$).

Figure 2. Mood-related parameters. Scores for (A) body esteem, n=72 at baseline and n=63 at 3-months for the placebo group and n=76 at baseline and n=68 at 3-months for the probiotic group, (B) anxiety (state and trait), n=72 at baseline and n=63 at 3-months for the placebo group and n=77-78 at baseline and n=69 at 3-months for the probiotic group, (C) depression, n=72 at baseline and n=63 at 3-months for the placebo group and n=74 at baseline and n=66 at 3-months for the probiotic group, (D) perceived stress, n=72 at baseline and n=63 at 3-months for the placebo group and n=78 at baseline and n=69 at 3-months for the probiotic group, (E) gastrointestinal symptoms, n=72 at baseline and n=63 at 3-months for the placebo group and n=78 at baseline and n=69 at 3-months for the probiotic group. Data are represented as Mean ± SD, Within-group significance (*= $p \leq 0.05$, **= $p<0.01$).

Figure 3. Absolute quantification of probiotic in fecal samples. Specific probiotic strain quantification measured by qPCR before and after the intervention. n=30 for placebo group and n=37 for probiotic group.

4.11 Figures

Figure 1

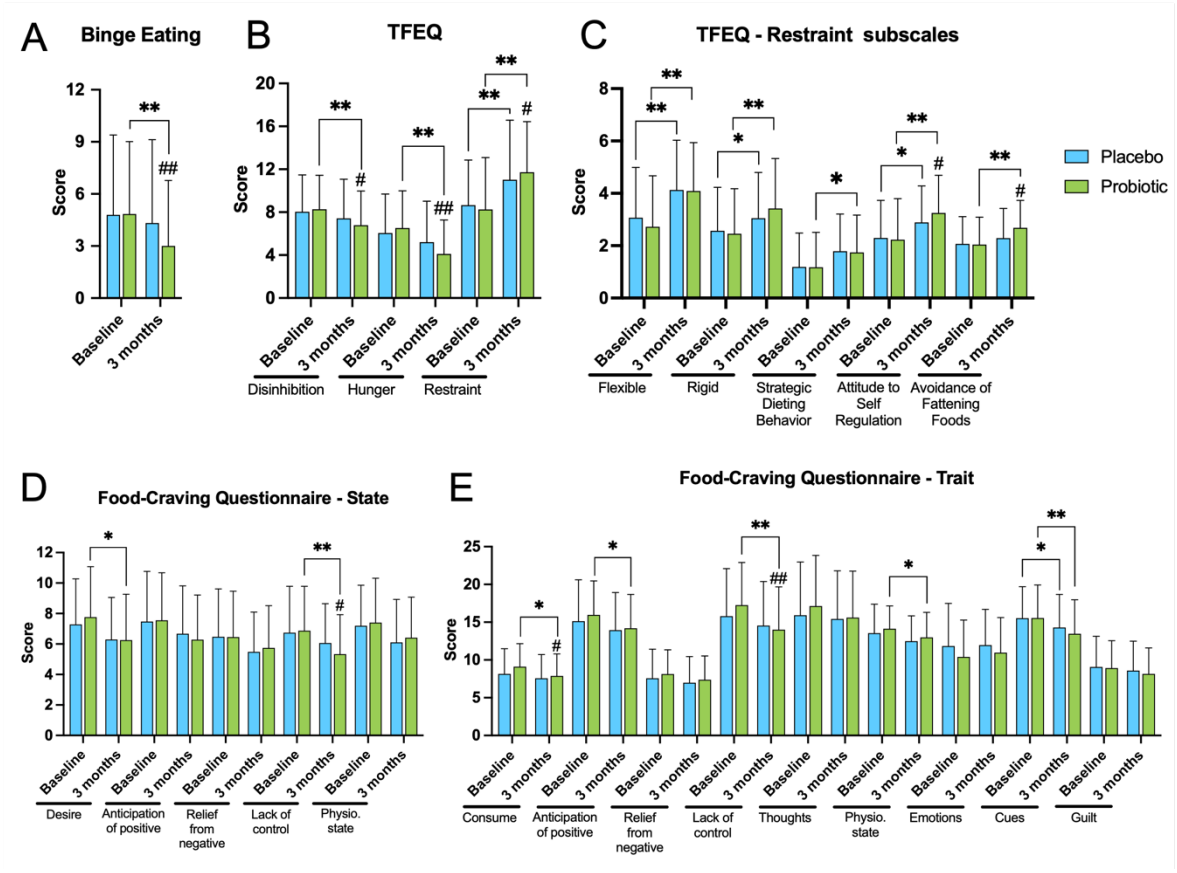


Figure 2

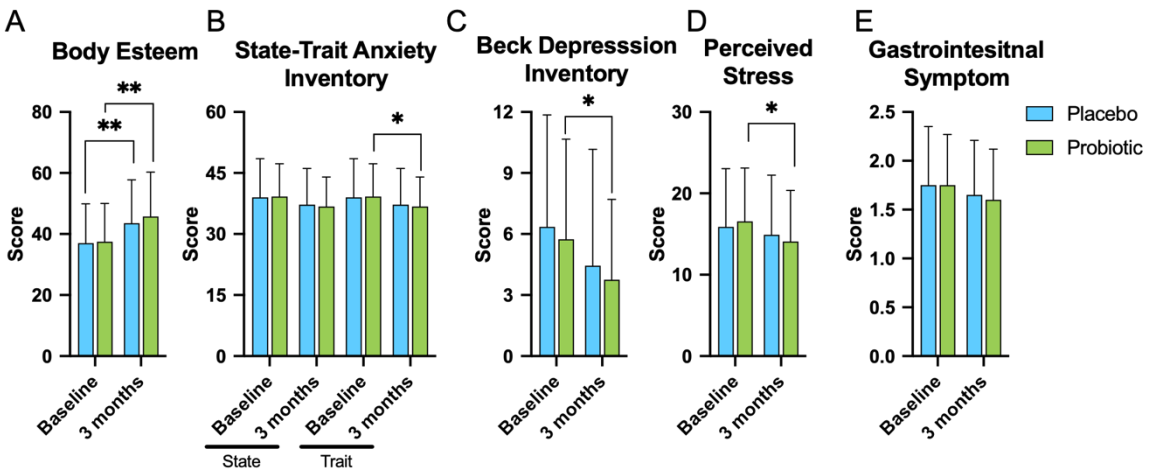
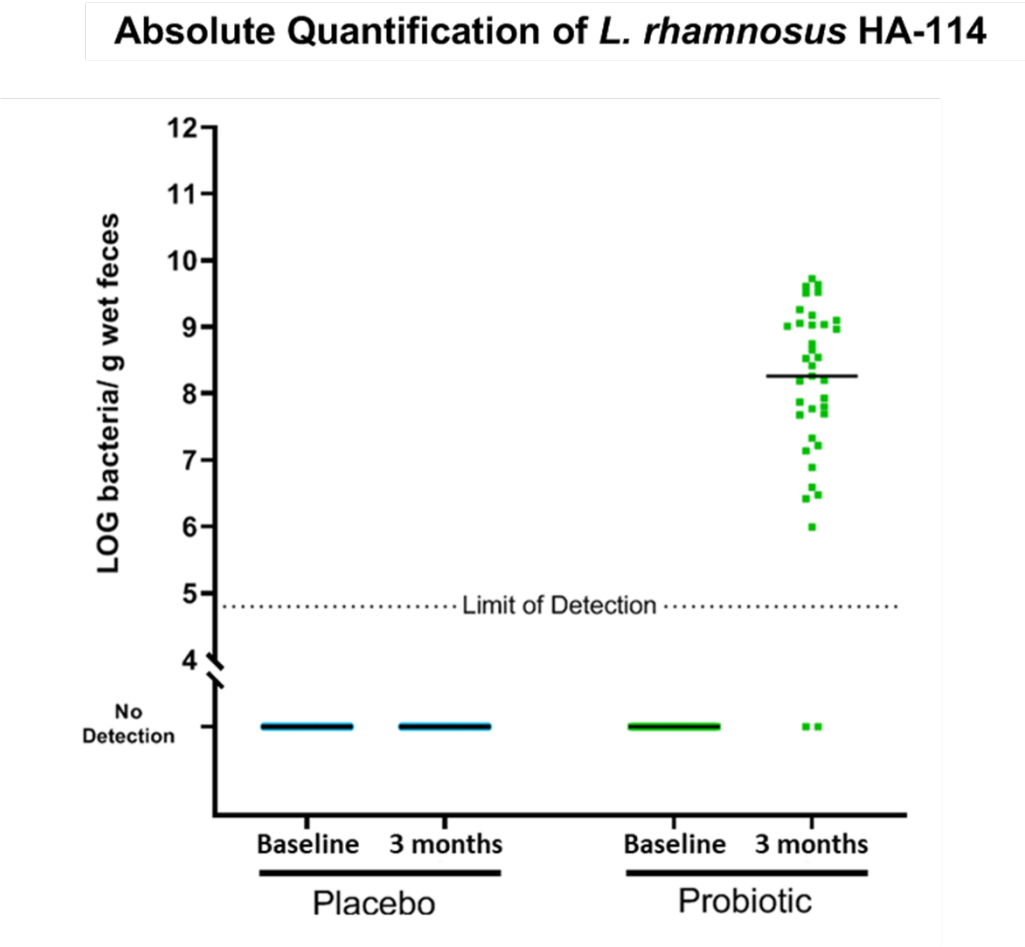
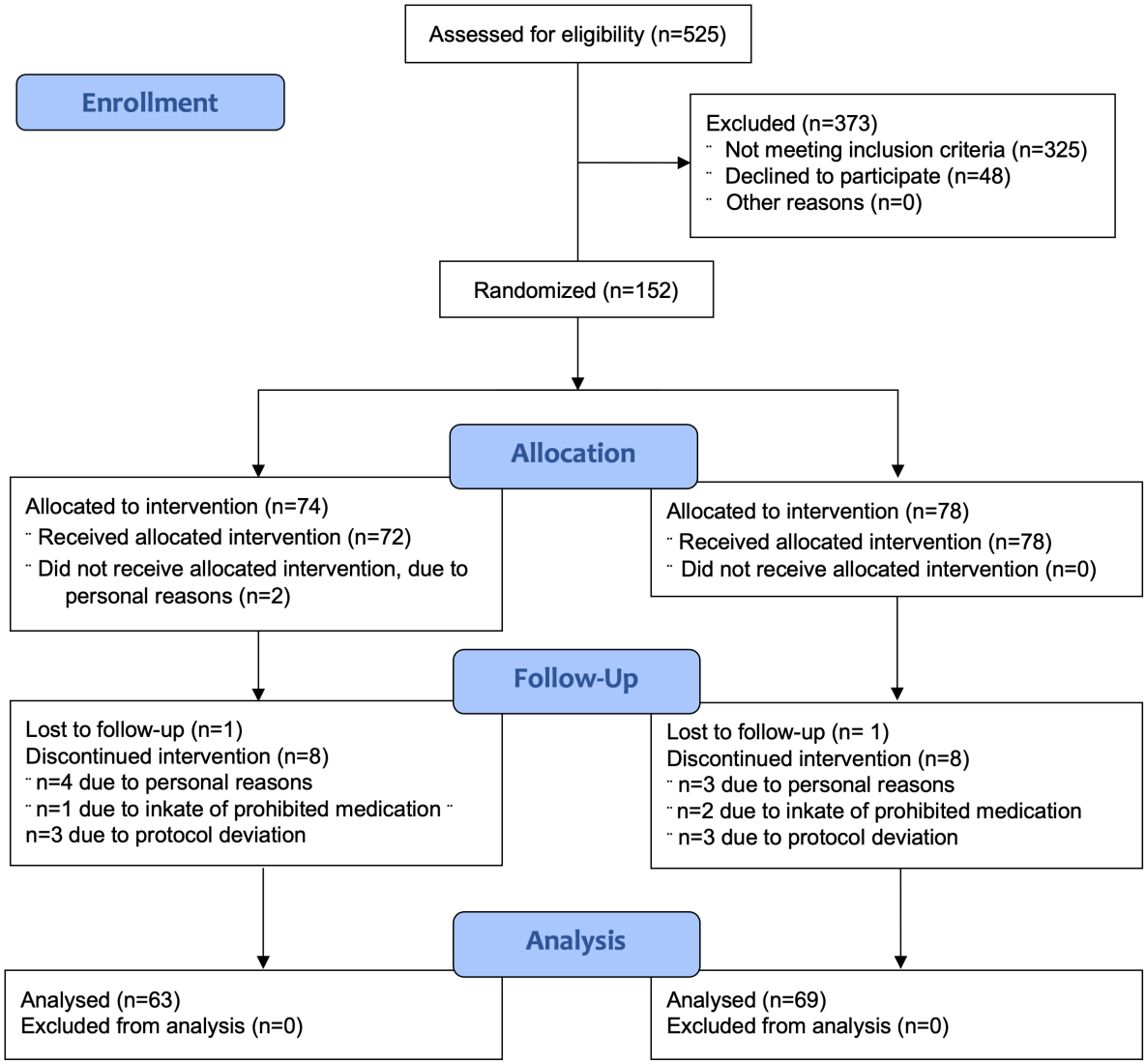

A
Body Esteem
B
State-Trait Anxiety Inventory
C
Beck Depresssion Inventory
D
Perceived Stress
E
Gastrointesitnal Symptom
Score
Baseline
3 months
State
Trait
Placebo
Probiotic

Figure 3


Absolute Quantification of L. rhamnosus HA-114
LOG bacteria/ g wet feces
12
11
10
9
8
7
6
5
4
Limit of Detection
No Detection
Baseline
3 months
Baseline
3 months
Placebo
Probiotic

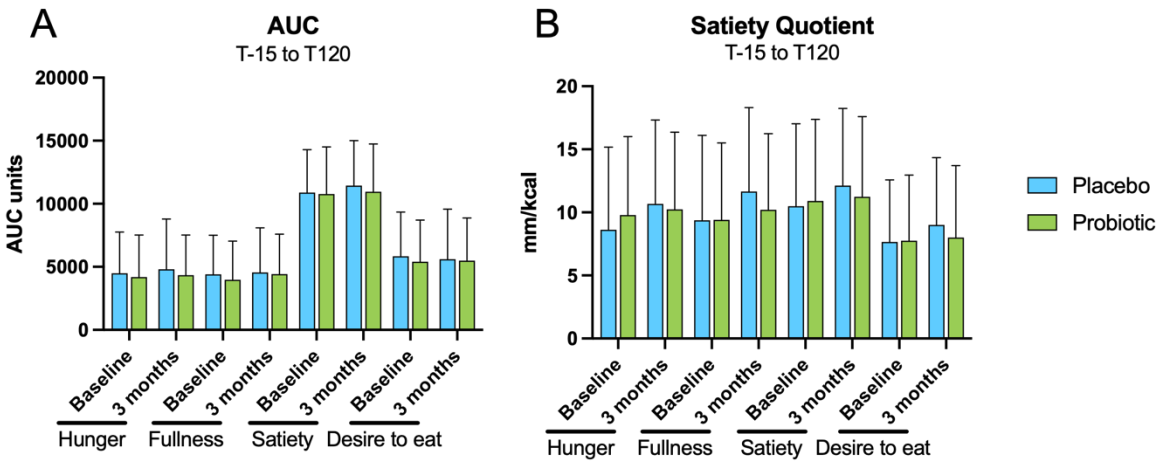
4.12 Supplementary Material

Supplementary Figure 1



Supp. Figure 1. CONSORT flow chart of the study protocol.

Supplementary Figure 2



Supp Fig 2. Visual analog scales for hunger, fullness, satiety and desire to eat. (A) Area under the curve for the standardized breakfast meal, n=69-72 at baseline and n=60-61 at 3-months for the placebo group and n=74 at baseline and n=65-66 at 3-months for the probiotic group and (B) satiety quotient for the standardized breakfast meal, n=72 at baseline and n=63 at 3-months for the placebo group and n=78 at baseline and n=68-69 at 3-months for the probiotic group. Data are represented as Mean ± SD.

Discussion

L'année 2021 marquant la 100^{e} année de la découverte de l'insuline [372], rappelle que malgré l'avancement constant des connaissances, certaines découvertes peuvent révolutionner la façon dont on comprend et traite une maladie. Sans avoir eu un impact direct aussi important, la compréhension du rôle du microbiote intestinal et de la relation avec son hôte est selon moi un élément clé qui redéfinira le traitement de nombreuses maladies chroniques, telles que le diabète, certains types de cancers, la dépression, etc. À la suite des projets inclus dans cette thèse, plusieurs sujets connexes seront abordés dans la section qui suit, dont les habitudes alimentaires, les approches personnalisées, les modèles, la reproductibilité et la transposabilité des résultats de recherche. De plus, des perspectives spécifiques découlant des travaux effectués et générales seront discutées.

1. Changements des habitudes alimentaires et impact sur la santé métabolique

Les aliments sont la somme des composantes (nutriments, polluants, agents de conservations, etc.) qu'ils contiennent, mais doivent aussi être considérés comme un tout, puisque la structure et la matrice alimentaire ont aussi un rôle important au niveau de l'absorption des nutriments. Par exemple, à quantité égale, l'absorption du calcium du lait comparativement à celui d'une boisson de soya fortifiée, sera plus grande en consommant le lait, puisque la molécule est davantage biodisponible dans cette matrice [373].

Les habitudes alimentaires, autrefois influencées par la disponibilité des ressources et les saisons, ne dépendent plus de ces facteurs dans les populations industrialisées grâce à l'abondance des aliments et l'augmentation de l'efficacité de l'industrie agroalimentaire. L'utilisation de pesticides, d'outils automatisés ou encore de sélection génétique pour la production ou la résistance permet d'accroître la quantité d'aliments produits. Par contre, plusieurs études ont tenté de démontrer que l'agriculture biologique a un impact positif sur la santé, donc que malgré l'augmentation de la production, certaines mesures d'optimisation devraient être diminuées pour favoriser les bénéfices des aliments sur la santé humaine et sur l'environnement [374].

Un exemple particulièrement connu des impacts néfastes de l'utilisation de produits chimiques pour favoriser une plus grande production agricole est l'utilisation de l'insecticide nommé Dichlorodiphenyltrichloroethane (DDT). Au début des années 70, cette utilisation a grandement

diminué due à un mouvement environnementaliste initié, entre autres, par la publication du livre *Silent Spring* de Rachel Carson en 1962. Le DDT est maintenant reconnu comme un perturbateur endocrinien néfaste pour la santé animale et humaine [51]. Il peut également induire des changements épigénétiques transmissibles sur plusieurs générations [375] et est encore présent dans l'environnement, l'alimentation et détectable dans notre sang malgré le fait que son utilisation soit bannie depuis des décennies, puisqu'il fait partie de la catégorie des POP, étudiés dans le chapitre 1.

Depuis quelques années, l'alimentation à base végétale gagne énormément en popularité, notamment pour l'impact direct sur la santé du consommateur. Les effets bénéfiques comparés aux aliments d'origine animale peuvent être expliqués par plusieurs facteurs, qui sont liés aux chapitres 1, 2 et 3. Tout d'abord, les végétaux contiennent beaucoup moins de POP [356] et autres polluants lipophiles qui s'accumulent majoritairement dans les tissus animaux [376]. Ensuite, les végétaux sont sources de fibres et de polyphénols, donc de prébiotiques ayant des propriétés bénéfiques bien connues sur la santé à travers le microbiote intestinal [227]. De plus, les types de macronutriments qu'ils contiennent, donc les glucides, lipides et sources de protéines semblent avoir des impacts favorables sur la santé par rapport aux sources de macronutriments retrouvés dans certaines sources animales, telles que la viande [185, 192]. Finalement, outre les effets positifs sur la santé du consommateur, un enjeu mis de l'avant pour l'alimentation végétale est également la composante environnementale, puisque la pollution liée à la production végétale est moins importante que celle liée à la production animale [377].

Les aliments fermentés sont fabriqués grâce à la croissance microbienne et aux conversions enzymatiques souhaitées des composants alimentaires [378] et ce processus peut ajouter la présence de bactéries potentiellement probiotiques aux aliments [379]. La fermentation de végétaux étant présente dans de nombreuses cultures depuis des décennies, principalement pour la conservation des aliments, est au cœur de l'alimentation de certaines populations. Par exemple le kimchi, un condiment à base de chou fermenté, qui est consommé en Corée du Sud accompagne tous les repas [380]. De plus, certaines bactéries en soi peuvent être une source de protéines, qui pourrait être bénéfiques pour la santé [381] qui, comme d'autres sources alternatives telles que les insectes, pourraient avoir un potentiel prometteur [382]. Bien qu'il est possible de fermenter des produits d'origine animale, dont les produits laitiers fermentés, qui sont associés à un phénotype métabolique positif [383], le potentiel de fermentation d'aliments d'origine végétale est plus diversifié, puisqu'il est possible de fermenter de nombreux grains, fruits, légumes et légumineuses [378].

Outre les quantités et la qualité des aliments consommés, d'autres facteurs sont à prendre en considération lorsqu'on parle d'habitudes alimentaires, dont l'environnement dans lequel les aliments sont consommés et les comportements alimentaires. Ces facteurs sont d'ailleurs discutés dans le nouveau guide alimentaire Canadien 2019, qui recommande notamment de savourer ses aliments et de manger en bonne compagnie. Bien que ces facteurs soient souvent plus difficiles à étudier et moins quantitatifs que les mesures de quantités d'aliments consommés, ils sont quand même importants à prendre en considération et à comprendre. Dans le chapitre 4, l'environnement visuel, sonore et olfactif dans lequel les repas standardisés étaient consommés était très contrôlé, pour éviter d'influencer les mesures de sensations de satiété.

Une autre nuance importante à faire est l'impact des saines habitudes de vie comparativement au poids, sur la santé. En effet, un important mouvement contre la discrimination basée sur le poids, qu'elle soit dans la vie de tous les jours, dans un contexte professionnel [384] ou médical [385] a débuté dans les dernières années. En effet, de nombreuses études ont montré des corrélations entre le poids et une pléiotropie de maladies, mais des études plus récentes montrent que le type de graisse accumulée (notamment viscérale par rapport à sous-cutanée) [386] et les habitudes de vie incluant l'alimentation, la pratique d'activité physique [387] sont de meilleurs prédicteurs sur le développement de maladies cardiovasculaires que l'IMC d'un individu. Pour cette raison, dans le chapitre 4, l'activité physique était mesurée même si elle ne faisait pas l'objet de notre étude, pour éviter d'induire une valeur confondante.

Globalement, la majorité des évidences sont en faveur d'une alimentation riche en végétaux et peu transformée, telle que la diète méditerranéenne, pour avoir des bénéfices au niveau du microbiote intestinal et de la santé cardio-métabolique [37]. Par contre, un nouveau niveau d'évidence, grâce à l'accès à de grandes cohortes caractérisées pour une multitude de facteurs permet de considérer la nutrition personnalisée comme étant plus adéquate par rapport à des recommandations générales pour l'ensemble de la population [90].

2. Personnalisation des interventions nutritionnelles et médicales

Le concept de médecine et de nutrition personnalisées est de plus en plus populaire, surtout pour le traitement de maladies chroniques [40]. Un traitement par rapport à un autre pourrait donc être favorisé selon la génétique ou la composition du microbiote intestinal d'un individu [388]. En plus de ces paramètres très précis, d'autres sont importants pour déterminer les recommandations que des

professionnels de la santé donnent à leurs patients, notamment, le sexe, l'âge, le mode de vie, l'origine ethnique, la motivation, etc. L'environnement et la nourriture traditionnelle devraient aussi être prise en compte. Par exemple, il a été démontré que la consommation de maaqtaq de beluga contribuerait à protéger les Inuits contre l'absorption du méthyl mercure grâce à la sélénonéine, un nutriment présent dans cet aliment [389]. De plus, si le traitement ou le changement d'alimentation prescrit est trop difficile à adopter pour le patient, il y a peu de chances qu'il soit respecté et il a été montré qu'en contexte de perte de poids, c'est l'assiduité à une diète et non la diète en soi qui expliquerait le plus grand changement de masse corporelle [83].

D'autre part, la majorité des études faites ne prennent pas en compte plusieurs principes d'équité, diversité et inclusion. Par exemple, lors de l'utilisation de modèles animaux, la très grande majorité des études cardio-métaboliques est faite en très grande majorité sur des mâles, alors qu'il est bien connu que le développement de pathologies n'est pas du tout le même entre l'homme et la femme, notamment les manifestations de problèmes cardiovasculaires [390]. Il est donc primordial de favoriser l'utilisation de modèles animaux des deux sexes, mais aussi la reproduction des résultats d'interventions cliniques sur des cohortes indépendantes et diversifiées, selon l'âge, le sexe, l'origine ethnique, etc.

Des approches personnalisées pour favoriser certaines bactéries au sein du microbiote intestinal pourraient donc être pertinentes à effectuer avant le début d'un traitement, pour assurer son succès. En ce qui concerne le microbiote intestinal, il est maintenant connu que de nombreux médicaments, tels que la metformine, ont un impact sur sa composition. En effet, le microbiote intestinal contribue à la régulation du diabète de ce médicament [142]. Dans le domaine du cancer, il a été démontré que la présence ou l'absence de certaines bactéries intestinales pouvait influencer la réponse aux traitements [391] ainsi que les effets secondaires ressentis [392].

Au niveau des transplantations fécales, leur potentiel pour enrayer les infections au *c. difficile* résistance aux antibiotiques a été montré [393], ce qui a ouvert les possibilités thérapeutiques de cette technique à d'autres domaines [394]. Cependant, les premières études dans le domaine de l'obésité montrent des résultats mitigés [395, 396] et certaines questions telles que comment choisir le bon donneur, assurer une bonne colonisation sont à considérer pour assurer un effet optimal pour le receveur. De plus, la réponse, du moins sur la résistance à l'insuline, pourrait également dépendre du microbiote de base du receveur de la FMT [274]. Dans le cadre du chapitre 2, nous avons démontré que l'importance des conditions d'hébergements sur le phénotype observé à la suite d'une FMT. En

effet, même si certaines bactéries ont bien colonisé l'intestin des souris dans les deux secteurs, nous avons observé un phénotype hépatique très différent entre les conditions d'hébergement. Ces résultats renforcent le besoin de mieux comprendre les conditions nécessaires à l'implantation d'une FMT, pour assurer un succès de cette technique non seulement chez la souris, mais également chez l'humain. De plus, cette approche pourrait éventuellement permettre d'évaluer l'efficacité ou la réponse de certains traitements de façon personnalisée en essayant les différents traitements sur des souris avec un phénotype similaire et le microbiote du patient.

3. Modèles, reproductibilité et transposabilité des résultats chez l'humain

Puisqu'il est impossible de tester toutes les hypothèses scientifiques directement chez l'humain, l'utilisation de modèles est essentielle à l'avancement des connaissances dans le domaine de la santé. Par contre, tous les modèles viennent avec leurs limites, dont il est important d'être conscient. Dans le domaine des maladies métaboliques et du microbiote intestinal, les rongeurs sont très utilisés [397], puisqu'ils ont des tissus et caractéristiques similaires à l'humain, comme ce sont des mammifères. En revanche, de plus en plus de nouveaux modèles, ayant des avantages différents, que ce soit au niveau éthique, technique, etc. sont utilisés, mais ceux-ci comportent également leurs limites. Par exemple, des modèles *in vitro* de structure tridimensionnelle comme les organoïdes ou encore les systèmes digestifs reproduits, comme le SHIME®, les modèles *ex vivo* permettant d'isoler un seul tissu, par exemple le foie, d'autres modèles *in vitro*, comme le poisson zèbre, *c. elegans*. Finalement les modèles *in silico* sont de plus en plus populaires, puisqu'ils tentent de prédire des réactions biologiques sans avoir à utiliser le vivant, grâce aux avancées dans le domaine de l'intelligente artificielle.

Tel que mentionné plus tôt, le modèle du rongeur reste le plus courant, mais une amélioration continue du potentiel de ce modèle est également observée dans plusieurs domaines. Par exemple, en optimisant les diètes utilisées ou encore à travers les modèles génétiques qui peuvent être induits. Dans le cadre de cette thèse, le chapitre 3 fait référence à l'utilisation d'une nouvelle diète, qui permet d'induire un phénotype plus sévère et qui se rapproche du phénotype humain, sans avoir à utiliser un modèle génétique. Une autre façon de plus en plus populaire est de tenter d'humaniser ce modèle, notamment en le colonisant avec un microbiote intestinal humain ou encore en lui donnant des cellules immunitaires humaines [398]. L'humanisation des diètes pour étudier des phénotypes chez les rongeurs permettrait d'obtenir des résultats avec un plus grand potentiel translationnel, qui pourrait être combiné à la colonisation des animaux avec un microbiote intestinal d'origine humaine.

Peu importe le modèle utilisé, un aspect primordial est d'assurer un potentiel translationnel à l'humain. Ceci peut être effectué en assurant la reproductibilité des résultats, qu'ils soient positifs ou négatifs et leur diffusion, pour permettre à d'autres de bâtir sur ces nouvelles connaissances. Par exemple, un article montrant que l'ajout d'une transplantation fécale à un changement de l'alimentation chez des sujets ayant un syndrome métabolique n'accentue pas les effets bénéfiques de la diète seule [399]. Ces résultats négatifs permettent tout de même de tirer plusieurs conclusions et d'évaluer les différents paramètres de l'étude qui pourraient expliquer l'absence de différence, par exemple la courte durée de l'étude, qui n'était que deux semaines.

4. Perspectives

Des perspectives communes et très générales sont applicables aux différents chapitres de cette thèse, mais des perspectives à chaque projet sont aussi à considérer. La première perspective, commune aux trois premiers chapitres, réalisés chez l'animal, est que ces protocoles devraient être reproduits chez la souris femelle, puisqu'ils ont tous été effectués chez le mâle.

4.1 Perspectives par chapitre

La première perspective pour le chapitre 1 portant sur l'effet détoxifiant d'un extrait de canneberge sur l'exposition endogène aux POP, tout d'abord, serait de reproduire l'étude et d'essayer de mieux comprendre les mécanismes d'action qui expliquent le phénotype observé. En effet, l'hypothèse est que le microbiote a un rôle à jouer dans cette interaction entre le supplément et l'excrétion des polluants. Identifier quelles bactéries précisément ont contribué à cet effet, notamment par la présence d'enzymes impliquées dans la dégradation des polluants permettrait de les considérer comme de potentiels probiotiques. En effet, dans l'environnement, lorsqu'il y a un déversement de ces types de polluants, la technique la plus efficace pour les dégrader est la bioremédiation [400], donc la dégradation par des microorganismes. Les deux façons d'obtenir cet effet est de supplémenter le milieu avec les microorganismes, mais aussi de leur fournir tous les substrats nécessaires pour bien proliférer.

Sur l'aspect translationnel, chez l'humain, il serait intéressant d'aller voir si une modulation du microbiote par différentes interventions ou supplémentations, telles que la canneberge, en contexte de perte de poids peut également affecter les quantités de polluants retrouvés en circulation. Globalement, l'objectif à long terme de ces travaux serait de trouver une façon simple et sécuritaire

d'augmenter l'excrétion et la dégradation des polluants lors de grandes expositions, qu'elles soient endogènes, par la perte de poids, ou exogènes.

Pour le chapitre 2, portant sur différentes conditions d'hébergement affectant le phénotype de souris axéniques à la suite de leur colonisation, encore une fois, reproduire ces résultats et tester d'autres conditions d'hébergement seraient selon moi les premières étapes à poursuivre. En effet, ce projet démontre l'importance des détails techniques lors de protocole de transplantation fécale, qui sont de plus en plus utilisés pour démontrer le rôle causal du microbiote intestinal. Par contre, une principale lacune de cette technique est qu'elle n'est pas du tout standardisée et qu'il y a donc une très grande variabilité entre les différentes équipes de recherche qui l'utilisent [401]. Un manque de standardisation diminue la possibilité d'obtenir des résultats robustes et reproductibles, qui sont essentiels pour permettre des effets transposables à l'humain. De ce fait, il serait important d'identifier les paramètres méthodologiques optimaux de cette technique et de s'assurer que ceux-ci soient respectés dans le domaine. Cela nous permettra également de mieux comprendre les paramètres clés qui influencent la colonisation du microbiote, un sujet qui peut avoir des répercussions importantes pour les jeunes enfants, en particulier ceux nés par césarienne [113].

Pour le chapitre 3, portant sur les sources de protéines, ce projet aura des perspectives à deux niveaux différents. Tout d'abord, à la suite de la démonstration claire de l'impact des sources de protéines sur le développement de l'obésité et de la résistance à l'insuline chez la souris, il serait intéressant de voir si la substitution de certaines sources de protéines dans l'alimentation humaine peut également avoir cet effet. Par exemple, est-ce que les substituts de produits laitiers à base de soya sont meilleurs pour la santé métabolique que les produits d'origine, dont le lait et le yogourt.

Ensuite, il est primordial de mieux comprendre les mécanismes d'action impliqués dans les effets métaboliques des différentes sources de protéines, qu'ils passent par le microbiote intestinal ou non. Dans le chapitre 3, nous avons montré que par transplantation fécale, on pouvait reproduire une partie des effets, mais pas l'entièreté. De plus, les protéines sont majoritairement catabolisées et absorbées dans le petit intestin, mais on voit quand même des changements importants dans les bactéries et les métabolites microbiens mesurés dans les échantillons fécaux des animaux. Il est donc logique de penser que certains des modes d'actions sont dépendants du microbiote intestinal et d'autres sont indépendants.

Le potentiel des BCFA sur l'augmentation de la production de glucose et la résistance à l'insuline a été montré *in vitro* sur des hépatocytes, mais il reste à déterminer si ces métabolites ont aussi un impact sur le foie entier, notamment avec des expériences *in vivo* et *ex vivo.* Le TMAO est également une piste intéressante, puisqu'il a été associé aux désordres cardiovasculaires [402], mais qu'on le retrouve dans le poisson, dont la consommation a été associée à une bonne santé cardio-métabolique [403]. La quantité d'azote, souvent assumée comme équivalente dans les différentes sources de protéine est une autre hypothèse qui mériterait d'être étudiée davantage. L'excès d'azote pourrait contribuer à la surcharge mitochondriale et du cycle de l'urée dans le foie, ce qui accentuerait l'inflammation et la résistance à l'insuline dans ce tissu. Finalement, une augmentation claire et reproduite de la bactérie *Akkermansia muciniphila* chez les animaux consommant de la caséine comme unique source de protéine par rapport aux animaux consommant le mix de protéine ouvre également le potentiel qu'une partie des effets positifs de cette source protéique soit due à un effet prébiotique sur *Akkermansia*, dont plusieurs mécanismes d'action protecteurs contre les désordres métaboliques ont été démontrés [148, 404].

Les perspectives du chapitre 4 se divisent également en deux parties. Tout d'abord, au niveau mécanistique, mieux comprendre la cause des effets psychologiques observés. Plusieurs hypothèses, notamment la production de sérotonine par le probiotique ou la modification du profil d'acides biliaires, qui peuvent avoir un impact sur le système nerveux central [405] seraient à tester. De plus, il serait intéressant de mesurer l'impact de la supplémentation de *L. rhamnosus* HA-114 sur une population particulièrement vulnérable, soit des sujets qui souffrent de dépression et d'obésité. Le dernier facteur qui serait intéressant de mieux comprendre est les effets à long terme, notamment si les changements induits sur une période de 12 semaines persistent dans le temps et influencent davantage la santé métabolique et psychologique, ou encore s'il est nécessaire de prendre le probiotique en continue pour continuer d'avoir des effets bénéfiques.

4.2 Perspectives générales

De façon globale, l'étude d'interventions nutritionnelles sur le microbiote intestinal, dans différents contextes permet de faire avancer l'état des connaissances des interactions complexes impliquées dans les effets observés. Cependant, les interventions nutritionnelles et supplémentation en prébiotiques, probiotiques, etc. sont considérées comme non ciblées, puisqu'elles ne sont pas sélectives à une bactérie ou une fonction bactérienne. Grâce aux avancées technologiques, plusieurs interventions ciblées commencent à être mises au point [44]. Les exemples les plus concrets sont la thérapie phagique, des composés pharmaceutiques ayant des effets sélectifs sur le métabolisme

bactérien, l'application de la technique CRISPR-Cas9 ou encore des bactéries bio-ingénérées. Cette dernière technique pourrait notamment permettre de créer des probiotiques personnalisés selon l'individu qui le consomme et les effets désirés.

Un autre secteur d'étude très intéressant est les conditions de l'environnement dans lequel les bactéries se trouvent, notamment la quantité de nutriments, de mucus, de molécules immunitaires de l'hôte, le pH, la température, le taux d'oxygène, le temps de transit, etc. Ces facteurs ont certainement un impact sur la composition du microbiote intestinal et inversement sont impactés par celui-ci, ce qui peut grandement influencer l'équilibre entre l'hôte et les bactéries qu'il contient.

Finalement, il est primordial de garder en tête que dans toutes interactions complexes, il faut déterminer si les changements observés sont seulement des corrélations ou si un lien causal peut être établi. La causalité est clé puisque des nouvelles recommandations ou thérapies peuvent découler de ces résultats. En ce qui concerne le microbiote intestinal et son impact sur l'hôte, les molécules microbiennes sont la meilleure façon d'établir un lien causal à ce jour [406].

Conclusion

Cette thèse contribue à l'avancement des connaissances dans la compréhension des interactions complexes entre l'alimentation, le microbiote intestinal et la santé métabolique. À travers les différents chapitres, des résultats *in vitro, in vivo* et cliniques ont été montrés et la combinaison de ces différents niveaux d'évidences contribue à démontrer la validité des résultats. En effet, je pense qu'il est très important de combiner différentes méthodes expérimentales pour montrer l'efficacité d'interventions nutritionnelles ainsi que les mécanismes d'actions impliqués.

Plusieurs perspectives avec des objectifs clés dans différents aspects en lien avec l'obésité et le rôle du microbiote intestinal sont discutés. Ces projets portent sur la perte de poids et la libération de polluants, les facteurs environnementaux impliqués dans la colonisation du microbiote, la prévention du développement de l'obésité et de la résistance à l'insuline et finalement la diminution de comportements alimentaires néfastes et de paramètres psychologiques tels que le stress, l'anxiété et la dépression.

Concrètement, cette thèse contribue à notre compréhension de différentes composantes de l'alimentation humaine en contexte de désordres métaboliques, notamment les protéines alimentaires, les polyphénols de petits fruits et les polluants. En ajoutant ces composantes aux connaissances notamment sur les fibres, les glucides, les lipides et les additifs alimentaires, il serait concevable de réfléchir à une nouvelle diète pour les modèles animaux, représentative de l'alimentation humaine et favorable au développement de désordres métaboliques et d'une dysbiose. De plus, une meilleure compréhension des facteurs influençant la colonisation de l'intestin par les bactéries provenant d'une transplantation fécale permettra aussi de réguler les modèles murins humanisées, soient colonisés avec des échantillons fécaux humains. La combinaison d'une diète humanisée et la colonisation du microbiote de nos modèles par des échantillons humains permettrait une meilleure transposabilité des résultats chez l'humain.

Ces travaux et ceux qui en découleront auront certainement un impact sur différents aspects de prévention et de traitement de l'obésité et des désordres métaboliques, en utilisant tout le potentiel du microbiote intestinal, pour lequel il reste encore beaucoup à comprendre et à découvrir.

Bibliographie

1. Wharton, S., et al., *L'obésité chez l'adulte : ligne directrice de pratique clinique.* Canadian Medical Association Journal, 2020. **192**(49): p. E1757-E1775.
2. Lee, D.H., et al., *Chlorinated persistent organic pollutants, obesity, and type 2 diabetes.* Endocr Rev, 2014. **35**(4): p. 557-601.
3. WHO, *World Health Statistics 2021*. 2021. p. 121.
4. Razak, F., et al., *Ethnic differences in the relationships between obesity and glucose-metabolic abnormalities: a cross-sectional population-based study.* Int J Obes (Lond), 2005. **29**(6): p. 656-67.
5. Mulligan, A.A., et al., *Changes in waist circumference and risk of all-cause and CVD mortality: results from the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) cohort study.* BMC Cardiovasc Disord, 2019. **19**(1): p. 238.
6. Myint, P.K., et al., *Body fat percentage, body mass index and waist-to-hip ratio as predictors of mortality and cardiovascular disease.* Heart, 2014. **100**(20): p. 1613-9.
7. Lemieux, I., et al., *Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men?* Circulation, 2000. **102**(2): p. 179-84.
8. Amato, M.C. and C. Giordano, *Visceral adiposity index: an indicator of adipose tissue dysfunction.* Int J Endocrinol, 2014. **2014**: p. 730827.
9. Amato, M.C., V. Guarnotta, and C. Giordano, *Body composition assessment for the definition of cardiometabolic risk.* J Endocrinol Invest, 2013. **36**(7): p. 537-43.
10. Saely, C.H., K. Geiger, and H. Drexel, *Brown versus white adipose tissue: a mini-review.* Gerontology, 2012. **58**(1): p. 15-23.
11. Almandoz, J.P., et al., *Spillover of Fatty acids during dietary fat storage in type 2 diabetes: relationship to body fat depots and effects of weight loss.* Diabetes, 2013. **62**(6): p. 1897-903.
12. Despres, J.P. and I. Lemieux, *Abdominal obesity and metabolic syndrome.* Nature, 2006. **444**(7121): p. 881-7.
13. Neeland, I.J., et al., *Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement.* The Lancet Diabetes & Endocrinology, 2019. **7**(9): p. 715-725.
14. Tchernof, A. and J.P. Despres, *Pathophysiology of human visceral obesity: an update.* Physiol Rev, 2013. **93**(1): p. 359-404.
15. Neeland, I.J., et al., *Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults.* Obesity (Silver Spring), 2013. **21**(9): p. E439-47.
16. Carpentier, A.C., et al., *Brown Adipose Tissue Energy Metabolism in Humans.* Front Endocrinol (Lausanne), 2018. **9**: p. 447.
17. Ikeda, K., P. Maretich, and S. Kajimura, *The Common and Distinct Features of Brown and Beige Adipocytes.* Trends Endocrinol Metab, 2018. **29**(3): p. 191-200.
18. Blondin, D.P., et al., *Increased brown adipose tissue oxidative capacity in cold-acclimated humans.* J Clin Endocrinol Metab, 2014. **99**(3): p. E438-46.
19. Calder, P.C., et al., *Dietary factors and low-grade inflammation in relation to overweight and obesity.* Br J Nutr, 2011. **106 Suppl 3**: p. S5-78.
20. Cinti, S., et al., *Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans.* J Lipid Res, 2005. **46**(11): p. 2347-55.
21. Trayhurn, P., *Hypoxia and adipose tissue function and dysfunction in obesity.* Physiol Rev, 2013. **93**(1): p. 1-21.
22. Cani, P.D., et al., *Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity.* Gut Microbes, 2012. **3**(4): p. 279-88.
23. Bluher, M., *Obesity: global epidemiology and pathogenesis.* Nat Rev Endocrinol, 2019. **15**(5): p. 288-298.

24. Chami, N., et al., *The role of polygenic susceptibility to obesity among carriers of pathogenic mutations in MC4R in the UK Biobank population.* PLoS Med, 2020. **17**(7): p. e1003196.
25. Bouchard, C., *Genetics of Obesity: What We Have Learned Over Decades of Research.* Obesity (Silver Spring), 2021. **29**(5): p. 802-820.
26. Stunkard, A.J., T.T. Foch, and Z. Hrubec, *A Twin Study of Human Obesity.* JAMA, 1986. **256**(1): p. 51-54.
27. Bouchard, C., et al., *The response to long-term overfeeding in identical twins.* N Engl J Med, 1990. **322**(21): p. 1477-82.
28. Hainer, V., et al., *A twin study of weight loss and metabolic efficiency.* Int J Obes Relat Metab Disord, 2001. **25**(4): p. 533-7.
29. Ozanne, S.E., *Epigenetic signatures of obesity.* N Engl J Med, 2015. **372**(10): p. 973-4.
30. Ling, C. and T. Ronn, *Epigenetics in Human Obesity and Type 2 Diabetes.* Cell Metab, 2019. **29**(5): p. 1028-1044.
31. King, S.E. and M.K. Skinner, *Epigenetic Transgenerational Inheritance of Obesity Susceptibility.* Trends Endocrinol Metab, 2020. **31**(7): p. 478-494.
32. Ost, A., et al., *Paternal diet defines offspring chromatin state and intergenerational obesity.* Cell, 2014. **159**(6): p. 1352-64.
33. Deshpande, S.S., et al., *High-fat diet-induced and genetically inherited obesity differentially alters DNA methylation profile in the germline of adult male rats.* Clin Epigenetics, 2020. **12**(1): p. 179.
34. Vohl, M.C., M.M. Malagon, and B. Ramos-Molina, *Editorial: Dietary Factors, Epigenetics and Their Implications for Human Obesity.* Front Endocrinol (Lausanne), 2020. **11**: p. 601.
35. Ideraabdullah, F.Y. and S.H. Zeisel, *Dietary Modulation of the Epigenome.* Physiol Rev, 2018. **98**(2): p. 667-695.
36. Canada, S. *Guide alimentaire canadien*. 2019; Available from: https://guide-alimentaire.canada.ca/fr/.
37. Estruch, R., et al., *Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts.* N Engl J Med, 2018. **378**(25): p. e34.
38. de Lorgeril, M., et al., *Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease.* Lancet, 1994. **343**(8911): p. 1454-9.
39. Tonstad, S., et al., *Type of vegetarian diet, body weight, and prevalence of type 2 diabetes.* Diabetes Care, 2009. **32**(5): p. 791-6.
40. Zeevi, D., et al., *Personalized Nutrition by Prediction of Glycemic Responses.* Cell, 2015. **163**(5): p. 1079-1094.
41. Li, S., et al., *Physical activity attenuates the genetic predisposition to obesity in 20,000 men and women from EPIC-Norfolk prospective population study.* PLoS Med, 2010. **7**(8).
42. Agus, A., K. Clement, and H. Sokol, *Gut microbiota-derived metabolites as central regulators in metabolic disorders.* Gut, 2020.
43. Koh, A. and F. Backhed, *From Association to Causality: the Role of the Gut Microbiota and Its Functional Products on Host Metabolism.* Mol Cell, 2020. **78**(4): p. 584-596.
44. Fan, Y. and O. Pedersen, *Gut microbiota in human metabolic health and disease.* Nat Rev Microbiol, 2021. **19**(1): p. 55-71.
45. Turnbaugh, P.J., et al., *An obesity-associated gut microbiome with increased capacity for energy harvest.* Nature, 2006. **444**(7122): p. 1027-31.
46. Liang, Y., et al., *New insight into the mechanism of POP-induced obesity: Evidence from DDE-altered microbiota.* Chemosphere, 2020. **244**: p. 125123.
47. Dirinck, E., et al., *Obesity and persistent organic pollutants: possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls.* Obesity (Silver Spring), 2011. **19**(4): p. 709-14.
48. Ridaura, V.K., et al., *Gut microbiota from twins discordant for obesity modulate metabolism in mice.* Science, 2013. **341**(6150): p. 1241214.
49. Lee, Y.M., et al., *Persistent organic pollutants in adipose tissue should be considered in obesity research.* Obes Rev, 2017. **18**(2): p. 129-139.

50. Petrakis, D., et al., *Endocrine Disruptors Leading to Obesity and Related Diseases.* Int J Environ Res Public Health, 2017. **14**(10).
51. Heindel, J.J., R. Newbold, and T.T. Schug, *Endocrine disruptors and obesity.* Nat Rev Endocrinol, 2015. **11**(11): p. 653-61.
52. Eckel, R.H., S.M. Grundy, and P.Z. Zimmet, *The metabolic syndrome.* The Lancet, 2005. **365**(9468): p. 1415-1428.
53. Powell-Wiley, T.M., et al., *Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association.* Circulation, 2021. **143**(21): p. e984-e1010.
54. Kahn, S.E., R.L. Hull, and K.M. Utzschneider, *Mechanisms linking obesity to insulin resistance and type 2 diabetes.* Nature, 2006. **444**(7121): p. 840-6.
55. Younossi, Z., et al., *Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention.* Nat Rev Gastroenterol Hepatol, 2018. **15**(1): p. 11-20.
56. Grundy, S.M., et al., *Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement.* Circulation, 2005. **112**(17): p. 2735-52.
57. James, D.E., J. Stöckli, and M.J. Birnbaum, *The aetiology and molecular landscape of insulin resistance.* Nature Reviews Molecular Cell Biology, 2021.
58. Petrick, H.L., et al., *Adipose Tissue Inflammation Is Directly Linked to Obesity-Induced Insulin Resistance, while Gut Dysbiosis and Mitochondrial Dysfunction Are Not Required.* Function, 2020. **1**(2).
59. Newsholme, P., L. Brennan, and K. Bender, *Amino Acid Metabolism, -Cell Function, and Diabetes.* Diabetes, 2006. **55**(Supplement 2): p. S39-S47.
60. Tremblay, F., et al., *Overactivation of S6 kinase 1 as a cause of human insulin resistance during increased amino acid availability.* Diabetes, 2005. **54**(9): p. 2674-84.
61. Shum, M., et al., *Pharmacological inhibition of S6K1 increases glucose metabolism and Akt signalling in vitro and in diet-induced obese mice.* Diabetologia, 2016. **59**(3): p. 592-603.
62. Tremblay, F., et al., *Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance.* Proc Natl Acad Sci U S A, 2007. **104**(35): p. 14056-61.
63. Newgard, C.B., *Interplay between lipids and branched-chain amino acids in development of insulin resistance.* Cell Metab, 2012. **15**(5): p. 606-14.
64. White, P.J., et al., *Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export.* Mol Metab, 2016. **5**(7): p. 538-551.
65. White, P.J. and C.B. Newgard, *Branched-chain amino acids in disease.* Science, 2019. **363**(6427): p. 582-583.
66. McCoin, C.S., T.A. Knotts, and S.H. Adams, *Acylcarnitines--old actors auditioning for new roles in metabolic physiology.* Nat Rev Endocrinol, 2015. **11**(10): p. 617-25.
67. Schooneman, M.G., et al., *Acylcarnitines: reflecting or inflicting insulin resistance?* Diabetes, 2013. **62**(1): p. 1-8.
68. Schertzer, J.D. and T.K.T. Lam, *Peripheral and central regulation of insulin by the intestine and microbiome.* Am J Physiol Endocrinol Metab, 2021. **320**(2): p. E234-E239.
69. Ferguson, D. and B.N. Finck, *Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus.* Nat Rev Endocrinol, 2021.
70. Jensen, T., et al., *Fructose and sugar: A major mediator of non-alcoholic fatty liver disease.* J Hepatol, 2018. **68**(5): p. 1063-1075.
71. Schultz, A., et al., *Hepatic adverse effects of fructose consumption independent of overweight/obesity.* Int J Mol Sci, 2013. **14**(11): p. 21873-86.
72. Van Gaal, L.F., I.L. Mertens, and C.E. De Block, *Mechanisms linking obesity with cardiovascular disease.* Nature, 2006. **444**(7121): p. 875-80.
73. Wang, Y., et al., *Association between obesity and kidney disease: a systematic review and meta-analysis.* Kidney Int, 2008. **73**(1): p. 19-33.

74. Donohoe, C.L., et al., *Emerging Concepts Linking Obesity with the Hallmarks of Cancer.* Trends Endocrinol Metab, 2017. **28**(1): p. 46-62.
75. Klenov, V.E. and E.S. Jungheim, *Obesity and reproductive function: a review of the evidence.* Curr Opin Obstet Gynecol, 2014. **26**(6): p. 455-60.
76. Rubino, F., et al., *Joint international consensus statement for ending stigma of obesity.* Nat Med, 2020. **26**(4): p. 485-497.
77. Iceta, S., et al., *Cognitive function in binge eating disorder and food addiction: A systematic review and three-level meta-analysis.* Prog Neuropsychopharmacol Biol Psychiatry, 2021. **111**: p. 110400.
78. Spahlholz, J., et al., *Obesity and discrimination - a systematic review and meta-analysis of observational studies.* Obes Rev, 2016. **17**(1): p. 43-55.
79. Tapking, C., et al., *Influence of Body Mass Index and Gender on Stigmatization of Obesity.* Obes Surg, 2020. **30**(12): p. 4926-4934.
80. Jackson, S.E. and A. Steptoe, *Obesity, perceived weight discrimination, and hair cortisol: a population-based study.* Psychoneuroendocrinology, 2018. **98**: p. 67-73.
81. Wharton, S., et al., *L'obésité chez l'adulte : ligne directrice de pratique clinique.* CMAJ, 2020. **192**(49): p. E1757-E1775.
82. Gardner, C.D., et al., *Effect of Low-Fat vs Low-Carbohydrate Diet on 12-Month Weight Loss in Overweight Adults and the Association With Genotype Pattern or Insulin Secretion: The DIETFITS Randomized Clinical Trial.* JAMA, 2018. **319**(7): p. 667-679.
83. Dansinger, M.L., et al., *Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial.* Jama, 2005. **293**(1): p. 43-53.
84. Srivastava, G. and C. Apovian, *Future Pharmacotherapy for Obesity: New Anti-obesity Drugs on the Horizon.* Curr Obes Rep, 2018. **7**(2): p. 147-161.
85. O'Neil, P.M., et al., *Efficacy and safety of semaglutide compared with liraglutide and placebo for weight loss in patients with obesity: a randomised, double-blind, placebo and active controlled, dose-ranging, phase 2 trial.* The Lancet, 2018. **392**(10148): p. 637-649.
86. Arterburn, D.E., et al., *Benefits and Risks of Bariatric Surgery in Adults: A Review.* JAMA, 2020. **324**(9): p. 879-887.
87. Ribaric, G., J.N. Buchwald, and T.W. McGlennon, *Diabetes and weight in comparative studies of bariatric surgery vs conventional medical therapy: a systematic review and meta-analysis.* Obes Surg, 2014. **24**(3): p. 437-55.
88. Redondo, M., I. Hernandez-Aguado, and B. Lumbreras, *The impact of the tax on sweetened beverages: a systematic review.* Am J Clin Nutr, 2018. **108**(3): p. 548-563.
89. Julia, C., F. Etilé, and S. Hercberg, *Front-of-pack Nutri-Score labelling in France: an evidence-based policy.* The Lancet Public Health, 2018. **3**(4).
90. Salvia, M.G., *The Look AHEAD Trial: Translating Lessons Learned Into Clinical Practice and Further Study.* Diabetes Spectr, 2017. **30**(3): p. 166-170.
91. Sender, R., S. Fuchs, and R. Milo, *Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans.* Cell, 2016. **164**(3): p. 337-40.
92. Turnbaugh, P.J., et al., *The human microbiome project.* Nature, 2007. **449**(7164): p. 804-10.
93. Gilbert, J.A., et al., *Current understanding of the human microbiome.* Nat Med, 2018. **24**(4): p. 392-400.
94. Canfora, E.E., et al., *Gut microbial metabolites in obesity, NAFLD and T2DM.* Nat Rev Endocrinol, 2019. **15**(5): p. 261-273.
95. Araujo, J.R., et al., *Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity.* Biochimie, 2017. **141**: p. 97-106.
96. Zoetendal, E.G., et al., *The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates.* ISME J, 2012. **6**(7): p. 1415-26.
97. Martinez-Guryn, K., et al., *Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids.* Cell Host Microbe, 2018. **23**(4): p. 458-469 e5.

98. Daniel, N., E. Lecuyer, and B. Chassaing, *Host/microbiota interactions in health and diseases-Time for mucosal microbiology!* Mucosal Immunol, 2021.
99. Mar Rodriguez, M., et al., *Obesity changes the human gut mycobiome.* Sci Rep, 2015. **5**: p. 14600.
100. Nash, A.K., et al., *The gut mycobiome of the Human Microbiome Project healthy cohort.* Microbiome, 2017. **5**(1): p. 153.
101. Camarillo-Guerrero, L.F., et al., *Massive expansion of human gut bacteriophage diversity.* Cell, 2021. **184**(4): p. 1098-1109 e9.
102. Yang, K., et al., *Alterations in the gut virome in obesity and type 2 diabetes mellitus.* Gastroenterology, 2021.
103. Da Silva, H.E., et al., *Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance.* Sci Rep, 2018. **8**(1): p. 1466.
104. Vivarelli, S., et al., *Gut Microbiota and Cancer: From Pathogenesis to Therapy.* Cancers (Basel), 2019. **11**(1).
105. Nishida, A., et al., *Gut microbiota in the pathogenesis of inflammatory bowel disease.* Clin J Gastroenterol, 2018. **11**(1): p. 1-10.
106. Singh, R.K., et al., *Influence of diet on the gut microbiome and implications for human health.* J Transl Med, 2017. **15**(1): p. 73.
107. Choi, H., M.C. Rao, and E.B. Chang, *Gut microbiota as a transducer of dietary cues to regulate host circadian rhythms and metabolism.* Nat Rev Gastroenterol Hepatol, 2021.
108. Voigt, R.M., et al., *Circadian Rhythm and the Gut Microbiome.* Int Rev Neurobiol, 2016. **131**: p. 193-205.
109. Vujkovic-Cvijin, I., et al., *Host variables confound gut microbiota studies of human disease.* Nature, 2020. **587**(7834): p. 448-454.
110. Asnicar, F., et al., *Microbiome connections with host metabolism and habitual diet from 1,098 deeply phenotyped individuals.* Nat Med, 2021. **27**(2): p. 321-332.
111. Kennedy, K.M., et al., *Fetal meconium does not have a detectable microbiota before birth.* Nat Microbiol, 2021.
112. Petersen, C., et al., *A rich meconium metabolome in human infants is associated with early-life gut microbiota composition and reduced allergic sensitization.* Cell Reports Medicine, 2021. **2**(5).
113. Backhed, F., et al., *Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life.* Cell Host Microbe, 2015. **17**(5): p. 690-703.
114. Roswall, J., et al., *Developmental trajectory of the healthy human gut microbiota during the first 5 years of life.* Cell Host Microbe, 2021. **29**(5): p. 765-776 e3.
115. Rothschild, D., et al., *Environment dominates over host genetics in shaping human gut microbiota.* Nature, 2018. **555**(7695): p. 210-215.
116. von Schwartzenberg, R.J., et al., *Caloric restriction disrupts the microbiota and colonization resistance.* Nature, 2021.
117. Wibowo, M.C., et al., *Reconstruction of ancient microbial genomes from the human gut.* Nature, 2021.
118. Kennedy, E.A., K.Y. King, and M.T. Baldridge, *Mouse Microbiota Models: Comparing Germ-Free Mice and Antibiotics Treatment as Tools for Modifying Gut Bacteria.* Front Physiol, 2018. **9**: p. 1534.
119. Le Roy, T., et al., *Comparative Evaluation of Microbiota Engraftment Following Fecal Microbiota Transfer in Mice Models: Age, Kinetic and Microbial Status Matter.* Front Microbiol, 2018. **9**: p. 3289.
120. Daft, J.G., et al., *Cross-fostering immediately after birth induces a permanent microbiota shift that is shaped by the nursing mother.* Microbiome, 2015. **3**: p. 17.
121. Davey Smith, G. and G. Hemani, *Mendelian randomization: genetic anchors for causal inference in epidemiological studies.* Hum Mol Genet, 2014. **23**(R1): p. R89-98.
122. Sanna, S., et al., *Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases.* Nat Genet, 2019. **51**(4): p. 600-605.
123. Krautkramer, K.A., J. Fan, and F. Backhed, *Gut microbial metabolites as multi-kingdom intermediates.* Nat Rev Microbiol, 2021. **19**(2): p. 77-94.

124. Cani, P.D., et al., *Microbial regulation of organismal energy homeostasis.* Nat Metab, 2019. **1**(1): p. 34-46.
125. Gupta, A., V. Osadchiy, and E.A. Mayer, *Brain-gut-microbiome interactions in obesity and food addiction.* Nat Rev Gastroenterol Hepatol, 2020. **17**(11): p. 655-672.
126. Breit, S., et al., *Vagus Nerve as Modulator of the Brain-Gut Axis in Psychiatric and Inflammatory Disorders.* Front Psychiatry, 2018. **9**: p. 44.
127. Morais, L.H., H.L.t. Schreiber, and S.K. Mazmanian, *The gut microbiota-brain axis in behaviour and brain disorders.* Nat Rev Microbiol, 2021. **19**(4): p. 241-255.
128. Koh, A., et al., *From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites.* Cell, 2016. **165**(6): p. 1332-1345.
129. Rooks, M.G. and W.S. Garrett, *Gut microbiota, metabolites and host immunity.* Nat Rev Immunol, 2016. **16**(6): p. 341-52.
130. Canfora, E.E., J.W. Jocken, and E.E. Blaak, *Short-chain fatty acids in control of body weight and insulin sensitivity.* Nat Rev Endocrinol, 2015. **11**(10): p. 577-91.
131. Juanola, O., et al., *Circulating levels of butyrate are inversely related to portal hypertension, endotoxemia, and systemic inflammation in patients with cirrhosis.* FASEB J, 2019. **33**(10): p. 11595-11605.
132. Frampton, J., et al., *Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function.* Nat Metab, 2020. **2**(9): p. 840-848.
133. Dalile, B., et al., *The role of short-chain fatty acids in microbiota-gut-brain communication.* Nat Rev Gastroenterol Hepatol, 2019. **16**(8): p. 461-478.
134. Frost, G., et al., *The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism.* Nat Commun, 2014. **5**: p. 3611.
135. Byrne, C.S., et al., *The role of short chain fatty acids in appetite regulation and energy homeostasis.* Int J Obes (Lond), 2015. **39**(9): p. 1331-8.
136. Needham, B.D., R. Kaddurah-Daouk, and S.K. Mazmanian, *Gut microbial molecules in behavioural and neurodegenerative conditions.* Nat Rev Neurosci, 2020. **21**(12): p. 717-731.
137. Singh, V., et al., *Dysregulated Microbial Fermentation of Soluble Fiber Induces Cholestatic Liver Cancer.* Cell, 2018. **175**(3): p. 679-694 e22.
138. Koh, A., et al., *Microbially Produced Imidazole Propionate Impairs Insulin Signaling through mTORC1.* Cell, 2018. **175**(4): p. 947-961 e17.
139. Arumugam, M., et al., *Enterotypes of the human gut microbiome.* Nature, 2011. **473**(7346): p. 174-80.
140. Molinaro, A., et al., *Imidazole propionate is increased in diabetes and associated with dietary patterns and altered microbial ecology.* Nat Commun, 2020. **11**(1): p. 5881.
141. Koh, A., et al., *Microbial Imidazole Propionate Affects Responses to Metformin through p38gamma-Dependent Inhibitory AMPK Phosphorylation.* Cell Metab, 2020. **32**(4): p. 643-653 e4.
142. Wu, H., et al., *Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug.* Nat Med, 2017. **23**(7): p. 850-858.
143. Derrien, M., et al., *Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium.* Int J Syst Evol Microbiol, 2004. **54**(Pt 5): p. 1469-1476.
144. Cani, P.D. and W.M. de Vos, *Next-Generation Beneficial Microbes: The Case of Akkermansia muciniphila.* Front Microbiol, 2017. **8**: p. 1765.
145. Everard, A., et al., *Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity.* Proc Natl Acad Sci U S A, 2013. **110**(22): p. 9066-71.
146. Dao, M.C., et al., *Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology.* Gut, 2016. **65**(3): p. 426-36.
147. Depommier, C., et al., *Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study.* Nat Med, 2019. **25**(7): p. 1096-1103.
148. Plovier, H., et al., *A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice.* Nat Med, 2017. **23**(1): p. 107-113.

149. Cani, P.D., *Human gut microbiome: hopes, threats and promises.* Gut, 2018. **67**(9): p. 1716-1725.
150. Seregin, S.S., et al., *NLRP6 Protects Il10(-/-) Mice from Colitis by Limiting Colonization of Akkermansia muciniphila.* Cell Rep, 2017. **19**(4): p. 733-745.
151. Le Roy, T., et al., *Dysosmobacter welbionis is a newly isolated human commensal bacterium preventing diet-induced obesity and metabolic disorders in mice.* Gut, 2021.
152. Le Roy, T., et al., *Dysosmobacter welbionis gen. nov., sp. nov., isolated from human faeces and emended description of the genus Oscillibacter.* Int J Syst Evol Microbiol, 2020. **70**(9): p. 4851-4858.
153. Miquel, S., et al., *Faecalibacterium prausnitzii and human intestinal health.* Curr Opin Microbiol, 2013. **16**(3): p. 255-61.
154. Pedersen, H.K., et al., *Human gut microbes impact host serum metabolome and insulin sensitivity.* Nature, 2016. **535**(7612): p. 376-81.
155. Stanislawski, M.A., et al., *Gut microbiota phenotypes of obesity.* NPJ Biofilms Microbiomes, 2019. **5**(1): p. 18.
156. Korem, T., et al., *Bread Affects Clinical Parameters and Induces Gut Microbiome-Associated Personal Glycemic Responses.* Cell Metab, 2017. **25**(6): p. 1243-1253 e5.
157. Bar, N., et al., *A reference map of potential determinants for the human serum metabolome.* Nature, 2020. **588**(7836): p. 135-140.
158. Vojinovic, D., et al., *Relationship between gut microbiota and circulating metabolites in population-based cohorts.* Nat Commun, 2019. **10**(1): p. 5813.
159. Zeevi, D., et al., *Structural variation in the gut microbiome associates with host health.* Nature, 2019. **568**(7750): p. 43-48.
160. Jie, Z., et al., *The Baseline Gut Microbiota Directs Dieting-Induced Weight Loss Trajectories.* Gastroenterology, 2021.
161. Zhao, L., et al., *A Glucagon-Like Peptide-1 Receptor Agonist Lowers Weight by Modulating the Structure of Gut Microbiota.* Front Endocrinol (Lausanne), 2018. **9**: p. 233.
162. Anhe, F.F., et al., *The Gut Microbiota as a Mediator of Metabolic Benefits after Bariatric Surgery.* Can J Diabetes, 2017. **41**(4): p. 439-447.
163. Seganfredo, F.B., et al., *Weight-loss interventions and gut microbiota changes in overweight and obese patients: a systematic review.* Obes Rev, 2017. **18**(8): p. 832-851.
164. Liu, R., et al., *Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention.* Nat Med, 2017. **23**(7): p. 859-868.
165. Cani, P.D., et al., *Metabolic endotoxemia initiates obesity and insulin resistance.* Diabetes, 2007. **56**(7): p. 1761-72.
166. Cani, P.D., et al., *Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice.* Diabetes, 2008. **57**(6): p. 1470-81.
167. Neves, A.L., et al., *Metabolic endotoxemia: a molecular link between obesity and cardiovascular risk.* J Mol Endocrinol, 2013. **51**(2): p. R51-64.
168. Gomes, J.M.G., J.A. Costa, and R.C.G. Alfenas, *Metabolic endotoxemia and diabetes mellitus: A systematic review.* Metabolism, 2017. **68**: p. 133-144.
169. Plovier, H. and P.D. Cani, *Microbial Impact on Host Metabolism: Opportunities for Novel Treatments of Nutritional Disorders?* Microbiol Spectr, 2017. **5**(3).
170. Anhe, F.F., et al., *Metabolic endotoxemia is dictated by the type of lipopolysaccharide.* Cell Rep, 2021. **36**(11): p. 109691.
171. Winer, D.A., et al., *The Intestinal Immune System in Obesity and Insulin Resistance.* Cell Metab, 2016. **23**(3): p. 413-26.
172. Peterson, L.W. and D. Artis, *Intestinal epithelial cells: regulators of barrier function and immune homeostasis.* Nat Rev Immunol, 2014. **14**(3): p. 141-53.
173. Tilg, H., et al., *The intestinal microbiota fuelling metabolic inflammation.* Nat Rev Immunol, 2020. **20**(1): p. 40-54.
174. Khan, S., et al., *Emerging concepts in intestinal immune control of obesity-related metabolic disease.* Nat Commun, 2021. **12**(1): p. 2598.

175. Massier, L., et al., *Adipose tissue derived bacteria are associated with inflammation in obesity and type 2 diabetes.* Gut, 2020. **69**(10): p. 1796-1806.
176. Anhe, F.F., et al., *Type 2 diabetes influences bacterial tissue compartmentalisation in human obesity.* Nat Metab, 2020. **2**(3): p. 233-242.
177. Suppli, M.P., et al., *Hepatic microbiome in healthy lean and obese humans.* JHEP Rep, 2021. **3**(4): p. 100299.
178. Ha, C.W.Y., et al., *Translocation of Viable Gut Microbiota to Mesenteric Adipose Drives Formation of Creeping Fat in Humans.* Cell, 2020. **183**(3): p. 666-683 e17.
179. Jensen, B.A. and A. Marette, *Microbial translocation in type 2 diabetes: when bacterial invaders overcome host defence in human obesity.* Gut, 2020. **69**(10): p. 1724-1726.
180. Ayoub-Charette, S., et al., *Different Food Sources of Fructose-Containing Sugars and Fasting Blood Uric Acid Levels: A Systematic Review and Meta-Analysis of Controlled Feeding Trials.* J Nutr, 2021.
181. Southgate, D.A., *Digestion and metabolism of sugars.* The American Journal of Clinical Nutrition, 1995. **62**(1): p. 203S-210S.
182. Chassaing, B., et al., *Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome.* Nature, 2015. **519**(7541): p. 92-6.
183. Sacks, F.M., et al., *Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates.* N Engl J Med, 2009. **360**(9): p. 859-73.
184. Bel Lassen, P., et al., *Protein supplementation during an energy-restricted diet induces visceral fat loss and gut microbiota amino acid metabolism activation: a randomized trial.* Sci Rep, 2021. **11**(1): p. 15620.
185. Liisberg, U., et al., *The protein source determines the potential of high protein diets to attenuate obesity development in C57BL/6J mice.* Adipocyte, 2016. **5**(2): p. 196-211.
186. Liisberg, U., et al., *Intake of a Western diet containing cod instead of pork alters fatty acid composition in tissue phospholipids and attenuates obesity and hepatic lipid accumulation in mice.* J Nutr Biochem, 2016. **33**: p. 119-27.
187. Myrmel, L.S., et al., *The Impact of Different Animal-Derived Protein Sources on Adiposity and Glucose Homeostasis during Ad Libitum Feeding and Energy Restriction in Already Obese Mice.* Nutrients, 2019. **11**(5).
188. Holm, J.B., et al., *Diet-induced obesity, energy metabolism and gut microbiota in C57BL/6J mice fed Western diets based on lean seafood or lean meat mixtures.* J Nutr Biochem, 2016. **31**: p. 127-36.
189. Lavigne, C., A. Marette, and H. Jacques, *Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats.* Am J Physiol Endocrinol Metab, 2000. **278**(3): p. E491-500.
190. Lavigne, C., et al., *Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats.* Am J Physiol Endocrinol Metab, 2001. **281**(1): p. E62-71.
191. Pilon, G., et al., *Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat-fed rats.* Metabolism, 2011. **60**(8): p. 1122-30.
192. Ijaz, M.U., et al., *Beef, Casein, and Soy Proteins Differentially Affect Lipid Metabolism, Triglycerides Accumulation and Gut Microbiota of High-Fat Diet-Fed C57BL/6J Mice.* Front Microbiol, 2018. **9**: p. 2200.
193. Ouellet, V., et al., *Dietary cod protein improves insulin sensitivity in insulin-resistant men and women: a randomized controlled trial.* Diabetes Care, 2007. **30**(11): p. 2816-21.
194. Ouellet, V., et al., *Dietary cod protein reduces plasma C-reactive protein in insulin-resistant men and women.* J Nutr, 2008. **138**(12): p. 2386-91.
195. Bel Lassen, P., et al., *Protein Intake, Metabolic Status and the Gut Microbiota in Different Ethnicities: Results from Two Independent Cohorts.* Nutrients, 2021. **13**(9).
196. National Research Council Subcommittee on Laboratory Animal, N., in *Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995*. 1995, National Academies Press (US) © 1995 by the National Academy of Sciences. All rights reserved.: Washington (DC).

197. Chevrier, G., et al., *Chapter 18 - Impact of Dietary Proteins on Energy Balance, Insulin Sensitivity and Glucose Homeostasis: From Proteins to Peptides to Amino Acids*, in *The Molecular Nutrition of Amino Acids and Proteins*, D. Dardevet, Editor. 2016, Academic Press: Boston. p. 241-264.
198. Tremblay, F. and A. Marette, *Amino acid and insulin signaling via the mTOR/p70 S6 kinase pathway. A negative feedback mechanism leading to insulin resistance in skeletal muscle cells.* J Biol Chem, 2001. **276**(41): p. 38052-60.
199. Khamzina, L., et al., *Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance.* Endocrinology, 2005. **146**(3): p. 1473-81.
200. Tremblay, F., et al., *Activation of the mammalian target of rapamycin pathway acutely inhibits insulin signaling to Akt and glucose transport in 3T3-L1 and human adipocytes.* Endocrinology, 2005. **146**(3): p. 1328-37.
201. Newgard, C.B., et al., *A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance.* Cell Metab, 2009. **9**(4): p. 311-26.
202. White, P.J., et al., *The BCKDH Kinase and Phosphatase Integrate BCAA and Lipid Metabolism via Regulation of ATP-Citrate Lyase.* Cell Metab, 2018. **27**(6): p. 1281-1293.e7.
203. White, P.J., et al., *Muscle-Liver Trafficking of BCAA-Derived Nitrogen Underlies Obesity-Related Glycine Depletion.* Cell Rep, 2020. **33**(6): p. 108375.
204. Walejko, J.M., et al., *Branched-chain α-ketoacids are preferentially reaminated and activate protein synthesis in the heart.* Nat Commun, 2021. **12**(1): p. 1680.
205. Maltais-Payette, I., et al., *Circulating glutamate concentration as a biomarker of visceral obesity and associated metabolic alterations.* Nutr Metab (Lond), 2018. **15**: p. 78.
206. Yamakado, M., et al., *Plasma amino acid profile is associated with visceral fat accumulation in obese Japanese subjects.* Clin Obes, 2012. **2**(1-2): p. 29-40.
207. Lehn-Stefan, A., et al., *Elevated Circulating Glutamate Is Associated With Subclinical Atherosclerosis Independently of Established Risk Markers: A Cross-Sectional Study.* J Clin Endocrinol Metab, 2021. **106**(2): p. e982-e989.
208. Fernstrom, J.D., *Branched-chain amino acids and brain function.* J Nutr, 2005. **135**(6 Suppl): p. 1539s-46s.
209. Reuter, S.E. and A.M. Evans, *Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects.* Clin Pharmacokinet, 2012. **51**(9): p. 553-72.
210. Mihalik, S.J., et al., *Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity.* Obesity (Silver Spring), 2010. **18**(9): p. 1695-700.
211. Sun, L., et al., *Early Prediction of Developing Type 2 Diabetes by Plasma Acylcarnitines: A Population-Based Study.* Diabetes Care, 2016. **39**(9): p. 1563-70.
212. Dodd, D., et al., *A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites.* Nature, 2017. **551**(7682): p. 648-652.
213. Arnoriaga-Rodriguez, M., et al., *Obesity Impairs Short-Term and Working Memory through Gut Microbial Metabolism of Aromatic Amino Acids.* Cell Metab, 2020. **32**(4): p. 548-560 e7.
214. Arnoriaga-Rodriguez, M., et al., *Obesity-associated deficits in inhibitory control are phenocopied to mice through gut microbiota changes in one-carbon and aromatic amino acids metabolic pathways.* Gut, 2021.
215. Tiihonen, K., A.C. Ouwehand, and N. Rautonen, *Effect of overweight on gastrointestinal microbiology and immunology: correlation with blood biomarkers.* Br J Nutr, 2010. **103**(7): p. 1070-8.
216. Granado-Serrano, A.B., et al., *Faecal bacterial and short-chain fatty acids signature in hypercholesterolemia.* Sci Rep, 2019. **9**(1): p. 1772.
217. Koeth, R.A., et al., *Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis.* Nat Med, 2013. **19**(5): p. 576-85.
218. Gibson, G.R., et al., *Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics.* Nat Rev Gastroenterol Hepatol, 2017. **14**(8): p. 491-502.

219. Guo, X., et al., *Polyphenol Levels Are Inversely Correlated with Body Weight and Obesity in an Elderly Population after 5 Years of Follow Up (The Randomised PREDIMED Study).* Nutrients, 2017. **9**(5).
220. Anhe, F.F., et al., *A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice.* Gut, 2015. **64**(6): p. 872-83.
221. Anhe, F.F., et al., *Arctic berry extracts target the gut-liver axis to alleviate metabolic endotoxaemia, insulin resistance and hepatic steatosis in diet-induced obese mice.* Diabetologia, 2018. **61**(4): p. 919-931.
222. Rodriguez-Daza, M.C., et al., *Berry Polyphenols and Fibers Modulate Distinct Microbial Metabolic Functions and Gut Microbiota Enterotype-Like Clustering in Obese Mice.* Front Microbiol, 2020. **11**: p. 2032.
223. Morissette, A., et al., *Blueberry proanthocyanidins and anthocyanins improve metabolic health through a gut microbiota-dependent mechanism in diet-induced obese mice.* Am J Physiol Endocrinol Metab, 2020. **318**(6): p. E965-E980.
224. Paquette, M., et al., *Strawberry and cranberry polyphenols improve insulin sensitivity in insulin-resistant, non-diabetic adults: a parallel, double-blind, controlled and randomised clinical trial.* Br J Nutr, 2017. **117**(4): p. 519-531.
225. Capomolla, A.S., et al., *Atherogenic Index Reduction and Weight Loss in Metabolic Syndrome Patients Treated with A Novel Pectin-Enriched Formulation of Bergamot Polyphenols.* Nutrients, 2019. **11**(6).
226. Cires, M.J., et al., *The Gastrointestinal Tract as a Key Target Organ for the Health-Promoting Effects of Dietary Proanthocyanidins.* Frontiers in Nutrition, 2017. **3**(57).
227. Anhe, F.F., et al., *Host-Microbe Interplay in the Cardiometabolic Benefits of Dietary Polyphenols.* Trends Endocrinol Metab, 2019. **30**(6): p. 384-395.
228. Pandey, K.B. and S.I. Rizvi, *Plant polyphenols as dietary antioxidants in human health and disease.* Oxid Med Cell Longev, 2009. **2**(5): p. 270-8.
229. Girard, C., et al., *Cooking and co-ingested polyphenols reduce in vitro methylmercury bioaccessibility from fish and may alter exposure in humans.* Sci Total Environ, 2018. **616-617**: p. 863-874.
230. Koudoufio, M., et al., *Insight into Polyphenol and Gut Microbiota Crosstalk: Are Their Metabolites the Key to Understand Protective Effects against Metabolic Disorders?* Antioxidants (Basel), 2020. **9**(10).
231. Anhe, F.F., et al., *Triggering Akkermansia with dietary polyphenols: A new weapon to combat the metabolic syndrome?* Gut Microbes, 2016. **7**(2): p. 146-53.
232. Anhe, F.F., et al., *Treatment with camu camu (Myrciaria dubia) prevents obesity by altering the gut microbiota and increasing energy expenditure in diet-induced obese mice.* Gut, 2019. **68**(3): p. 453-464.
233. Hill, C., et al., *Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic.* Nat Rev Gastroenterol Hepatol, 2014. **11**(8): p. 506-14.
234. Le Barz, M., et al., *Probiotics as Complementary Treatment for Metabolic Disorders.* Diabetes Metab J, 2015. **39**(4): p. 291-303.
235. Aoki, R., et al., *A proliferative probiotic Bifidobacterium strain in the gut ameliorates progression of metabolic disorders via microbiota modulation and acetate elevation.* Sci Rep, 2017. **7**: p. 43522.
236. Alard, J., et al., *Beneficial metabolic effects of selected probiotics on diet-induced obesity and insulin resistance in mice are associated with improvement of dysbiotic gut microbiota.* Environ Microbiol, 2016. **18**(5): p. 1484-97.
237. Degirolamo, C., et al., *Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice.* Cell Rep, 2014. **7**(1): p. 12-8.
238. Bron, P.A., et al., *Can probiotics modulate human disease by impacting intestinal barrier function?* Br J Nutr, 2017. **117**(1): p. 93-107.

239. Sato, J., et al., *Probiotic reduces bacterial translocation in type 2 diabetes mellitus: A randomised controlled study.* Sci Rep, 2017. **7**(1): p. 12115.
240. Al-Sadi, R., et al., *Lactobacillus acidophilus Induces a Strain-specific and Toll-Like Receptor 2-Dependent Enhancement of Intestinal Epithelial Tight Junction Barrier and Protection Against Intestinal Inflammation.* Am J Pathol, 2021. **191**(5): p. 872-884.
241. Macho Fernandez, E., et al., *Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide.* Gut, 2011. **60**(8): p. 1050-9.
242. Cavallari, J.F., et al., *Muramyl Dipeptide-Based Postbiotics Mitigate Obesity-Induced Insulin Resistance via IRF4.* Cell Metab, 2017. **25**(5): p. 1063-1074 e3.
243. da Silva, S.T., C.A. dos Santos, and J. Bressan, *Intestinal microbiota; relevance to obesity and modulation by prebiotics and probiotics.* Nutr Hosp, 2013. **28**(4): p. 1039-48.
244. Kristensen, N.B., et al., *Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials.* Genome Med, 2016. **8**(1): p. 52.
245. Morovic, W. and C.R. Budinoff, *Epigenetics: A New Frontier in Probiotic Research.* Trends Microbiol, 2021. **29**(2): p. 117-126.
246. Licciardi, P.V., et al., *Epigenome targeting by probiotic metabolites.* Gut Pathog, 2010. **2**(1): p. 24.
247. Ghadimi, D., et al., *Epigenetic imprinting by commensal probiotics inhibits the IL-23/IL-17 axis in an in vitro model of the intestinal mucosal immune system.* J Leukoc Biol, 2012. **92**(4): p. 895-911.
248. Vahamiko, S., et al., *The impact of probiotic supplementation during pregnancy on DNA methylation of obesity-related genes in mothers and their children.* Eur J Nutr, 2018.
249. Sarkar, A., et al., *Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals.* Trends Neurosci, 2016. **39**(11): p. 763-781.
250. Long-Smith, C., et al., *Microbiota-Gut-Brain Axis: New Therapeutic Opportunities.* Annu Rev Pharmacol Toxicol, 2020. **60**: p. 477-502.
251. Tremblay, A., et al., *The effects of psychobiotics on the microbiota-gut-brain axis in early-life stress and neuropsychiatric disorders.* Prog Neuropsychopharmacol Biol Psychiatry, 2021. **105**: p. 110142.
252. Labarre, A., et al., *Probiotic Lacticaseibacillus rhamnosus HA-114 suppresses age-dependent neurodegeneration via mitochondrial beta-oxidation.* Nature Portfolio - Preprint, 2020.
253. Choi, B.S., et al., *Potential therapeutic applications of the gut microbiome in obesity: from brain function to body detoxification.* Int J Obes (Lond), 2020. **44**(9): p. 1818-1831.
254. Sanchez, M., et al., *Effects of a Diet-Based Weight-Reducing Program with Probiotic Supplementation on Satiety Efficiency, Eating Behaviour Traits, and Psychosocial Behaviours in Obese Individuals.* Nutrients, 2017. **9**(3).
255. Zhang, L., et al., *Persistent Organic Pollutants Modify Gut Microbiota-Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation.* Environ Health Perspect, 2015. **123**(7): p. 679-88.
256. Masumoto, S., et al., *Non-absorbable apple procyanidins prevent obesity associated with gut microbial and metabolomic changes.* Sci Rep, 2016. **6**: p. 31208.
257. Selma, M.V., et al., *The gut microbiota metabolism of pomegranate or walnut ellagitannins yields two urolithin-metabotypes that correlate with cardiometabolic risk biomarkers: Comparison between normoweight, overweight-obesity and metabolic syndrome.* Clin Nutr, 2018. **37**(3): p. 897-905.
258. Chen, Z., et al., *Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity.* J Clin Invest, 2014. **124**(8): p. 3391-406.
259. Markowiak, P. and K. Slizewska, *Effects of Probiotics, Prebiotics, and Synbiotics on Human Health.* Nutrients, 2017. **9**(9).
260. Schneeberger, M., et al., *Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice.* Sci Rep, 2015. **5**: p. 16643.
261. Dao, M.C., et al., *Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology.* Gut, 2015.

262. Chelakkot, C., et al., *Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions.* Exp Mol Med, 2018. **50**(2): p. e450.
263. Plovier, H., et al., *A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice.* Nat Med, 2016.
264. Ashrafian, F., et al., *Akkermansia muciniphila-Derived Extracellular Vesicles as a Mucosal Delivery Vector for Amelioration of Obesity in Mice.* Front Microbiol, 2019. **10**: p. 2155.
265. Cavallari, J.F., et al., *Muramyl Dipeptide-Based Postbiotics Mitigate Obesity-Induced Insulin Resistance via IRF4.* Cell Metab, 2017. **25**(5): p. 1063-1074.e3.
266. Bailey, C. and C. Day, *Metformin: its botanical background.* Practical Diabetes International, 2004. **21**(3): p. 115-117.
267. Shin, N.R., et al., *An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice.* Gut, 2014. **63**(5): p. 727-35.
268. de la Cuesta-Zuluaga, J., et al., *Metformin Is Associated With Higher Relative Abundance of Mucin-Degrading Akkermansia muciniphila and Several Short-Chain Fatty Acid-Producing Microbiota in the Gut.* Diabetes Care, 2017. **40**(1): p. 54-62.
269. Adeshirlarijaney, A., et al., *Amelioration of metabolic syndrome by metformin associates with reduced indices of low-grade inflammation independently of the gut microbiota.* Am J Physiol Endocrinol Metab, 2019.
270. Allegretti, J.R., et al., *The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications.* The Lancet, 2019. **394**(10196): p. 420-431.
271. Leshem, A., N. Horesh, and E. Elinav, *Fecal Microbial Transplantation and Its Potential Application in Cardiometabolic Syndrome.* Front Immunol, 2019. **10**: p. 1341.
272. Aron-Wisnewsky, J., K. Clement, and M. Nieuwdorp, *Fecal Microbiota Transplantation: a Future Therapeutic Option for Obesity/Diabetes?* Curr Diab Rep, 2019. **19**(8): p. 51.
273. Zhang, Z., et al., *Impact of Fecal Microbiota Transplantation on Obesity and Metabolic Syndrome-A Systematic Review.* Nutrients, 2019. **11**(10).
274. Kootte, R.S., et al., *Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition.* Cell Metab, 2017. **26**(4): p. 611-619 e6.
275. Gundling, F., et al., *Patient perception and approval of faecal microbiota transplantation (FMT) as an alternative treatment option for obesity.* Obesity Science & Practice, 2019. **5**(1): p. 68-74.
276. Cummings, D.E. and F. Rubino, *Metabolic surgery for the treatment of type 2 diabetes in obese individuals.* Diabetologia, 2018. **61**(2): p. 257-264.
277. Liu, H., et al., *Role of gut microbiota, bile acids and their cross-talk in the effects of bariatric surgery on obesity and type 2 diabetes.* J Diabetes Investig, 2018. **9**(1): p. 13-20.
278. Aron-Wisnewsky, J., J. Dore, and K. Clement, *The importance of the gut microbiota after bariatric surgery.* Nat Rev Gastroenterol Hepatol, 2012. **9**(10): p. 590-8.
279. van Greevenbroek, M.M., C.G. Schalkwijk, and C.D. Stehouwer, *Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences.* Neth J Med, 2013. **71**(4): p. 174-87.
280. Lizarbe, B., et al., *High-fat diet consumption alters energy metabolism in the mouse hypothalamus.* Int J Obes (Lond), 2019. **43**(6): p. 1295-1304.
281. Thaler, J.P., et al., *Obesity is associated with hypothalamic injury in rodents and humans.* J Clin Invest, 2012. **122**(1): p. 153-62.
282. Waise, T.M.Z., et al., *One-day high-fat diet induces inflammation in the nodose ganglion and hypothalamus of mice.* Biochem Biophys Res Commun, 2015. **464**(4): p. 1157-1162.
283. Stranahan, A.M., et al., *Blood-brain barrier breakdown promotes macrophage infiltration and cognitive impairment in leptin receptor-deficient mice.* J Cereb Blood Flow Metab, 2016. **36**(12): p. 2108-2121.
284. Johnson, P.M. and P.J. Kenny, *Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats.* Nat Neurosci, 2010. **13**(5): p. 635-41.
285. Valles-Colomer, M., et al., *The neuroactive potential of the human gut microbiota in quality of life and depression.* Nat Microbiol, 2019. **4**(4): p. 623-632.

286. Lin, P., et al., *Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder.* J Affect Disord, 2017. **207**: p. 300-304.
287. Hassan, A.M., et al., *High-fat diet induces depression-like behaviour in mice associated with changes in microbiome, neuropeptide Y, and brain metabolome.* Nutr Neurosci, 2018: p. 1-17.
288. Strandwitz, P., et al., *GABA-modulating bacteria of the human gut microbiota.* Nat Microbiol, 2019. **4**(3): p. 396-403.
289. Asano, Y., et al., *Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice.* Am J Physiol Gastrointest Liver Physiol, 2012. **303**(11): p. G1288-95.
290. Yano, J.M., et al., *Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis.* Cell, 2015. **161**(2): p. 264-76.
291. Arentsen, T., et al., *The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior.* Mol Psychiatry, 2017. **22**(2): p. 257-266.
292. Faith, M.S., et al., *Evidence for prospective associations among depression and obesity in population-based studies.* Obes Rev, 2011. **12**(5): p. e438-53.
293. Zheng, P., et al., *Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism.* Mol Psychiatry, 2016. **21**(6): p. 786-96.
294. Bruce-Keller, A.J., et al., *Obese-type gut microbiota induce neurobehavioral changes in the absence of obesity.* Biol Psychiatry, 2015. **77**(7): p. 607-15.
295. Jiang, H., et al., *Altered fecal microbiota composition in patients with major depressive disorder.* Brain Behav Immun, 2015. **48**: p. 186-94.
296. Aizawa, E., et al., *Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder.* J Affect Disord, 2016. **202**: p. 254-7.
297. Chen, Z., et al., *Comparative metaproteomics analysis shows altered fecal microbiota signatures in patients with major depressive disorder.* Neuroreport, 2018. **29**(5): p. 417-425.
298. Kelly, J.R., et al., *Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat.* J Psychiatr Res, 2016. **82**: p. 109-18.
299. Moloney, G.M., et al., *Microbial regulation of hippocampal miRNA expression: Implications for transcription of kynurenine pathway enzymes.* Behav Brain Res, 2017. **334**: p. 50-54.
300. Soto, M., et al., *Gut microbiota modulate neurobehavior through changes in brain insulin sensitivity and metabolism.* Mol Psychiatry, 2018. **23**(12): p. 2287-2301.
301. Gal, Z., et al., *[Anxiety and depression - the role of blood-brain barrier integrity].* Neuropsychopharmacol Hung, 2019. **21**(1): p. 19-25.
302. Hargrave, S.L., et al., *Western diets induce blood-brain barrier leakage and alter spatial strategies in rats.* Behav Neurosci, 2016. **130**(1): p. 123-35.
303. Davidson, T.L., et al., *The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood-brain barrier integrity differ for diet-induced obese and diet-resistant rats.* Physiol Behav, 2012. **107**(1): p. 26-33.
304. Braniste, V., et al., *The gut microbiota influences blood-brain barrier permeability in mice.* Sci Transl Med, 2014. **6**(263): p. 263ra158.
305. Alcock, J., C.C. Maley, and C.A. Aktipis, *Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms.* Bioessays, 2014. **36**(10): p. 940-9.
306. Duca, F.A., et al., *Increased oral detection, but decreased intestinal signaling for fats in mice lacking gut microbiota.* PLoS One, 2012. **7**(6): p. e39748.
307. Swartz, T.D., et al., *Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota.* Br J Nutr, 2012. **107**(5): p. 621-30.
308. Sclafani, A. and K. Ackroff, *Role of gut nutrient sensing in stimulating appetite and conditioning food preferences.* Am J Physiol Regul Integr Comp Physiol, 2012. **302**(10): p. R1119-33.

309. DiLeone, R.J., J.R. Taylor, and M.R. Picciotto, *The drive to eat: comparisons and distinctions between mechanisms of food reward and drug addiction.* Nat Neurosci, 2012. **15**(10): p. 1330-5.
310. Wu, C., et al., *Altered Dopamine Synaptic Markers in Postmortem Brain of Obese Subjects.* Front Hum Neurosci, 2017. **11**: p. 386.
311. Delbes, A.S., et al., *Prebiotics Supplementation Impact on the Reinforcing and Motivational Aspect of Feeding.* Front Endocrinol (Lausanne), 2018. **9**: p. 273.
312. Nettleton, J.E., et al., *Low-Dose Stevia (Rebaudioside A) Consumption Perturbs Gut Microbiota and the Mesolimbic Dopamine Reward System.* Nutrients, 2019. **11**(6).
313. Bernard, A., et al., *A Preventive Prebiotic Supplementation Improves the Sweet Taste Perception in Diet-Induced Obese Mice.* Nutrients, 2019. **11**(3).
314. Alabduljader, K., et al., *Ecological momentary assessment of food perceptions and eating behavior using a novel phone application in adults with or without obesity.* Eat Behav, 2018. **30**: p. 35-41.
315. Rezzi, S., et al., *Human metabolic phenotypes link directly to specific dietary preferences in healthy individuals.* J Proteome Res, 2007. **6**(11): p. 4469-77.
316. Sanmiguel, C.P., et al., *Surgically Induced Changes in Gut Microbiome and Hedonic Eating as Related to Weight Loss: Preliminary Findings in Obese Women Undergoing Bariatric Surgery.* Psychosom Med, 2017. **79**(8): p. 880-887.
317. Burokas, A., et al., *Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice.* Biol Psychiatry, 2017. **82**(7): p. 472-487.
318. Vaghef-Mehrabany, E., et al., *Calorie restriction in combination with prebiotic supplementation in obese women with depression: effects on metabolic and clinical response.* Nutr Neurosci, 2019: p. 1-15.
319. Logan, A.C. and M. Katzman, *Major depressive disorder: probiotics may be an adjuvant therapy.* Med Hypotheses, 2005. **64**(3): p. 533-8.
320. Dinan, T.G., C. Stanton, and J.F. Cryan, *Psychobiotics: a novel class of psychotropic.* Biol Psychiatry, 2013. **74**(10): p. 720-6.
321. Abildgaard, A., et al., *Probiotic treatment protects against the pro-depressant-like effect of high-fat diet in Flinders Sensitive Line rats.* Brain Behav Immun, 2017. **65**: p. 33-42.
322. Osadchiy, V., et al., *Correlation of tryptophan metabolites with connectivity of extended central reward network in healthy subjects.* PLoS One, 2018. **13**(8): p. e0201772.
323. Laurans, L., et al., *Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health.* Nat Med, 2018. **24**(8): p. 1113-1120.
324. Jennis, M., et al., *Microbiota-derived tryptophan indoles increase after gastric bypass surgery and reduce intestinal permeability in vitro and in vivo.* Neurogastroenterol Motil, 2018. **30**(2).
325. Tuomainen, M., et al., *Associations of serum indolepropionic acid, a gut microbiota metabolite, with type 2 diabetes and low-grade inflammation in high-risk individuals.* Nutr Diabetes, 2018. **8**(1): p. 35.
326. Saez-Lara, M.J., et al., *Effects of Probiotics and Synbiotics on Obesity, Insulin Resistance Syndrome, Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease: A Review of Human Clinical Trials.* Int J Mol Sci, 2016. **17**(6).
327. Koppel, N., V. Maini Rekdal, and E.P. Balskus, *Chemical transformation of xenobiotics by the human gut microbiota.* Science, 2017. **356**(6344).
328. Arrebola, J.P., et al., *Differential contribution of animal and vegetable food items on persistent organic pollutant serum concentrations in Spanish adults. Data from BIOAMBIENT.ES project.* Sci Total Environ, 2018. **634**: p. 235-242.
329. Pestana, D., et al., *Persistent organic pollutant levels in human visceral and subcutaneous adipose tissue in obese individuals--depot differences and dysmetabolism implications.* Environ Res, 2014. **133**: p. 170-7.
330. Ngwa, E.N., et al., *Persistent organic pollutants as risk factors for type 2 diabetes.* Diabetol Metab Syndr, 2015. **7**: p. 41.

331. Reaves, D.K., et al., *Persistent organic pollutants and obesity: are they potential mechanisms for breast cancer promotion?* Endocr Relat Cancer, 2015. **22**(2): p. R69-86.
332. Lim, J.E., et al., *Body concentrations of persistent organic pollutants and prostate cancer: a meta-analysis.* Environ Sci Pollut Res Int, 2015. **22**(15): p. 11275-84.
333. Nadal, A., et al., *Endocrine-disrupting chemicals and the regulation of energy balance.* Nat Rev Endocrinol, 2017. **13**(9): p. 536-546.
334. Marushka, L., et al., *Association between fish consumption, dietary omega-3 fatty acids and persistent organic pollutants intake, and type 2 diabetes in 18 First Nations in Ontario, Canada.* Environ Res, 2017. **156**: p. 725-737.
335. Lee, Y.M., et al., *Prospective associations between persistent organic pollutants and metabolic syndrome: a nested case-control study.* Sci Total Environ, 2014. **496**: p. 219-225.
336. Gauthier, M.S., et al., *The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype.* J Clin Endocrinol Metab, 2014. **99**(6): p. E1061-6.
337. Hue, O., et al., *Increased plasma levels of toxic pollutants accompanying weight loss induced by hypocaloric diet or by bariatric surgery.* Obes Surg, 2006. **16**(9): p. 1145-54.
338. Jansen, A., et al., *Increased levels of persistent organic pollutants in serum one year after a great weight loss in humans: Are the levels exceeding health based guideline values?* Sci Total Environ, 2018. **622-623**: p. 1317-1326.
339. Cheikh Rouhou, M., et al., *Adverse effects of weight loss: Are persistent organic pollutants a potential culprit?* Diabetes Metab, 2016. **42**(4): p. 215-23.
340. Pelletier, C., P. Imbeault, and A. Tremblay, *Energy balance and pollution by organochlorines and polychlorinated biphenyls.* Obes Rev, 2003. **4**(1): p. 17-24.
341. Imbeault, P., et al., *Weight loss-induced rise in plasma pollutant is associated with reduced skeletal muscle oxidative capacity.* Am J Physiol Endocrinol Metab, 2002. **282**(3): p. E574-9.
342. Pelletier, C., et al., *Associations between Weight Loss-Induced Changes in Plasma Organochlorine Concentrations, Serum T3 Concentration, and Resting Metabolic Rate.* Toxicological Sciences, 2002. **67**(1): p. 46-51.
343. Tremblay, A., et al., *Thermogenesis and weight loss in obese individuals: a primary association with organochlorine pollution.* Int J Obes Relat Metab Disord, 2004. **28**(7): p. 936-9.
344. Vizcaino, E., et al., *Transport of persistent organic pollutants across the human placenta.* Environ Int, 2014. **65**: p. 107-15.
345. Lee, M.H., et al., *Association between serum persistent organic pollutants and DNA methylation in Korean adults.* Environ Res, 2017. **158**: p. 333-341.
346. Rusiecki, J.A., et al., *Global DNA hypomethylation is associated with high serum-persistent organic pollutants in Greenlandic Inuit.* Environ Health Perspect, 2008. **116**(11): p. 1547-52.
347. Acharya, N., et al., *Polycyclic aromatic hydrocarbons in breast milk of obese vs normal women: Infant exposure and risk assessment.* Sci Total Environ, 2019. **668**: p. 658-667.
348. Iszatt, N., et al., *Environmental toxicants in breast milk of Norwegian mothers and gut bacteria composition and metabolites in their infants at 1 month.* Microbiome, 2019. **7**(1): p. 34.
349. Wang, D., et al., *In utero and lactational exposure to BDE-47 promotes obesity development in mouse offspring fed a high-fat diet: impaired lipid metabolism and intestinal dysbiosis.* Arch Toxicol, 2018. **92**(5): p. 1847-1860.
350. Chen, L., et al., *Acute exposure to PBDEs at an environmentally realistic concentration causes abrupt changes in the gut microbiota and host health of zebrafish.* Environ Pollut, 2018. **240**: p. 17-26.
351. Petriello, M.C., et al., *Dioxin-like PCB 126 increases intestinal inflammation and disrupts gut microbiota and metabolic homeostasis.* Environ Pollut, 2018. **242**(Pt A): p. 1022-1032.
352. Zhan, J., et al., *Pectin reduces environmental pollutant-induced obesity in mice through regulating gut microbiota: A case study of p,p'-DDE.* Environ Int, 2019. **130**: p. 104861.
353. Hoffman, J.B., M.D. Flythe, and B. Hennig, *Environmental pollutant-mediated disruption of gut microbial metabolism of the prebiotic inulin.* Anaerobe, 2019. **55**: p. 96-102.

354. Natividad, J.M., et al., *Impaired Aryl Hydrocarbon Receptor Ligand Production by the Gut Microbiota Is a Key Factor in Metabolic Syndrome.* Cell Metab, 2018. **28**(5): p. 737-749 e4.
355. Petriello, M.C., et al., *Modulation of persistent organic pollutant toxicity through nutritional intervention: emerging opportunities in biomedicine and environmental remediation.* Sci Total Environ, 2014. **491-492**: p. 11-6.
356. Arguin, H., et al., *Impact of adopting a vegan diet or an olestra supplementation on plasma organochlorine concentrations: results from two pilot studies.* Br J Nutr, 2010. **103**(10): p. 1433-41.
357. Ta, C.A., et al., *Binding capacity of various fibre to pesticide residues under simulated gastrointestinal conditions.* Food Chem Toxicol, 1999. **37**(12): p. 1147-51.
358. Sera, N., et al., *Binding effect of polychlorinated compounds and environmental carcinogens on rice bran fiber.* J Nutr Biochem, 2005. **16**(1): p. 50-8.
359. Morita, K. and K. Tobiishi, *Increasing effect of nori on the fecal excretion of dioxin by rats.* Biosci Biotechnol Biochem, 2002. **66**(11): p. 2306-13.
360. Aislabie, J.M., N.K. Richards, and H.L. Boul, *Microbial degradation of DDT and its residues - A review.* New Zealand Journal of Agricultural Research, 2010. **40**: p. 269-282.
361. Murinova, S., K. Dercova, and H. Dudasova, *Degradation of polychlorinated biphenyls (PCBs) by four bacterial isolates obtained from the PCB-contaminated soil and PCB-contaminated sediment.* International Biodeterioration & Biodegradation, 2014. **91**: p. 52-59.
362. De, S., S. Ghosh, and S.K. Dutta, *Congener specific polychlorinated biphenyl metabolism by human intestinal microbe Clostridium species: Comparison with human liver cell line-HepG2.* Indian J Microbiol, 2006. **46**(3): p. 199-207.
363. Lee, H.S., et al., *Associations among organochlorine pesticides, Methanobacteriales, and obesity in Korean women.* PLoS One, 2011. **6**(11): p. e27773.
364. Jandacek, R.J. and P. Tso, *Enterohepatic circulation of organochlorine compounds: a site for nutritional intervention.* J Nutr Biochem, 2007. **18**(3): p. 163-7.
365. Fader, K.A., et al., *2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-elicited effects on bile acid homeostasis: Alterations in biosynthesis, enterohepatic circulation, and microbial metabolism.* Sci Rep, 2017. **7**(1): p. 5921.
366. Wahlstrom, A., et al., *Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism.* Cell Metab, 2016. **24**(1): p. 41-50.
367. Jeun, J., et al., *Hypocholesterolemic effects of Lactobacillus plantarum KCTC3928 by increased bile acid excretion in C57BL/6 mice.* Nutrition, 2010. **26**(3): p. 321-30.
368. Skinner, M.K., *Endocrine disruptors in 2015: Epigenetic transgenerational inheritance.* Nat Rev Endocrinol, 2016. **12**(2): p. 68-70.
369. Krautkramer, K.A., et al., *Diet-Microbiota Interactions Mediate Global Epigenetic Programming in Multiple Host Tissues.* Mol Cell, 2016. **64**(5): p. 982-992.
370. Choi, B.S., et al., *A polyphenol-rich cranberry extract protects against endogenous exposure to persistent organic pollutants during weight loss in mice.* Food Chem Toxicol, 2020. **146**: p. 111832.
371. Choi, B.S., 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**(1): p. 3377.
372. Sims, E.K., et al., *100 years of insulin: celebrating the past, present and future of diabetes therapy.* Nat Med, 2021. **27**(7): p. 1154-1164.
373. Heaney, R.P., et al., *Bioavailability of the calcium in fortified soy imitation milk, with some observations on method.* Am J Clin Nutr, 2000. **71**(5): p. 1166-9.
374. Reganold, J.P. and J.M. Wachter, *Organic agriculture in the twenty-first century.* Nat Plants, 2016. **2**: p. 15221.
375. Skinner, M.K., et al., *Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity.* BMC Medicine, 2013. **11**(1): p. 228.
376. Myrmel, L.S., et al., *Macronutrient composition determines accumulation of persistent organic pollutants from dietary exposure in adipose tissue of mice.* J Nutr Biochem, 2016. **27**: p. 307-16.

377. Poore, J. and T. Nemecek, *Reducing food's environmental impacts through producers and consumers.* Science, 2018. **360**(6392): p. 987-992.
378. Marco, M.L., et al., *The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods.* Nat Rev Gastroenterol Hepatol, 2021. **18**(3): p. 196-208.
379. Dimidi, E., et al., *Fermented Foods: Definitions and Characteristics, Impact on the Gut Microbiota and Effects on Gastrointestinal Health and Disease.* Nutrients, 2019. **11**(8).
380. Patra, J.K., et al., *Kimchi and Other Widely Consumed Traditional Fermented Foods of Korea: A Review.* Front Microbiol, 2016. **7**: p. 1493.
381. Jensen, B.A.H., et al., *Lysates of Methylococcus capsulatus Bath induce a lean-like microbiota, intestinal FoxP3(+)RORgammat(+)IL-17(+) Tregs and improve metabolism.* Nat Commun, 2021. **12**(1): p. 1093.
382. Jantzen da Silva Lucas, A., et al., *Edible insects: An alternative of nutritional, functional and bioactive compounds.* Food Chem, 2020. **311**: p. 126022.
383. Fernandez, M.A. and A. Marette, *Novel perspectives on fermented milks and cardiometabolic health with a focus on type 2 diabetes.* Nutr Rev, 2018. **76**(Supplement_1): p. 16-28.
384. Ji, Y., et al., *Weight Bias 2.0: The Effect of Perceived Weight Change on Performance Evaluation and the Moderating Role of Anti-fat Bias.* Front Psychol, 2021. **12**: p. 679802.
385. Friedman, C., H. Feldner, and L. VanPuymbrouck, *Anti-Fat Biases of Occupational and Physical Therapy Assistants.* Occup Ther Health Care, 2021: p. 1-21.
386. Shah, R.V., et al., *Visceral adiposity and the risk of metabolic syndrome across body mass index: the MESA Study.* JACC Cardiovasc Imaging, 2014. **7**(12): p. 1221-35.
387. Arsenault, B.J., et al., *Effect of exercise training on cardiometabolic risk markers among sedentary, but metabolically healthy overweight or obese post-menopausal women with elevated blood pressure.* Atherosclerosis, 2009. **207**(2): p. 530-3.
388. Kolodziejczyk, A.A., D. Zheng, and E. Elinav, *Diet-microbiota interactions and personalized nutrition.* Nat Rev Microbiol, 2019. **17**(12): p. 742-753.
389. Achouba, A., et al., *Selenoneine is a major selenium species in beluga skin and red blood cells of Inuit from Nunavik.* Chemosphere, 2019. **229**: p. 549-558.
390. Regitz-Zagrosek, V. and G. Kararigas, *Mechanistic Pathways of Sex Differences in Cardiovascular Disease.* Physiol Rev, 2017. **97**(1): p. 1-37.
391. Sanchez-Alcoholado, L., et al., *Relationships of Gut Microbiota Composition, Short-Chain Fatty Acids and Polyamines with the Pathological Response to Neoadjuvant Radiochemotherapy in Colorectal Cancer Patients.* Int J Mol Sci, 2021. **22**(17).
392. Andrews, M.C., et al., *Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade.* Nat Med, 2021. **27**(8): p. 1432-1441.
393. Ashraf, M.F., et al., *Fecal Microbiota Transplantation in Patients With Recurrent Clostridium difficile Infection: A Four-Year Single-Center Retrospective Review.* Gastroenterology Res, 2021. **14**(4): p. 237-243.
394. Liptak, R., B. Gromova, and R. Gardlik, *Fecal Microbiota Transplantation as a Tool for Therapeutic Modulation of Non-gastrointestinal Disorders.* Front Med (Lausanne), 2021. **8**: p. 665520.
395. Allegretti, J.R., et al., *Effects of Fecal Microbiota Transplantation With Oral Capsules in Obese Patients.* Clin Gastroenterol Hepatol, 2020. **18**(4): p. 855-863.e2.
396. Proenca, I.M., et al., *Fecal microbiota transplantation improves metabolic syndrome parameters: systematic review with meta-analysis based on randomized clinical trials.* Nutr Res, 2020. **83**: p. 1-14.
397. Hugenholtz, F. and W.M. de Vos, *Mouse models for human intestinal microbiota research: a critical evaluation.* Cell Mol Life Sci, 2018. **75**(1): p. 149-160.
398. Turnbaugh, P.J., et al., *The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice.* Science Translational Medicine, 2009. **1**(6): p. 6ra14-6ra14.
399. Koopen, A.M., et al., *Effect of Fecal Microbiota Transplantation Combined With Mediterranean Diet on Insulin Sensitivity in Subjects With Metabolic Syndrome.* Frontiers in Microbiology, 2021. **12**.

400. Horvathova, H., K. Laszlova, and K. Dercova, *Bioremediation of PCB-contaminated shallow river sediments: The efficacy of biodegradation using individual bacterial strains and their consortia.* Chemosphere, 2018. **193**: p. 270-277.
401. Gheorghe, C.E., et al., *Investigating causality with fecal microbiota transplantation in rodents: applications, recommendations and pitfalls.* Gut Microbes, 2021. **13**(1): p. 1941711.
402. Tang, W.H.W., D.Y. Li, and S.L. Hazen, *Dietary metabolism, the gut microbiome, and heart failure.* Nat Rev Cardiol, 2019. **16**(3): p. 137-154.
403. Kris-Etherton, P.M., et al., *Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease.* Circulation, 2002. **106**(21): p. 2747-57.
404. Yoon, H.S., et al., *Akkermansia muciniphila secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice.* Nat Microbiol, 2021.
405. Mertens, K.L., et al., *Bile Acid Signaling Pathways from the Enterohepatic Circulation to the Central Nervous System.* Front Neurosci, 2017. **11**: p. 617.
406. Chaudhari, S.N., M.D. McCurry, and A.S. Devlin, *Chains of evidence from correlations to causal molecules in microbiome-linked diseases.* Nature Chemical Biology, 2021.