Morphologie de l'adipocyte humain :
Méthodologie, dysfonction adipeuse et altérations cardiométaboliques

Mémoire

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Sous la direction de

Dr André Tchernof
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Résumé

L'accumulation excessive de gras viscéral est un marqueur majeur des altérations cardiométaboliques associées à l'obésité, telles que les dyslipidémies, la résistance à l'insuline et l'inflammation chronique de faible intensité. À l'inverse, pour un même niveau d'adiposité, l'accumulation préférentielle de tissu sous-cutané semble conférer un effet neutre, voire protecteur. Plusieurs mécanismes peuvent contribuer à l'apparition de l'obésité viscérale et à sa relation avec les facteurs de risque cardiométabolique. L'objectif général de ce mémoire est d'examiner la contribution de l'alimentation et de la morphologie des adipocytes à la physiopathologie de l'obésité viscérale chez l'humain. Pour atteindre cet objectif, nous avons d'abord effectué une revue de la littérature sur la modulation de la distribution des graisses par les composants nutritionnels. Cette étude a révélé que l'effet des nutriments sur la distribution des graisses survenait en grande partie via son effet sur la masse grasse totale. Nous avons ensuite réalisé une analyse critique de la littérature sur l'hypertrophie adipocytaire comme un marqueur de la dysfonction du tissu adipeux. Cette étude a confirmé que la taille adipocytaire des dépôts sous-cutané et viscéral est un excellent prédicteur des altérations du profil lipidique et de l'homéostasie du glucose et de l'insuline, indépendamment des mesures d'adiposité totale. Nous avons également constaté que chaque technique de mesure de la taille adipocytaire générait des résultats variables, et ce, sur tout l'intervalle des valeurs d'indice de masse corporelle. Enfin, dans une étude originale visant à comparer trois techniques de mesure de la taille adipocytaire, nous avons démontré que le choix d'une technique n'influençait pas de manière prononcée les associations entre la taille adipocytaire et les facteurs de risque cardiométabolique ainsi que les associations avec les différentes mesures d'adiposité, régionales ou totales. En conclusion, l'hypertrophie de l'adipocyte viscéral humain est une mesure importante dans l'évaluation du risque associé à l'obésité.

Abstract

Excess accumulation of visceral fat is a major marker of the cardiometabolic alterations associated with obesity such as dyslipidemia, insulin resistance and chronic low-grade inflammation. Conversely, for the same level of adiposity, preferential accumulation of subcutaneous adipose tissue has a neutral or protective effect. Several factors may contribute to the development of visceral adiposity and its relationship with cardiometabolic risk factors. The general objective of this master thesis is to examine the contribution of diet and the morphology of adipocytes in the pathophysiology of visceral obesity in humans. To achieve this goal, we first conducted a literature analysis on the modulation of fat distribution by nutritional components. This analysis revealed that the effect of nutrients on body fat distribution was mediated in large part by its effect on total fat accumulation. Second, we conducted a critical review of the literature on adipocyte hypertrophy as a marker of adipose tissue dysfunction. This study confirmed that adipocyte size of both subcutaneous and visceral depots is a strong predictor of alterations in the lipid profile and in glucose-insulin homeostasis, independently of adiposity. We also found that each of the measurement techniques for fat cell sizing generated variable results on the entire range of body mass index values. Third, in an original study aimed at comparing three measurement methods for cell sizing, we reported that the choice of technique had little impact on the associations between adipocyte size and cardiometabolic risk factors as well as the various adiposity indices, regional or total. In conclusion, human visceral adipocyte hypertrophy is an important measure to evaluate the risk associated with obesity.

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Liste des abréviations et des sigles

3T3-L1: Lignée cellulaire préadipocytaire embryonnaire de souris, *mouse embryonic preadipocyte cell line*

25(OH)D: 25-hydroxy vitamine D, *25-hydroxy vitamin D*

α-SMA: Actine des muscles lisses alpha, *alpha smooth muscle actin*

AGL: Acides gras libres, *free fatty acids*

ap2: Protéine adipocytaire 2, *adipocyte protein 2*

ATGL: Lipase des triglycérides adipeux, *adipose triglyceride lipase*

ApoB: Apolipoprotéine B, *apolipoprotein B*

ASP: Protéine qui stimule l'acylation, *acylation stimulating protein*

ATP-III: Panneau de traitement des adultes III, *adult treatment panel*

BCAA: Acides aminés branchés, *branched-chain amino acids*

BMI: Indice de masse corporelle, *body mass index*

BCKD: Complexe alpha-cétoglutarate déshydrogénase, *branched-chain α-keto acid dehydrogenase*

C/EBPα: CCAAT/enhancer-binding protein alpha

CD: Digestion à la collagenase, *collagenase digestion*

CD11b: Cluster de differentiation 11b (Marqueur de macrophage et de neutrophile), *cluster of differenciation 11b (Macrophage and neutrophil cell marker)*

CD11c: Cluster de differentiation 11c (Marqueur de macrophage et de neutrophile), *cluster of differenciation 11c (Dendritic cell and macrophage cell marker)*

CD31: Cluster de differentiation 31 (Marqueur de cellule endothéliale), *cluster of differenciation 31 (Endothelial cell marker)*

CD68: Cluster de differentiation 68 (Marqueur de macrophage), *cluster of differenciation 68 (Macrophage cell marker)*

CETP: Protéine de transfert des esters de cholestérol, *cholesteryl ester transfer protein*

CLA: Acide linoléique conjugué, *conjugated linoleic acid*

CLS: Structures en forme de couronne; *crown-like structures*

CT: Tomographie axiale, *computed tomography*

DHA: Acide docosahexaénoïque, *docosahexaenoic acid*

DXA: Absorptiométrie biphotonique à rayons X, *dual-energy X-ray absorptiometry*

ECMS: Enquête canadienne sur les mesures de la santé, *Canadian Health Measures Survey*

EPA: Acide eicosapentaénoïque, *eicosapentaenoic acid*

FA: Acides gras, *fatty acids*

FCS: Taille adipocytaire, *fat cell size*

GI: Indice glycémique, *glycemic index*

GL: Charge glycémique, *glycemic load*

GLUT4: Transporteur de glucose 4 sensible à l'insuline, *glucose transporter type 4*

HC: Circonférence des hanches, *hip circumference*

HDL: Lipoprotéines de haute densité, *high density lipoproteins*

H&E: Hématoxyline et éosine, *hematoxylin & eosin*

HIF-α: Facteur induit par l'hypoxie, *hypoxia*-inducible factor 1

HIS: Analyse histologique, *histological analysis*

HL: Lipase hépatique, *hepatic lipase*

HOMA-IR: Évaluation du modèle d'homéostasie de la résistance à l'insuline, *homeostatic model assessment of insulin resistance*

hsCRP: Protéine C-réactive mesurée par méthode ultrasensible, *high-sensitive C-reactive protein*

HSL: Lipase hormonosensible, *hormone-sensitive lipase*

IGF-1: Facteur de croissance 1 analogue à l'insuline, *insulin-like growth factor 1*

IKK-β: Inhibiteur du facteur nucléaire kappa B, *inhibitor of nuclear factor kappa B kinase subunit beta*

IL-6: Interleukine-6, *interleukin 6*

IMC: Indice de masse corporelle, *body mass index*

IRS-1: Substrat 1 du récepteur de l'insuline, *insulin receptor substrate 1*

IUPAC: Union internationale de chimie pure et appliquée, *International Union of Pure and Applied Chemistry*

JNK: Kinase C-Jun du N-terminal, *C-Jun N-terminal kinase*

LCT: Triglycérides à longues chaînes, *long-chain triglycerides*

LDL: Lipoprotéines de faible densité, *low density lipoproteins*

LPL: Lipoprotéine lipase, *Lipoprotein lipase*

LPS: Lipopolysaccharide, *lipopolysaccharide*

MAPK: Protéine kinase activée par les mitogènes, *mitogen-activated protein kinase*

MCT: Triglycérides à moyennes chaînes, *medium-chain triglycerides*

MD: Diète méditerranéenne, *mediterranean diet*

MEDS: Score méditerranéen, *mediterranean score*

MeSH: Titre de sujets médicaux, *medical subject heading*

MRI: Imagerie par résonance magnétique, *magnetic resonance imaging*

MUFA: Acides gras monoinsaturés, *monounsaturated fatty acids*

Myf5: Facteur myogénique 5, *myogenic factor 5*

NAFLD: Stéatose hépatique non alcoolique, *non-alcoholic fatty liver disease*

NASH: Stéatohépatite non alcoolique, *non-alcoholic steatohepatitis*

NCEP: Programme national d'éducation sur le cholestérol, *National Cholesterol Education Program*

NF-κB: Facteur nucléaire kappa B, *nuclear factor-kappa B*

NIH: Instituts américains de la santé, *National Institutes of Health*

NGT: Tolérance au glucose normale, *normal glucose tolerant*

OM: Omental, *omental*

OMS: Organisation mondiale de la Santé, *World Health Organization*

OS: Fixation à l'acide osmique, *osmium tetroxide fixation*

PAI-1: Inhibiteur de l'activateur du plasminogène 1, *plasminogen activator inhibitor 1*

PCOS: Syndrome des ovaires polycystiques, *polycystic ovary syndrome*

PDGFRβ: Récepteur du facteur de croissance d'origine plaquettaire, *beta-type platelet-derived growth factor receptor*

PECAM: Molécule d'adhésion des cellules endothéliales aux plaquettes, *platelet endothelial cell adhesion molecule*

PKC: Protéine kinase C, *protein kinase C*

PLIN: Périlipine (protéine associée aux gouttelettes lipidiques), *perilipin (lipid droplet-associated protein)*

PPARγ: Récepteur activé par les prolifférateurs de peroxysomes gamma, *peroxisome proliferator-activated receptor gamma*

Pref-1: Facteur de préadipocyte 1, *preadipocyte factor-1*

PUFA: Acides gras polyinsaturés, *polyunsaturated fatty acids*

RC: Rapport de cotes, *odds ratio*

RR: Risque relatif, *relative risk*

SAT: Tissu adipeux sous-cutané, *subcutaneous adipose tissue*

SC: Sous-cutané, *subcutaneous*

Sca1: Antigène des cellules souches 1; *stem cells antigen-1*

SFA: Acides gras saturés, *saturated fatty acids*

SMD: Différence moyenne standard, *standard mean difference*

SSB: Boissons sucrées, *sugar-sweetened beverages*

SVF: Fraction stroma-vasculaire, *stroma-vascular fraction*

T2DM: Diabète mellitus de type 2, *type 2 diabetes mellitus*

ToF-SIMS: Spectrométrie de masse des ions secondaires en temps de vol, *time-of-flight secondary ion mass spectrometry*

TNFα: Facteur de nécrose tumorale alpha, *tumor necrosis factor alpha*

TZDs: Thiazolidinediones, *thiazolidinediones*

UCP1: Protéine découplante mitochodriale 1, *mitochondrial uncoupling protein 1*

VAT: Tissu adipeux viscéral, *visceral adipose tissue*

VEGF: Facteur de croissance de l'endothélium vasculaire, *vascular endothelial growth factor*

VEGFA: Facteur de croissance de l'endothélium vasculaire A, *vascular endothelial growth factor A*

VEGF-R2: Récepteur 2 du facteur de croissance de l'endothélium vasculaire, *vascular endothelial growth factor receptor 2*

vWF: Facteur de von Willebrand , *von Willebrand Factor*

VLDL: Lipoprotéines de très faible densité, *very low density lipoproteins*

WC: Circonférence de la taille, *waist circumference*

WHR: Ratio taille-hanches, *waist-to-hip ratio*

WMD: Différence moyenne pondérée, *weighted mean difference*

Wt1: Protéine tumorale Wilms, *Wilms tumor protein*

Zfp423: Protéine à doigts de zinc 423, *zinc finger protein 423*

Un expert c'est quelqu'un qui a fait toutes ses erreurs dans un champ réduit d'application
-Bohr

Remerciements

Vous aurez compris que je ne peux pas commencer cette section Remerciements sans souligner mon mentor scientifique, mon directeur de recherche Dr André Tchernof qui a su m'épauler dès la fin de mon baccalauréat pour la poursuite de mes études graduées. Je tiens à le remercier pour son écoute, sa légendaire disponibilité, son relativisme toujours positif, son talent de vulgarisateur.

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Avant-propos

Au cours de ma maîtrise, j'ai étudié la distribution des graisses chez l'humain sous plusieurs aspects. J'ai rédigé un chapitre de livre sur la modulation de la distribution du tissu adipeux par l'alimentation chez l'humain. Il est présenté au **Chapitre 1** de ce mémoire et a pour titre : *Diet as a potential modulator of body fat distribution*. Pour ce chapitre, j'ai mis en place et participé à la collecte des données de la littérature et à la rédaction du manuscrit. J'aimerais souligner l'aide de Geneviève B. Marchand, nutritionniste, à la collecte et à la rédaction du manuscrit, ainsi qu'au mentorat du Dr Tchernof lors de l'élaboration et de la révision finale du manuscrit. Ce chapitre a été soumis à la révision par les pairs. Il est présentement sous presse et sera le chapitre 6 du livre *Nutrition and Cardiometabolic Health* (*CRC Press, Taylor & Francis group, Krauss, Bray, Bergeron, Siri-Tarino Editors*).

J'ai également rédigé un article de type revue de la littérature sur l'hypertrophie adipocytaire comme marqueur de la dysfonction du tissu adipeux ainsi que des maladies métaboliques. Il a été publié en août 2015 sous le titre *Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction* dans le journal avec comité de révision par les pairs *Critical Reviews in Clinical Laboratory Sciences*. Cet article est présenté dans le **Chapitre 2** de ce mémoire. Pour ce manuscrit, j'ai participé à la mise en place et à la collecte des données provenant de la littérature, à l'analyse des données qui ont permis la création de figures originales et à la rédaction du manuscrit. J'aimerais souligner l'apport de Jennifer Labrecque, alors stagiaire d'été, pour son aide lors de la collecte préliminaire des données ainsi que l'apport du Dr Andréanne Michaud pour la révision critique du manuscrit. Je tiens aussi à remercier Dr Katherine Cianflone pour son support financier ainsi que sa supervision et le mentorat du Dr André Tchernof durant la rédaction de cet article.

Cette revue de la littérature a mené à la rédaction d'un article original intitulé : *Comparative analysis of three human adipocyte size measurement methods and their relevance for cardiometabolic risk*. Il est présenté dans le **Chapitre 3** de ce mémoire. Cet article a été resoumis pour publication après révision. Concernant ce manuscrit, j'ai participé à la collecte des données en laboratoire, à l'analyse

statistique et à l'interprétation des données. J'ai également élaboré les tableaux et les figures et rédigé en entier le manuscrit. J'aimerais remercier Dr Andréanne Michaud pour sa collaboration lors de l'interprétation des données, ainsi que le Drs Alain Géloën et Hubert Vidal de l'Université Claude Bernard Lyon 1 pour la génération d'une partie des données expérimentales. Merci aussi à Mélissa Pelletier pour son aide précieuse lors de la réalisation des expériences en laboratoire et son travail de coordination clinique. Je tiens aussi à remercier le gynécologue Gaétan Paris et toute l'équipe de chirurgie du Centre Hospitalier de l'Université Laval pour la supervision des aspects médicaux de cette étude et la collecte des échantillons de tissus adipeux. Finalement, j'aimerais remercier mon directeur de recherche, Dr Tchernof pour son soutien lors de la rédaction de ce manuscrit.

Introduction

1. Obésité humaine

1.1 Généralités

1.1.1 Définitions, Épidémiologie, Étiologie

L'obésité est une maladie reconnue depuis 1997 par l'Organisation mondiale de la Santé (OMS) (1). Celle-ci se définit par une accumulation excessive de tissu adipeux pouvant atteindre plus de 50% de la masse totale d'un individu (2). L'obésité est une pathologie très hétérogène caractérisée par plusieurs phénotypes distincts. Deux paramètres ont une influence primordiale sur la physiopathologie de l'obésité, soit l'accumulation de gras et sa localisation anatomique (3).

La prévalence mondiale de l'obésité a doublé depuis 1980 pour atteindre près de 600 millions d'individus (4). Les dernières données de l'OMS indiquent qu'en plus, près de 40% de la population mondiale souffre d'embonpoint (4). Au Canada, l'Enquête canadienne sur les mesures de la santé (ECMS) a permis de recueillir plusieurs informations populationnelles sur le surpoids et l'obésité, et ce, de manière standardisée. Les dernières données auto-rapportées nous indiquent une certaine stabilisation durant les cinq dernières années de la prévalence de l'obésité bien que celle-ci demeure élevée (5). Plus de 60% de la population nationale est en surpoids ou obèse et cette tendance est un peu plus élevée chez les hommes (**Figure 1**) (5). Par contre, les femmes sont plus nombreuses dans les catégories d'obésité sévère (classe 2 et 3).

L'excès de tissu adipeux résulte de plusieurs facteurs physiologiques, génétiques, épigénétiques, sociaux, comportementaux et psychologiques (6). La balance énergétique positive, l'environnement *in utero* et certaines hormones semblent affecter la prise de poids de manière marquée (6).

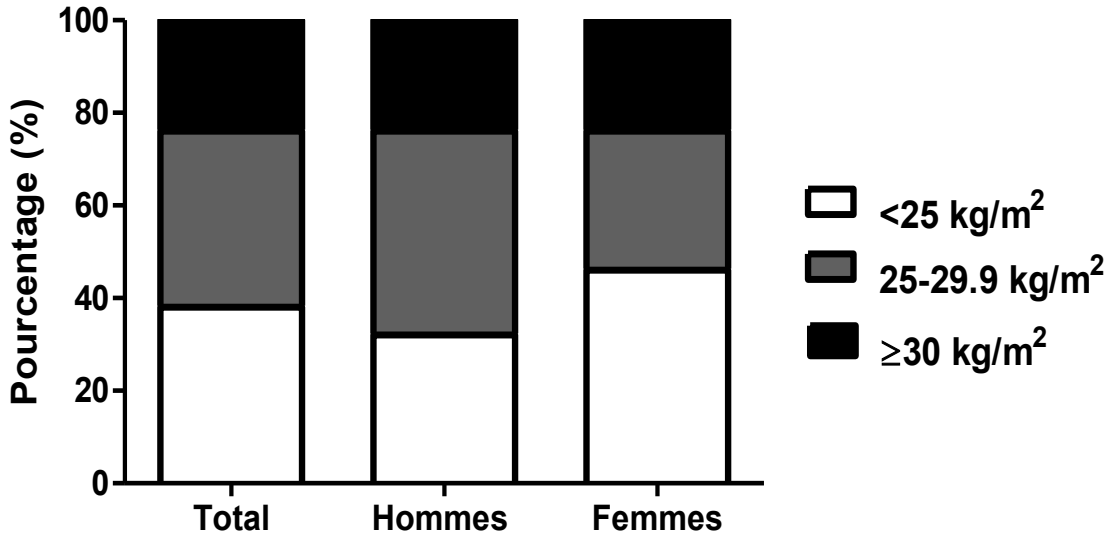

Figure 1. Pourcentage de la population canadienne pour chacune des catégories d'indice de masse corporelle (IMC).
Source : Rapport conjoint de l'Agence de la santé publique du Canada et de l'Institut canadien d'information sur la santé) (5)

1.1.2 Mesures d'adiposité

Plusieurs outils sont utilisés, autant par les chercheurs que les cliniciens, afin de mesurer adéquatement et à faible coût l'excédent de masse grasse. L'outil le plus utilisé en analyse populationnelle est l'indice de masse corporelle (IMC), qui consiste à diviser la masse (kg) par la taille en mètres au carré (m^2) (7). Cette mesure simple présente plusieurs avantages : faible coût, rapidité d'exécution et prédiction efficace du risque de maladies chroniques (7). De plus, cette méthode classifie les individus selon des catégories (sous-poids, normal, embonpoint et obésité (classe I, II et III)) qui sont reconnues et acceptées par la communauté internationale (8). Cependant, cette mesure possède des lacunes majeures. Elle ne permet pas de faire la distinction entre la masse maigre et la masse grasse. Elle ne tient donc pas compte des différences d'accumulation des graisses entre les hommes et les femmes, les jeunes et les personnes âgées, les divers groupes ethniques ou de certaines populations particulières comme les athlètes de haut niveau (7, 9, 10). C'est pourquoi plusieurs équipes de recherche prônent l'utilisation du tour de taille en complémentarité avec l'IMC en clinique pour déceler le surpoids et/ou l'obésité en

plus du risque de maladies chroniques qui les accompagne (7, 9, 11-16). En effet, la circonférence de la taille est un excellent indicateur de l'accumulation de gras au niveau abdominal, particulièrement du tissu adipeux viscéral (17). Tout comme l'IMC, elle est facile à mesurer, se fait pratiquement à coût nul et possède des valeurs seuils. Néanmoins, ces dernières varient selon le sexe et la population étudiée. Les États-Unis et le Canada ont adopté les valeurs proposées par le panneau de traitement des adultes (ATP-III; *Adult Treatment Panel*) du Programme national d'éducation sur le cholestérol (NCEP; *National Cholesterol Education Program*) soit 88 cm et 102 cm pour les femmes et les hommes respectivement (18). L'Europe a choisi l'exemple proposé par l'OMS, soit 80 cm et 94 cm pour un risque élevé et 88 cm et 102 cm pour un risque substantiellement plus élevé, chez les femmes et les hommes respectivement (19). Dans le même ordre d'idées, la méthode de mesure du tour de taille n'est pas standardisée. L'OMS recommande de prendre la mesure à mi-chemin entre la crête iliaque et la dernière côte flottante, alors que les Instituts américains de la santé (NIH; *National Institutes of Health*) propose de la prendre juste au-dessus de la crête iliaque pour limiter le nombre de repères anatomiques (18, 19). Les Japonais préconisent la mesure au niveau de l'ombilic et certaines équipes utilisent la mesure au point le plus mince entre les deux repères anatomiques proposés par l'OMS (19). Une autre variable très utilisée est le ratio taille-hanche, qui mesure aussi l'obésité abdominale et peut également donner des indices sur le profil de distribution gynoïde ou androïde des individus. On considère généralement que le rapport est élevé lorsqu'il dépasse 0,85 chez les femmes et 0,9 chez les hommes (20). Comme cet indice requiert la prise de deux mesures distinctes, elle est moins précise et plus susceptible aux erreurs (20). L'utilisation d'un ratio diminue l'information obtenue. Par exemple, deux individus ayant deux IMC très différents pourraient avoir le même ratio taille-hanche. Tout comme la circonférence de la taille, cette mesure devient moins précise pour les IMC dépassant 35 kg/m^2 (20). Une autre méthode est la mesure des plis cutanés, particulièrement utilisée chez les enfants étant donné son caractère peu invasif. Tout comme les autres mesures anthropométriques, elle est relativement rapide, peu coûteuse et peut s'effectuer facilement en cabinet sans matériel lourd et

encombrant. Des aires spécifiques, en utilisant un étrier adapté peuvent être ainsi mesurées (i.e. les plis subscapulaire, bicipital, tricipital, abdominal, supra-iliaque et inguino-crural) (21). La masse grasse totale peut ensuite être estimée par des équations validées dans la littérature (22, 23). Elle n'est cependant pas aussi reproductible que les autres méthodes et certaines équations utilisées ne tiennent pas compte de l'obésité du bas du corps, pouvant ainsi mal classifier certains individus (20). D'autres mesures sont aussi utilisées pour déterminer la composition corporelle, particulièrement en recherche, comme l'absorption biphotonique à rayons X (DXA) (24), la bioimpédance électrique (25, 26), le déplacement d'air par pléthysmographie (27), la méthode de dilution par hydrométrie (28) et la pesée hydrostatique (20, 29). Pour la mesure très spécifique de certains dépôts adipeux, particulièrement au niveau abdominal, la tomographie axiale et la résonnance magnétique sont deux outils très utilisés dans la littérature (30-32). Il s'agit des techniques les plus précises pour la quantification du tissu adipeux (33). La prochaine section de ce mémoire portera sur l'obésité abdominale et viscérale.

1.2 Obésité viscérale

1.2.1 Distribution des graisses

Les travaux du pionnier Jean Vague ont mis en évidence deux patrons de distributions des graisses, soit le profil gynoïde, associé à une accumulation de gras préférentielle dans le bas du corps et le profil androïde, lié à une accumulation de gras abdominal (34, 35). Ces patrons de distribution sont observés chez l'humain en présence ou en absence d'obésité. De manière générale, les femmes présentent le plus souvent un profil gynoïde, tandis que les hommes accumulent préférentiellement les graisses au niveau abdominal (3, 31, 35). Ce dimorphisme sexuel explique d'ailleurs, en partie, la différence observée dans le risque de maladies cardiovasculaires entre les hommes et les femmes (35). Tel qu'initialement rapporté par Jean Vague, le profil androïde est davantage lié à une détérioration du profil de risque métabolique que le profil gynoïde, et ce, pour le même niveau d'adiposité (**Figure 2**) (34, 36).

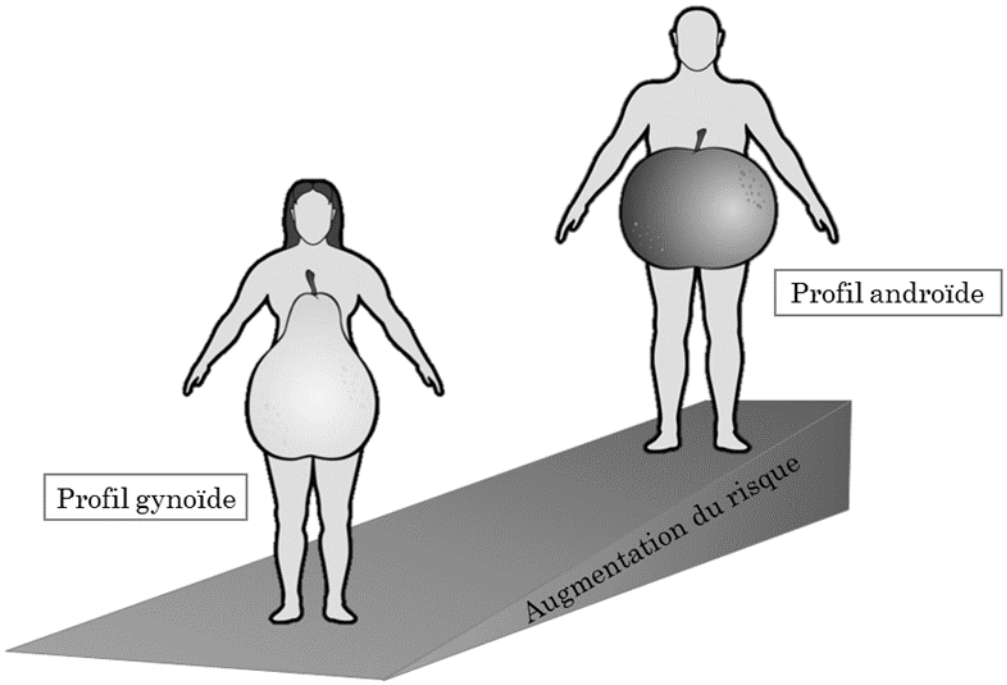

Figure 2. Le profil d'accumulation différentielle des graisses en fonction du sexe chez l'humain est lié à une augmentation du risque cardiométabolique chez les hommes. Les profils pomme et poire, fréquemment utilisés, sont également représentés.
Source : Figure adaptée de Vague, 1947 (34).

Bien que les femmes présentent plus souvent un phénotype gynoïde, certaines femmes accumulent les graisses préférentiellement au niveau abdominal, par des mécanismes partiellement élucidés. Par exemple, dans la cohorte Oxford BioBank, formée d'hommes et de femmes en bonne santé apparente de 30 à 50 ans, deux de ces femmes ont été appariées pour la masse maigre, la masse grasse et l'âge, mais présentant une différence au niveau des sites d'accumulation des graisses (36) (**Figure 3**).

Ce dimorphisme sexuel, présent chez l'humain pourrait être expliqué en grande partie par l'effet des hormones. Les œstrogènes seraient des modulateurs importants de la localisation du tissu adipeux dans la région sous-cutanée chez la

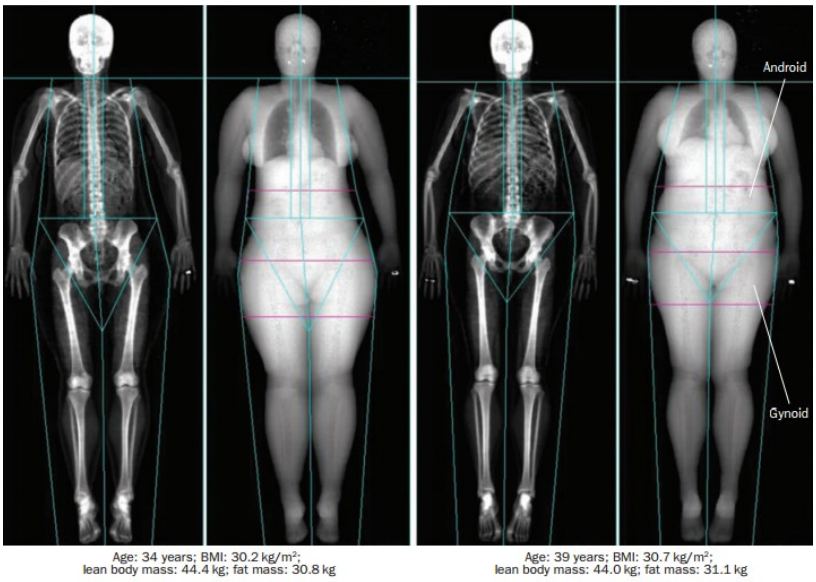

Figure 3. Composition corporelle mesurée par absorptiométrie biphotonique à rayons X chez deux femmes obèses appariées pour l'âge, la masse maigre et la masse grasse totale.
Distribution gynoïde (à gauche) et distribution androïde (à droite). Figure adaptée de Karpe et Pinnick, 2015 (36).

femme. Les études les plus parlantes sur ce phénomène sont celles réalisées chez les transsexuels. Chez les hommes traités avec de l'estradiol, les auteurs ont observé une augmentation du gras sous-cutané, tandis que chez les femmes traitées avec de la testostérone, la distribution des graisses a complètement changé, passant d'une accumulation périphérique à centrale (37-39). Comme l'accumulation préférentielle de gras au niveau viscéral est liée à plusieurs altérations métaboliques (discutées à la section suivante), il demeure important d'étudier les différents facteurs pouvant y jouer un rôle (voir **Section 1.2.3**).

1.2.2 Conséquences de l'obésité viscérale

L'excès de tissu adipeux amène et/ou reflète la présence de nombreux problèmes de santé majeurs touchant plusieurs systèmes du corps humain (cardiovasculaire, pulmonaire, nerveux, musculo-squelettique, tégumentaire, gastro-intestinal, génito-urinaire et rénal). Dans cette section, les principales altérations physiologiques associées à l'obésité viscérale seront décrites brièvement.

Dyslipidémie

L'obésité viscérale est étroitement associée à une détérioration du profil lipidique, par exemple, chez le patient à jeun, on observe une augmentation des acides gras libres (AGL), des triglycérides circulants et de l'apolipoprotéine B (ApoB), une diminution du cholestérol dans les HDL (lipoprotéines de haute densité) et une augmentation de la population de particules LDL (lipoprotéines de faible densité) et HDL petites et denses (40). La dysfonction des tissus adipeux (décrite à la section 2 de ce mémoire) augmente en phase postprandiale la disponibilité des substrats énergétiques, dont les AGL, ce qui stimule la production de VLDL (lipoprotéines de très faible densité) riches en triglycérides. Ce phénomène diminue l'activité de la lipoprotéine lipase (LPL) dans le tissu adipeux et dans le muscle squelettique et diminue également la lipolyse vasculaire des chylomicrons, induisant l'hypertriglycéridémie (41, 42). En présence d'hypertriglycéridémie, les VLDL transfèrent aux LDL et aux HDL des triglycérides en échange d'esters de cholestérol via l'activité de la protéine de transfert des esters de cholestérol (CETP) (43). Ces triglycérides sont par la suite hydrolysés au foie par la lipase hépatique (HL), diminuent le volume des particules LDL et HDL (44). L'activité de la HL est associée au risque cardiovasculaire ainsi qu'à l'aire de tissu adipeux viscéral, indépendamment de la masse grasse totale, soulignant encore une fois le lien prépondérant de ce dépôt avec la physiopathologie de l'obésité (45-47). Les particules LDL plus denses ont une clairance diminuée, favorisant leur attachement à la paroi vasculaire et le développement d'une plaque d'athérosclérose (48).

Résistance à l'insuline et diabète de type 2

L'insulinorésistance apparaît lorsque les tissus insulinosensibles ont une réponse diminuée voire défectueuse à l'insuline. De plus, la production de glucose du foie n'est pas inhibée, ce qui résulte en une augmentation de la néoglucogenèse. Le captage des molécules de glucose par le tissu adipeux et le muscle est déficient, contribuant aussi à l'hyperglycémie. L'adiposité viscérale est un facteur de risque de la résistance à l'insuline et donc du diabète de type 2 (49-53). L'impact des taux élevés d'AGL circulants sur le métabolisme ne se limite pas à une détérioration du

profil lipidique. En effet, les AGL peuvent altérer la signalisation de l'insuline via diverses voies de transduction dans plusieurs tissus tels que le foie, le tissu adipeux et le pancréas. Par exemple, les AGL peuvent activer certaines kinases comme la protéine kinase C (PKC), la protéine kinase activée par les mitogènes (MAPK; *mitogen-activated protein kinase*), l'inhibiteur du facteur nucléaire kappa B (IKK-β; *inhibitor of nuclear factor kappa B kinase subunit beta*) et la kinase C-Jun du N-terminal (JNK; *C-Jun N-terminal kinase*). Ces dernières peuvent altérer la signalisation de l'insuline, par exemple par la phosphorylation de certains résidus sérine du substrat 1 du récepteur de l'insuline (IRS-1; *insulin receptor substrate 1*) (54). Certaines adipokines sécrétées de manière plus importante dans le tissu adipeux viscéral que dans le sous-cutané peuvent également altérer la signalisation de l'insuline. Par exemple, plusieurs études suggèrent que l'interleukine-6 (IL-6), le facteur de nécrose tumorale alpha (TNFα; *tumor necrosis factor alpha*), la protéine qui stimule l'acylation (ASP; *acylation stimulating protein*) et l'inhibiteur de l'activateur du plasminogène 1 (PAI-1; *plasminogen activator inhibitor*) jouent un rôle dans la physiopathologie du diabète de type 2 (55-59). Les altérations des voies signalétiques peuvent aussi diminuer l'expression et la translocation du transporteur de glucose 4 sensible à l'insuline (GLUT4; *glucose transporter type 4*) à la membrane des adipocytes et des cellules musculaire et contribuent à augmenter la glycémie par une baisse du captage du glucose circulant par ces cellules (60).

Maladies cardiovasculaires

La circonférence de la taille est maintenant reconnue comme étant un facteur de risque des maladies cardiovasculaires indépendant de l'IMC (61). La formation de plaques d'athérosclérose est liée à la dyslipidémie de l'obésité viscérale décrite ci-haut. Brièvement, les macrophages résidents de la paroi vasculaire captent le cholestérol-LDL oxydé et deviennent des cellules spumeuses formant ainsi une plaque d'athérosclérose qui peut éventuellement se détacher, former un caillot et arrêter le flux sanguin, causant la mort des cellules à proximité (62). L'athérosclérose est donc l'une des causes de maladie coronarienne, de thrombose et d'accident vasculaire cérébral.

L'hypertension est aussi directement associée à l'accumulation de gras viscéral et à une circonférence de taille élevée (63). En effet, dans l'obésité les niveaux d'angiotensine II et d'aldostérone sont considérablement augmentés (64). Aussi, une augmentation du gras viscéral près des reins peut aussi affecter la fonction rénale, entre autres, par la compression de plusieurs structures telles que la médullaire interne du rein, ce qui diminue la capacité de filtration rénale (65).

Cancer

L'accumulation de graisse au niveau viscéral augmente de façon considérable le risque de plusieurs types de cancer, tels que le cancer colorectal, du sein, de l'œsophage, de l'endomètre, du pancréas, du rein, de la thyroïde et de la vessie, et ce, indépendamment de l'IMC (66-71). En effet, dans une méta-analyse récente comprenant 11 111 participants, la cote du cancer colorectal chez les individus ayant plus de 100 ou plus de 130 cm^2 de tissu adipeux viscéral était1,67 [IC à 95% : 1,29-2,16] fois celle chez le groupe n'ayant pas d'obésité viscérale (66). D'autres études montrent que l'excès de tissu adipeux viscéral augmente aussi la mortalité liée au cancer (72-74). Dans une autre méta-analyse, le risque de morbidité lié au cancer des sujets sans obésité viscérale était plus bas que celui des sujets montrant de l'obésité viscérale [RR=0,15; IC à 95% : 0,10-0,21] (75). Pour ce qui est du cancer du sein, l'adiposité générale, telle que définie par l'IMC, est un facteur de risque chez les femmes post-ménopausées tandis que la relation est moins claire pour ce qui est des femmes pré-ménopausées (76). Néanmoins, un ratio taille-hanche élevé est associé avec un risque élevé de cancer du sein, que ce soit avant ou après la ménopause. En effet, le risque de cancer du sein des femmes ayant un ratio taille-hanche élevé est plus élevé que celui des femmes ayant un plus petit ratio-taille hanche, chez les pré- (RC=1,79; [IC à 95% :1,22-2,62]) et les post-ménopausées (RC=1,50; [IC à 95% :1,10-2,04]) (67). Les mécanismes qui sous-tendent le lien entre l'adiposité et le risque ou la mortalité reliées aux cancers ne sont pas tous bien connus et compris. L'hyperinsulinisme et les autres facteurs de croissance analogues à l'insuline, l'inflammation chronique de faible intensité et plusieurs cytokines, les hormones sexuelles, certaines enzymes clés comme l'aromatase, le

stress oxydatif, l'hypoxie et certains médiateurs lipidiques sont présentement à l'étude (77). Plusieurs facteurs confondants sont aussi à prendre en compte, par exemple l'activité physique, l'historique de gain de poids et plusieurs aspects de la diète peuvent avoir des effets autant sur le risque de cancer que sur l'adiposité viscérale.

Syndrome métabolique

Le syndrome métabolique, précédemment nommé syndrome X, fait référence à plusieurs facteurs de risque des maladies cardiovasculaires et métaboliques, tel que le diabète de type 2. Ces facteurs comprennent : 1) une obésité abdominale i.e. viscérale (telle que déterminée par la mesure du tour de taille); 2) une dyslipidémie (un haut taux circulant de triglycérides et une diminution du cholestérol-HDL); 3) une tension systolique et diastolique élevée et 4) une glycémie augmentée (due à une résistance à l'insuline progressive) (18). Comme le premier critère diagnostique du syndrome métabolique est un tour de taille élevé (18), il est évident que le tissu adipeux viscéral est un marqueur-clé de toutes les altérations liées au syndrome métabolique. Même chez les individus minces, un tour de taille plus élevé que celui prédit est un marqueur de dysfonction (78). Les mécanismes reliant le syndrome métabolique (insulinorésistance, dyslipidémie, maladies cardiovasculaires) à l'obésité viscérale seront été décrits en détail dans la section sur les fonctions du tissu adipeux.

Stéatose hépatique

La stéatose hépatique (NAFLD; *non-alcoholic fatty liver disease*) est l'accumulation de triglycérides dans les hépatocytes. La stéatose est suivie par la stéatohépatite non alcoolique (NASH; *non-alcoholic steatohepatitis*) caractérisée par de l'inflammation et de la fibrose subséquente qui peuvent mener à la cirrhose du foie (79). Il est connu que les deux tiers des personnes obèses ont une stéatose hépatique (80). Encore une fois, les niveaux d'ASP et d'angiotensinogène, augmentés dans l'obésité, particulièrement dans l'obésité abdominale, sont

associés avec la résistance à l'insuline dans la NAFLD et leur diminution est liée à une amélioration de la fonction hépatique (81, 82).

1.2.3 Étiologie de l'obésité viscérale

Tel que discuté à la section **1.2.1**, le sexe biologique semble jouer un rôle majeur dans la répartition des graisses chez l'humain, effet expliqué en partie par les hormones sexuelles et par une capacité de stockage des lipides différentes. En effet, même si les femmes sont en partie protégées d'une circonférence de taille élevée par leur capacité plus grande à entreposer les surplus énergétiques dans les dépôts sous-cutanés périphériques, elles sont également plus nombreuses que les hommes à dépasser les recommandations de Santé Canada sur le tour de taille (**Figure 4**) (5). Ce phénomène peut être expliqué en partie par le fait que les critères semblent beaucoup plus sévères chez les femmes que chez les hommes, mais démontrent aussi une tendance lourde non négligeable soit que les femmes ont un risque de plus en plus grand de développer une maladie cardiométabolique.

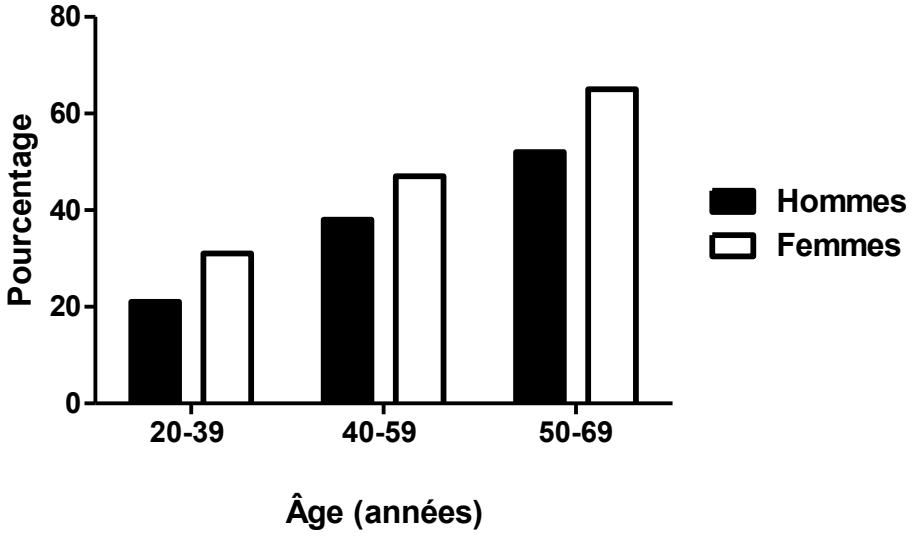

Figure 4. Pourcentage de la population canadienne qui dépasse les recommandations de Santé Canada sur la circonférence de la taille en fonction de l'âge.
Source : Rapport conjoint de l'Agence de la santé publique du Canada et de l'Institut canadien d'information sur la santé (5).

Néanmoins, plusieurs autres facteurs tels que l'âge, la génétique, l'origine ethnique, les glucocorticoïdes, l'hormone de croissance, la sédentarité ainsi que l'alimentation aurait un impact non négligeable sur l'accumulation de gras viscéral chez l'humain (16).

L'alimentation, comme potentiel modulateur de la distribution des graisses chez l'humain sera traitée en détail au **Chapitre 1** de ce mémoire. Brièvement, l'alimentation a d'abord été liée à l'accumulation de gras viscéral par son impact sur le bilan énergétique (83). En effet, un bilan énergétique positif est lié à une augmentation de la masse totale, par son effet sur la masse grasse. De plus, en contexte de perte de poids à la suite d'une diète ou de la pratique d'activité physique ou d'une combinaison des deux, la perte de masse grasse survient principalement au niveau du dépôt adipeux viscéral (84). Cependant, cet effet du bilan énergétique n'est pas spécifique à l'accumulation viscérale et serait davantage lié à d'autres patrons de distribution des graisses, tel qu'un profil gynoïde (85). La communauté scientifique s'est alors penchée sur le contenu en macronutriments et en certains nutriments pour tenter d'expliquer l'apport de l'alimentation à l'étiologie de l'obésité viscérale. Cependant, la littérature est disparate et aucune synthèse formelle n'a été réalisée avec l'objectif d'identifier des effets qui seraient indépendants de l'impact sur le bilan d'énergie et l'adiposité totale. Le **Chapitre 1** de ce mémoire présente les résultats de notre analyse critique de la littérature sur cette question.

2. Tissu adipeux

2.1 Évolution et origine

Le tissu adipeux se forme tout d'abord *in utero* et son expansion/régénération se poursuit tout au long de la vie (86). Cependant, chez l'humain et la plupart des mammifères, l'organogénèse est différente selon la localisation anatomique du tissu. Le premier tissu adipeux à se former est le tissu adipeux crânio-facial, provenant de la crête neurale (neuroectoderme) (87) suivi du tissu adipeux abdominal et finalement périphérique, suivant le déroulement normal de l'embryogenèse chez l'humain (88). Ces données sont différentes de celles observées chez la souris chez laquelle le tissu adipeux viscéral apparaît vers la fin de la parité et même après la naissance (86). Les dépôts sous-cutané et viscéral proviennent du mésoderme latéral et se forment en suivant le développement du système vasculaire, plus précisément des capillaires (89). Ces observations sont corroborées par les études récentes de deux groupes qui ont mis en évidence des marqueurs de cellules endothéliales (CD31+/PECAM+) et murales (péricytes) (α-SMA+/PDGFRβ+) dans les préadipocytes (90, 91). Le tissu adipeux brun, quant à lui, provient d'une lignée mésenchymateuse différente, du mésoderme paraxial qui est Myf5+, caractérisée comme myogénique (92). Le mésoderme ventral pourrait contribuer lui aussi au *pool* de préadipocytes de la cavité abdominale. En effet, les cellules mésothéliales peuvent se différencier en adipocytes *in vitro* (93). De plus, le tissu adipeux cardiaque peut se développer à partir de l'épicarde, le tissu mésothélial du cœur *in vivo* (93). Chez la souris, les cellules mésothéliales sont caractérisées par l'expression du gène Wt1 et d'autres marqueurs connus des préadipocytes (CD29, CD34, Sca1) (93). Wt1 est d'ailleurs un gène candidat permettant de différencier durant le développement les deux dépôts majeurs, viscéral et sous-cutané (94). D'autres gènes candidats sont d'ailleurs à l'étude afin de faire la distinction entre les dépôts. CD10 est seulement exprimé dans le tissu sous-cutané et CD200 est exprimé à la hauteur de 80% dans le tissu adipeux viscéral et à 20% dans le sous-cutané (95). Cependant, l'expression de ces marqueurs et d'autres gènes prometteurs ne se limitent pas aux préadipocytes rendant la signature génique plus

complexe à développer. Les marqueurs récemment découverts, le facteur de préadipocyte 1 (Pref-1; *preadipocyte factor 1*) et la protéine à doigts de zinc 423 (Zfp423; *zinc finger protein*) (90, 96), seraient fortement liés au potentiel adipogénique des préadipocytes, ne montrent pas de différences d'expression entre les deux dépôts. Il pourrait s'agir en fait de marqueurs d'adipogenèse plus avancés, cest-à-dire des préadipocytes engagés plus loin dans la voie de différenciation.

Les préadipocytes provenant du tissu viscéral ou sous-cutané ont donc des marqueurs cellulaires distincts concordant avec leurs fonctions et leur capacité adipogénique respectives. Par exemple, les thiazolidinediones (TZDs) ont un effet adipogénique seulement dans les préadipocytes sous-cutanés, et ce, même si l'expression du récepteur activé par les proliférateurs de peroxysomes gamma (PPARγ; *peroxisome proliferator-activated receptor gamma*) est similaire entre les adipocytes viscéraux et sous-cutanés (97). Les deux dépôts ne diffèrent pas seulement en termes d'origine, mais aussi en termes de composition tissulaire, ce qui sera abordé à la section suivante.

2.2 Composition tissulaire

Le tissu adipeux est un tissu conjonctif très hétérogène. Il est aussi riche en composants acellulaires telles que les fibres réticulaires (principalement de collagène) formant la matrice extracellulaire du tissu, qui varie selon l'adiposité et le dépôt étudié (98, 99). Il est non seulement composé de cellules très spécialisées, soit les adipocytes, qui forment de 30 à 50% (en nombre) du tissu (100), mais aussi d'une fraction stroma-vasculaire regroupant des cellules endothéliales, des cellules murales, des fibroblastes, des cellules souches, des préadipocytes ainsi que des cellules immunitaires du système inné (macrophages, mastocytes, éosinophiles, neutrophiles) et du système acquis (lymphocytes, cellules dendritiques) (**Figure 5**) (101, 102). De plus, la proportion des différentes cellules composant la fraction stroma-vasculaire varie aussi selon le dépôt, ainsi que selon le degré d'obésité (100, 103). Le tissu sous-cutané contient plus de cellules progénitrices d'adipocytes, tandis que le tissu viscéral est davantage vascularisé (104). Les deux tissus sont également différentiellement innervés (104). Le tissu adipeux viscéral,

particulièrement le dépôt omental contient aussi des régions fortement concentrées en cellules immunitaires, les «*milky spots*», qui sont absentes des tissus sous-cutanés (105-107). Le tissu adipeux viscéral contient également des cellules mésothéliales, contrairement au tissu sous-cutané (94, 108, 109). Ces deux dernières caractéristiques, ajoutée aux multiples données de la littérature indiquant que l'accumulation de macrophages et la sécrétion de cytokines pro-inflammatoires sont plus abondantes dans le tissu viscéral que dans le tissu sous-cutané (110-113), font de ce premier un tissu beaucoup plus susceptible à l'inflammation chronique de faible intensité associée à l'obésité. De manière similaire, le tissu adipeux viscéral contient un nombre plus important de structures en forme de couronne (CLS; *crown-like structure*), constituées d'adipocytes nécrosés entourés de macrophages (114, 115). Ainsi, la localisation anatomique de ces dépôts et leur nature intrinsèque jouent un rôle important dans leurs fonctions, ce qui sera discuté dans les deux sections suivantes.

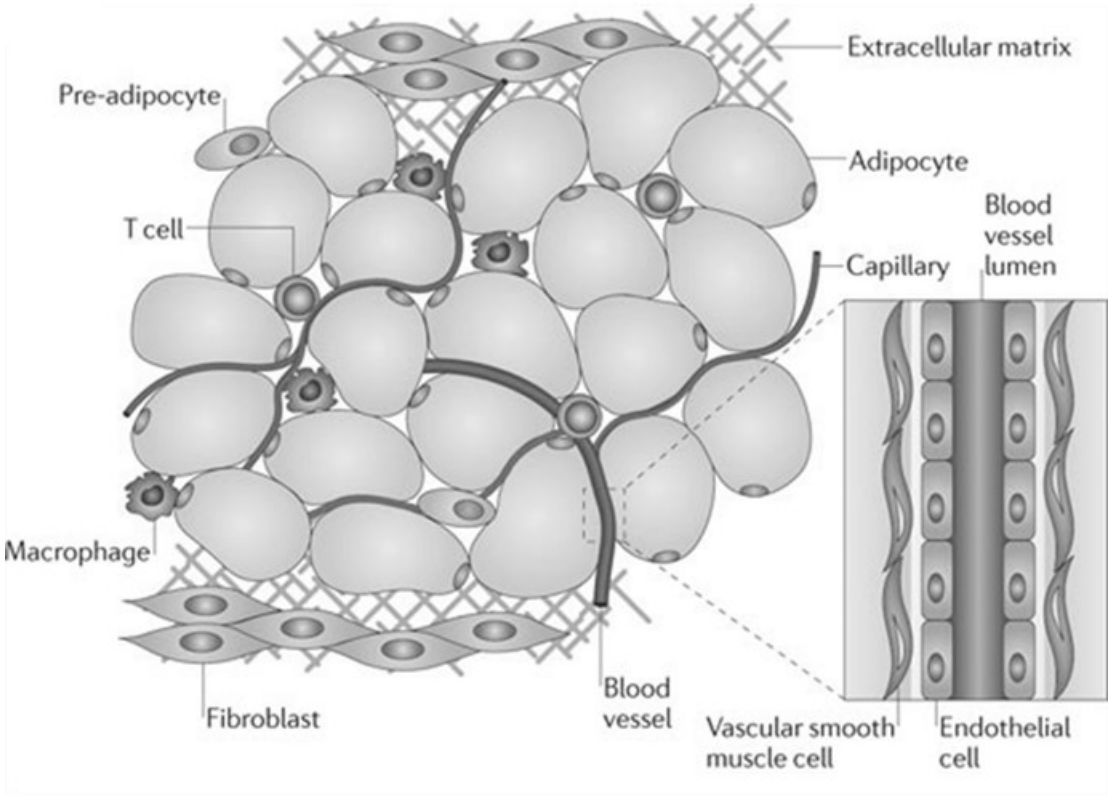

Figure 5. Composition cellulaire du tissu adipeux blanc.
Figure tirée de Ouchi, 2011 (101).

2.3 Classification

Les organismes unicellulaires comme les levures sont en mesure d'accumuler des triglycérides dans certaines conditions physiologiques. Les invertébrés, comme les modèles *C. elegans* ou encore *Drosophila melanogaster*, accumulent de l'énergie sous forme de triglycérides dans des cellules s'apparentant à celles du foie, localisées près du tractus intestinal et du système lymphatique (116). Ces cellules spécialisées ont aussi une fonction endocrine et sécrètent divers peptides ayant des fonctions similaires à la leptine et au facteur de croissance 1 analogue à l'insuline (IGF-1; *insulin-like growth factor 1*) (117, 118). Chez l'humain et chez les mammifères, le tissu adipeux a évolué vers un tissu extrêmement spécialisé pouvant former plus de 50% de la masse totale chez l'obèse (2).

Le tissu adipeux se classifie tout d'abord en deux types histologiques et fonctionnels très distincts, soit le tissu adipeux blanc et le tissu adipeux brun. L'adipocyte blanc est constitué d'une seule gouttelette lipidique de taille variable qui peut occuper jusqu'à 95% du volume de la cellule (119). Cette dernière effectue une pression sur le cytoplasme, qui forme une mince couche entre la gouttelette lipidique et la membrane cellulaire et le noyau de la cellule, lui donnant une forme en croissant de lune qui est caractéristique de l'adipocyte (119) (**Figure 6**).

L'adipocyte blanc, peu après sa différentiation terminale, accumule des lipides intracellulaires dans différentes vésicules qui fusionnent et prennent de l'expansion assez rapidement, contrairement à l'adipocyte brun généralement de forme polygonale, qui demeure multiloculaire (119). Cette différence dans l'organisation des lipides intracellulaires et de la taille pourrait découler de l'expression de la protéine S-100 exprimée chez les adipocytes blancs matures et qui diminuerait la rigidité du cytosquelette et donc l'expansion de la gouttelette lipidique (120).

L'adipocyte brun quant à lui présente un nombre important de mitochondries et est caractérisé par l'expression de la protéine découplante mitochondriale 1 (UCP1; *mitochondrial uncoupling protein 1*) (121). Il s'agit aussi d'un tissu fortement innervé et ayant une excellente vascularisation (122). On pensait jusqu'à assez récemment

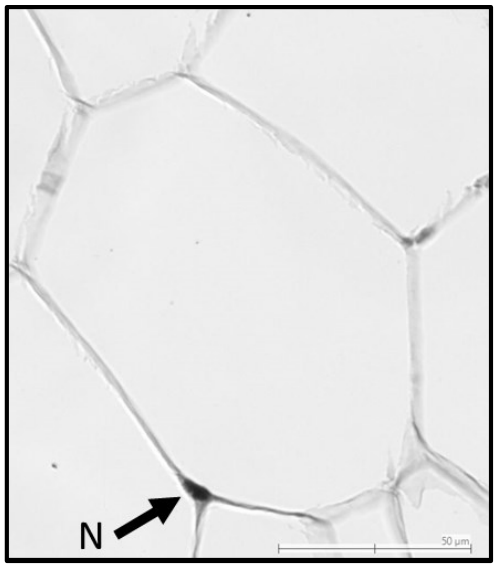

Figure 6. Représentation microscopique d'un adipocyte blanc avec son noyau (N) en forme de demi-lune.
Source : Données non-publiées, Laforest et Tchernof.

que ce tissu était seulement présent chez les nouveau-nés et les petits rongeurs. Quoique ce tissu soit très peu présent chez l'adulte, des études récentes de tomographie par émission de positrons ont montré sa présence et son activité dans certaines régions telles que le cou, le médiastin et la région supraclaviculaire (123). La quantité et l'activité du tissu adipeux brun peuvent être stimulées par l'activation du système nerveux sympathique ou en réponse au froid (124-126).

Le tissu adipeux se classifie aussi selon sa localisation anatomique (**Figure 7**) (inspirée de (127)). Tel qu'illustré à la **Figure 7**, le tissu adipeux se regroupe principalement en deux grandes catégories, soit le tissu viscéral qui représente 20% de la masse grasse chez l'homme et 5-8% de la masse grasse chez la femme (128), et le tissu sous-cutané. Chez l'humain, le tissu adipeux viscéral est composé des dépôts mésentérique, rétropéritonéal, périrénal, pararénal et omental. Le tissu adipeux mésentérique se situe dans le tissu conjonctif tout au long de l'intestin. Le tissu rétropéritonéal se situe entre les vertèbres et la paroi abdominale postérieure (119). Chez l'humain, les reins sont pourvus de deux dépôts adipeux; le périrénal séparé du rétropéritonéal par un pli péritonéal et le pararénal séparé du périrénal par le fascia rénal. Le dépôt omental constitue le grand omentum, soit la structure

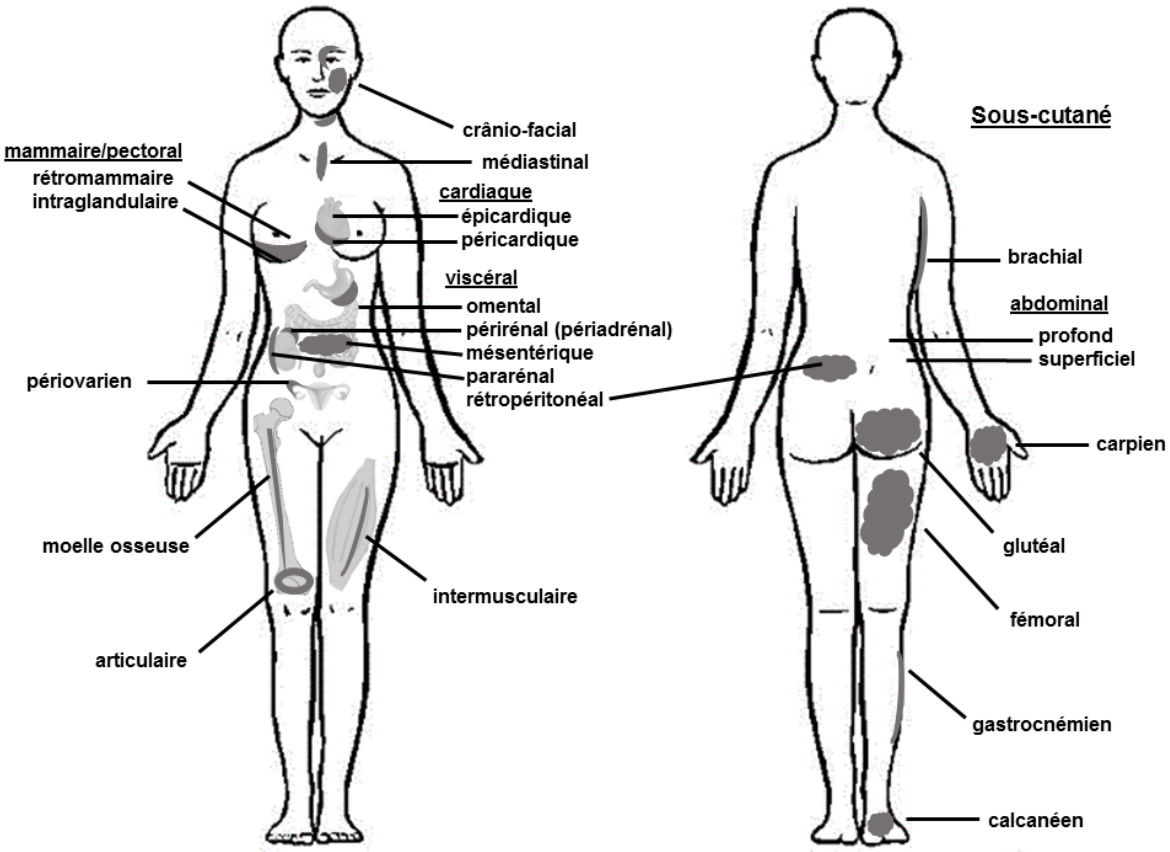

Figure 7. Localisation anatomique des dépôts adipeux blancs chez l'humain.
Figure inspirée de Cook et Cowan, 2009 (127).

commençant près de la grande courbure de l'estomac et qui forme un tablier recouvrant les intestins. Ce dépôt est quasi imperceptible chez les rongeurs. En effet, chez ceux-ci, le principal dépôt adipeux est le tissu gonadique et il s'agit donc du plus étudié. Ce dépôt est également présent chez l'humain, mais seulement chez la femme (tissu adipeux périovarien).

Pour ce qui est du tissu adipeux sous-cutané, il est situé sous la peau sur pratiquement toute la surface du corps. Les dépôts les plus importants sont ceux situés au niveau brachial, glutéal, fémoral et gastrocnémien ainsi qu'au niveau abdominal où il se sépare en deux compartiments, le profond et le superficiel, ces derniers étant séparés par un fascia. Les dépôts situés au niveau des mains (carpien) et au niveau du talon (calcanéen) sont davantage des dépôts de support et de forme. Quoiqu'habituellement classifié comme un tissu sous-cutané, le tissu adipeux mammaire fait lui-même l'objet d'une classification secondaire. Il est

composé du tissu sous-cutané de l'épiderme, du tissu intraglandulaire (localisé près des glandes mammaires) et du tissu rétromammaire près du muscle pectoral (129). Le tissu adipeux cardiaque est souvent classifié comme un dépôt ectopique. Il se sépare en deux groupes, soit le tissu adipeux épicardique, situé entre le myocarde et l'épicarde et nourri par les artères coronaires et le tissu péricardique, qui entoure le cœur dans le médiastin (130). D'autres dépôts ne se retrouvent pas dans ces catégories, soit le tissu adipeux crânio-facial, articulaire, médiastinal, intermusculaire ainsi que la moelle osseuse jaune.

Dans le cadre de ce mémoire, les dépôts adipeux qui seront discutés dans les sections suivantes seront le tissu adipeux viscéral, plus précisément le dépôt omental, ainsi que le tissu sous-cutané abdominal. En effet, chacun des dépôts possède des caractéristiques fonctionnelles différentes qui se traduisent par une morphologie qui leur est propre. L'intérêt d'étudier les dépôts abdominaux provient des études déjà discutées liant l'obésité abdominale viscérale aux complications cardiométaboliques de l'obésité.

Comme nous ne pouvons pas dissocier la structure de la fonction, la prochaine section traitera des diverses fonctions du tissu adipeux en mettant l'accent sur les principales différences entre les dépôts.

2.4 Fonction

Le tissu adipeux a longtemps été considéré comme un organe quasi inerte ne possédant qu'une seule fonction, l'entreposage et la libération des lipides. Depuis la découverte de la leptine en 1994 (131), il est maintenant reconnu qu'il s'agit d'un tissu complexe, avec plusieurs fonctions endocrines, paracrines et autocrines. La **Figure 8** énumère les principales fonctions du tissu adipeux ainsi que certains processus métaboliques dans lesquels le tissu adipeux est activement impliqué. Même si la plupart des dépôts adipeux chez l'humain partagent *grosso modo* les mêmes fonctions, celles-ci diffèrent particulièrement en termes d'intensité et de mécanistique.

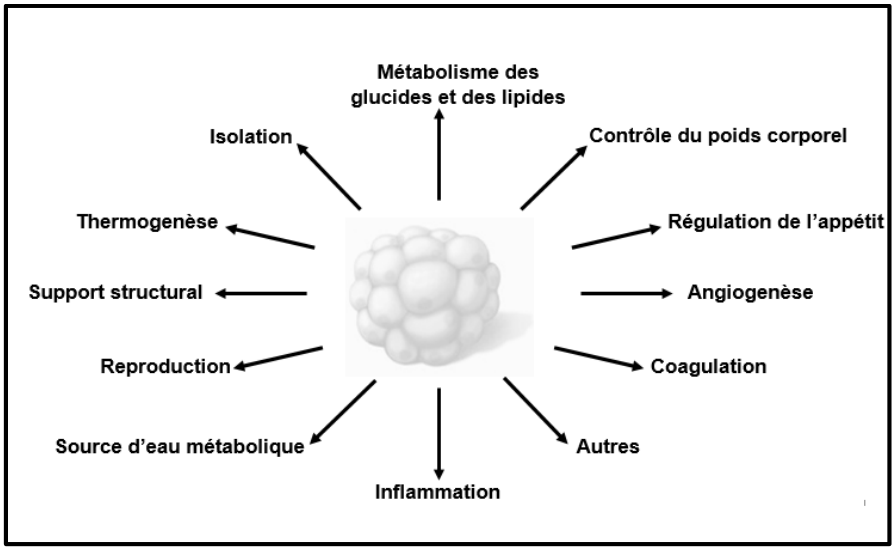

Figure 8. Principales fonctions et divers processus métaboliques du tissu adipeux.
Figure inspirée de Trayhurn, 2013 (132).

Le rôle principal du tissu adipeux est lié au métabolisme des lipides et des glucides. Son rôle est d'entreposer les acides gras sous la forme préférentielle de triglycérides et selon les besoins énergétiques, de les hydrolyser via l'action des lipases afin de libérer les acides gras dans la circulation afin qu'ils soient oxydés dans les mitochondries des organes cibles. De par son transporteur de glucose sensible à l'insuline (GLUT4), le tissu adipeux peut aussi augmenter son absorption post-prandiale du glucose et entreposer l'excédent énergétique. En période post-prandiale, le tissu adipeux viscéral est plus actif métaboliquement que le tissu sous-cutané. En effet, le tissu adipeux viscéral capte davantage d'AGL que le tissu sous-cutané par unité de masse (133). De plus, plusieurs études rapportent une activité accrue de la LPL, qui hydrolyse les triglycérides des VLDL et des chylomicrons, dans le dépôt adipeux viscéral, particulièrement chez les hommes, alors que chez les femmes cette relation est moins claire et probablement atténuée par la plus grande capacité d'entreposage des femmes dans le tissu sous-cutané (40). Pour ce qui est du captage du glucose au niveau basal ou en réponse à l'insuline, les adipocytes viscéraux capteraient aussi davantage de molécules de glucose que ce soit au niveau basal ou en réponse à l'insuline (134-137).

Outre son rôle majeur dans le métabolisme des lipides et des glucides, le tissu adipeux contribue aussi à la régulation du poids corporel, donc au bilan énergétique, ainsi qu'à la régulation de l'appétit et de la satiété via les actions combinées de la leptine, de l'adiponectine et de plusieurs autres hormones. Comme les populations de cellules de la fraction stroma-vasculaire diffèrent entre les dépôts et que celles-ci sont des sources majeures d'adipokines, des différences majeures sont présentes entre les deux dépôts. Par exemple, la leptine, une des hormones de satiété (131), est exprimée et sécrétée de manière plus importante dans le dépôt sous-cutané, reflétant davantage la masse grasse totale (138). De plus, l'activité β-adrénergique plus élevée dans le dépôt viscéral inhibe la production de la leptine et de l'adiponectine (139-144).

D'autres protéines sécrétées par le tissu adipeux englobent un large éventail de processus métaboliques, comme l'angiogenèse (VEGF; *vascular endothelial growth factor*), la coagulation (PAI-1) et la régulation de la pression sanguine (angiotensinogène) (145-152). Le tissu adipeux joue également un rôle primordial dans la reproduction et dans le métabolisme des hormones stéroïdiennes (153). Après la ménopause, le tissu adipeux devient la source principale d'œstrogènes (154, 155). Les œstrogènes influencent grandement plusieurs aspects du métabolisme et de la fonction du tissu adipeux (156). En effet, ceux-ci ont un effet sur la régulation de la balance énergétique puisqu'ils augmentent la sensibilité à la leptine (157). De plus, les œstrogènes exogènes diminuent l'activité de la LPL (158, 159), augmentent l'activité de la lipase hormonosensible (HSL; *hormone-sensitive lipase)* (159) et augmentent l'adipogenèse, particulièrement dans le tissu sous-cutané (158). Le tissu adipeux sert aussi de support structural, particulièrement au niveau du visage, des pieds et des mains. Il sert également d'isolant thermique au niveau de la peau (sous-cutané) et il produit de la chaleur, par thermogenèse (tissu adipeux brun). De manière intéressante, chez certaines espèces, le tissu adipeux est une source essentielle d'eau métabolique, c'est-à-dire d'eau provenant de l'oxydation des macronutriments (160). En effet, plusieurs espèces désertiques et certains mammifères marins nécessitent une source d'eau alternative à celle présente dans leur environnement (160).

Finalement le tissu adipeux et son expansion dans l'obésité, comme site principal d'entreposage de l'excédent énergétique contribue, jusqu'à un certain point, à la protection contre la lipotoxicité du foie, du cœur, du pancréas, du muscle et des autres dépôts ectopiques (40).

2.5 Dysfonction du tissu adipeux

La dysfonction des tissus adipeux est au cœur des hypothèses formulées pour expliquer le lien entre l'obésité viscérale et les comorbidités associées à l'obésité. Le tissu adipeux dysfonctionnel est caractérisé par l'altération de ses fonctions, soit par une capacité d'entreposage et de gestion des lipides altérée (lipolyse, lipogenèse, adipogenèse, hypertrophie adipocytaire), par une homéostasie du glucose et de l'insuline altérée, un profil pro-inflammatoire (recrutement de cellules immunitaires et synthèse d'adipocytokines augmentée) et par un remodelage de la matrice extracellulaire (angiogenèse et fibrose). Les hypothèses qui font le lien entre les composantes de la dysfonction du tissu adipeux, l'obésité viscérale et les altérations métaboliques seront décrites dans cette section.

Un des scénarios repose sur les propriétés intrinsèques du tissu adipeux viscéral et sa localisation anatomique. Tel que précédemment décrit, le tissu adipeux viscéral a une réponse hyper-lipolytique de dégradation des triglycérides intracellulaires. Il est également résistant à l'action anti-lipolytique de l'insuline. Ces phénomènes augmentent la libération locale d'AGL. Ces derniers sont directement acheminés au foie, car le dépôt viscéral est drainé par la veine porte, contrairement au dépôt sous-cutané abdominal, qui est drainé par les veines caves inférieures et supérieures. Le surplus d'AGL au foie augmente la néoglucogenèse, peut augmenter la production de VLDL (et d'ApoB) (voir **section 1.2.2**) et diminuer la dégradation de l'insuline par le foie (161-163). Ce dérèglement du métabolisme du foie par l'augmentation des AGL pourrait aussi expliquer en partie l'association entre l'obésité viscérale et la stéatose hépatique (75, 76)

Cette hypothèse pour expliquer le lien entre les altérations métaboliques et l'obésité viscérale n'est pas supportée par plusieurs études récentes. En effet, les AGL en circulation proviendraient principalement du tissu adipeux sous-cutané dans une proportion de plus de 85% (164). Plusieurs études de l'équipe du Dr Jensen à la clinique Mayo ont démontré que la graisse sous-cutanée abdominale est le site principal de libération des AGL et que le tissu adipeux viscéral n'est pas le contributeur le plus important de l'élévation des AGL en circulation, et cela chez les

femmes et les hommes, avec ou sans diabète (164-168). Cependant, dans l'obésité, la contribution de la lipolyse du dépôt viscéral au *pool* d'AGL dans la veine porte augmente, passant de 10% à 50% (168). Ces résultats démontrent que c'est bien l'excès de tissu adipeux et non sa présence en tant que telle qui est tributaire des altérations métaboliques. Ces conclusions sont supportées par les études de transplantation de tissu adipeux. En effet, des études de transplantation autologue de tissu adipeux sous-cutané dans la cavité abdominale chez des souris en santé ont montré une réduction de la masse totale (masse maigre et masse grasse), de la masse grasse, de la glycémie, des niveaux d'insuline à jeun, ainsi que de la sensibilité à l'insuline à la suite de clamp hyperinsulinémique-euglycémique (169-171). Il n'y avait pas de différence dans le profil des souris lorsque celles-ci subissaient une transplantation autologue de tissu viscéral dans la région sous-cutanée (169-171). Dans le même ordre d'idée, dans le cadre d'une étude en chirurgie bariatrique, l'équipe suédoise de Johan Hoffstedt et de Peter Arner a démontré que le profil de résolution des comorbidités n'était pas amélioré ni empiré à la suite d'une omentectomie totale durant une dérivation gastrique Roux-en-Y (172). Ces résultats tendent à démontrer que c'est bien l'excès de gras viscéral et non la présence en tant que tel de tissu adipeux dans la cavité abdominale qui est lié aux altérations métaboliques de l'obésité de par ses propriétés intrinsèques.

D'autre part, des études effectuées chez le chien par l'équipe du Dr Bergman ont montré qu'à la suite de l'inhibition des récepteurs β3-adrénergiques avec du bupranolol, il y avait une baisse de 50% des AGL en circulation systémique après un jeûne de 24 heures (173). De plus, cette baisse semblait attribuable à la disparition complète des fluctuations rapides de lipolyse observées lors de l'infusion avec la solution saline (1 heure avant le début de l'infusion de bupranolol), ces fluctuations représentant 40% des AGL en circulation (173). Comme les récepteurs β-adrénergiques sont exprimés de façon plus importante dans le tissu adipeux viscéral que dans le tissu adipeux sous-cutané et qu'ils sont plus sensibles aux agonistes β-adrénergiques, cette étude démontre que l'apport accru au foie en AGL par le tissu adipeux repose sur des concepts concrets, mais que ce ne serait pas le seul contributeur des altérations métaboliques associées à l'obésité (128, 174-176).

La seconde hypothèse exploite la fonction endocrine du tissu adipeux. Tel que décrit précédemment, le tissu adipeux viscéral comprend davantage de cellules immunitaires et leur recrutement accru dans l'obésité pourrait conduire à l'exacerbation du profil pro-inflammatoire déjà caractéristique du dépôt viscéral (145, 177). Le relâchement de cytokines inflammatoires acheminées directement au foie pourrait conduire aux altérations métaboliques telles que la résistance à l'insuline (145, 161). En effet, plusieurs adipocytokines altèrent la signalisation de l'insuline (55-59).

Plusieurs études chez l'animal pointent vers un rôle majeur du profil sécrétoire du tissu adipeux viscéral dans la pathogenèse des altérations cardiométaboliques. Patsouris et collaborateurs ont utilisé un modèle de souris transgénique, conditionnellement CD11c (marqueur de macrophages M1; pro-inflammatoire) négatives à la suite d'injection de toxine diphtérique (178). Ils ont montré que lorsque ces souris sont soumises à une diète riche en gras, le nombre de CLS ainsi que les niveaux systémiques et locaux de cytokines pro-inflammatoires sont diminués en comparaison avec les souris sauvages, soumises à la même diète, et ce, sans différence sur le gain de poids (178). Les souris CD11c conditionnellement négatives avaient aussi une meilleure sensibilité à l'insuline, tant au niveau du muscle, que du foie et du tissu adipeux (mesurée par clamp euglycémique) (178).

Par ailleurs, d'autres travaux tendent à démontrer un rôle important des cellules immunitaires et des cytokines pro-inflammatoires dans l'homéostasie du tissu adipeux et dans l'adipogenèse du tissu adipeux. L'équipe du Dr Scherer au Texas a développé dans les dernières années un modèle de souris transgénique, l'*adipochaser mouse* qui permet de visualiser la génération de nouvelles cellules adipocytaire ou l'hypertrophie des cellules préexistantes (179). Ce modèle de souris a permis de mettre en évidence que des injections de lipopolysaccharide (LPS) directement dans le tissu adipeux induisaient la différentiation adipocytaire (180). Dans le même ordre d'idées, chez des souris transgéniques exprimant le récepteur du TNFα sans activité intrinsèque, sur diète riche en gras, les adipocytes étaient hypertrophiques et il y avait augmentation de la fibrose (déterminée par coloration

au trichrome de Masson) (180). De plus, ces souris étaient résistantes à l'action de l'insuline et présentaient des niveaux faibles d'adiponectine (180). Ces souris avaient une masse plus petite que les souris contrôles, cependant la taille adipocytaire moyenne était similaire entre les deux groupes (180). Ce dernier résultat semble pointer vers un défaut de l'adipogenèse chez les souris transgéniques soumises à une diète riche en gras. La dualité des rôles de l'inflammation dans les complications métaboliques de l'obésité peut s'expliquer par la très fine modulation de ce système et de l'importance de son équilibre, autant local que systémique. En fait, la présence de cellules immunitaires dans les dépôts viscéraux est essentielle pour filtrer les endotoxines pouvant provenir du tractus digestif, telle que démontré par la présence de plusieurs organes lymphoïdes secondaires dans cette région, dont les *milky spots* (106).

Finalement, une accumulation de gras au niveau viscéral pourrait aussi être causée par un défaut de stockage dans le tissu adipeux sous-cutané. En effet, le tissu sous-cutané est le site préférentiel d'entreposage des lipides en situation de bilan énergétique positif. De plus, le tissu adipeux sous-cutané a un potentiel adipogénique plus élevé que le tissu adipeux viscéral, du moins *in vitro* (181). Par contre, une étude récente de l'équipe du Dr Scherer, effectuée avec un nouveau modèle de souris transgénique qui permet de suivre l'adipogènese *in vivo* (Adipochaser), tend à démontrer qu'en situation de gain de poids induit par une diète riche en gras, le tissu adipeux viscéral prend de l'expansion par adipogenèse alors que le tissu sous-cutané croît principalement par hypertrophie (179). Cependant, il faut souligner que chez la souris, le dépôt viscéral étudié est le dépôt gonadique et qu'il s'agit du dépôt le plus grand en importance chez la souris, alors que chez l'humain, le dépôt le plus volumineux est le dépôt sous-cutané. D'ailleurs, chez les femmes, on observe que les adipocytes sous-cutanés sont davantage hypertrophiques que ceux du dépôt omental, et ce, sur tout l'intervalle des valeurs d'IMC (182). Donc, une augmentation du tissu adipeux viscéral pourrait survenir lorsque le dépôt sous-cutané n'est plus en mesure de gérer les nouveaux influx de lipides. Cette incapacité à gérer le surplus énergétique augmenterait les AGL en circulation et donc à leur déposition dans les tissus ectopiques tels que le muscle,

le cœur, le foie et le pancréas. Il semblerait que la déposition de lipides dans les tissus ectopiques se produise avant l'apparition des complications métaboliques (183, 184). Une étude prospective (le projet FATe) est en cours en Espagne depuis 2015, chez des patients subissant une chirurgie laparoscopique élective (chirurgie bariatrique, cholécystectomie, chirurgie d'une hernie abdominale ou d'une hernie hiatale oesophagienne), minces à obèses, pour lesquels des biopsies de tissu adipeux, des échantillons sanguins ainsi que des mesures précises d'adiposité abdominale (tomographie axiale) seront obtenus (185). Cette étude devrait permettre de déterminer les facteurs et possiblement des marqueurs de l'incapacité du tissu sous-cutané à entreposer efficacement les lipides (185). Plusieurs études chez l'animal et chez l'humain supportent le scénario de l'incapacité du tissu sous-cutané à prendre de l'expansion dans l'obésité comme mécanisme engendrant les complications métaboliques liées à l'adiposité viscérale. Tout d'abord, les études de cas de lipodystrophie démontrent que les altérations métaboliques observées dans l'obésité sont également présentes chez les humains et les souris ayant une incapacité de stocker l'excédent énergétique. La lipodystrophie partielle chez l'humain est caractérisée par une diminution du tissu adipeux sous-cutané et une accumulation de gras au niveau intra-abdominal (186). L'obésité et la lipodystrophie augmentent toutes deux le risque d'insulinorésistance (49-53, 186). La clé est donc dans l'entreposage efficace des lipides afin de prévenir la lipotoxicité. C'est par ce mécanisme que le facteur de transcription PPARγ, acteur majeur de la lipogenèse et de l'adipogenèse, améliore la sensibilité à l'insuline (187). D'ailleurs, quelques patients ayant une mutation de PPARγ ont été décrits dans des études de cas et sont non seulement partiellement lipodystrophiques, mais sont également résistants à l'insuline, hypertendus et ont un foie gras (188-191). Chez l'animal, il a été démontré que les souris n'exprimant pas PPARγ avaient une réduction marquée de leur masse adipeuse (192-195). De manière intéressante, les souris ayant la même mutation que celle observée dans certains cas chez l'humain (P467L) ne présentaient pas d'altérations de leur métabolisme et pas de lipodystrophie (196). Par contre, lorsque ces souris étaient également déficientes en leptine (*ob*/*ob*), celles-ci étaient incapables d'entreposer l'excédent énergétique et souffraient

d'insulinorésistance de manière plus prononcée que les souris seulement *ob*/*ob* classiques (196). Parallèlement, la capacité adipogénique du tissu sous-cutané *in vitro* diminue avec l'augmentation de l'IMC et de la circonférence de la taille (181, 197-199). De plus, les femmes obèses ont un pourcentage réduit de préadipocytes engagés dans la voie de différenciation dans la fraction stroma-vasculaire de leur dépôt sous-cutané comparativement aux femmes minces (200). De surcroît, une étude de notre laboratoire a démontré qu'une capacité réduite de différenciation des préadipocytes sous-cutanés est corrélée à l'hypertrophie des adipocytes viscéraux et donc à une augmentation du gras viscéral, et ce, indépendamment de l'IMC (201). Dans cette même étude, la capacité réduite de différenciation était associée à un bilan lipidique détérioré c'est-à-dire à une augmentation des triglycérides plasmatiques, du contenu en lipides et en triglycérides des VLDL ainsi qu'à une glycémie à jeun plus élevée (201).

Bien que plusieurs études transversales supportent l'hypothèse d'une incapacité d'expansion du tissu adipeux comme un mécanisme important, possiblement causal des complications métaboliques liées à l'obésité viscérale, celles-ci ne permettent pas conclure à une relation de cause à effet. Dans cet ordre d'idées, l'étude de suralimentation de huit semaines chez l'humain de Johannsen et collaborateurs a démontré que la présence de plus gros adipocytes n'était pas tributaire des dérangements métaboliques observés à la suite du gain de poids (202). En fait, les individus caractérisés par une plus petite moyenne de taille adipocytaire au départ ont vu leur sensibilité à l'insuline diminuée de façon plus importante, et ce, sans différence de gain de poids et d'aire de tissu adipeux viscéral entre les groupes (202). Cependant, les auteurs ont pu déterminer que les participants ayant une taille adipocytaire plus élevée au départ sont aussi ceux qui ont eu la plus grande augmentation dans le nombre de nouveaux adipocytes dans le tissu sous-cutané en réponse à la suralimentation (202). D'autre part, les individus ayant une taille adipocytaire moyenne plus petite au départ sont ceux chez qui la masse totale du tissu adipeux sous-cutané a le plus augmentée. Dans une autre étude réalisée par McLaughlin et al., les individus insulinorésistants qui avaient de plus gros adipocytes au départ avaient aussi la plus grande proportion de petites cellules (203). Seuls les

participants insulinosensibles (avec de plus petits adipocytes en moyenne) ont augmenté leur masse grasse viscérale à la suite de la suralimentation (quatre semaines) (203). Leur pourcentage de petits adipocytes a aussi diminué (203). Seul le groupe insulinorésistant a eu une augmentation significative de l'aire de tissu adipeux sous-cutané abdominale. Le gain de poids entre les deux groupes était similaire. Les résultats de ces deux dernières études sont donc en partie incompatibles avec la théorie d'expansion du tissu adipeux. La relation entre l'obésité viscérale et les altérations métaboliques associées à l'obésité est donc expliquée en partie par les hypothèses formulées, mais la relation causale reste à démontrer.

2.6 Hypertrophie adipocytaire

L'altération des fonctions des adipocytes (décrites dans la section précédente) est étroitement liée à la morphologie des adipocytes. Le tissu adipeux peut prendre de l'expansion par deux mécanismes ou par une combinaison des deux: 1) l'hyperplasie des adipocytes, soit une augmentation du nombre par un recrutement de cellules progénitrices et 2) l'hypertrophie des cellules adipeuses, soit une augmentation de la taille des cellules préexistantes. Même si les deux voies mènent à une augmentation de la masse grasse totale, les données de la littérature suggèrent que l'hypertrophie des adipocytes est étroitement liée aux troubles cardiométaboliques associés à l'obésité, contrairement à l'hyperplasie du tissu adipeux qui aurait un effet protecteur, et ce, pour le même niveau d'adiposité (40). En effet, l'incapacité du tissu sous-cutané à entreposer des lipides de façon efficace en augmentant le nombre de cellules adipeuses est l'une des théories discutées dans la littérature pour expliquer le lien entre l'obésité viscérale et les complications métaboliques associées (40). L'hypertrophie du tissu adipeux et ses liens avec les diverses conditions physiopathologiques seront traités en détail dans l'article présenté au **Chapitre 2** de ce mémoire.

Même si plusieurs opinions ont été formulées dans la littérature concernant la taille adipocytaire et certaines altérations métaboliques, plusieurs associations restent à valider. Un des points de discussion concerne le site de prélèvement du tissu

adipeux. En effet, la variation de la taille adipocytaire chez un même individu/animal selon la localisation anatomique du dépôt adipeux a été observée dès 1968 (204). L'hypertrophie des adipocytes dans les deux dépôts les plus étudiés chez l'humain (viscéral et sous-cutané abdominal) ne génère pas systématiquement des associations similaires. Ce point sera discuté plus en détail dans le **Chapitre 2** de ce mémoire. Un autre point de divergence entre les études est la technique de mesure de la taille des adipocytes. Il apparaît donc d'une importance primordiale dans le cadre de ce mémoire de décrire en détail chacune des méthodes utilisées dans la littérature pour mesurer la taille adipocytaire. Il faut souligner, pour le lecteur, qu'il n'existe pas de mesure étalon-or pour mesurer la taille des adipocytes. Dans cette section, les trois techniques les plus fréquemment utilisées pour mesurer la taille des adipocytes matures seront décrites en détail ainsi que leurs variantes les plus communes. D'autres techniques émergentes ou peu fréquentes seront aussi discutées brièvement.

2.6.1 Méthode enzymatique : Digestion à la collagénase

Cette méthode a été décrite pour la première fois par Dr Rodbell comme un moyen efficace et assez rapide de séparer les adipocytes matures de la fraction stroma-vasculaire (205). Cette technique repose sur le postulat que la matrice extracellulaire du tissu adipeux est formée principalement de collagène. En incubant une biopsie de tissu adipeux avec une solution contenant une enzyme dégradant le collagène (la collagénase), les adipocytes se libèrent du tissu. Il est alors possible de séparer la fraction stroma-vasculaire des adipocytes libérés grâce à leur propriété de flottaison, car leur masse volumique est plus petite que l'eau en raison de leur contenu cytoplasmique riche en lipides. Ces cellules peuvent être isolées facilement par aspiration de la fraction stroma-vasculaire et à la suite de quelques lavages (206). Plusieurs modifications ont été apportées au protocole original de Rodbell afin d'optimiser la pureté et la viabilité des cellules isolées. Il est maintenant pratique courante d'utiliser des contenants de plastique ou de silicone au lieu de contenants de verre pour isoler les adipocytes, ce qui permet de limiter le nombre de cellules endommagées (207, 208). Aussi, l'ajout d'adénosine a permis de limiter le nombre d'adipocytes éclatés à la suite de la digestion (209). Une autre étape a été éliminée

par quelques laboratoires, soit la centrifugation durant la récupération des adipocytes matures, ce qui a été rapporté comme pouvant nuire à la récupération des petites cellules (210). Il faut cependant noter que cette méthode peut induire une sous-représentation des petits adipocytes qui ont un degré de flottabilité moindre dû à leur faible contenu lipidique. Après l'isolation, des photographies des adipocytes matures sont prises en utilisant un microscope à contraste de phase en déposant les cellules adipeuses sur un hémocytomètre ou une lame. Certaines équipes colorent les cellules (e.g. bleu de méthylène, bleu de trypan, BODIPY live) afin de vérifier leur viabilité ou encore pour différencier les gouttelettes lipidiques des adipocytes (205, 211, 212). Afin d'augmenter le nombre d'échantillons à traiter durant un même laps de temps, certaines équipes ont effectué un protocole de fixation des adipocytes matures libérés à la suite d'une digestion à la collagénase à l'aide d'une solution de formaldéhyde (10% v/v) (212, 213).

2.6.2 Méthode chimique : Fixation à l'acide osmique

En 1894, un histopathologiste allemand du nom de Richard Altmann a publié dans son recueil histologique que l'acide osmique (nom IUPAC : tétroxyde d'osmium, formule brute : OsO_4) noircit l'acide oléique ainsi que le triglycéride trioléine formé d'une molécule de glycérol et de trois acides oléiques (214). En 1959, des pathologistes américains ont introduit l'usage du tampon s-collidine (nom IUPAC : 2,4,6-Triméthylpyridine, formule brute : $C_8H_{11}N$) (215). L'avantage de cette solution tampon est sa très longue stabilité à température pièce, ainsi que l'augmentation de la perméabilité du tissu, ce qui facilite la fixation de larges blocs de spécimens. Il s'agit également d'un meilleur tampon au pH 7,4 que la solution de véronal-acétate précédemment utilisée (215). L'acide osmique comme fixateur a d'abord été exploitée en microscopie électronique étant donné sa capacité à fixer efficacement les structures délicates (216, 217). La technique proprement dite de fixation *in situ* des adipocytes matures à l'aide d'une solution d'acide osmique et de tampon s-collidine a été développée par Hirsch et Gallian (218). Ces derniers ont publié quelques techniques pour quantifier la taille des adipocytes. Leur approche de départ consistait à isoler les cellules selon la technique de Rodbell et de fixer ensuite les cellules afin de pouvoir les mesurer dans un autre temps et ce, pour augmenter

le nombre d'échantillons traités. Comme ils observaient une grande perte d'adipocytes due à la rupture de leurs membranes, ils ont expérimenté la fixation du tissu entier avec l'acide osmique suivie de la quantification par compteur Coulter Multisizer. Ce dernier utilise la résistance électrique produite lors du passage des particules pour générer un diamètre.

Quoique depuis les travaux d'Altmann il est reconnu que le tétroxyde d'osmium fixe les lipides, son mécanisme d'action n'a pas été complètement élucidé. Il a été observé à de multiples reprises que l'acide osmique colore les acides gras insaturés et non les saturés. Cette observation a permis de formuler l'hypothèse que cet acide réagit avec les alcènes et de rejeter l'hypothèse selon laquelle le tétroxyde d'osmium réagit avec une forte affinité avec la tête polaire des acides gras et des triglycérides (219). La plus forte affinité pour l'acide osmique a été observée avec l'acide oléique et son triglycéride dérivé, ainsi qu'avec d'autres acides gras insaturés (e.g. acide linolénique, acide linoléique) (219). Une étude récente a utilisé la spectrométrie de masse des ions secondaires en temps de vol (ToF-SIMS) afin de valider la colocalisation de l'acide osmique avec différents types de lipides en microscopie électronique (220). Les résultats de cette étude démontrent une réduction marquée des protéines et des phospholipides après incubation avec l'acide osmique, ainsi que la conservation des acides gras (220). Tel qu'attendu, la colocalisation de l'acide osmique était plus importante avec les acides gras insaturés (C18:1, C18:2) que les autres acides gras saturés (C14 et C16) ainsi que les acides gras insaturés à moyenne chaîne (C16:1) (220).

Depuis la publication des travaux de Hirsch et Gallian, plusieurs équipes ont apporté des modifications importantes, soit au protocole de fixation, soit par rapport à l'analyse des courbes de distribution générées. L'ajout d'urée 10 mM et de Triton X-100 (0,01% v/v) lors des étapes de lavage ont ainsi permis de diminuer l'agglomération des cellules adipeuses post-fixation (221). Aussi, certaines équipes utilisent des filtres permettant d'obtenir de très petits adipocytes (≥6 µm) ce qui constitue une amélioration par rapport au protocole d'origine qui discriminait les cellules en deçà de 25 µm (221, 222). Cependant, il faut noter que plusieurs équipes

fixent un seuil de détection entre 15 et 25 µm de diamètre pour éviter la contamination avec d'autres types cellulaires. D'ailleurs, l'ouverture de 400 µm utilisée avec le compteur Coulter Multisizer III/IV est précise pour les tailles adipocytaires variant de 8 à 240 µm tel que rapporté par le fabricant, mais pour les différents groupes de recherche, cette précision est vraie seulement pour les cellules allant de 20 à 240 µm de diamètre (223-232). Les données de taille adipocytaire générées par le compteur Coulter Multisizer peuvent être analysées de façon brute ou par la génération de courbes de distribution pour chaque échantillon par des logiciels tels que R ou SAS (231-233). Ces courbes sont le plus souvent bimodales et consistent en une population principale de cellules formant une courbe gaussienne qui est précédée par une courbe exponentielle, formée des petites cellules. Plusieurs mesures peuvent être dérivées de ces courbes de distribution. Par exemple, le nadir exprime la fréquence la plus petite entre les deux populations de cellules, le mode i.e. la fréquence maximale de la population gaussienne, la moyenne, la médiane, la largeur et la hauteur de la courbe gaussienne, le pourcentage des cellules sous ou au-dessus du nadir et le ratio des petites cellules sur les grosses cellules (231, 232). De plus, comme la courbe de distribution des tailles générée est assez précise, plusieurs équipes de recherche se sont intéressées, chez l'animal, au changement de cette distribution en fonction de l'âge, du statut nutritionnel et du stade de développement (223, 224, 226, 234, 235) ou récemment chez l'humain à la suite d'une diète hypercalorique (203). Cette technique a permis de mettre en évidence une population de très petites cellules (sous le nadir) surreprésentée chez les personnes obèses, particulièrement chez celles ayant un diabète de type 2 ou un syndrome métabolique (225, 231, 232).

2.6.3 Analyse histomorphométrique du tissu adipeux inclus dans la paraffine

L'analyse par microscopie de coupes histologiques colorées à l'hématoxyline et à l'éosine (H&E) de divers tissus a été publiée pour la première fois en 1878 (236, 237). Ce n'est que quelques années plus tard que la fixation à la formaline et le montage subséquent en paraffine a été décrit (238, 239). Ce n'est qu'en 1899 que paraît dans le *Journal of Anatomy and Physiology* la combinaison de la fixation, du montage et de la coloration pour l'observation de coupes de tissus (240). La taille

des adipocytes a été mesurée pour la première fois sur des coupes histologiques en 1953 (241, 242). Ce n'est qu'après l'apparition de la méthode enzymatique de Rodbell où l'importance de la taille des adipocytes a été mise en lumière, que les analyses histomorphométriques ont vraiment débuté (243-246). Ces analyses requièrent des techniques de laboratoire courantes et faciles d'exécution, des réactifs présentant peu de dangerosité, ainsi que de l'équipement de laboratoire standard. Brièvement, la technique consiste à fixer un échantillon de tissu adipeux dans une solution de formaldéhyde (formaline) d'une concentration variant de 4 à 10% (v/v) durant une période allant de 24 à 72 heures. La quantité de solution est entre 15 et 20% de la masse du tissu à fixer. Ensuite, le tissu est déshydraté par des bains d'éthanol de concentrations croissantes et des bains d'éthanol pur afin de conserver sa morphologie. Par la suite, le tissu est immergé dans des bains de xylène ou de toluène pour éliminer l'éthanol et est imprégné de paraffine chauffée afin de le solidifier. Il est par la suite inclus dans un moule de paraffine. Des sections de tissu sont déposées des lames chargées après une coupe réalisée avec un microtome. Avant la coloration, la lame peut être séchée à 45°C pendant une heure ou pendant 24 heures à température de la pièce.

Selon les équipes, la concentration et le type des agents de fixation, le temps de fixation, l'épaisseur des coupes et la coloration de base peuvent varier. En plus de ces différences, plusieurs biais sont associés avec cette technique. L'échantillonnage du tissu adipeux est très important car la distribution cellulaire doit être représentative, ce qui n'est pas toujours le cas chez les obèses ou encore chez les enfants (247). De plus, il faut considérer que les cellules ne montrent pas toujours leur diamètre le plus grand et qu'elles peuvent aussi, sous l'effet du fixatif, réduire significativement de taille (247-249). Cette technique, malgré ses nombreux biais, est la seule qui permet d'observer la structure du tissu adipeux *in situ*. Cette méthode permet également la mesure de plusieurs marqueurs cellulaires par immunohistochimie ou par immunofluorescence.

Pour mesurer la taille sur les coupes histologiques, plusieurs équipes ont développé des méthodes semi-automatisées ou complètement automatisées. Cependant,

certaines particularités de ces méthodes font en sorte qu'elles demeurent inapplicables à grande échelle. Par exemple, la méthode décrite par Osman et collaborateurs nécessite l'analyse de 16 champs représentatifs, ce qui n'est pas toujours possible pour l'analyse de la taille adipocytaire (250). Ensuite, la méthode de Chen et Farese amène une sous-estimation de la taille due à l'exclusion des cellules touchant les côtés de l'image analysée (251). L'utilisation du logiciel libre ImageJ par Galarraga et al., a mené au développement du module d'extension Adiposoft (252). Malgré son aspect prometteur, il n'a été cité que 2 fois depuis 2012, ce qui peut s'expliquer, en partie, par le retrait du lien pour l'utiliser de manière gratuite par les concepteurs (252). Le logiciel Adcount développé à la clinique Mayo été très utilisé par l'équipe l'ayant publié, mais très peu par d'autres équipes (253). La méthode proposée par une équipe de Yale utilise la propriété d'autofluorescence de la coloration H&E après exposition à la lumière provenant du filtre Texas Red (533-588 nm) (254). Cette technique nécessite une caméra prenant des images à très haute résolution, un microscope à fluorescence, ainsi que le logiciel libre Cell Profiler 2.0 et le pipeline correspondant (254). Cette technique semble être montée en popularité, de par son utilisation facile et rapide. Néanmoins, chacune de ces méthodes est imparfaite en raison du fait que les membranes des adipocytes sont souvent incomplètes/brisées sur les coupes histologiques, ce qui nécessite toujours une analyse *a posteriori* des échantillons. Aussi, il y a toujours la présence d'artefacts qui est difficile à considérer et à éliminer. De plus, ces méthodes semi-automatiques utilisent toujours la surface visible des adipocytes. Ceci amène de manière automatique une sous-estimation de la taille des adipocytes qui peut être diminuée par l'utilisation du diamètre le plus grand de chaque cellule.

La méthode histomorphométrique devrait être privilégiée dans les études rétrospectives où le tissu a déjà été fixé ou dans les cas où les analyses subséquentes nécessitent l'utilisation de coupes de tissus comme l'immunohistochimie et l'immunofluorescence. L'analyse histomorphométrique peut aussi être très utile pour caractériser le phénotype d'une nouvelle souris transgénique ou encore pour comparer, de façon rapide et peu coûteuse, deux types d'alimentation ou de supplémentation entre des groupes d'individus/animaux.

2.6.4 Autres méthodes

Microscopie électronique à balayage

Cette méthode très spécialisée permettant de voir de très petits adipocytes n'est pas très utilisée dans la littérature. En effet, lors de la mesure de la taille adipocytaire moyenne, il n'y a pas raison d'être aussi précis. De plus, cette technique nécessite un apprentissage laborieux et le coût de la technologie est très élevé pour les quelques avantages qu'elle peut procurer, tels que l'acquisition rapide de l'image et la visualisation de l'ultrastructure de l'adipocyte. Cette technique a été utilisée pour la première fois par une équipe canadienne (255) et une seconde fois par une équipe de Boston (256), mais reste d'un usage marginal.

Cytométrie en flux

La cytométrie en flux est très utilisée en clinique pour caractériser les cellules sanguines selon leur phénotype et aussi selon leur taille. Plusieurs équipes ont aussi caractérisé les cellules de la fraction stroma-vasculaire du tissu adipeux avec cette technique. Cependant, cette méthode reste marginale pour mesurer la taille des adipocytes en raison de plusieurs problèmes d'ordre logistique. Certains adipocytes sont tous simplement trop gros ou trop fragiles pour cette technologie, ce qui génère une très grande perte de cellules et donc un nombre très limité de cellules mesurées tel que rapporté par les équipes ayant réalisé la mesure de la taille adipocytaire par cytométrie en flux (257-259).

En conclusion, l'hypertrophie de l'adipocyte viscéral humain est une mesure importante dans l'évaluation du risque associé à l'obésité. Plusieurs aspects reliés à la physiopathologie de l'obésité viscérale et de l'adipocyte sont encore peu connus. Ce mémoire portera sur une sélection de ces aspects, soit l'impact de la nutrition dans l'étiologie de l'obésité viscérale (**Chapitre 1**), la morphologie et la taille adipocytaire comme marqueur d'un tissu adipeux dysfonctionnel (**Chapitre 2** et **3**) et l'importance de la technique de mesure de la taille adipocytaire (**Chapitre 3**). Le chapitre de livre formant le **Chapitre 1** est la première revue de littérature qui a étudié le lien entre les macronutriments et certains nutriments particuliers et

l'adiposité viscérale déterminée par tomographie axiale, DXA, imagerie par résonance magnétique et par d'autres mesures substitutives telles que le tour de taille et le ratio taille-hanches. Le **Chapitre 2** constitue la première analyse critique répertoriant les études sur la taille adipocytaire chez l'humain et ses associations avec les altérations cardiométaboliques. Enfin, le **Chapitre 3** est le premier article qui analyse la taille adipocytaire provenant des trois techniques de mesure les plus utilisées dans la même population, caractérisée par des mesures d'adiposité totale et régionales ainsi que par les principaux facteurs de risque cardiométabolique.

Objectifs et hypothèses

Objectif général

L'objectif général de ce mémoire est d'examiner la distribution des graisses chez l'humain sous les aspects nutritionnels, morphologiques et techniques en relation avec les altérations cardiométaboliques associées à l'obésité viscérale.

Notre hypothèse générale est que la distribution des graisses est modulée en partie par la nutrition et par la variation de la capacité d'entreposage des lipides des divers compartiments adipeux et que ces différents aspects influencent l'accumulation de gras au niveau viscéral et les complications cardiométaboliques associées.

Les chapitres de ce mémoire sont au nombre de trois. Chaque objectif spécifique est examiné dans un chapitre de ce mémoire.

Objectifs spécifiques

Chapitre 1 : Nutrition et adiposité

L'objectif spécifique de ce chapitre est de répertorier et d'analyser les déterminants nutritionnels possibles de la distribution des graisses chez l'humain, plus spécifiquement l'accumulation de gras au niveau abdominal et viscéral. Nous émettons l'hypothèse que certains nutriments peuvent moduler l'accumulation de gras abdominal, en grande partie par leurs effets sur le bilan d'énergie et sur la masse grasse totale.

Chapitre 2 : Hypertrophie adipocytaire

L'objectif spécifique du **Chapitre 2** est d'évaluer la littérature disponible sur l'hypertrophie adipocytaire en tant que marqueur de la dysfonction des tissus adipeux. Nous émettons l'hypothèse que les effets délétères de l'accumulation de gras au niveau viscéral sont expliqués par une capacité d'expansion limitée du tissu adipeux sous-cutané qui se reflète par une hypertrophie adipocytaire dans les dépôts sous-cutané et viscéral.

Chapitre 3 Analyse comparative de trois techniques de mesure de la taille adipocytaire chez l'humain: importance de la technique de mesure et association avec les facteurs de risque cardiométabolique

L'article original du **Chapitre 3** fait suite aux conclusions du **Chapitre 2** en ce qui a trait à la technique de mesure de la taille des adipocytes matures dans les dépôts abdominaux humains. Il a donc comme objectifs spécifiques : 1) de quantifier la différence dans la taille adipocytaire selon la méthode de mesure utilisée; et 2) de vérifier si la taille adipocytaire dérivée de chacune des méthodes est associée de manière similaire aux mesures d'adiposité et aux variables liées au risque cardiométabolique. Nous émettons l'hypothèse que la taille médiane des adipocytes mesurée à la suite d'une analyse histomorphométrique est plus petite que celle obtenue à la suite d'une digestion à la collagénase ou d'une fixation à l'acide osmique, mais que les corrélations entre la taille adipocytaire et l'adiposité, la distribution des graisses ainsi que les variables cardiométaboliques sont similaires entre les méthodes.

Chapitre 1 : Nutrition et adiposité

Sofia Laforest, Geneviève B. Marchand et André Tchernof

Le chapitre de livre composant ce chapitre du mémoire s'intitule:

Diet as a potential modulator of body fat distribution

Ce chapitre sera publié comme le chapitre 6 du livre :

Nutrition and Cardiometabolic Health

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Il est présentement sous presse.

Résumé

L’obésité abdominale est liée au risque cardiométabolique de manière plus étroite que l’excès d’adiposité comme tel. L’accumulation de gras au niveau abdominal, particulièrement dans le dépôt viscéral, est étroitement liée à des anomalies métaboliques telles que la résistance à l'insuline et l'hypertriglycéridémie, alors que pour un niveau donné d’adiposité totale, l’accumulation préférentielle de tissu adipeux sous-cutané a des effets neutres, voire protecteurs. Les mécanismes de régulation de distribution des graisses sont complexes et demeurent mal compris. Nous avons étudié la contribution potentielle des composants alimentaires sur la distribution de la graisse corporelle indépendamment du poids et de l'adiposité totale. Les types et les quantités de matières grasses, les glucides et la charge glycémique, les protéines, ainsi que des composants alimentaires spécifiques avec des effets bioactifs qui pourraient affecter l'accumulation de gras ont été examinés en détail. Selon la littérature disponible, il n'y a aucune preuve concrète qu'un seul nutriment module directement l’accumulation de gras viscéral et par extension, la distribution des dépôts adipeux. La plupart des études disponibles sur l'alimentation et la distribution des graisses semblent pointer vers un effet non spécifique sur l'accumulation de graisse totale. Par contre, certaines études ont identifié des composants alimentaires potentiellement liés à un patron d’accumulation des graisses délétère (augmentation de la graisse viscérale/abdominale ou du tour de taille) par exemple, le fructose, en particulier sous la forme de boissons sucrées, les acides gras *trans*, ainsi que la consommation élevée d'alcool et les diètes composées principalement de mets préparés et de restauration rapide.

Chapter 6: Diet as a potential modulator of body fat distribution

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Keywords: body fat distribution, fat mass, central adiposity, waist circumference, macronutrient distribution, dietary pattern, protein intake, fat intake, carbohydrates intake.

Abstract

Abdominal obesity has been reported to be more closely related to cardiometabolic risk than excess adiposity *per se*. Specifically, high abdominal fat accumulation, especially in the visceral depot, is closely linked to metabolic abnormalities such as insulin resistance and hypertriglyceridemia, whereas for a similar body fat mass, subcutaneous fat accretion seems to confer a neutral/protective effect. Mechanisms regulating body fat distribution are complex and remain poorly understood. We reviewed the potential contribution of dietary components on body fat distribution independent of body weight and total adiposity. Types and amounts of fat, carbohydrates and glycemic load, proteins, as well as specific food components with bioactive functions that could affect fat accretion were examined in detail. According to available literature, there is no concrete evidence that a single nutrient directly modulates visceral fat accretion and, by extension body fat distribution. Most available studies on diet and body fat distribution seem to point toward a non-specific effect on total fat accumulation. Yet, some studies identified dietary components potentially linked to a detrimental fat accretion pattern (increased visceral/abdominal fat or waist circumference), including fructose, especially in the form of sugar-sweetened beverages, *trans* fatty acids, as well as high alcohol intake and refined/fast-food diets.

Abbreviations

25(OH)D: 25-hydroxy vitamin D; ap2: Adipocyte protein 2; BCAA: Branched-chain amino acids; BMI: Body mass index; BCKD: Branched-chain α-keto acid dehydrogenase; C/EBPα: CCAAT/enhancer-binding protein alpha; CLA: Conjugated linoleic acid; CT: Computed tomography; DHA: Docosahexaenoic acid; DXA: Dual energy X-ray absorptiometry; EPA: Eicosapentaenoic acid; FA: Fatty acids; GI: Glycemic index; GL: Glycemic load; GLUT4: Glucose transporter type 4; HC: Hip circumference; hsCRP: High-sensitive C-reactive protein; LCT: Long-chain triglycerides; LPL: Lipoprotein lipase; MCT: Medium-chain triglycerides; MD: Mediterranean diet; MEDS: Mediterranean score; MRI: Magnetic resonance imaging; MUFA: Monounsaturated fatty acids; NF-κB: Nuclear factor-kappa B; PPAR: Peroxisome proliferator-activated receptor; PPARγ: Peroxisome proliferator-activated receptor gamma; PUFA: Polyunsaturated fatty acids; SAT: Subcutaneous adipose tissue; SFA: Saturated fatty acids; SMD: Standard mean difference; SSB: Sugar-sweetened beverages; TNFα: Tumor necrosis factor alpha; VAT: Visceral adipose tissue; WC: Waist circumference; WHR: Waist-to-hip ratio; WMD: Weighted mean difference.

Introduction

Obese individuals have an increased risk of developing coronary heart disease, hypertension, type 2 diabetes and several types of cancer (reviewed in (Haslam and James 2005)). However, the risk of developing these conditions is closely related to body fat distribution patterns. More specifically, numerous studies now support the notion that excess visceral adipose tissue (VAT) accumulation is more closely related to alterations in cardiometabolic health, whereas for any given level of total adiposity, preferential subcutaneous adipose tissue (SAT) accumulation has protective or neutral effects (reviewed in (Tchernof and Despres 2013)). Molecular mechanisms affecting lipid storage sites have not yet been completely elucidated. Hormones, genetic or epigenetic factors, and possibly diet may all partly contribute to human body fat distribution patterns (Berry et al. 2013).

The present chapter reviews scientific evidence on the modulation of body fat distribution, with visceral fat accumulation as the key indicator, by dietary factors such as macronutrients, food patterns and other particular nutrients or food items. The major question to be addressed is whether nutritional factors can specifically modulate body fat distribution patterns or visceral fat accumulation beyond what could be accomplished solely by modulating energy intake and total body fat stores (**Figure 6.1**).

We have reviewed articles found in the PubMed database with search terms related to fat accumulation: body fat distribution, peripheral adiposity, visceral fat, abdominal fat, subcutaneous fat, and body composition. They were individually combined with nutritional keywords such as lipids, carbohydrates, proteins, diet, nutrients and nutrition. A total of 224 journal articles were kept after removing duplicates. The articles were selected depending on the following specific criteria: 1) emphasis was placed on human studies; and 2) body fat distribution had to be measured by computed tomography (CT), magnetic resonance imaging (MRI), dual energy X-ray absorptiometry (DXA), ultrasound or waist and/or hip circumference (WC/HC). Reviews were excluded. Systematic reviews and meta-analyses were included.

Relevant articles from the reference list of identified papers were added. In total, 170 studies were retained.

Macronutrients and their relevance for body fat distribution

Lipids

Types of fatty acids

Fatty acids (FA) are often classified in three broad groups, namely saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). In the typical Western diet, C4 to C18 are the major SFA. They are found primarily in animal foods and oils derived from tropical fruits. MUFA are generally associated with the Mediterranean diet (MD) (see section on MD) considering that oleic acid, the major component of olive oil, is the primary MUFA in this common diet. Nuts (almonds, pecans, peanuts, cashew nuts) are also rich in MUFA. PUFA comprise a large group, including the essential FA, that is linoleic (C18:2n-6) and linolenic (C18:3n-3) acids. PUFA also refer to n-3 FA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). PUFA can be found in corn oil, sunflower oil and seafood. *Trans* FA, usually formed by commercial incomplete hydrogenation of MUFA, are also a component of the Western diet and are known to increase cardiovascular disease risk (Mozaffarian et al. 2006).

Larson et al. reported a positive association between body fat mass and total fat, SFA, MUFA and PUFA intake independent of nonfat energy intake in men and women (Larson et al. 1996). In regression analyses, SFA intake was a better predictor of body fat mass than total fat intake. VAT and SAT areas, adjusted for nonfat energy intake, were positively associated with total fat, SFA and MUFA (Larson et al. 1996). All these associations became non-significant when the authors adjusted for total body fat mass (Larson et al. 1996). Their results suggest no apparent association between the fatty acid content of the diet and body fat distribution.

In a longitudinal study in African-Americans and Hispanic-Americans, intake of SFA, MUFA and PUFA was not associated with 5-year changes in VAT or SAT area (Hairston et al. 2012). Consistent with these results, data from a Danish cohort followed for 5 years failed to show an association between the change in WC, a

surrogate measure of VAT, and total intake of fat (Halkjaer et al. 2006). In a randomized crossover study with overweight men, fat mass in both the trunk and limbs was increased in the SFA diet group and decreased in the MUFA diet group after 4 weeks, without differences in total energy intake (Piers et al. 2003). Replacement of PUFA or carbohydrates by *trans* FA increased WC in men during a 9-year follow-up, whereas there was no difference when the substitution was done with SFA or MUFA (Koh-Banerjee et al. 2003). Forouhi and collaborators found no association between intake of SFA, MUFA or PUFA and WC (Forouhi et al. 2009). In a randomized 1-year control trial, participants were provided low fat dietary advice from dieticians and half received 30 g of walnuts per day to increase their consumption of PUFA (Tapsell et al. 2009). Compliance was monitored by a validated diet history interview, 3-days food records coupled with an analysis of erythrocyte fatty acid composition. The control group preferentially lost VAT at three months while the walnut group lost SAT (Tapsell et al. 2009). There was a trend ($p=0.08$) at baseline for a higher SAT area in the walnut group, which can partially explain this difference (Tapsell et al. 2009). At 12 months, the walnut group had lost more body fat than the control group, but the difference did not reach statistical significance (Tapsell et al. 2009).

Taken together, the above-mentioned studies suggest that the link between fatty acid composition of the diet and abdominal or visceral fat accretion seems to be mediated by its association with overall adiposity levels.

Medium-chain triglycerides vs long-chain triglycerides

Medium-chain triglycerides (MCT) have unique properties which distinguish them from long-chain triglycerides (LCT). These characteristics seem to confer beneficial metabolic effects. MCT, after cleavage to glycerol and FA, are transported directly to the liver by the portal vein, bypassing incorporation into chylomicrons and the lymphatic system (Poppitt et al. 2010). Unlike LCT, the MCT do not require the carnitine acyltransferase enzyme to be incorporated into the mitochondria, allowing more rapid β-oxidation, a phenomenon that has been linked with greater energy expenditure and less weight gain (Bach and Babayan 1982). MCT may also increase

satiety after a meal (Poppitt et al. 2010, Krotkiewski 2001, Van Wymelbeke et al. 1998). Generally, MCT comprise FA from 6 to 10 and even 12 carbons, since lauric acid (C12:0), structurally classified as an LCT, shows properties that are similar to caprylic (C8:0) and capric (C10:0) acids. MCT are found primarily in vegetable oils such as coconut (71%) and palm kernel (48%) (Health Canada Nutrient File, 2010).

Many studies have been conducted to elucidate the effect of LCT replacement by MCT on body weight, body fat mass, WC, SAT and VAT. Bueno and collaborators, in a recent meta-analysis found that replacement of LCT by MCT (at least 5 g) in 6 randomized control trials reduces WC in both men and women (Weighted mean difference (WMD):-1.78 cm [95% CI, -2.4 to -1.1]) (Bueno et al. 2015). In another meta-analysis, a similar result was reported for WC (WMD:-1.46 cm [95% CI -2.04 to -0.87]) (Mumme and Stonehouse 2015). In addition, total SAT and VAT were also investigated. Replacement of LCT by MCT (<1% to 24% of energy intake) in the diet led to a moderate loss of both SAT and VAT (Standard mean difference (SMD): -0.46 [95% CI -0.64 to -0.27] and -0.55 [95% CI -0.75 to -0.34], respectively), suggesting a greater loss of VAT in these trials. Overall, these results strongly suggest that consumption of MCT may be favorable, not only for body composition, but also toward healthier body fat distribution. Further studies are needed to verify the relationship between MCT and reduced VAT area independent of other adiposity measurements.

n-3 fatty acids

Consumption of n-3 FA, namely EPA and DHA, found principally in fatty fish and components of the MD (see section on MD) has been linked with improvements in insulin resistance and cardiovascular risk factors (Wang et al. 2006, Li 2015). Preliminary results from animal studies showed possible beneficial effects, independent of body weight. In rats, seven weeks of a diet with 17.4% EPA and 10.1% DHA (% of total FA) reduced VAT accumulation with no difference in body weight compared to lard-fed rats (high-fat regimen used as control) (Rokling-Andersen et al. 2009). In human studies, some authors found that supplements of EPA/DHA reduce WC (Bender et al. 2014, Bays et al. 2009, Crochemore et al. 2012,

DeFina et al. 2011, Kunesova et al. 2006, Munro and Garg 2013, 2012, Thorsdottir et al. 2007). In a group of 30 Japanese men and women with coronary heart disease, a supplement of 1800 mg administered over 6 months decreased SAT and VAT assessed by CT, but only the latter was also associated with the expected increase of plasma EPA level (Sato et al. 2014). However, the effect of n-3 fatty acids was not reported to be independent of changes in body weight and fat mass in the above mentioned studies. Further studies are needed to confirm their effect on body fat distribution in humans.

Conjugated linoleic acid

Conjugated linoleic acid (CLA) supplements have been of interest following reports of their anticancer and anti-inflammatory properties as well as a potential role in modulating body fat mass (Pariza 2004, Silveira et al. 2007). CLA occurs naturally and is found primarily in ruminant meat and dairy products (Steinhart, Rickert, and Winkler 2003). It is synthetically produced from sunflower and safflower oil in supplements (Pariza, Park, and Cook 2001). The estimated daily intake of CLA is 0.36 g per day for women and 0.43 g per day for men, according to the German Nutrition Study (Steinhart, Rickert, and Winkler 2003). CLA comprises a group of 28 isomers which present two conjugated *cis* or *trans* dienes, primarily on positions C9, C11 or C10 and C12. CLA is well absorbed in free FA form or as digested triglycerides when compared to ethyl ester (Fernie et al. 2004). Poor palatability was reported when CLA was ingested as free FA (Fernie et al. 2004). CLA has multiple effects on adipose tissue metabolism. It has been linked to decreased fat cell size and small cell proliferation (Brown and McIntosh 2003, Tsuboyama-Kasaoka et al. 2000, Evans, Brown, and McIntosh 2002). Other reports showed adverse effects of CLA such as a decrease of preadipocyte differentiation via reduced peroxisome proliferator-activated receptor (PPARγ) and CCAAT/enhancer-binding protein alpha (C/EBPα) activity (Brown et al. 2003, Kang et al. 2003), activation of the nuclear factor-kappa B (NF-κB) pathway and subsequent expression of tumor necrosis factor alpha (TNFα) (Chung et al. 2005). Impaired insulin signaling after CLA supplementation was also reported in animal models and was linked to the impact

of TNFα on the expression of key adipogenic genes such as glucose transporter type 4 (GLUT4), lipoprotein lipase (LPL) and adipocyte protein 2 (ap2) (Chung et al. 2005). Recent reports (Evans, Brown, and McIntosh 2002) suggested differential effects of the two most studied CLA isomers, *trans*10*cis*12 and *cis*9*trans*11, the latter being associated with body composition (reduction in body fat and increase of lean body mass) and the other with anticarcinogenic properties.

Some studies reported significant weight loss with CLA supplements (range 0.59 to 6.8 g per day) and a fat-free mass increase (Watras et al. 2007, Syvertsen et al. 2007, Smedman and Vessby 2001, Gaullier et al. 2007, Gaullier et al. 2004, Blankson et al. 2000), while others found no effect (Tricon et al. 2004, Taylor et al. 2006, Moloney et al. 2004, Whigham et al. 2004, Malpuech-Brugere et al. 2004, Kreider et al. 2002, Mougios et al. 2001, Benito et al. 2001, Zambell et al. 2000, Berven et al. 2000, Joseph et al. 2011). WC was not significantly reduced in a meta-analysis of randomized control trials on CLA supplementation (including 534 subjects) over at least 6 months of treatment (Onakpoya et al. 2012). In contrast, in a randomized control trial following women with the metabolic syndrome on a hypocaloric diet, women taking placebo reduced their WC whereas women receiving CLA supplements did not (Carvalho, Uehara, and Rosa 2012). Earlier fat loss occurred in women taking the CLA supplement. However, final body fat loss was similar at the end of the 14-week trial (Carvalho, Uehara, and Rosa 2012). In obese women supplemented with 3.4 g/day of CLA, Gaullier and collaborators noted a decrease in waist-to-hip ratio (WHR) and observed that fat loss was primarily due to loss of leg fat and not abdominal fat (Gaullier et al. 2007). No change in WC was observed in another randomized control trial involving overweight and obese men and women supplemented with 3 g of CLA during 12 weeks (Laso et al. 2007). However, the investigators reported a trend toward reduced trunk fat mass assessed by DXA in the overweight group only (Laso et al. 2007). In overweight and obese men, no differences were observed in CT-assessed VAT and SAT areas after a 4-week supplementation with ~2.6g CLA/day (Desroches et al. 2005). Similar results were obtained in men following a resistance training program, where VAT loss was not related to CLA supplementation (Adams et al. 2006). To summarize these

previous studies, some but not all studies show that CLA may induce fat loss, possibly by increasing lipolysis and apoptosis in adipose tissue (Pariza 2004). However, data are lacking regarding the capacity of naturally found CLA to modulate body fat distribution toward a healthier pattern. In fact, studies previously discussed used supplements including 0.59 up to 6.8 g of CLA, which is 1.3 to 14 times greater than general daily intake (Steinhart, Rickert, and Winkler 2003). Studies are also needed to assess the safety of CLA supplements, particularly in individuals at risk for type 2 diabetes. Since both major isomers appear to present extensive differences in functional properties, specific characterization is also required.

Carbohydrates

Percentage of energy intake as carbohydrates

No cross-sectional study has reported a positive or negative association between total carbohydrate intake and VAT accumulation in either young or adult individuals (Bailey et al. 2010, Davis et al. 2009, Lagou et al. 2011, Larson et al. 1996, Stallmann-Jorgensen et al. 2007).

Glycemic index/load

Glycemic index (GI) and glycemic load (GL) have been extensively studied. High GI and GL diets are generally high in energy, they may be less satiating and increase insulin secretion, possibly contributing to hyperinsulinemia (Esfahani et al. 2009). In a prospective cohort study of adult Danes Hare-Bruun and collaborators reported that individuals consuming a high GI diet had higher intakes of fat and added sugars as well as lower protein and fiber intake while those with a high GL diet consumed less fat and more carbohydrates such as added sugars and dietary fiber (Hare-Bruun, Flint, and Heitmann 2006). These data suggest that high GI and GL may contribute to cardiometabolic risk.

In men, GI and GL were not associated with any of the adiposity indices measured in this prospective cohort (body weight, percent body fat, waist and hip circumferences) (Hare-Bruun, Flint, and Heitmann 2006). In women, body weight and percent body fat changes over 6 years were positively correlated with GI,

whereas a trend was found with change in WC (2 cm) (p=0.07) (Hare-Bruun, Flint, and Heitmann 2006). Conversely, there was a borderline negative association between change in WC and GL among women (-0.5 cm for a 10% increase of GL, p=0.06), suggesting a favorable effect of this diet on central adiposity (Hare-Bruun, Flint, and Heitmann 2006). On the other hand, GI and GL were not associated with WHR in either men or women in two cross-sectional studies comprising 8703 subjects (Rossi et al. 2010, Liese et al. 2005). In two other cross-sectional studies, WHR was positively associated with GI and GL in men only (Mosdol et al. 2007, Toeller et al. 2001).

A recent systematic review showed that information was insufficient to ascertain the relationship between GI, GL and adiposity measurements in children (Rouhani et al. 2014). In fact, there is only one prospective cohort in children on this topic (DONALD study) (Buyken et al. 2008). This study found no association between GI and GL and body mass index (BMI) z-score when the entire study population was considered (Buyken et al. 2008). In fact, only one cross-sectional study reported an association between GI and waist z-score independently of age, sex, BMI and education of the parents as well as residuals of diet variables (energy, protein, fat, carbohydrate, fiber, GL) (Barba et al. 2012). In that study, GI was the only nutritional variable to be associated with WC (Barba et al. 2012). Overall, there is insufficient evidence at this time to conclude on the potential benefit of a low GI/GL diet on central obesity, in either children or adults.

Discrepancy in findings among studies could be explained partially by methodology in assessing adiposity measurements or dietary intake. Some limitations in the use of GI and GL should also be acknowledged. GI and GL are often used as commutable variables, whereas GL takes into account the amount of carbohydrates in one portion whereas GI does not (Venn and Green 2007). The large variation in GI measurements, individual variability, unclear impact of mixed meals and food transformation make difficult to study GI and GL (Venn and Green 2007).

Fructose and sugar-sweetened beverages

Added sweeteners and fructose have raised particular concern for public health (Lustig, Schmidt, and Brindis 2012). Hepatic fructose metabolism, which differs from that of glucose, leads to a host of metabolic alterations associated with increased plasma triglycerides and glycaemia, as reviewed in (Tappy et al. 2010), and Chapters 3 & 17-19 of this textbook. Fructose and sugar-sweetened beverages (SSB) have also been studied in relation to visceral fat accumulation. As described in Chapter 3, Stanhope and collaborators characterized differences in central fat accumulation measured by CT in men and women consuming a fructose- or glucose-sweetened beverage providing 25% of energy for 10 weeks (Stanhope et al. 2009). For a similar weight gain in both groups, consumption of a fructose-sweetened beverage increased visceral and total fat accumulation whereas consumption of a glucose sweetened beverage did not. The mechanisms underlying this effect are still unclear but may involve depot-specific modulation of lipogenic enzymes (Stanhope et al. 2009). In a cross-sectional study of 791 Caucasian men and women, VAT and SAT mass (measured by MRI) were not associated with SSB consumption; however their intake was positively correlated with an increased ratio of VAT-to-total-abdominal fat area after adjustment for confounding variables (Odegaard et al. 2012). In an observational study combining the Framingham Heart Study and the Third Generation Cohort (n=2596), men tended to consume more SSB than women (Ma et al. 2014). Of note, whereas mean BMI and fasting glycaemia were not different across categories of SSB consumption (none; >1/month and <1 per week; >1/week and <1 per day; and >1 a day), the prevalence of dyslipidemia increased as SSB consumption increased (Ma et al. 2014). The association between VAT area and SSB consumption only became significant after adjustment for SAT area (Ma et al. 2014). In a 6-month, randomized control intervention study, overweight or obese men and women were given 1 of 4 drinks (sucrose-sweetened regular cola, aspartame-sweetened diet cola, 1.7% fat milk-isocaloric to the regular cola- or water) to assess changes in VAT area (Maersk et al. 2012). Whereas total fat mass did not differ across beverage groups, the increase in VAT area was higher in the regular

cola group when compared to the other groups; the same was observed for total cholesterol and triglyceride levels (Maersk et al. 2012).

Davis and collaborators found no association between SSB consumption and VAT accumulation in young overweight Latino youth followed for two years (Davis et al. 2009). However, added sugar and SSB intakes were similar at baseline and follow-up. In a cross-sectional study in teenagers, VAT but not SAT area was associated with total fructose consumption (Pollock et al. 2012).

Available studies suggest that SSB and/or fructose may modulate body fat distribution and promote accumulation of VAT. Additional studies are required to further document this phenomenon.

Protein

Percentage of energy intake as protein

Even though standard nutritional guidelines promote lower percentage of energy from protein than from fat and carbohydrate, many weight loss programs promote adherence to a moderate-high protein intake diet (Abete et al. 2010). There is growing evidence that higher intake of protein may increase weight loss through increased satiety and thermogenesis, as reviewed in (Halton and Hu 2004) and in Chapters 17, 24-25 of this textbook. High protein diets have been proposed to counteract the reduction in fat free mass often observed in weight loss trials (Leidy et al. 2007, Farnsworth et al. 2003). Whether protein intake modulates abdominal fat accumulation will be discussed in this section.

Several randomized weight loss trials found favorable outcomes with increases in total protein intake; however these studies often included an exercise program, which made it difficult to assess the specific nutritional impact. Arciero et al. investigated the effect of exercise and percentage of energy as protein on body composition in overweight/obese men and women. They found that a 40% carbohydrate and 40% protein diet decreased abdominal fat, measured by DXA by more than 25% over 3 months, whereas a standard diet (50-55% carbohydrates, 15-

20% proteins) reduced abdominal fat by only 7.5%. Of note, the exercise training program was more vigorous in the high-protein group and included high-intensity resistance and cardiovascular training, whereas the standard diet group performed moderate cardiovascular training (Arciero et al. 2006). In a subgroup of individuals followed for 1 year, abdominal fat was not different from baseline in the high-protein group and was reduced in the standard diet group, which could reflect the difficulty of maintaining long-term lifestyle modifications such as adherence to a high-protein diet (Arciero et al. 2006). In another randomized control trial there was no difference in abdominal fat measured by DXA in groups assigned either to high (40-46%) or standard (15-20%) protein diets for 12 weeks (Clifton, Bastiaans, and Keogh 2009). Interestingly, improvements in cardiometabolic risk factors were greater in those with high circulating triglyceride concentrations at baseline on the high-protein diet when compared to those on the standard protein diet (Clifton, 2009). Similar results (no difference in fat mass measured by DXA) were obtained in obese women assigned to diets of various macronutrient distributions (15, 50 or 63% of energy as protein) and a training program over 14 weeks (Kerksick et al. 2010). In a recent meta-analysis comparing low/standard (<20%/20-30%) vs. high (>30%) protein randomized weight loss clinical trials, the mean decrease in WC was 1.66 cm greater following high-protein diets than after the low/standard protein diets (n=1214) (Santesso et al. 2012). Cardiometabolic risk factors such as systolic and diastolic blood pressure, total triglycerides and fasting insulin were also significantly lower, and HDL-cholesterol levels were higher, as expected with the decrease in WC (Santesso et al. 2012).

In sum, there is little evidence to date that high protein intake is associated with reduced VAT accumulation, independent of exercise. In fact, results from 6 observational studies including 2393 individuals found no association between overall protein intake and VAT area measured by CT scan or MRI after adjustment for age, race, sex, baseline VAT and SAT and energy intake (Bailey et al. 2010, Davis et al. 2009, Hairston et al. 2012, Kondoh et al. 2014, Lagou et al. 2011, Stallmann-Jorgensen et al. 2007).

Branched-chain amino acids

There is growing evidence that obese individuals have a deteriorated plasma amino acid profile, including elevated levels of branched-chain amino acid (BCAA). This increase could reflect dysfunctional basic metabolism and altered catabolic pathways in adipose tissue. Newgard and collaborators reported that rats fed a protein-enriched diet had higher levels of plasma BCAA metabolites (Newgard et al. 2009). However, in a study from our group conducted in women, no direct correlation was found between dietary intake of amino acids, assessed by dietary records and circulating amino acid levels (Boulet et al. 2015). There was also no association between plasma BCAA and dietary BCAA (documented by food records or as part of an intervention) in other studies (Tai et al. 2010, Piccolo et al. 2015). Available data suggest that in humans, relationships between circulating BCAA and obesity, body fat distribution or insulin resistance are more closely related to lower activity of branched-chain α-keto acid dehydrogenase (BCKD) enzyme complex such as BCKDE1α leading to diminished tissue BCAA catabolism independent of dietary BCAA intake (Boulet et al. 2015, Lynch and Adams 2014).

Dietary patterns

Interest on diet patterns has grown over the past decades (Van Horn 2011). Dietary behavior as a whole has been suggested to contribute to a larger extent to chronic diseases than could single nutrients (Kant 2010). This section synthesizes information on the relevance of specific dietary patterns or diets, types of foods or other dietary indices for abdominal fat accumulation and cardiometabolic risk factors.

Dietary patterns

Grouping food into categories allows the assessment of various eating behaviors, or dietary patterns (see description in **Table 6.1**) which may differ according to gender, education level, ethnicity and culture (McNaughton et al. 2007, Newby et al. 2003). Villegas et al. reported that the number of men and women from south Ireland (n=1473) with a high WC and WHR was higher in the 'alcohol and convenience foods' dietary pattern than in the 'prudent diet' (Villegas et al. 2004). Unexpectedly, WC and WHR were lower among those adopting the 'traditional diet' (participants with the highest intake of non-alcoholic beverages, refined cereals, butter, whole milk and sweets) than those following the 'prudent diet' (Villegas et al. 2004). These associations were not significant for BMI, suggesting that being part of the 'alcohol and convenience food' group relates more closely to central adiposity than to overall obesity level. In another study, African-American men with a higher 'southern pattern diet' score had a higher WC and higher CT-measured VAT area; similar trends were observed between WC and the 'fast food pattern' (Liu et al. 2013). No significant association was detected between these adiposity parameters and the 'prudent pattern diet'. In a Brazilian study (Vilela et al. 2014), consuming a 'western diet' was positively associated with WHR and WC after adjustment for BMI in women, while a positive association between WHR and a trend with WC was also observed in the 'regional traditional diet' group (high in rice, beans, tubers, meat, eggs, coffee and sugar). In female Iranian teachers, lower total and central obesity was observed in the higher quintile of the 'healthy pattern diet' while the opposite relationship was found in the 'western and Iranian diet' (Esmaillzadeh and Azadbakht 2008).

Data from Iranian participants with abnormal glucose homeostasis suggest that the 'western diet' is associated with central and total obesity, whereas the 'high-fat dairy pattern' is associated only with total obesity level (Amini et al. 2012). A cross-sectional study in Mexican individuals identified three general dietary patterns: 'westernized', 'high animal protein/fat' and 'prudent'. The 'westernized' and 'high animal protein/fat patterns' were positively associated with percent total fat body and percent abdominal body fat measured by DXA while the 'prudent diet' was related to a lower percent body fat (Denova-Gutierrez et al. 2011). Taken together the above studies suggest that specific 'unfavorable' dietary patterns (such as the 'fast-food pattern', for example) may influence abdominal fat but the effect seems dependent on concurrent gain in body weight or fat mass.

A small number of longitudinal studies investigated the long-term impact of various dietary patterns on body fat distribution. One study reported an inverse correlation between a 'mixed diet pattern' (**Table 6.1**) and WC, but not BMI after adjustment for confounders in men, while the 'fruit, vegetables and dairy pattern' (which shares similarities with the 'prudent pattern' used in other studies) was associated with a decrease in both BMI and WC in women (McNaughton et al. 2007). In healthy men and women participating in the Baltimore Longitudinal study (n=459), subjects in the 'white bread pattern' subgroup had a mean annual WC change of +1.32 cm which was 3 times greater than participants in the 'healthy pattern' subgroup (Newby et al. 2003).

Although many studies have reported associations between dietary patterns and obesity level, few convincingly demonstrated that they may be linked to body fat distribution profiles. Several features of these studies may underlie this conclusion. First, there are numerous differences in population characteristics consuming a particular diet (e.g. smoking status, ethnicity, sex, education level, physical activity), some of which are strong modulators of body fat distribution (reviewed in (Tchernof and Despres 2013)). Moreover, the frequent use of cross-sectional designs, the variability in the populations examined, inconsistencies in the dietary patterns

identified as well as limitations and variation in the methodology used for dietary assessment make direct comparisons of available studies difficult.

Food item subgroups

Another method to assess the impact of diet is to segregate food items in various subgroups according to their nature or composition. The association between the relative weight of food subgroups in the diet and physiological variables can be subsequently analyzed. Several large cross-sectional studies (>1000 participants) have examined the relative distribution of food groups as a function of WC. One of these studies (McCarthy et al. 2006) including adults from Northern Ireland observed significant positive correlations between WC, but not BMI, and food categories like pastries and cake, whole-milk, cream and desserts (which included cream, puddings, chilled desserts and ice cream), meat as well as alcoholic beverages. A negative correlation was also reported between WC and intake of rice and pasta. Data from 1519 adults participating in the National Diet and Nutrition Survey of British adults showed positive associations between WC and portion sizes of various food groups including whole milk, chips and processed potatoes, sweets as well as soft drinks (Kelly et al. 2009). Portion sizes of these food groups were not correlated with BMI (Kelly et al. 2009). A Swedish study including 6069 men and women examined the association between food types and WC or HC (Krachler et al. 2006). In women, a preferential fat accumulation in the hips and thighs was associated with increased consumption of vegetable oil, pasta and 1.5% fat milk, whereas central fat accumulation was associated with higher consumption of hamburgers, potatoes, French fries and soft drinks. In men, vegetable oil, pasta and 1.5-3% milk was associated with gluteofemoral fat accumulation, while central fat accumulation was positively associated with increased beer, but not wine, consumption. Results of the 12-year follow-up of the Framingham Offspring/Spouse cohort study showed that obesity-specific nutritional risk score (based on eleven components including total energy, energy density, carbohydrate, protein, fiber, calcium, alcohol, and total, MUFA, PUFA and SFA) was related to abdominal obesity in women (Wolongevicz et al. 2010).

A Danish monozygotic co-twin case-control study evaluated the impact numerous diet components on body fat distribution (Hasselbalch et al. 2010). A negative association between WC and vegetable oil intake was found in men (Hasselbalch et al. 2010). The prospective EPIC study evaluated annual WC changes in relation with food groups (Romaguera et al. 2011). A high consumption of fruits and dairy products (including milk, yogurt and cheese; irrespective of fat content) coupled to low intake of white bread, processed meat, margarine and soft drinks was associated with lower increases in WC for a given BMI (Romaguera et al. 2011).

Although available studies could suggest detrimental effects of a more refined or fast food type of diet on surrogate measures of VAT, no firm conclusion can be reach about specific food items and body fat distribution, taking into account the relative paucity of data, the heterogeneity of food patterns and populations examined, the disparities in the results and in the various adjustments that were performed (or not performed).

Mediterranean diet

As discussed in Chapter 11, Mediterranean diets (MD) are characterized mainly by high consumption of vegetables, fruits, whole grains, olive oil, nuts, a moderate intake of fatty fish, dairy and alcohol -mostly wine- as well as low consumption of red meat and sweets. This pattern received increasing scientific attention with the development of a Mediterranean score (MEDS) by Trichopoulou and collaborators, in which a diet including these components was shown to positively affect life expectancy in a prospective cohort study (Trichopoulou et al. 1995). In the growing literature of the past decades, many studies have addressed adherence to the MD and body fat distribution.

In the EPIC-PANACEA PROJECT, a cross-sectional study including 497,308 individuals from 10 European countries, a higher MEDS was associated with lower WC for a given BMI in both women and men (Romaguera et al. 2009). No significant association was found between the MEDS and BMI (Romaguera et al. 2009). In a large prospective case-cohort study in five European countries, a higher MEDS was

associated with a reduction in WC, adjusted for BMI, at a 6.8 years median follow-up (Roswall et al. 2014). A longitudinal study with a mean follow-up of 7 years from the Framingham Offspring Cohort showed MEDS-related improvement in several features of the metabolic syndrome, including WC, after adjustment for BMI and change in BMI (Rumawas et al. 2009). These results are consistent with those of the SU.VI.MAX (*Supplémentation en Vitamines et Minéraux AntioXydants*) study cohort (Kesse-Guyot et al. 2013). Similarly, a 9-year follow-up of 3058 Spanish men and women showed that adherence to the MD was negatively associated with increases in WC (Funtikova et al. 2014).

In contrast to the above reports, a cross-sectional Lebanese study found that although the classical MEDS was associated with lower WC independent of BMI, these results were not replicated with a customized MEDS adapted for the Lebanese population. With the custom score, both BMI and WC were lower with a higher score, suggesting that the MD is associated with lower total and central adiposity (Issa et al. 2011). This result is consistent with findings of Boghossian et al. on 258 pre-menopausal women (Boghossian et al. 2013). A high MEDS was, indeed, significantly associated with lower BMI, lower waist and hip circumferences and lower body fat mass assessed by DXA (Boghossian et al. 2013). Negative associations between a higher MEDS and overall adiposity were replicated in other studies (Schroder et al. 2010, Panagiotakos et al. 2006).

Another study including 23,597 participants from the EPIC cohort showed that when energy intake was not controlled for, the MEDS was associated with a marginal increase in BMI and WC (Trichopoulou et al. 2005). For example, in the Greek cohort of the EPIC study, the MD was directly linked to increased energy intake (Ferro-Luzzi, James, and Kafatos 2002). Conversely, an Italian study reported no association between BMI or WHR and adherence to the major characteristics of the MD (Rossi et al. 2008). In a recent nutritional intervention study in a non-Mediterranean population at risk for cardiovascular diseases, both men and women significantly increased their MEDS and, as a result, decreased the energy density of their diet and thus reduced their energy intake (Leblanc et al. 2015). At the end of

the intervention period (12 weeks), both genders had significantly lower WC and BMI, along with other improvements in metabolic indicators such as HDL-C (in men only) (Leblanc et al. 2015). Reports of nutritional data may need to be standardized across population studies to assess the apparent favorable impact of the MD on body fat distribution and/or overall adiposity.

One study reported lower weight gain with a higher MEDS for participants with 1 or 2 minor alleles of the *TCF7L2* gene, which has been related to diabetes. No interaction was observed between this gene, MD and central obesity (Roswall et al. 2014). Moreover, in the Prevención con Dieta Mediterránea (PREDIMED) clinical trial, participants with the minor 12Ala allele of the PPARγ gene increased their WC significantly more than non-carriers of this allele, while no difference in BMI was noted. Yet, this result was only observed in the control group advised to follow a low-fat diet, not in the two Mediterranean groups. Therefore, the MD appears to protect against the detrimental effects of the 12A1a PPARγ minor allele (Razquin et al. 2009). It has been proposed that the highly concentrated MUFA and PUFA of the MD could modulate PPARγ activation (Xu et al. 1999).

While available data point toward a possible specific reduction of central adiposity with the MD, this issue is still controversial in the literature. Adaptation of the original MEDS to local populations may also complicate study comparisons. WC was generally used in large cohort studies, but additional imaging studies would be required to better assess the impact of MD on abdominal fat compartments.

Other nutrients or food items

A wide selection of specific nutrients or food items has been studied in relation to body fat distribution and will be briefly addressed in this section. Extensive discussion of the mechanisms underlying their possible impact on abdominal obesity is beyond the scope of this chapter.

Alcohol

Data on alcohol consumption and obesity are conflicting. Alcohol provides 7.1 kcal/g, yet some beverages contain active biomolecules such as polyphenols (e.g. red wine), which can positively impact cardiometabolic risk factors. Existence of the 'beer belly' is a widely spread popular notion and heavy alcohol use also leads to liver disease and other metabolic alterations. Studies on alcohol consumption and abdominal obesity are briefly reviewed here.

In Japanese men, only a trend between VAT area and alcohol intake was noted (Kondoh et al. 2014). However, a significant inverse relationship was found with abdominal subcutaneous fat along with a positive association with the VAT/SAT ratio. In age-adjusted regression analyses, alcohol intake was strongly and positively associated with VAT area (Kondoh et al. 2014). Similar results were reported by Larson and collaborators in a cohort of men and women from the USA. VAT adjusted for SAT was positively associated with alcohol intake (as a binary variable) whereas SAT adjusted for VAT was negatively associated with alcohol consumption (Larson et al. 1996). In the Framingham Heart Study, relationships between alcohol intake and VAT or SAT were studied separately in both sexes (Molenaar et al. 2009). Men whose intake was greater than 14 drinks per week (equivalent to >24g ethanol/day) and women with intake greater than 7 drinks per week (>12g ethanol/day) were identified as heavy drinkers whereas the remaining individuals were classified as light and moderate drinkers (Molenaar et al. 2009). In women but not in men, SAT area was higher in light-to-moderate drinkers, whereas only in men VAT area was higher among the heavy drinkers (Molenaar et al. 2009). Another study reported that central obesity in women, assessed by DXA, was associated with low levels of alcohol consumption which is consistent with the notion that SAT is increased

preferentially in women gaining weight (Greenfield et al. 2003). In men from the Normative Aging Study, there was a trend toward a positive association between the WHR and alcohol consumption (Troisi et al. 1991). Brandhagen and collaborators reported associations between types of alcoholic beverages and measures of central adiposity in both men and women of the Swedish Obese Subjects (SOS) study (Brandhagen et al. 2012). With their fully adjusted model in men, consumption of spirits was positively associated with percentage body fat, sagittal diameter and WC, whereas beer consumption was not associated with any of these measurements (Brandhagen et al. 2012). In contrast, in women, wine and total alcohol intake were negatively associated with percent body fat (Brandhagen et al. 2012).

In overweight young adults, alcohol consumption in both sexes was not associated with, nor predictive of, VAT or SAT area (Bailey et al. 2010). However, as reported by the authors, cafeteria-based diet assessment may not reflect usual alcohol consumption in that cohort. In abdominally obese men, consumption of 40 g of ethanol (450 mL of red wine) daily over a 4-week trial did not result in increased body weight, abdominal or subcutaneous fat contents as determined by ultrasound (Beulens et al. 2006), but led to increased adiponectin secretion. Data from the EPIC study showed a 0.02 cm increase in WC per 5% increase of alcohol intake (as a proportion of total energy intake) for a given BMI only in women (Romaguera et al. 2010).

In sum, most studies show that alcohol consumption may be linked to increases in total fat mass as well as depot-specific abdominal fat storage that may be a reflection of the propensity of each sex to store fat either in central (men) or peripheral (women) compartments.

Dairy products, calcium and vitamin D

Many epidemiological findings suggest a favorable antiobesogenic effect of dairy products, likely related to high calcium and vitamin D intake (Loos et al. 2004, Jacqmain et al. 2003, Pereira et al. 2002, Zemel et al. 2000). A recent meta-analysis found that daily increase in dairy intake (550 to 1000 mg of additional calcium) was

associated with a lower WC (-2.43 cm, 95% CI: -3.42, -1.44) (Abargouei et al. 2012) compared to control groups in energy restriction studies but not in studies without energy restriction (WC: -2.19 cm, 95% CI: -8.02, 2.66). In a randomized weight loss trial where obese men and women consumed yogurt or placebo in the form of a sugar-free gelatin snack, there was a more pronounced weight loss in the yogurt group. Furthermore, trunk fat loss was 81% higher than that of the control group, and there was a 4 cm reduction in WC. The authors proposed increased fat cell lipolysis as a potential mechanism, as supported by an increase in plasma glycerol observed only in participants who consumed yogurt (Zemel et al. 2005). In a retrospective study including overweight and obese Australians, there was an inverse relationship between total dairy food intake and WC after adjustment for age, sex and total energy intake (Murphy et al. 2013). After adjustment for total dairy food intake, dairy protein and dairy calcium were still negatively associated with WC and DXA-measured abdominal fat (Murphy et al. 2013). In a recent randomized control trial, men and women taking calcium and vitamin D supplementation in orange juice had greater decreases in VAT area than controls treated with orange juice without supplementation after adjustment for baseline total abdominal area (SAT+VAT) to consider for baseline group differences (Rosenblum et al. 2012). Consistent with these results, Bush et al. reported that premenopausal women gained 2.7 cm^2 less visceral fat assessed by CT scan for each 100 mg/day increase of total dietary calcium over a 1-year period (Bush et al. 2010). In postmenopausal women, total dietary calcium intake was negatively associated with abdominal fat mass and percent body fat, but when adjusted for total energy intake only percent body fat was still associated with calcium intake. Non-significant associations were also reported between calcium intake and BMI, WHR and WC (Heiss, Shaw, and Carothers 2008).

Vitamin D has been proposed to play a role in adipocyte differentiation, however its effect remains unclear (Kong and Li 2006). Our team reported that women with higher plasma levels of 25(OH)D had smaller omental adipocytes, and lower VAT and SAT areas determined by CT (Caron-Jobin et al. 2011). In another study, overweight and obese women supplemented with 25 µg of vitamin D3 for 3 months showed no difference in WC and weight compared to controls (Salehpour et al.

2012). Wamberg and collaborators reported no difference in SAT and VAT areas (determined by MRI) in obese men and women after 26 weeks of supplementation with 175 µg of vitamin D3 compared to controls, and no difference in the cardiometabolic risk factors including the homeostasis model assessment of insulin resistance (HOMA-IR), high-sensitivity C-reactive protein (hsCRP), plasma lipids and blood pressure (Wamberg et al. 2013). In chronic kidney disease patients, levels of 25(OH)D decreased as BMI increased and were also lower in diabetic patients. However, there was no association between vitamin D status and SAT or VAT areas as well as body fat mass (Figuiredo-Dias et al. 2012). The apparent null effect of vitamin D supplementation alone may point toward a combined action of dairy protein and/or calcium in the modulation of body fat distribution.

Soy and isoflavones

In the Soy Health Effects (SHE) study, intake of the most common isoflavone subtypes (genistein and daidzein) was assessed with a food-frequency questionnaire in 208 postmenopausal women (Goodman-Gruen and Kritz-Silverstein 2003). Women with higher genistein intake had lower BMI, fat mass and WC than women who reported no intake of isoflavones but they were also generally more active (Goodman-Gruen and Kritz-Silverstein 2003). In two randomized control trials, postmenopausal women taking a daily shake with added soy protein and isoflavones for 3 months gained less total and SAT area than women in the casein placebo group (Sites et al. 2007, Christie et al. 2010). VAT gain was not different between the two groups (Sites et al. 2007, Christie et al. 2010). Different results were obtained in randomized control trials on exercise, isoflavones and weight loss in postmenopausal women (Choquette et al. 2011, Maesta et al. 2007). While exercise had a favorable effect on WC, HC and body fat mass, no apparent additive nor synergetic effect of isoflavones was detected (Choquette et al. 2011, Maesta et al. 2007). In a weight loss trial comparing the effect of soy vs. milk protein only those in the milk protein group experienced a reduction in BMI and body weight (Takahira et al. 2011). Decreased WC was observed in both groups but only the milk group had significant decreases in both abdominal SAT and VAT area (Takahira et al. 2011).

Overall, a specific effect of soy or isoflavones on visceral fat accumulation is improbable.

Dietary fiber and whole grains

Dietary fiber and whole grain intake has been associated with body composition in many studies. McKeown and collaborators reported inverse relationships between intake of whole grain cereal fiber and percent trunk fat mass as well as percent body fat in an elderly population (60 to 80 yrs old) (McKeown et al. 2009). Of note, they did not find an association between these measurements and total fiber intake (McKeown et al. 2009). Interestingly, those in the highest quartile category of dietary fiber and/or whole grain intake were also the group with the higher energy intake (McKeown et al. 2009). Two other observational studies found no association between whole grain intake and VAT or SAT areas (Davis et al. 2009, Stallmann-Jorgensen et al. 2007).

Total fiber intake was negatively associated with VAT measured by MRI in two other studies (n=644), but SAT was not measured (Davis et al. 2009, Parikh et al. 2012). Results from CT scans in overweight young adults and middle-aged men and women from the United States showed no association between VAT or SAT and total fiber intake (Bailey et al. 2010, Larson et al. 1996). In US Latino teenagers, VAT area was associated with insoluble fiber but not soluble fiber intake (Davis et al. 2009). On the other hand, in overweight African-American and Latino adults, soluble fiber intake was negatively associated with VAT area, whereas insoluble fiber intake was not (Hairston et al. 2012). The effects of high fiber/whole grain diet on body fat distribution do not seem to be mediated by reduced energy intake as the vast majority of the above-mentioned studies adjusted for energy intake.

Vitamins A and C

Excess storage of fat is associated with increased fatty acid oxidation leading to production of larger amounts of free radicals, thus inducing a greater use of antioxidant molecules such as vitamins A and C. Therefore, low plasma levels of

vitamin A and C could be associated with low-grade chronic inflammation related to obesity, especially in the visceral depot (Zulet et al. 2008).

In healthy young adults, high vitamin A intake was negatively associated with WC and WHR as well as numerous cardiometabolic risk variables such as fasting glycaemia, insulin and blood pressure (Zulet et al. 2008). However a relationship between vitamin A intake and VAT or SAT accumulation was not found in overweight young adults (Bailey et al. 2010).

In women, higher intake of ascorbic acid was associated with lower WC (Choi et al. 2013) and lower odds ratio of having a WHR ≥ 0.84 (Azadbakht and Esmaillzadeh 2008). In men from the same study, no association was found between WC and vitamin C intake (Choi et al. 2013).

Probiotics and the gut microbiota

There is increasing evidence linking the gut microbiota to total adiposity (Rosenbaum, Knight, and Leibel 2015). A complete review of such evidence is beyond the scope of this book chapter. Of note, a probiotic bacteria (LG2055) naturally present in human gut microbiota led to significant decreases of adipocyte hypertrophy in rats (Sato et al. 2008). More recently, the same team showed a reduction in VAT and SAT areas as well as WC, BMI and body fat mass in a randomized control trial of healthy men and women supplementing their habitual diet with fermented milk containing LG2055 vs. placebo (fermented milk without LG2055) (Kadooka et al. 2010). Emerging studies on the human gut microbiota will eventually allow assessing its potential role in body fat distribution.

Infant feeding practices

Other possible dietary influences on body fat distribution patterns may include early-life nutrition. Many studies have linked infant feeding practices or even maternal diet during pregnancy with the onset of obesity in the offspring, and several mechanisms were proposed to explain this association (reviewed in (Lecoutre and Breton 2014, 2015) and Chapter 28 of this textbook). However, only a few studies have directly investigated infant feeding practice or maternal diet vs. body fat distribution patterns of the offspring independently of maternal BMI, socio-economic status and other confounding variables.

Whether breastfeeding is related to offspring adiposity later in life is still a matter of debate. Systematic reviews have concluded that exclusive breastfeeding for at least 6 months is protective against obesity (Kramer and Kakuma 2012). After adjustment for confounding variables, this relation was attenuated or abolished (Kramer and Kakuma 2012). Only a few studies have investigated duration of breastfeeding as a function of offspring body fat distribution. In a retrospective study of 442 children (EPOCH study), breastfeeding for less than 6 months or for more than 6 months had no differential impact on the accumulation of MRI-assessed VAT or SAT, sum of skinfolds and BMI of the offspring (Crume et al. 2012). However, a protective effect of breastfeeding was apparent for those in the upper percentiles of BMI, VAT and SAT after adjustment for confounding variables (Crume et al. 2012).

In the Generation R prospective study, children who were breastfed exclusively for at least 4 months had lower total body fat mass and peripheral fat compared to no breastfeeding at 6 months (Durmus et al. 2012). Children who were never or non-exclusively breastfed until four months had higher subcutaneous central fat at 24 months of age compared to exclusive breastfeeding (Durmus et al. 2012). However, there was no difference in body fat distribution related to timing of the introduction of solids foods (Durmus et al. 2012). Similarly, no difference in body fat distribution was found in the same children at 6 years of age, after adjustment for confounding variables (Durmus et al. 2014). In fact, neither timing of solid food introduction nor use of formula were associated with total skinfolds in children between 4 and 5 years

of age in two other independent studies (Zive et al. 1992, van der Grond et al. 1991). Similar observations were made in the HELENA cohort including 3528 adolescents (Rousseaux et al. 2014). Of note, investigators of the HELENA cohort showed a trend for a protective effect of breastfeeding for those in the upper percentile of skinfold thickness and waist-to-height ratio, in line with previous results (Crume et al. 2012).

Opposite results were obtained in a Brazilian study of 185 children between four and seven years. Breastfeeding duration was positively associated with percent body fat assessed by DXA, but not with WC or fat mass localized in the abdominal region. Much like the previously-cited study (Durmus et al. 2014), timing of solid food introduction was not associated with BMI, WC, total body fat mass or central adiposity (Magalhaes et al. 2012). In young infants, introduction of cereals or meat at 5 months of age did not modulate adiposity at 9 months of age (Tang and Krebs 2014). Regarding maternal macronutrient intake, a study in a large cohort showed that fetal adiposity was highest in the abdominal region of infants from mothers with low dietary intake of protein (<16%), irrespective of carbohydrate or fat intakes, and this was caused by an increase of SAT accumulation measured by ultrasound at 19, 25, 30 and 36 week. Maternal diet was assessed by a validated 74-items food-frequency questionnaire during the early and late pregnancy periods. Change in the diet was then compared to changes in foetus adiposity. On the other hand, VAT accumulation (assessed by ultrasound) was higher when maternal calories from protein represented more than 20% energy and this was linked with carbohydrate intake, which was diminished, thereby increasing the protein-to-carbohydrate ratio. Ultrasound-measured mid-thigh fat in these foetuses was related to maternal intakes that were high in fat, low in carbohydrates and intermediary in protein. These studies identify a plausible mechanism by which maternal protein intake can module infant body fat distribution *in utero* (Blumfield et al. 2012). In another study of pregnant adolescents, intake of total and added sugar was the best predictor of ultrasound-measured fetal abdominal fat thickness during the last trimester of pregnancy. In this cohort, a U-shaped curve was observed between energy-adjusted carbohydrate intake and abdominal fat, pointing again to an ideal maternal carbohydrate intake.

Of note, gestational weight gain was not related to fat accretion in the foetus for these women (Whisner et al. 2015). A Danish study examined maternal intake of animal and vegetable protein in relation to BMI and WC of offspring at 20 years of age (Maslova et al. 2014). Offspring BMI was related to maternal protein intake during pregnancy but WC showed no significant association (Maslova et al. 2014). In the ROLO study, maternal protein intake was not associated with newborn anthropometric measurements, but SFA intake in the second and third trimester was related to offspring waist circumference and the waist-length ratio whereas there was a negative trend between maternal PUFA intake in the third trimester and abdominal circumference at birth (Horan et al. 2014). Consistent with these results, low level of circulating PUFA in newborns was positively associated with higher central fat (Sanz et al. 2014). Finally, in a randomized control trial modulating the ratio of n6-n3 FA in the diet of pregnant women had no effect on body fat distribution in infants at birth, 6 weeks, 4 and 12 months (Hauner et al. 2012).

Overall, there is growing evidence that maternal diet modulates body fat distribution in the offspring independently of a number of confounding factors. A moderate protein (>16 to <20% energy) diet, and PUFA intake appears to limit abdominal fat accretion whereas SFA intake seems linked to detrimental adiposity profiles. However, some important facts must be acknowledged. Investigators often make statistical adjustment for energy intake in children, obliterating the fact that prenatal and postnatal nutrition may impact appetite and the desire for particular foods. Age of adiposity rebound, i.e. the age between 3 and 7 years where BMI-for age starts to increase after it reaches its lowest point, is not always considered in available studies which could account for some differences in adiposity between groups.

Conclusion

Most available studies on diet and body fat distribution seem to point toward a non-specific effect on fat accumulation or protection from (VAT, SAT, both or total adiposity). Nutrients potentially linked to a detrimental fat accretion pattern (increased VAT, abdominal fat or WC) include fructose consumption, especially in the form of SSB, *trans* fatty acids, as well as high alcohol consumption and refined/fast food diets. On the other hand, some nutrients (MCT, calcium, fiber, whole grains) and dietary patterns (MD, prudent diet) have been found to reduce adiposity in epidemiological studies and there is growing evidence supporting these notions through randomized control trials. Whether these effects emerge from depot specific impact in adipose tissue compartments remains unclear. Therefore, there is no clear evidence that a single nutrient directly modulates visceral fat accretion and, by extension body fat distribution. However, some nutrients and/or diet patterns may affect energy balance and subsequent weight gain or loss, which will be reflected in changes in regional fat accumulation as a function of the prevailing genetic, epigenetic and hormonal milieu (**Figure 6.1**). In this context, responses to nutrients or diets present remarkable inter-individual variability most likely resulting from interactions with a plethora of physiological factors. Future studies on this topic should aim to identify subpopulations of responders and non-responders with the goal of unraveling biological modulators of the response. Considering the emerging impact of early life nutrition, prevention strategies may also have to focus on maternal nutrition and perinatal feeding.

Table

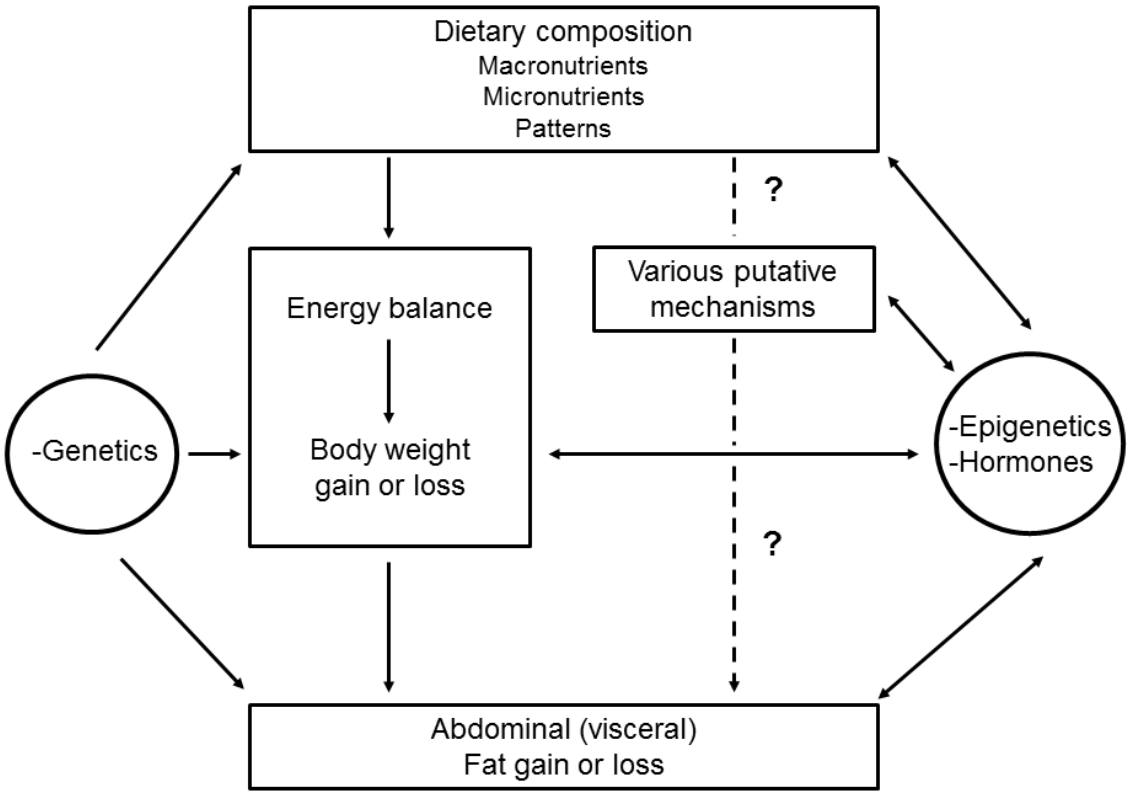
Table 6.1: Characteristics of the main diet patterns reported.

Diet pattern	Description[1]	Studies
Prudent or healthy	High in fruits, vegetables, white meats or fish, nuts, vegetable oils and whole grains.	(Villegas et al. 2004) (Esmaillzadeh and Azadbakht 2008) (Denova-Gutierrez et al. 2011) (Amini et al. 2012) (Liu et al. 2013) (Vilela et al. 2014)
Western	High in refined grains, red meats, butter, eggs, hydrogenated fats, soft drinks and sweets.	(Esmaillzadeh and Azadbakht 2008) (Denova-Gutierrez et al. 2011) (Amini et al. 2012) (Vilela et al. 2014)
Alcohol and convenience foods	High intake in alcohol and meats with low consumption of fruits, vegetables and whole grains.	(Villegas et al. 2004)
Southern	Traditional rural southern US foods: high consumption of beans, legumes, corn products, fried fish and chicken, margarine and butter.	(Liu et al. 2013)
Fast-food	High in sugar, fast-foods and salty snacks.	(Liu et al. 2013)
Traditional (Iranian)	High consumption of grains, potato, tea, hydrogenated fats and legumes.	(Esmaillzadeh and Azadbakht 2008)
Mixed diet	High consumption of fruits, vegetables, low-fat yogurt and soy milk, but high in sweets.	(McNaughton et al. 2007)

[1]description can vary according to the study and population

Figure Heading

Figure 6.1. Potential impact of dietary factors on abdominal-visceral fat gain or loss



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Chapitre 2 : Hypertrophie adipocytaire

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Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction

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Résumé

L'obésité est une maladie hétérogène qui est associée à plusieurs comorbidités telles que le diabète de type 2, les maladies cardiovasculaires et certains types de cancer. Plusieurs études ont examiné le rôle du tissu adipeux dysfonctionnel dans la pathogenèse de l'obésité en mettant en évidence les propriétés contrastantes des divers compartiments de tissus adipeux, parfois avec des résultats contradictoires. Le tissu adipeux dysfonctionnel est caractérisé par l'élargissement ou l'hypertrophie des cellules adipeuses pré-existantes, qui contribuent à l'augmentation du risque cardiométabolique, indépendamment du niveau d'obésité. Dans cet article, nous analyserons de façon critique la littérature sur la capacité prédictive de la taille des cellules adipocytaires en termes de maladies métaboliques et de dysfonctionnement du tissu adipeux chez l'humain. De nombreuses études démontrent que l'augmentation de la taille des cellules adipeuses est un prédicteur important d'un profil lipidique altéré et des altérations de l'homéostasie du glucose et de l'insuline, indépendamment des mesures d'adiposité. La contribution de l'adiposité viscérale à ces associations semble être d'une importance particulière. Cependant, les études disponibles ne sont pas unanimes et de nombreux aspects spécifiques des différents dépôts modulent la relation entre l'augmentation de la taille des cellules adipeuses et le risque cardiométabolique, ou encore le dysfonctionnement du tissu adipeux. Les paramètres méthodologiques, entre autres l'approche utilisée pour exprimer les données, peuvent représenter des facteurs de confusion importants dans ces études. Des travaux supplémentaires devraient prendre en compte que la relation entre la taille des adipocytes et les indices d'adiposité communs est non-linéaire, en particulier lorsqu'elles atteignent des valeurs plus élevées. Nous proposons que l'hypertrophie adipocytaire et l'accumulation excessive de tissu adipeux viscéral peuvent représenter des marqueurs importants d'une capacité hyperplasique limitée dans les tissus adipeux sous-cutanés, qui à son tour est associée à la présence de nombreuses altérations cardiométaboliques.

Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction

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Abstract

Obesity is a heterogeneous disease and is associated with comorbidities such as type 2 diabetes mellitus, cardiovascular diseases and cancer. Several studies have examined the role of dysfunctional adipose tissue in the pathogenesis of obesity, highlighting the contrasting properties and impact of distinct adipose tissue compartments, sometimes with contradictory results. Dysfunctional adipose tissue involves enlargement, or hypertrophy, of pre-existing fat cells, which is thought to confer increases in cardiometabolic risk, independent of the level of obesity *per se*. In this article we critically analyze available literature which examined the ability of adipocyte cell size to predict metabolic disease and adipose tissue dysfunction in humans. Many studies demonstrate that increased fat cell size is a significant predictor of blood lipid profiles and glucose-insulin homeostasis alterations independent of adiposity indices. The contribution of visceral adiposity to these associations appears to be of particular importance. However, available studies are not unanimous and many fat depot-specific aspects of the relationship between increased fat cell size and cardiometabolic risk or adipose tissue dysfunction are still unresolved. Methodological factors such as the approach used to express the data, may represent significant confounders in these studies. Additional studies should consider the fact that the relationship between fat cell size and common adiposity indices is non-linear, particularly when reaching the obese range. We propose that adipocyte hypertrophy and excess visceral adipose tissue accumulation may represent strong markers of limited hyperplasic capacity in subcutaneous adipose tissues, which in turn is associated with the presence of numerous cardiometabolic alterations.

Abbreviations and Glossary

3T3-L1: Mouse embryonic preadipocyte cell line; ApoB: apolipoprotein B; ATGL: adipose triglyceride lipase; BFM: body fat mass; BMI: body mass index; CD11b: macrophage and neutrophil cell marker; CD11c: dendritic cell and macrophage cell marker; CD31: endothelial cell marker; CD68: macrophage cell marker; DEXA: dual-energy X-ray absorptiometry; FCS: fat cell size; GLUT4: Glucose transporter type 4; HOMA-IR: homeostatic model assessment of insulin resistance; HIF-α: hypoxia-inducible factor 1; HSL: hormone-sensitive lipase; IGF-1: insulin-like growth factor 1; IL-6:interleukin 6; IRS-1: insulin receptor substrate 1; MeSH: Medical Subject Heading; NAFLD: non-alcoholic fatty liver disease; NF-κB: nuclear factor kappa B; NGT: normal glucose tolerant; OM: omental; PCOS: polycystic ovarian syndrome; PLIN: perilipin (lipid droplet-associated protein); SC : subcutaneous; SVF: stroma-vascular fraction; T2DM: type 2 diabetes mellitus; TNF-α: tumor necrosis factor alpha; VEGFA: vascular endothelial growth factor A; VEGF-R2: vascular endothelial growth factor receptor 2; vWF: von Willebrand Factor; WHR: waist-to-hip ratio

Introduction

Over one third of the world population is now struggling with overweight or obesity and the disease burden frequently associated with these conditions (1). Obesity is a well-known risk factor of metabolic diseases such as type 2 diabetes mellitus (T2DM), dyslipidemia, coronary heart disease, hypertension, non-alcoholic fatty liver disease (NAFLD) and stroke (2). It has also been linked with dementia, obstructive sleep apnea and numerous types of cancer (2). However, a significant proportion of obese individuals, 15 to 30% depending on the populations examined and the definition of metabolic health, do not develop alterations associated to obesity, at least in the short term (3, 4). These subsets of apparently healthy obese subjects are named metabolically healthy obese.

Differences in body fat distribution likely account for many discrepancies between metabolically healthy and unhealthy individuals. Jean Vague proposed as early as 1947 that patients with upper-body obesity had an increased risk for metabolic diseases, while individuals with lower-body (femoral-gluteal) obesity had a lower risk of alterations (5). Since then, body fat distribution indices have been recognized as stronger predictors of metabolic alterations compared to overall obesity, emphasizing their impact on global metabolic health (2). Specifically, studies have shown that excess fat accumulation on visceral anatomical structures such as the greater omentum or mesentery is a strong predictor of a detrimental metabolic status; whereas accumulation of gluteal and femoral fat is viewed as protective when total adiposity is accounted for (2). In fact, despite a high body fat mass, low visceral fat accumulation is a major feature of the metabolically healthy profile observed in some obese individuals (6, 7).

Adipose tissue expansion in a given fat compartment occurs through adipocyte hyperplasia, hypertrophy or a combination of both. Recruitment of new cells through differentiation of preadipocytes (hyperplasia) is now generally considered as a mechanism that protects against metabolic alterations. Indeed, adipocyte hyperplasia is associated with preferential accumulation of subcutaneous (SC) adipose tissue and the gynoid phenotype (8-12). On the other hand, visceral

adipocytes and the android fat distribution phenotype are more commonly associated with adipocyte hypertrophy, that is, enlargement of existing fat cells (8, 9, 11, 12).

Increased fat cell size (FCS) has been demonstrated to be associated with metabolic impairment in patients as early as the 70s (13, 14). In 1979 (15), adipocyte hypertrophy was proposed as a valuable parameter to characterize metabolic disturbances. Since then, FCS has been linked to numerous variables assessing adipose tissue biology and also with metabolic alterations independently of total adiposity (9, 11, 16-25). To our knowledge, studies on the potential role of adipocyte hypertrophy as a biological marker of metabolic disease and adipose tissue dysfunction have never been reviewed in detail. The present article proposes an extensive analysis of the studies on this topic and demonstrates that adipocyte size may, indeed, represent an important determinant of adipose tissue dysfunction and a potential marker of pathologies that may or may not be linked with total adiposity.

Literature search

To perform this critical analysis, the first step was to determine all possible Medical Subject Heading (MeSH) terms to be used in searches on the PubMed database. The term «adipocyte» was combined with the following keywords: size, hypertrophy, white, area, diameter, morphology, cellularity, dysregulation and volume. The same approach was then repeated but this time using the term «fat cell» instead of «adipocyte». The articles were selected depending on the following specific criteria: 1) emphasis was placed on human studies directly relevant to the research topic; and 2) only articles on white adipose tissue were selected. Relevant articles from the reference list of identified papers were added. In total, 172 studies were retained. They were published between 1970 and March 2015. A record of the articles was kept and they were listed according to the research criteria used to find them. An exhaustive analysis of the population, the methods and major findings of each study was performed. We further selected the studies that had analyzed the ability of FCS to predict obesity-related metabolic complications or parameters of adipose tissue function. The latter studies represent the main body of literature used for this article.

Analyses of cell size variations among sexes, obesity degrees as assessed by body mass index (BMI) and cell sizing techniques were performed using 76 publications in which the variables of interest were available. FCS measurements in these studies were converted to the same unit. Units of volume (µL, pL and μm^3) and units of surface (μm^2) were converted to a diameter in micrometer (µm) assuming a spherical or circular cell shape. Units of mass (µg) were first converted to volume using the density of triolein (density=0.915 g/mL) and then to cell diameter in µm.

Methods to measure fat cell size

This section provides a brief overview of the main techniques used to assess FCS. We focus mainly on the most commonly used: collagenase digestion, osmium tetroxide fixation and histological analysis. We also provide analyses of FCS variation according to sex, obesity level and measurement technique.

Collagenase digestion

Collagenase digestion has been developed by Rodbell as a mean to separate mature adipocytes from the stroma-vascular fraction (SVF) (26). It is now used as the first step of many experiments such as cell cultures and cell sizing. Briefly, adipose tissue is digested by collagenase and mature adipocytes are separated from the SVF by floatation in an aqueous solution. Pictures of mature adipocytes can be taken with a phase contrast microscope to assess adipocyte size. This method has been the most frequently used. However, some drawbacks have prevented it from becoming a gold standard in determining FCS. Small adipocytes do not float as easily as the average-size cells, due to their low lipid content (27). Additionally, adipocytes tend to break in unfixed tissue because of their fragility (27). However the introduction of adenosine in the solution minimized this bias (28). Centrifugation during the floatation step can bias the recovery of small adipocytes, therefore this step can be omitted (29). Finally, blue methylene staining may be required to assess viability of the cells and facilitate identification of lipid droplets vs undamaged fat cells (26).

Osmium tetroxide fixation and Multisizer Counter analysis

This technique has been developed in 1968 by Hirsh and Gallian (30) on the basis that osmium tetroxide fixes intracellular lipids and allows staining of very fragile cell types. In brief, adipose tissue can be digested by collagenase and subsequently fixed by osmium tetroxide, or these steps can be simultaneous if a collidine-HCl solution is used to separate the SVF from mature adipocytes instead of collagenase. Adipocytes are then analyzed with a counter allowing cell size measurement by fluctuation of electrical resistance. This technique allows studying large distributions of adipocyte sizes as well as analyzing cell subpopulations like very small fat cells (31, 32). Even if this technique allows fixation of very small adipocytes (15-25µm), a threshold value (most often 25 µm) is used to discriminate between mature adipocytes and artefacts. However, multilobular fat cells tend to rupture during the fixation process which can lead to an underestimation of mean FCS, especially in obese individuals (31). This technique is also time-consuming, it comes at a relatively high price and requires proper handling of osmium tetroxide, a hazardous chemical (27, 30, 31).

Histological analysis

Adipocyte cell size analysis can also be performed on histological slides. Type and concentration of fixatives, paraffin-embedding, time of fixation, slice thickness and type of coloration may vary among laboratories. Of importance, this is the only technique that allows examination of global tissue architecture. However, many potential biases are inherent to this approach and many assumptions have to be made in order to assess cell size. Cell distribution has to be considered uniform, which may not be entirely true in obese and young individuals (27). Furthermore, fixation agents are known to induce significant shrinkage of the cells (33, 34). Likewise, it must be assumed that cells are perfect spheres showing their largest diameter (27). This technique has been extensively used recently due to the possibility of simultaneously performing immunostaining of various cellular markers.

Other techniques

Other methods have been used in various contexts to appreciate variation in FCS. Flow cytometry as well as scanning electron microscopy have been used by a few teams (35-39). These specialized methods are not in common use to measure FCS in clinical research.

Cell size analysis software

Promising semi-automatic methods have arisen with the purpose of limiting user-dependent biases and time of use (40-42). Information on a few completely automated programs has also been published (43, 44). Obstacles such as the time needed to obtain high-quality images may slightly complicate their use, along with the presence of the SVF and other tissue artefacts in some samples. Moreover, user-dependent variation still needs to be addressed.

Overall, among these approaches to assess FCS, none are without drawbacks. Characterization of very large and very small adipocytes remains a challenge for all methods available. Moreover, the number of adipocytes examined in each study varied from 50 to 20 000, which at times limits study comparisons. As a result, no single method has arisen in the literature as the gold standard for adipocyte cell sizing.

Population and methodological variation

Our survey of the literature demonstrates that adipocyte size varies between men and women as well as between fat depots (visceral vs. SC). Moreover, as a function of increasing obesity level, mean adipocyte size increases, reaching a plateau at a certain level of adiposity. Arner and colleagues have already shown that the relationship between SC FCS and body fat mass (BFM) is curvilinear in men and women (45). Moreover, adipocyte hypertrophy is negatively correlated with adipocyte hyperplasia, when adjusted for the predicted adipocyte volume at a given BFM, and subjects with higher fat cell size than predicted by the curve have lower rates of adipogenesis (45). This suggests an important impact of adipose

morphology, independent of BFM. However, a complete review of FCS variation and obesity indices such as BMI has not been published yet.

Using all the studies in which mean FCS and mean BMI were available, we summarize sex-, depot- and obesity-related variation in adipocyte size in humans and address potential differences related to the method used for analysis. As shown in **Figure 1A**, omental (OM) and abdominal SC FCS increase in both sexes with increasing obesity level as assessed by BMI and reach a plateau around BMI values of approximately 30 kg/m^2. When plotting data from men (**Figure 1B**) and women (**Figure 1C**) separately, we find that the plateau is reached at lower BMI values in men (approximately 25 kg/m^2) compared to women (approximately 35 kg/m^2). Moreover, OM FCS appears to be approximately 20% lower than SC FCS in women, whereas this depot difference is not apparent in men. These findings suggest a clear difference among sexes regarding the expansion of adipose tissue. Men are more prone to hypertrophic obesity, as the plateau is seen at lower BMI values. On the other hand, women show signs of both hypertrophic and hyperplasic expansion as obesity level increases. Similar observations can be made with both the SC and OM adipocyte size curves.

When examining FCS as a function of the measurement technique, we found that the use of histological slides generally leads to lower mean FCS by approximately 15% across all BMI values (**Figure 1D**). Collagenase digestion and osmium fixation appear to generate similar mean FCS (**Figure 1D**). These patterns can be observed in both depots as well. All techniques showed a general pattern of BMI-related increase, with a plateau reached at higher obesity levels.

In this analysis, population differences noted in FCS variation closely reflect those observed in many individual studies as reviewed here. Adipocyte size is clearly influenced by sex, anatomical localization and obesity levels. As discussed in the sections below, these variations need to be considered critically when examining the relationship between fat cell hypertrophy in a given fat compartment and metabolic diseases or parameters of adipose tissue function.

Adipocyte size as a potential biomarker of cardiometabolic alterations or disease endpoints independent of common adiposity indices

Common adiposity indices have been widely used to assess the presence of cardiometabolic risk factors in overweight or obese individuals. BMI is certainly the most widely used. However, it has its limitations. It does not take into account body fat distribution and only provides a crude assessment of body composition. Other anthropometric indices have been examined along with more invasive measurements of body fat distribution and/or body composition (2). An important question which has been addressed in many original publications is whether adipocyte size also predicts the metabolic complications frequently associated with obesity or abdominal obesity. Considering that FCS is strongly related to body composition and fat distribution, whether it predicts metabolic alterations independent of overall or regional adiposity has also been tested in a number of studies. The next sections provide a review of the studies that have addressed the link between FCS and cardiometabolic risk factors or disease endpoints.

Blood lipid profile

Imbeault et al. (46) reported that when matched for visceral adipose tissue area, abdominal but not femoral SC FCS predicted an altered lipid profile in men but not in women. Specifically, men with large adipocytes had hypertriglyceridemia and higher LDL-apolipoprotein B (ApoB) levels. Another study reported in patients with first-degree relatives of T2DM individuals that elevated SC FCS correlated with lower HDL-cholesterol concentration, but not with high total cholesterol or LDL-cholesterol concentrations when adjusted for BMI (20). In homozygous twins discordant for obesity level, after adjustment for total body fat mass (BFM), SC FCS correlated positively with LDL-cholesterol concentrations (47). Interestingly, in the obese twin characterized by adipocyte hypertrophy, higher LDL-cholesterol and lower HDL-cholesterol levels were found compared to the lean co-twin, but this difference was not found in twins characterized by hyperplasia. However, in both cases, the obese co-twin had 61% larger adipocytes than the lean twin (47). In obese women, Ledoux

et al. (21) did not find a correlation between FCS in either the SC or visceral depot and blood lipid concentrations when adjusting for BMI or waist-to-hip ratio (WHR).

An elegant study by Hoffsted et al. (16) showed in obese women that OM, but not SC FCS, was associated with plasma apolipoprotein B, total cholesterol, LDL-cholesterol and triglyceride concentrations independent of BMI, BFM and body fat distribution indices obtained by dual-energy X-ray absorptiometry (DEXA). In another study, visceral and SC fat cell volumes were positively correlated with triglyceride levels and negatively with HDL-cholesterol concentrations in bariatric patients after control for total BFM (9). In a sample of women ranging in adiposity from lean to moderately obese, after control for BMI, total BFM and visceral adipose tissue area measured by computed tomography, we found that OM FCS predicted triglyceride concentration whereas SC FCS did not (17). Yet, in female and male Indians, another group reported no significant correlation between total cholesterol and triglyceride concentrations and SC or OM FCS after adjustment for BMI, BFM, waist circumference, total abdominal adipose tissue area and SC adipose tissue area (48). However, in that particular study, associations between visceral adipose tissue accumulation and metabolic parameters were scarce as visceral adipose tissue only correlated with HDL-cholesterol and triglyceride concentrations in men. In sum, a number of studies have shown associations between high FCS either in the SC or visceral fat compartment and an altered lipid profile. Some of these associations appeared to be independent of total or abdominal adiposity in some cases. Further studies in both genders taking into account ethnicity as well as the OM and SC fat compartment are needed to generalize these findings.

Glucose homeostasis, insulin resistance and T2DM

The relationship between SC FCS and insulin resistance is well documented. A number of studies have shown a clear association between abdominal SC FCS and markers of insulin resistance, in both women and men (11, 15, 24, 45, 49-54). In overweight South African women with normal glucose tolerance (NGT), no link was found between abdominal or gluteofemoral SC FCS and insulin resistance markers such as basal plasma insulin, insulin area and glucose area after a 100 g oral

glucose load (55). Nevertheless, another study demonstrated that SC FCS was increased in obese with T2DM when compared with obese control subjects matched for BMI, age, sex and ethnicity (56). Also, when controlling for BMI, SC FCS was positively correlated with homeostatic model assessment of insulin resistance (HOMA-IR) and negatively with post-glucose insulin sensitivity (57).

This was challenged by McLaughlin et al. (58) who reported that mean diameter of the larger SC cells assessed with osmium tetroxide was not different between insulin resistant vs insulin sensitive patients (119 vs. 115 μm respectively). However, insulin resistant patients were characterized by a larger population of small cells (a high ratio of small-to-large cells), a feature that can only be assessed with the osmium technique and which could possibly reflect arrested development of the very small adipocytes toward fully mature cells (58). Another study found no link between SC FCS and HOMA-IR (21). On the other hand, in a sample of T2DM patients matched to NGT patients for BMI, Pasarica et al. found larger SC fat cells measured with osmium tetroxide in T2DM patients (124 μm diameter compared to 115 μm respectively) (59). In that study, there was no depletion of the small cell population in the diabetic subgroup and mean FCS was positively correlated with HOMA-IR. Similar results were obtained by Yang et al. (60). Mean fat cell volume was inversely correlated with insulin sensitivity measured by euglycemic, hyperinsulinemic clamp after adjustment for BMI in a sample of first-degree relatives of T2DM patients (60). In non-obese first-degree relatives of T2DM patients, SC FCS was negatively correlated with GLUT4 protein expression and positively with circulating insulin levels whereas BMI was not (61). We found that SC GLUT4 mRNA abundance was lower in women with hypertrophic obesity (62). Moreover, another group reported that after adjustment for BMI and percent body fat, SC FCS was associated with insulin resistance, but not with fasting glucose concentration in first-degree relatives of T2DM patients (20). Arner and colleagues found that in women characterized by SC adipocyte hypertrophy, adipocyte volume, adjusted for BFM, was an important and independent correlate of HOMA-index and fasting insulin levels (45).

Depending on how anthropometric adjustments are performed, SC FCS is not always an independent predictor of alterations in indices of glucose-insulin homeostasis. Our group reported that SC FCS was not a significant predictor of fasting glucose or fasting insulin in non-obese women when adjusted for adiposity and visceral adipose tissue area measured by computed tomography (17). The adjustment for variation in visceral adipose tissue area was critical in the latter analysis, raising the possibility that lack of control for this variable in other studies may have led to the finding of significant associations. Similarly, Azuma et al. (18) found in T2DM patients that increased FCS was associated with insulin resistance independent of total BFM, but when they adjusted for body fat distribution measurements (leg fat mass or trunk fat mass), the relationship became non-significant. These results are not unanimous. Glycemia, insulinemia and glucose disposal rate were associated with SC FCS independently of age, BMI, BFM or body fat distribution indices obtained by DEXA in a sample of obese women (16). Similar results were found in obese men and women, after adjustment for total body fat content (9). SC adipocyte hypertrophy also explained the difference in glucose disposal rate between South Asians and Caucasians, after control for total body fat content, intraperitoneal and SC fat (19). In another study, SC FCS was associated with markers of insulin resistance in NGT patients but not in T2DM patients, indicating that this relation may become non-linear when a certain degree of disease severity is reached (24). Such phenomena could contribute to the discrepancies noted among some studies.

The relationship between visceral FCS (OM adipocytes in most studies) and insulin resistance has also been of interest. Our group was the first to report a relationship between fasting insulin, HOMA-IR and OM FCS in non-obese women (17). However, once adjusted for adiposity and body fat distribution, there was no significant association. The close correlation between OM FCS and visceral adipose tissue area measured by computed tomography explained the latter finding. Similar results were obtained in female and male Indians when adjusting for BMI, BFM, waist circumference, total and SC adipose tissue area (48). Another study found an

association between fasting glycemia, HOMA-IR (63) and OM FCS; however statistical adjustment for anthropometric values was not performed in that study (52).

In obese patients, the association between visceral FCS and markers of insulin resistance remains uncertain. OM FCS seems to predict fasting glycemia, fasting insulin and HOMA-IR independent of BMI or WHR in a few studies (21-23). Hoffstedt et al. found that visceral adipocyte hypertrophy was not associated with plasma insulin, fasting glycemia and glucose disposal after control for age, BMI, BFM and DEXA-measured body fat distribution indices (16). The same group found that visceral fat cell volume (to a lesser extent than SC FCS) was correlated with insulin levels, insulin-induced glucose disposal and insulin sensitivity after control for total BFM, in both sexes (9). To add to these results, no significant relationship was found between OM FCS and HOMA-IR in non-diabetic obese adults, yet OM FCS was associated with these variables independently of BMI and WHR *in vitro* when glucose uptake in freshly isolated adipocytes stimulated by insulin was measured (24). In morbidly obese patients with insulin resistance, a greater proportion of adipocytes exceeding 100 µm diameter was also observed along with a lower proportion of small adipocytes compared to insulin sensitive patients (7, 52, 64). This relation was challenged recently by van Beek and colleagues (65), who reported no difference in adipocyte size between subjects with T2DM compared to those with NGT.

Overall, many studies have reported significant associations between SC FCS and various markers of insulin resistance. Some reported that this association was independent of concomitant elevations in total BFM, pointing toward a specific role of adipocyte hypertrophy, and perhaps adipose tissue dysfunction, as a marker of insulin resistance. On the other hand, most of the studies that adjusted their analysis for well-measured body fat distribution markers such as visceral adipose tissue accumulation have shown that the association between SC FCS and indices of insulin resistance was no longer significant. We propose that excess visceral adipose tissue accumulation and SC fat cell hypertrophy may represent markers of a common phenomenon: limited hyperplasic capacity of adipose tissues. Yet, the

relationship with visceral adipocyte hypertrophy remains equivocal in most studies. The non-linear nature of the relationship between FCS and obesity level or insulin resistance may contribute to explain discrepancies among the various studies that examined this relationship.

Metabolic syndrome features

Three studies examined the association between FCS and the number of metabolic syndrome features. In bariatric surgery patients, no difference was noted in FCS of patients with or without the metabolic syndrome (25, 66). The first study assessed the metabolic syndrome on the basis of the Harmonized criteria (67). Three features or more were required to qualify as metabolically unhealthy. The second study considered metabolic health status on the following criteria: 1) fasting glucose >100 mg/dL; 2) total insulin> 19 µU/mL; 3) triglycerides >150 mg/dL; 4) HDL-cholesterol <39 mg/dL; 5) systolic blood pressure >140 mmHg; and 6) diastolic blood pressure >90 mmHg. Patients were considered as metabolically unhealthy when at least one feature was present. Visceral adipocyte hypertrophy was observed in patients with low HDL-cholesterol levels and high fasting glucose concentrations in one of these studies (25). Also, the number of metabolic syndrome features increased with visceral FCS (25), consistent with another study (22) (adapted version of (68)) that associated adipocyte hypertrophy with a detrimental metabolic state in bariatric patients. The fact that only severely obese patients were studied may have led to low level associations between FCS and the number of metabolic syndrome features, considering that FCS values reach a plateau in the obese range. Another study reported that high mesenteric FCS was associated with a 1.79 increased in the risk of having metabolic syndrome (ATP III criteria) in overweight men (63). More studies are needed to assess whether FCS is associated with the onset of metabolic syndrome independent of adiposity.

NAFLD and liver fat accumulation

Six studies found an association between liver fat accumulation and SC adipocyte size (22, 47, 69-72). Two studies reported the opposite (73, 74). One of these studies

included a homogenous population of T2DM patients (74) and the other examined males before and after weight gain (73). When patients were matched for BMI, age, sex and/or BFM, SC adipocyte hypertrophy was an independent marker of liver fat (47, 69-72). Moreover, OM adipocyte hypertrophy could independently predict stages of NAFLD (22, 75). In one study, the extent of fatty liver disease was not predicted by FCS, but this finding was consistent with the lack of association between FCS and chronic intermittent hypoxia, the best and only predictor of liver disease stages in that sample (76).

Polycystic ovary syndrome (PCOS)

There are a few studies on adipocyte hypertrophy and PCOS in the literature. Two teams noted a 25% increase of SC FCS in PCOS women compared to controls when matched for age and BMI (77, 78). Another group found similar results for visceral adipocyte size, even if women with PCOS were younger than controls in this particular study (79). Moreover, in PCOS women, lipolysis responsiveness was reduced in SC and increased in visceral adipocytes when compared to controls (79). Additional studies are required to assess whether adipocyte hypertrophy in PCOS could represent the missing link between adipose tissue dysfunction and the metabolic alterations observed in this condition.

Cardiovascular endpoints

To our knowledge, there is no data available on FCS and cardiovascular endpoints. One study related visceral adipocyte volume positively to arterial stiffness, a known cardiovascular disease risk factor (80). Mean SC gluteal FCS has also been positively correlated with mean arterial blood pressure (81). Ledoux et al. found in patients with hypertension that mean OM and SC abdominal cell size were increased (21). Other studies did not report such an association.

FCS as a predictor of metabolic alterations in weight loss studies

A large number of studies has been performed on weight loss through various modalities (bariatric surgery, diet and/or exercise) and have documented the impact of such interventions on adipocyte size reductions. However only a few studies have

attempted to link the favorable metabolic effect of weight loss to reduced FCS. Some studies have shown that weight loss-induced decreases in FCS were associated with changes in plasma levels of leptin, adiponectin, glucose, insulin, triglycerides, cholesterol, LDL-cholesterol as well as with changes in HOMA-IR, glucose disposal rate and systolic/diastolic blood pressure independently of total BFM (70, 82-85).

Cancer

The link between obesity and the risk of mortality from cancer is well known (86, 87). However, the biological processes underlying this association are still being investigated. Breast adipose tissue has been of interest recently due to its relative proximity with cancer cells. Hypertrophic adipocytes can induce a permanent pro-tumorous microenvironment as a source of: 1) growth factors such as estrogen, IGF-1 and leptin; 2) constant energy supply; and 3) pro-inflammatory cytokines such as IL-6 and TNF-α. In fact, *in vitro* studies have shown that mature adipocytes are able to sustain and promote tumor growth (88). Therefore, hypertrophic mammary adipocytes could reflect dysfunctional adipose tissue contributing to a microenvironment that favors cancer growth. Only one team has examined FCS in mammary fat tissue in women with breast cancer and reported a positive correlation with BMI and other markers of inflammation such as increased NF-κB binding and number of macrophages (89). More studies are necessary to understand the link among obesity, FCS and breast cancer.

Adipose tissue dysfunction and hypertrophic adipocytes

An increasingly large body of evidence points toward dysfunctional adipose tissue as a major determinant of metabolic impairments in individuals with abdominal obesity. Hypertrophy of the adipose cells may represent a marker and perhaps to some extent a driver of adipose tissue dysfunction. After providing an overview of how FCS relates to metabolic abnormalities, we now review the basis of adipose tissue dysfunction and its relationship with adipocyte hypertrophy.

Lipid metabolism

The traditional belief has been that lipid metabolism is stimulated in large adipocytes, both from the standpoint of fatty acid uptake and fatty acid release through lipolytic pathways. For example, elegant studies comparing populations of small (35 μm) vs. large (50 μm) diameter adipocytes from the same anatomical location have shown that fatty acid synthase and lipoprotein lipase activities were increased in large compared to small adipocytes when expressed per number of cells (90). In that study, the lipolytic response to β-adrenergic agonist isoproterenol was also increased in large vs. small adipocytes. Interestingly, the beta(1)-integrin/ERKs signalling pathway was also activated in large adipocytes and had been proposed as a putative mechanism of the adaptation of adipose tissue functions to cell size (90, 91). Consistent with these results we found that adipocyte isoproterenol-stimulated lipolysis was higher in women with hypertrophic adipocytes, independent of overall adiposity and body fat distribution (62). Furthermore, Laurencikiene et al. demonstrated that hormone-induced lipolysis rates were increased in large (100 μm) compared to small (82 μm) diameter SC cells. They also observed that protein level of HSL, PLIN and ATGL was increased in large adipocytes (92). In contrast, opposite results were obtained in lean and obese children, in whom a significant negative correlation was found between basal lipolysis of isolated adipocytes (expressed per number of cells) and abdominal SC FCS (54). The latter association was lost upon statistical control for BMI and lipolytic responsiveness to isoproterenol was not associated with FCS (54). A study using an original fluorescence-based technique to assess lipid uptake in individual cells has reached opposite conclusions with SC

adipose tissue explants from monkeys under insulin-stimulated conditions (93). Specifically, small cells of the explants responded to insulin by increasing lipid uptake, whereas adipocytes with cell diameters >80-100 μm were insulin resistant. Data were expressed per cell area in that study. It was proposed that such a mechanism could protect adipocytes from lipid overload and restrict further expansion of adipose tissue (93). Additional studies in 3T3-L1 cells have shown highly dynamic lipid trafficking among cellular compartments and between lipid droplets (94). Interestingly, rates of exchange were lower in cells with larger lipid droplets compared to those with smaller lipid droplets, suggesting that cells with large lipid droplets are less efficient in transporting and possibly metabolizing fatty acids than those with small lipid droplets (94). An *in vivo* study was performed by the group of Jensen and collaborators to assess rates of lipid uptake in small, medium or large adipocytes with radioactive and stable-isotope-labelled fatty acids (95). Interestingly, when expressed per lipid weight, no difference in lipid uptake was found among small (83 μm), medium (103 μm) or large (117 μm) diameter adipocytes. On the other hand, when expressed per cell number, larger adipocytes had higher rates of lipid uptake (95). These experiments did not include insulin-stimulated conditions.

Considering the above-cited studies, it is difficult to reach firm conclusions regarding the impact of cell size on lipid metabolism in adipose tissue. Much confusion may arise from the method chosen to express the data. For example, in a study of diacylglycerol acyltransferase activity in isolated microsomal fractions from SC and OM adipose tissues, we have shown no relationship between maximal activity of this enzyme and adipocyte size when data were expressed per cell number (96). Yet, when expressed per mg of tissue, activity of the enzyme in OM tissue was lower in patients with excess visceral adipose tissue accumulation and large OM adipocytes (96). Consistent results were obtained in other *in vivo* studies by Votruba and collaborators showing that net lipid uptake from a meal expressed per adipose tissue weight but not per cell was decreased as a function of adipocyte size in both upper and lower body SC fat (97). These results suggest that a given amount of tissue seems to take up fewer fatty acids and synthesize triglyceride less efficiently when

adipocytes are larger, despite lack of a difference or increased metabolism on a per cell basis. The study by Serra and collaborators (98) also reported a positive relationship between abdominal SC FCS and adipose tissue heparin releasable lipoprotein lipase activity (expressed per number of cells) in the same depot, but only in obese women with a low relative accumulation of visceral fat. The authors concluded that high visceral fat accumulation relative to total abdominal fat reflected lower triglyceride storage capacity at least in the SC fat compartment (94). Inconsistencies among studies may also be explained by a loss of heterogeneity in insulin-stimulated lipid uptake of individual cells with increasing FCS and levels of obesity. This has been demonstrated by Varlamov and collaborators in white adipose tissue explants of rhesus macaques (99). Additional factors such as an obesity-related decrease in the ability to increase adipose tissue blood flow in response to a meal may also interfere with lipid storage in a given fat compartment (100), which could contribute to explain some of the discrepancies among *in vitro* and *in vivo* studies.

Glucose metabolism

Some studies have shown that there is no difference in small vs. large fat cells from the same patient for protein levels of IRS-1 and GLUT4 (101, 102), whereas another study reported increased GLUT4 protein in large vs small adipocytes from the same compartment (90). Insulin stimulation significantly increased GLUT4 translocation in small (81 μm) but not in large (114 μm) diameter cells from the same individual indicating an insulin resistant state consistent with the reduction of insulin-stimulated lipid uptake described in the previous section on lipid metabolism (101). Consistent with these results, we reported a decrease in mRNA transcript of GLUT4 in SC adipose tissue of women with hypertrophic OM adipocytes compared to those with adipocyte hyperplasia (62).

Adiponectin and leptin

Some studies have shown that expression and secretion of adipose-derived cytokines or adipokines may vary as a function of adipocyte size and location (103,

104). A full review of all cytokines is beyond the scope of this review. This section focuses on leptin and adiponectin, two adipokines secreted almost exclusively by adipocytes. Inflammatory cytokines will be discussed in the subsection on inflammation.

Many reports have shown a negative association between SC FCS and adiponectin release (19, 61, 69, 103, 105, 106), even after adjustment for SC adipose tissue area (19). One study reported no association between OM FCS or SC FCS and adiponectin levels (24). No association was found between SC FCS and serum adiponectin in another study in children (54). Another group reported that this association was non-significant when expressed as a function of cell surface (103). In South Asians, adiponectin decreased at lower obesity levels compared to Caucasians, likely due to ethnicity-related differences in FCS or in body fat distribution (69). Of interest, our group found that adiponectin release by isolated OM mature adipocytes (expressed as a function of lipid weight of the cell suspension) was reduced with increasing BFM and OM FCS (104). On the other hand, adiponectin release by SC fat cells remained unaffected by differences in total BFM or SC FCS (104).

Adipocytes appear to secrete more leptin as their size increases, at least for those derived from the SC fat compartment (19, 24, 103, 107-109). This is consistent with other studies showing that SC FCS is an independent predictor of plasma leptin concentration (24) and that SC FCS is the second most important predictor of leptin concentration after total BFM (107). SC FCS predicted 16.2% of the variance in leptin levels (109). SC FCS seems to be related to leptin levels, even when expressed per cell surface units (103) an association that is fully explained by differences in SC adipose tissue areas (19). However, in OM adipocytes, the association is equivocal. OM fat cells express lower levels of leptin mRNA (110) and it was thought that FCS and/or differences in tissue innervation contributed to this phenomenon. Recent studies showed no correlation (24) or even a negative relationship (111) between serum leptin levels and OM FCS.

Inflammation

Hypertrophic adipocytes overexpress NF-κB (112) and TNF-α (106, 113, 114) independent of BMI and total BFM (115). Other studies demonstrated that levels of inflammation marker C-reactive protein were correlated with the presence of larger adipocytes in obese individuals (51, 106, 114). However, this association was not found in lean individuals when they were analyzed separately from the obese subgroup (114).

The presence and number of adipose tissue macrophages in adipose tissues relate to its dysfunction and systemic inflammation. Macrophages in crown-like structures are often observed in adipose tissues of mice on a high fat diet, but their occurrence in human fat is far less common (116). We observed only a few crown-like structures in obese and more specifically in SC adipose tissue (116). No correlation was found between the number of crown-like structures and adipocyte hypertrophy in humans (65, 74, 76, 117).

We examined 40 women for whom SC and visceral adipose tissue was obtained and assessed the number of macrophages in the tissue using the CD68 marker and found positive correlations between percentage of macrophages in SC and OM adipose tissue as well as SC and OM FCS respectively (116). We also examined the CD11c and CD11b markers to distinguish subtypes of macrophages. We found that visceral and SC adipose tissue area was strongly correlated with CD11b and Cd11c expression in SC tissue, but not in visceral fat. Consistent with this result, OM and SC FCS were associated with expression of both CD11b and CD11c, but in SC tissue only. Increased SC FCS was associated positively with CD68+ macrophages in children. When FCS was categorized into tertiles, there was a threefold increase in the number of macrophages between the first (<116 um) and last (>130 um) FCS tertile (54). Further characterization of adipose tissue immune cells is needed to better understand how cell size relates to the low-grade, chronic inflammation state of obesity.

Hypoxia/Angiogenesis

Hypoxia and mitochondrial dysfunction could explain some of the mechanisms underlying adipose tissue dysfunction. Reactive oxygen species production could trigger an inflammatory response and cellular death. Hallgren et al. found that large diameter cells (88 μm) from both lean and obese individuals consumed more O_2 than small diameter cells (66 μm) regardless of the phenotype (118). This finding was not supported by recent work by Yin et al. who also showed that for lean patients, FCS increases consumption rate of O_2, but this relation was not present in obese patients (119). Moreover, obese patients had a lower O_2 consumption rate than their lean counterpart, regardless of FCS (119). This finding is consistent with a few other studies that found no link between FCS and angiogenic capacity as measured by expression of vWF, HIF-α or VEGFA (21, 61, 120). One study reported a positive association between FCS and VEGF-R2 expression and with the number of vessels per 10 adipocytes (121). We also examined women characterized by OM adipocyte hypertrophy and found higher expression of vWF and CD31 in both fat compartments compared to women with hyperplasia (62). Another study found a negative association between FCS (OM/SC) and capillary density (122). The relationship between dysfunctional adipocytes and angiogenesis necessitates further investigation since it appears in the obese phenotype, but its association with FCS remains unclear.

Adipogenesis

A complete review of adipogenesis is beyond the scope of this article and has already been done by our group (123). Adipogenic capacity is depot- and obesity-specific (123) and is apparently reduced in hypertrophic obesity (124).

Elegant studies by Arner and colleagues have shown that adipocyte hypertrophy is negatively correlated with adipocyte hyperplasia, when adjusted for the predicted adipocyte volume at a given BFM (45). Subjects with higher FCS than predicted by the regression curve had a lower rate of adipogenesis. These individuals also had a more deleterious metabolic profile. These results support the hypothesis that each

individual has a different plateau of maximum FCS and when it is reached, metabolic alterations arise.

In our recent analysis of published data, we confirmed the notion that adipogenesis is reduced in overweight and obese women with adipocyte hypertrophy (123). Moreover, in another study, (124), we initiated *in vitro* primary preadipocyte cultures of cells obtained from the SC and OM adipose tissues of 35 women. We assessed adipogenic capacity by measuring lipid accumulation (oil red staining) and G3PDH activity as a terminal differentiation marker. We found that lower SC preadipocyte differentiation capacity was related to increased OM FCS, excess visceral adipose tissue accumulation, high VLDL lipid content and slightly elevated fasting glycemia (124). These results are consistent with the notion that limited expandability of SC adipose tissue is related to adipocyte hypertrophy, particularly in the visceral fat compartment, and the concomitant presence of metabolic alterations.

Conclusions and perspectives

In conclusion, our analysis demonstrates that FCS may represent a significant biomarker of the cardiometabolic alterations related to obesity. In particular, many studies demonstrated that FCS predicts alterations in the blood lipid profile and glucose-insulin homeostasis independent of adiposity indices. The contribution of visceral adiposity to these associations seems to be of particular significance. We propose that excess visceral adipose tissue accumulation and adipocyte hypertrophy, either in the SC or visceral fat compartment depending on the studies, may represent strong markers of a common phenomenon: limited hyperplasic capacity of adipose tissues, which in turn associates with the presence of numerous cardiometabolic alterations (**Figure 2**).

However, we also emphasize that available studies are far from unanimous, in particular when addressing the relationship between FCS and parameters of adipose tissue function. A number of methodological factors such as the approach used to express the data may have confounded these analyses. Additional factors to be considered for future studies on this topic include analyses of cell subpopulations and the plateauing of cell size with increasing obesity levels. These issues are addressed below.

Most investigators have used average FCS as the primary variable in their analyses, which has become the default standard. However, this measure has been revealed to be incomplete when global tissue physiology is studied. Other approaches to better characterize the dynamics of cell populations have already been discussed, including osmium fixation and Multisizer Counting. This approach could represent a very useful method to characterize cell populations in more detail. Interestingly, the fraction of very small cells has become of interest in patients with severe obesity when it was found to be increased in the presence of the metabolic syndrome. Adipose tissue expansion could lead to changes in the various cell populations that may be missed when examining mean FCS. Analysis of cell size distribution patterns, of the ratio of small-to-large cells as well as minimal and maximal cell sizes are potential alternatives.

Another important factor to consider in analyses based on adipocyte size is the patient population under study. As shown in **Figure 1**, mean FCS increases with adiposity level and reaches a plateau at higher obesity levels. Accordingly, studies in obese and severely obese individuals have shown that mean FCS only slightly differs among these subgroups of patients. In these cases, it is expected that the predictive ability of FCS will be reduced. The relationship between FCS and adiposity indices becomes non-linear, which may limit the effectiveness of regression studies to control for the effect of adiposity. More importantly, the association between FCS and metabolic parameters may also become non-linear in the obese or very obese range. This phenomenon could indicate that the 'cardiometabolic pathology' has reached another stage, where adipose tissue storage capacity is very limited and other features such as ectopic fat accumulation will become better predictors of metabolic status than FCS *per se*. Further studies are needed to better understand the impact of adipocyte hypertrophy on metabolic health and the ways to manage it.

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Declaration of interest

The authors have nothing to disclose.

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Figure Headings

Figure 1: Mean FCS of abdominal subcutaneous (SC) and omental (OM) depot in 172 subgroups of individuals in 76 published studies. Sizes of the symbols reflect study samples size. Published studies on FCS were screened and inclusion criteria were availability of the BMI and FCS for the same subgroup. A total of 6207 individuals are included in the analysis. Non-linear regressions were determined taking study sample size into consideration. **A**. OM and SC FCS in both in males and females. **B**. OM and SC FCS as a function of BMI in males. **C**. OM and SC FCS as a function of BMI in females. **D**. FCS variation as a function of BMI and the technique used to assess FCS. Each curve combines OM and SC FCS. Collagenase digestion, n=4630; Osmium tetroxide fixation, n=1052; Histological analysis, n=2533.

Figure 2: Obesity is a multifactorial disease characterized by expansion of adipose tissue occurring through adipocyte hypertrophy (enlargement of pre-existing cells) or adipocyte hyperplasia (generation of new cells through adipogenesis). Limited expandability of adipose tissue through hyperplasia leads to increases in FCS (adipocyte hypertrophy), which represents a critical marker of central adiposity, adipose tissue dysfunction and concomitant metabolic disease risk.

Figure 1.

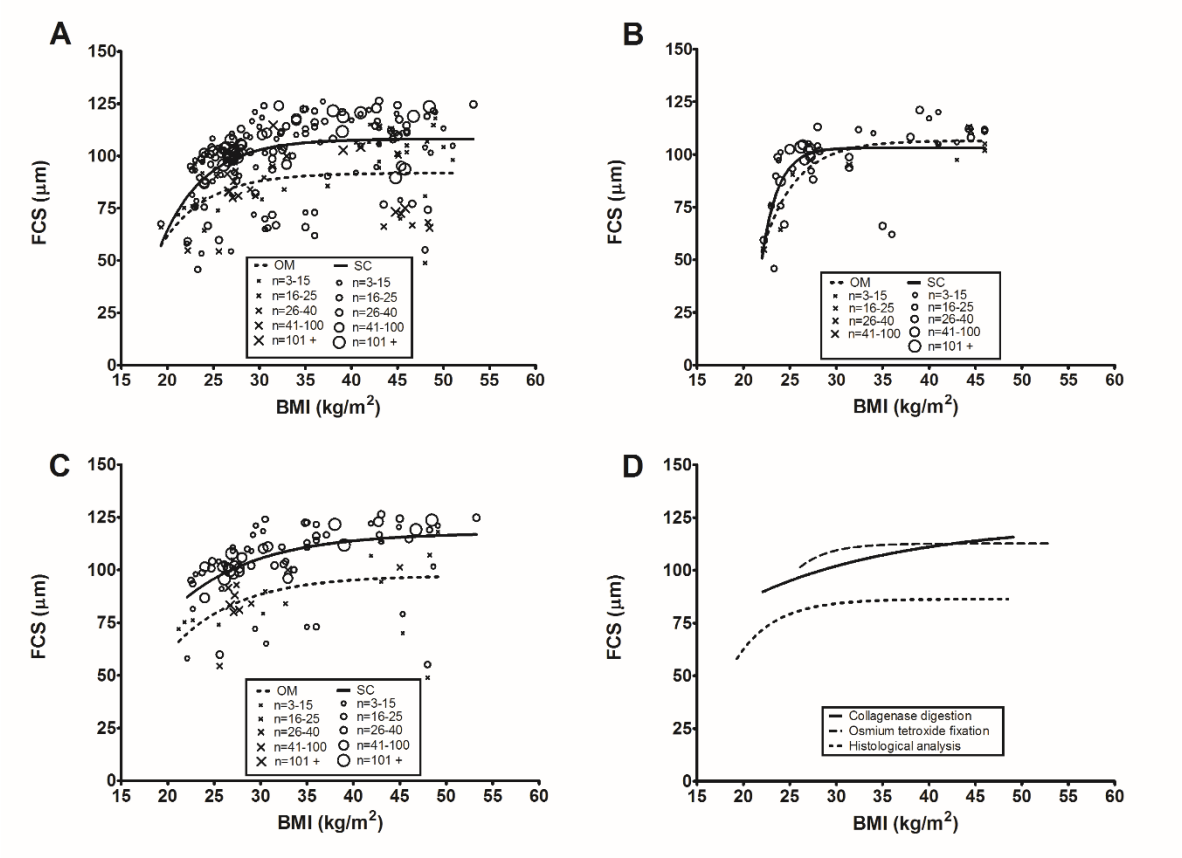
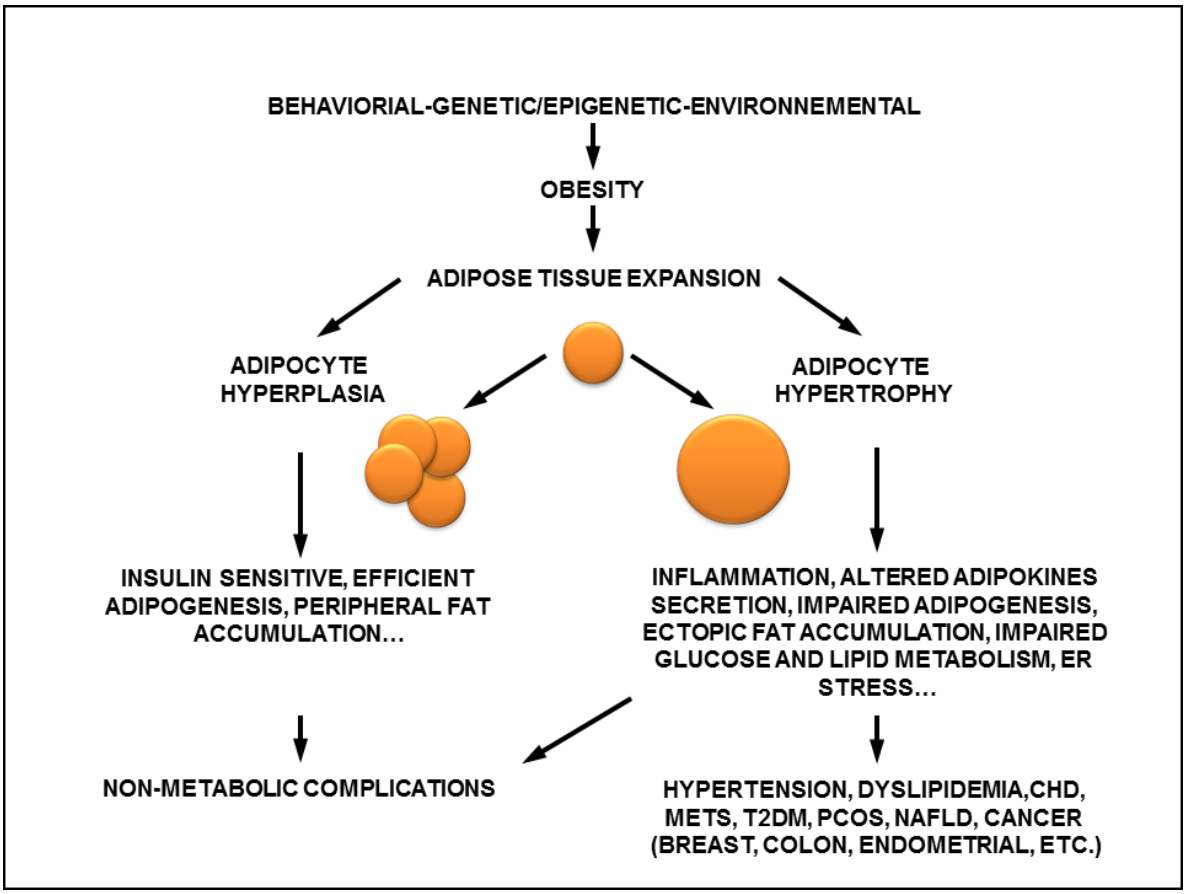

A
B
C
D
FCS (µm)
BMI (kg/m²)
OM
SC
n=3-15
n=16-25
n=26-40
n=41-100
n=101 +
Collagenase digestion
Osmium tetroxide fixation
Histological analysis

Figure 2.


BEHAVIORIAL-GENETIC/EPIGENETIC-ENVIRONNEMENTAL
OBESITY
ADIPOSE TISSUE EXPANSION
ADIPOCYTE HYPERPLASIA
ADIPOCYTE HYPERTROPHY
INSULIN SENSITIVE, EFFICIENT ADIPOGENESIS, PERIPHERAL FAT ACCUMULATION…
INFLAMMATION, ALTERED ADIPOKINES SECRETION, IMPAIRED ADIPOGENESIS, ECTOPIC FAT ACCUMULATION, IMPAIRED GLUCOSE AND LIPID METABOLISM, ER STRESS…
NON-METABOLIC COMPLICATIONS
HYPERTENSION, DYSLIPIDEMIA,CHD, METS, T2DM, PCOS, NAFLD, CANCER (BREAST, COLON, ENDOMETRIAL, ETC.)

Chapitre 3 : Analyse comparative de trois techniques de mesure de la taille adipocytaire chez l'humain: importance de la technique de mesure et association avec les facteurs de risque cardiométabolique

Sofia Laforest, Andréanne Michaud, Gaétan Paris, Mélissa Pelletier, Hubert Vidal, Alain Géloën et André Tchernof

L'article composant ce chapitre s'intitule:

Comparative analysis of three human adipocyte size measurement methods and their relevance for cardiometabolic risk

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Résumé

Objectif: Déterminer si les diamètres adipocytaires des trois méthodes de mesure sont associés de manière similaire aux mesures d'adiposité et aux variables cardiométaboliques.

Méthodes: Des échantillons chirurgicaux de tissu adipeux omental (OM) et sous-cutané abdominal (SC) ont été obtenus chez 54 femmes (âge 35-58 ans; IMC 20.9-41.1 kg/m^2). Le diamètre médian des adipocytes de la population cellulaire principale a été déterminé par digestion à la collagénase, par fixation au tétroxyde d'osmium et par analyse histomorphométrique. L'adiposité ainsi que et les facteurs de risque cardiométabolique ont été mesurés.

Résultats: Le diamètre adipocytaire mesuré par analyse histomorphométrique était systématiquement inférieur aux diamètres mesurés à la suite d'une digestion à la collagénase, alors que les cellules fixées par l'acide osmique étaient plus grosses ($p<0,0001$; pour tous). Les diamètres médians des adipocytes dérivés de toutes les méthodes étaient très bien intercorrélés ($r=0,46$ à $0,83$; $p<0,001$ pour tous). Des associations positives ont été observées entre le diamètre des adipocytes provenant de chacune des techniques et les mesures d'adiposité régionales ou totales ($p<0,01$; pour tous). Le diamètre adipocytaire OM a été positivement associé à la glycémie à jeun, l'insuline et l'HOMA-IR ($r=0,30$ à $0,52$; $p<0,05$ pour tous) le diamètre adipocytaire dérivé de la fixation à l'acide osmique étant le plus fort corrélat. Ce dernier a également été le meilleur corrélat de l'adiponectine et de la leptine plasmatique.

Conclusions: Bien que les techniques de mesure aient généré des différences systématiques dans la taille des adipocytes, les associations avec l'adiposité étaient peu affectées par la technique. La fixation au tétroxyde d'osmium a généré des associations plus fortes avec les facteurs de risque cardiométabolique que la digestion à la collagénase et l'analyse histomorphométrique.

Comparative analysis of three human adipocyte size measurement methods and their relevance for cardiometabolic risk

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Running Title: Measurement technique

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Study importance

What is already known about this subject?

- Adipocyte size is a recognized marker of adipocyte function and cardiometabolic risk
- Various methods to assess fat cell size have been used

What does your study add?

- Comparative analysis of three adipocyte size measurement methods generated systematic differences in adipocyte median diameter
- Associations with adiposity were only slightly affected by the technique
- Osmium fixation generated stronger associations with cardiometabolic risk factors than collagenase digestion and histological analysis

Abstract

Objective: To determine whether adipocyte diameters from three measurement methods are similarly associated with adiposity measurements and cardiometabolic variables.

Methods: Surgical samples of omental (OM) and abdominal subcutaneous (SC) adipose tissue were obtained in a sample of 60 women (age 35-58 years; BMI 20.3-41.1 kg/m^2). Median adipocyte diameter of the main cell population was determined by collagenase digestion, osmium tetroxide fixation and histological analysis. Adiposity and cardiometabolic risk factors were assessed.

Results: Adipocyte diameter was consistently smaller with formalin fixation than with collagenase digestion, whereas osmium-fixed cells were larger ($p<0.0001$, for all). Median adipocyte diameters derived from all methods were intercorrelated ($r=0.46$ to 0.83, $p<0.001$ for all). Positive associations were found between adipocyte diameter from all techniques and regional or total adiposity measurements ($p<0.01$ for all). OM adipocyte diameter was positively associated with fasting glucose, insulin and HOMA-IR ($r=0.30$ to 0.52, $p<0.05$ for all) with osmium-fixed cell size as a stronger correlate. Osmium-fixed cell diameter was also a better correlate of plasma adiponectin and leptin.

Conclusions: Although measurement techniques generated systematic differences in adipocyte size, associations with adiposity were only slightly affected by the technique. Osmium fixation generated stronger associations with cardiometabolic risk factors than collagenase digestion and histological analysis.

Introduction

Adipocyte size has been studied in humans and rodents for more than 50 years. Collagenase digestion, osmium tetroxide fixation and histological analysis have been used to assess fat cell size, yet no particular technique has emerged as the gold standard (1). Our recent analysis of the literature on fat cell size in humans showed that it generally increases as a function of obesity level, but that the three techniques generate different results in populations that are comparable (1). Histological analysis seems to generate lower mean fat cell size across all BMI values compared to the collagenase digestion and osmium fixation methods (1). However, our analysis also revealed that there is a large inter-individual variability in adipocyte size and no association with adiposity or metabolic abnormalities is observed at high BMI values, consistent with the fact that fat cell size tends to reach maximal values in patients with severe obesity (1).

Collagenase digestion has been developed by Rodbell (2) and is the most widely used technique to assess adipocyte size in humans. Adipose tissue is digested with collagenase to separate mature adipocytes from the stroma-vascular fraction by floatation. This method generates live cells, which makes it useful for functional cell assays. Limitations of this technique include the fact that small cells do not float easily due to their low lipid content, and that large cells are very fragile (3). However, the introduction of adenosine in the solution has possibly minimized this bias (4). Histological analysis can be used to assess adipocyte cell size in a retrospective manner. This method is frequently used when the primary investigation outcomes are adipose tissue morphology and other *in situ* markers. For example, this technique is useful for immunostaining experiments because adipocyte lipid droplets are easily seen with a perilipin antibody (5). Adipose tissue is rather fragile; therefore use of a fixating agent and necessity to cut samples may damage whole tissue architecture. Furthermore, the amount of time needed for analysis may limit the number of patients examined with either collagenase digestion or histological analysis. Post-fixation of collagenase-digested cells either with formaldehyde or

osmium tetroxide to postpone analysis can be performed to increase the number of samples treated (6).

If the primary investigation outcome is to assess fat cell population distributions, osmium tetroxide fixation followed by Multisizer counter analysis is the best option. This technique developed by Hirsch and Gallian (7) fixes intracellular lipids and allows the analysis of a substantially higher number of cells, limiting potential measurement bias. This method generates a bimodal distribution of adipocyte sizes, with subpopulations of very small and large adipocytes (8-10). Cell size distributions can be generated and analyzed by fitting an exponential-Gaussian formula to obtain parameters describing adipocyte subpopulations including very small adipocytes (11-14).

To our knowledge, no study has ever compared these three most commonly used techniques to assess fat cell size for their association with total and regional adiposity as well as cardiometabolic risk factors. Our objective was to investigate if there are differences in fat cell size related to the technique used. We also aimed to determine whether adipocyte diameters from these methods were similarly associated with adiposity measurements and cardiometabolic variables as there are still discrepancies in the literature on these correlations. Based on our previous analysis of the literature (1), we tested the hypothesis that median fat cell size obtained from histological analysis is lower than that of collagenase-digested and osmium tetroxide fixation approaches, yet all measurements are similarly correlated to adiposity and body fat distribution measurements as well as cardiometabolic risk variables.

Methods

Subjects and ethics statement

The study included 60 women (lean to obese) scheduled for gynecological surgery for total or subtotal abdominal hysterectomies at the Gynecology Unit of Laval University Medical Center. Ranges of age and BMI were 35.2 -59.4 years and 20.3-41.1 kg/m^2, respectively. Women were excluded if they had Cushing syndrome, hyperthyroidism, cancer, cardiovascular diseases, type 1 or 2 diabetes and if they reported weight loss or gain in the past year. The study was explained to each participant and a written consent was obtained. The study was approved by the Research Ethics Committees of Laval University Medical Center (C09-08-086).

Adiposity and body fat distribution measurements

On the morning of or a few days before surgery, body weight, height, body mass index (BMI) and waist circumference were measured. Participants also underwent a computed tomography (CT) exam (GE Light Speed 1.1 CT scanner, General Electric Medical Systems, Milwaukee, WI) at the L4L5 levels to assess visceral and abdominal subcutaneous (SC) adipose tissue areas as previously described (15). Total body fat mass and lean body fat mass were assessed by dual-energy X-ray absorptiometry (DXA) (Hologic QDR-4500A densitometer with whole-body fan beam software v8.26a:3-Hologic Inc., Bedford, MA).

Plasma lipid profile, glucose homeostasis and adipokine measurements

Fasting blood samples were collected on the morning of surgery after a 12h-overnight fast. From these samples, cholesterol and triglyceride levels in both plasma and lipoprotein fractions were measured (16). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin levels as previously described (17). Enzyme-linked immunosorbent assay was performed on these samples for the following adipokines: leptin (Human Leptin ELISA kit; EMD Millipore; Billerica, MA, USA), adiponectin (Human Adiponectin ELISA Kit; B-Bridge International Inc; Santa Clara, CA, USA), interleukin-6 (IL-6) (Human IL-6 Quantikine HS ELISA; R&D Systems; Minneapolis,

MN, USA) and tumor necrosis factor-α (TNF-α) (Human TNF-α Quantikine HS ELISA; R&D Systems; Minneapolis, MN, USA).

Adipose tissue sampling and fat cell size measurements

Abdominal subcutaneous (SC) and omental (OM) adipose tissues were collected at the site of the surgical incision and at the distal portion of the greater omentum, respectively. Samples were immediately separated into three subsamples for cell size analysis (~1g for collagenase digestion (CD), ~50-100 mg for formalin fixation and paraffin embedding H&E histological slides (HIS) and ~50 mg for osmium tetroxide fixation (OS)).

Collagenase digestion was performed on the first subsample of fresh tissue, as previously described (16). 0.1 µM adenosine was added in the Krebs-Ringer Henseleit (KRH) buffer to limit cell breakage. Fat cell diameter was measured on mature adipocyte suspension pictures captured using a phase contrast microscope attached to a camera and a computer interface (Scion Image Software, Scion Corporation, Frederick, MA, USA) as recommended in (18). The diameter of 250 adipocytes in each depot was measured. The second subsample of fresh tissue was fixed in a solution of osmium tetroxide, as described previously (19). Briefly, 50 mg of adipose tissue was incubated in osmium tetroxide collidine-HCL solution for at least 96 hours at room temperature. Samples were then rinsed with NaCl 0.9% for 24h and rinsed with 8 M urea for 48h. Isolated, fixed cells were resuspended in PBS 0.01% Triton X-100 and rinsed through a 250 µm nylon mesh. Cells were resuspended in glycerol and diluted into beakers containing Isoton II solution (Beckman Coulter, Villepinte, France) and analyzed using a Beckman Coulter Multisizer IV (Beckman Coulter, Villepinte, France) with a 400 µm aperture. The range of cell sizes analyzed was 25 to 240 µm. Cell size distributions were determined with at least 12 000 cells per sample and were analyzed by fitting an exponential (small cells)-Gaussian (large adipocytes) formula (non-linear least-squares function). Only the mode (center of the Gaussian peak), which is the median diameter of the large cells was used in the present analysis. Osmium tetroxide data were used in a previous publication on this topic (19). Finally, the last subsample of

fresh adipose tissue was fixed in formaldehyde and paraffin-embedded. Sections of 5 µm of OM and SC adipose tissues were mounted on the same slide and were stained with hematoxylin/eosin dyes. Whole slides were digitalized by scanning total area at 20X magnification and 0.24 µm/pixel resolution using a NanoZoomer Hamamatsu scanner (Hamamatsu Photonics KK, Systems Division). The smallest and largest diameters of each cells were manually measured on an average of 100 adipocytes per sample using CaloPix software (Tribvn, Chatillon, France). The mean of these two values was used in analyses.

The intra-observer coefficient of variation was 5.2% (95% confidence interval [CI], 1.6% to 8.8%) and 5.8% (95% confidence interval [CI], 3.2% to 8.4%) for n=8 OM and SC samples, respectively. The inter-observer coefficient of variation was 3.1% (95% CI, 1.5% to 4.8%) and 4.4% (95% CI, 0.1% to 8.9%) for n=8 OM and SC samples, respectively.

Statistical analyses

Student's paired t-tests were performed to examine depot differences for each measurement method. Differences among techniques and BMI categories were determined by mixed model analysis. Pearson correlation coefficients were computed to quantify associations between adipocyte median diameter for each technique and adiposity measurements as well as cardiometabolic risk variables. Non-normally distributed variables were log- or boxcox-transformed. All data are presented as mean ± SEM. Statistical analyses were performed with JMP software or SAS (SAS Institute Inc, Cary, NC, USA).

Results

Characteristics of the women

Table 1 shows the main characteristics of the study population. Women were covering a large range of obesity values according to BMI values spanning from 20.3 to 41.1 kg/m^2. The study sample included pre- and postmenopausal women with an average age of 47 years. Women were mostly overweight with a mean BMI of 27.1±4.4 kg/m^2 and presented evidence of abdominal fat accumulation according to their mean waist circumference value of 92.4 cm. They displayed a normal plasma lipid profile and glucose homeostasis values on average.

Differences in adipocyte size according to the measurement method

Average cell size distribution curves according to the three techniques are shown in **Figure 1**. **Figure 1A**, **C** and **E** show a Gaussian distribution for all techniques in both depots (OM and SC). Collagenase-digested and osmium-fixed cell distributions also presented a significant proportion of small cells, which appeared left of the Gaussian distribution. This feature was absent in the histological analysis, even when the number of cells analyzed was increased at 250 in a subsample of 10 participants (data not shown). As expected in this population composed exclusively of women, SC median adipocyte diameter was significantly higher compared to OM median adipocyte diameter for all techniques ($p<0.0001$) (**Figure 1B**, **D**, and **F**). Despite small differences in adipocyte distributions (**Figure 1**), all techniques were strongly intercorrelated in both depots as shown in **Figure 2**, with correlation coefficients ranging between 0.43 and 0.83 ($p<0.002$ for all).

Adipocyte size and obesity level

To assess the variation in fat cell size as a function of BMI category, a mixed-model analysis was performed. In each BMI category (lean, overweight and obese), osmium-fixed median adipocyte diameter was consistently larger and formalin-fixed median fat cell diameter was smaller than collagenase-digested median adipocyte diameter in both depots ($p<0.0001$, for all) (**Figure 3**). In OM adipose tissue, median adipocyte diameters of lean and overweight women were significantly different from

those of women with obesity ($p<0.01$), whereas the difference between lean and overweight women did not reach statistical significance ($p_{trend}=0.06$) (**Figure 3A**). Both, SC collagenase-digested and osmium-fixed median adipocyte diameters were increased through BMI categories ($p<0.05$, for all) (**Figure 3B**). Differences between lean and overweight women did not reach statistical significance for histological analysis of SC median adipocyte diameter ($p_{trend}=0.06$). Nevertheless, histological analysis of SC median adipocyte diameter was significantly higher in women with obesity compared to lean or overweight women ($p<0.05$). Using a correction factor for the formalin-fixed cell diameters generated median adipocyte diameters similar to those of the collagenase-digested cells for each depot and each BMI category (**Figure 4**) (20, 21). These results suggest that this correction factor could be used when comparing studies in which adipocyte size has been measured by collagenase digestion and histological analysis. Of note, osmium-fixed median adipocyte diameter was still larger than collagenase-digested and formalin-fixed median cell diameter possibly reflecting the space occupied by the osmium following fixation.

Adipocyte size and cardiometabolic risk factors

We then investigated whether the three measurement methods had an impact on the relationship between adipocyte size and adiposity indices and/or metabolic risk variables. As expected, OM median adipocyte diameter derived from all techniques was strongly and positively correlated with BMI, waist circumference, total body fat mass, total body fat percentage, trunk fat mass and adipose tissue areas ($p\leq0.05$) (**Table 2**). SC median adipocyte diameter was also strongly associated with these parameters regardless of the technique (**Table 2**) ($p\leq0.05$). OM median adipocyte size was positively associated with fasting glucose, fasting insulin as well as HOMA-IR, for all techniques ($p\leq0.05$) (**Table 3**). The strongest correlation coefficients were observed with osmium-fixed median cell sizes (**Table 3**). SC median adipocytes diameters from the histological and osmium techniques were also related to fasting insulin and HOMA-IR, although correlation coefficients were of slightly lower magnitude ($p\leq0.05$) (**Table 3**). SC median adipocyte diameters from all techniques were not associated with fasting glucose. OM median fat cell size from the three

techniques was also correlated with parameters of the plasma lipid profile, particularly VLDL-cholesterol and VLDL-triglyceride concentrations ($p \leq 0.05$) (**Table 3**). Significant associations were only found between osmium- or formalin-fixed median adipocyte diameter in OM adipose tissue with levels of HDL-cholesterol, total triglycerides as well as the total cholesterol-to-HDL-cholesterol ratio. In SC adipose tissue, the total cholesterol-to-HDL-cholesterol ratio was associated with median adipocyte diameter from the three methods. Plasma HDL-cholesterol levels were negatively correlated with collagenase-digested and osmium-fixed SC median cell diameters, whereas concentrations of VLDL-cholesterol, VLDL-triglyceride and total triglycerides were only associated with formalin-fixed SC median adipocyte diameter. Plasma leptin and adiponectin concentrations were both associated with OM and SC median fat cell size from all methods **(Table 3**). Although very stringent, Bonferroni correction showed that only associations between osmium-fixed median adipocyte diameter and cardiometabolic risk remained significant (data not shown). Associations between cell size measurements and adiposity indices were generally unaffected by Bonferroni correction (not shown).

Discussion

We investigated whether differences were found in fat cell size with three different techniques. We also examined how adipocyte diameter from these methods was associated with adiposity measurements and cardiometabolic risk variables. As expected, there was a difference in median fat cell size according to the method used. Histological median adipocyte diameter was lower and osmium tetroxide-measured median adipocyte diameter higher than that obtained by collagenase digestion in each BMI category in both the OM and SC depots. Yet, all techniques were strongly intercorrelated in both depots. Furthermore, median fat cell size from all techniques was, in general, similarly associated with adiposity values. However, osmium-fixed median adipocyte size generated stronger associations with cardiometabolic variables, especially glucose homeostasis parameter and plasma adipokine levels.

To our knowledge, this is the first study to clearly compare the three most widely used techniques to assess adipocyte size in two abdominal fat depots in a sample of healthy women well-characterized for body fat distribution and body composition examined by imaging methods (CT and DXA). Previous studies comparing fat cell measurement methods have been published (3, 8, 22, 23). However, these studies often compared a widely used method (for the time) with a novel method developed by the authors. For example, older literature often compared novel methods with ocular cell sizing of collagenase-digested cells, which has been replaced by picture analysis of isolated cells. It is noted across the literature that semi-automatic methods are not yet widely used among research teams, as the quality of the image is often a limitation. Furthermore, these methods still need significant input of the observer (final choice of cells counted, correction for incomplete membranes, decisions about the cells on the edge of the image, etc.). Considering these steps, semi-automated techniques may not be as fast as expected, or faster than manual analysis, as initially proposed. These notions support the rational of our study i.e to analyze variation in adipocyte size related to the measurement technique using simple, manual techniques.

Each technique presented a Gaussian curve and all three techniques and measurements were strongly intercorrelated in each fat compartment. However, as shown with the exponential curve, a population of small cells was observed in osmium-fixed cells and to a lesser extent in collagenase-digested cells. This subpopulation has been proposed to represent cells undergoing differentiation (11). Some studies also reported that small cells were more abundant in adipose tissue from subjects with obesity, which led to other hypotheses about their origin (19, 24, 25). These cells have been proposed to originate from multilocular cells that are ruptured during the fixation process or during the collagenase treatment, although there is no clear evidence of this phenomenon occurring (8, 26). McLaughlin et al. (11, 27) proposed that these cells may also represent immature adipocytes that are unable to store the excess dietary lipids. Other factors may explain the absence of small cells in histological analyses such the lower number of cells examined compared to other techniques and the difficulty to visualize small cells. Furthermore, the absence of blood vessels in the fields examined may induce this bias, since perivascular regions are thought to be the niche of adipose precursor cells (28, 29). Nevertheless, their presence among collagenase-digested cells, found both in our sample and in another animal study (24), provide convincing evidence that these small cells do represent lipid-containing adipocytes and not artefacts of the osmium tetroxide fixation method. A threshold value of 25 μm was applied in the osmium tetroxide method to discriminate cells from artefacts (19). The biological relevance of these small cells remains an open question.

As expected, median fat cell size in both depots varies as a function of obesity level regardless of the method. Central obesity was more strongly associated with fat cell size in OM and SC adipose tissue than overall adiposity. The chosen sampling sites may partly explain these results. SC adipocyte diameter was larger than OM adipocyte diameter with every method, reflecting the depot-specific difference in fat cell size reported in our previous studies in women (19, 30) and our detailed analysis of the literature (1). The association between visceral adiposity and cardiometabolic risk factors is well known (31). Here we showed for the first time in the same sample of women that adipocyte hypertrophy measured by three different techniques is

related to altered blood lipids and glucose homeostasis. OM and SC adipocyte diameter assessed by osmium fixation methods were more closely correlated to some of the metabolic variables than fat cell size assessed by collagenase digestion and histological analysis. These small differences between techniques may explain discrepancies observed among available studies for the association between adipocyte diameter and cardiometabolic variables (1). Furthermore, correlations between glucose homeostasis and fat cell size were stronger in OM compared to SC adipose tissue regardless of the technique used, as previously reviewed (1). Adipocyte hypertrophy in both depots may represent a critical determinant of cellular function and cardiometabolic risk (1).

Limitations of the study should be acknowledged. The first one is the sample population. Men were not studied and differences between male and female adipocyte characteristics in both depots have already been discussed in the literature (reviewed in (1)). Since sampling of both abdominal depots requires general anesthesia, recruitment of healthy men covering a large range of BMI values and age undergoing elective surgery without major metabolic alterations or chronic diseases such as cancer is a challenge in the scientific community. Women in this study were covering a large range of obesity level and the number of women with obesity or with severe obesity was low (n=13, 21.7% and n=1, 1.7%, respectively). However, participants were generally healthy apart from their gynecological condition. Another limitation of the study was the exclusion of women for whom adipose tissue architecture was too damaged to perform analysis after fixation and paraffin-embedding. A total of 15 (13%) slides were excluded which represents a significant amount and should be taken in consideration when small patient samples are investigated. In comparison, for collagenase digestion and osmium fixation methods, only two samples (1.7%) were excluded due to technical difficulties.

Conclusion

In conclusion, although histological analysis led to smaller adipocyte diameters and osmium tetroxide fixation led to higher adipocyte diameter than collagenase digestion in all BMI categories, associations between median adipocyte diameter and adiposity measurements was only slightly affected by the method used whereas osmium tetroxide fixation led to stronger associations with cardiometabolic risk factors. Each technique obviously has its advantages and disadvantages, which must be understood and acknowledged by investigators when designing their study (**Table 4**).

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Tables

Table 1: Characteristics of the 60 women of the study.

Variables	Mean	±	SD	Range (min-max)
Anthropometrics				
Age (yrs)	46.8	±	5.9	35.2-59.4
Weight (kg)	69.9	±	11.2	54.7-107.8
BMI (kg/m²)	27.1	±	4.4	20.3-41.1
Waist circumference (cm)	92.4	±	11.4	68.0-123.5
Body composition				
Total body fat percentage (%)	34.9	±	5.0	16.7-45.1
Total body fat mass (kg)	25.2	±	7.0	9.6-47.3
Lean body mass (kg)	36.0	±	5.0	36.0-58.9
Trunk fat mass (kg)	11.6	±	4.0	3.3-24.1
Trunk lean mass (kg)	24.4	±	2.7	18.5-31.1
Limb fat mass (kg)	13.0	±	3.6	5.4-24.0
Limb lean mass (kg)	17.4	±	2.4	12.5-24.6
Trunk fat mass/limb fat mass	0.9	±	0.2	0.5-1.4
Adipose tissue area (cm^2) [a]				
Total	407	±	140	92-725
Subcutaneous	311	±	103	71-568
Visceral	97	±	45	21-278
Plasma lipid profile				
Cholesterol (mmol/L)				
Total	4.91	±	0.82	3.21-6.99
VLDL	0.39	±	0.27	0.05-1.23
LDL	3.07	±	0.76	1.65-4.94
HDL	1.46	±	0.39	0.83-2.57
Triglycerides (mmol/L)				
Total	1.13	±	0.55	0.40-3.32
VLDL	0.65	±	0.48	0.12-2.75
LDL	0.22	±	0.07	0.11-0.40
HDL	0.26	±	0.07	0.14-0.49
Cholesterol/HDL-cholesterol	3.49	±	0.85	2.01-5.52
Glucose homeostasis				
Fasting glucose (mmol/L)	5.3	±	0.4	4.5-6.6
Fasting insulin (mmol/L) [b]	7.3	±	4.0	1.5-21.4
HOMA index [b]	1.7	±	1.0	0.3-5.0

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HOMA, homeostasis model assessment index; [a]n=59, [b]n=58,

Table 2: Pearson correlation coefficients between OM orSC adipocyte diameters and anthropometric variables.

	Omental adipose tissue			Subcutaneous adipose tissue		
Variables	**CD**	**HIS**	**OS**	**CD**	**HIS**	**OS**
Anthropometrics						
Weight	0.48 **	0.66 ***	0.57 ***	0.58 ***	0.47 **	0.59 ***
BMI	0.51 **	0.66 ***	0.57 ***	0.59 ***	0.47 **	0.60 ***
WC	0.61 ***	0.71 ***	0.66 ***	0.52 **	0.54 ***	0.63 ***
Body composition						
Total body fat percentage	0.34 #	0.66 ***	0.64 ***	0.62 ***	0.51 **	0.63 ***
Total body fat mass	0.45 *	0.72 ***	0.66 ***	0.65 ***	0.53 ***	0.66 ***
Lean body mass	0.45 *	0.49 **	0.37 *	0.37 *	0.32 #	0.38 *
Trunk fat	0.46 ***	0.69 ***	0.61 ***	0.60 ***	0.57 ***	0.62 ***
Limb fat	0.25	0.53 ***	0.46 **	0.52 **	0.36 *	0.48 **
Trunk fat mass/limb fat mass	0.36 #	0.36 *	0.33 #	0.27 #	0.43 *	0.34 #
Adipose tissue area						
Total	0.58 ***	0.82 ***	0.74 ***	0.61 ***	0.59 ***	0.70 ***
Visceral	0.62 ***	0.80 ***	0.78 ***	0.55 ***	0.49 **	0.68 ***
Subcutaneous	0.52 **	0.76 ***	0.68 ***	0.61 ***	0.59 ***	0.66 ***

#p<0.05, *p<0.01, **p<0.001, ***p<0.0001

BMI, body mass index; WC, waist circumference; CD, collagenase digestion; HIS, histological analysis; OS, osmium tetroxide fixation

Log10 or boxcox-transformed variables: weight, BMI, total body fat mass, fat-free mass, body composition (total body fat, total body fat mass, lean body mass, trunk fat, limb fat, trunk fat mass/limb), adipose tissue area (total, visceral and subcutaneous).

Table 3: Pearson correlation coefficients between OM or SC adipocyte diameters and metabolic variables.

	Omental adipose tissue			Subcutaneous adipose tissue		
Variables	**CD**	**HIS**	**OS**	**CD**	**HIS**	**OS**
Glucose homeostasis						
Fasting glucose	0.31#	0.30#	0.30#	0.25	0.12	0.21
Fasting insulin	0.40*	0.37#	0.50**	0.15	0.31#	0.31#
HOMA-IR	0.42*	0.39*	0.52**	0.18	0.30#	0.32#
Plasma lipid profile						
Total cholesterol	-0.08	0.04	-0.02	-0.16	0.05	-0.06
VLDL-C	0.33#	0.35*	0.34#	0.19	0.46**	0.21
LDL-C	-0.06	0.18	0.05	-0.06	-0.01	0.10
HDL-C	-0.21	-0.47**	-0.34#	-0.38*	-0.22	-0.42*
Total TG	0.25	0.30#	0.30#	0.24	0.45*	0.20
VLDL-TG	0.28#	0.31#	0.32#	0.25	0.45**	0.22
LDL-TG	0.17	0.36#	0.23	0.11	0.24	0.09
HDL-TG	0.15	0.04	0.07	0.00	0.17	-0.09
Total cholesterol to HDL-C ratio	0.16	0.51**	0.34#	0.30#	0.30#	0.39*
Adipokines/cytokines						
Leptin	0.37*	0.55***	0.60***	0.53***	0.53***	0.53***
Adiponectin	-0.40*	-0.48**	-0.57***	-0.28#	-0.17	-0.39*
TNF-α	-0.07	-0.08	-0.18	-0.04	-0.04	-0.04
IL-6	0.11	0.17	0.29#	0.20	0.08	0.32#

#p<0.05, *p<0.01, **p<0.001, ***p<0.0001

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HOMA, homeostasis model assessment index; CD, collagenase digestion; HIS, histological analysis; OS, osmium tetroxide fixation; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha

Log10 or boxcox-transformed variables: fasting insulin, HOMA-IR, VLDL-C, HDL-C, Total TG, VLDL-TG, LDL-TG, HDL-TG, leptin, adiponectin, TNF-α, IL-6.

Table 4: Summary of the characteristic of each method.

	Collagenase digestion (CD)	Histological analysis (HIS)	Osmium tetroxide fixation (OS)
Cost and handling of chemicals	Low cost	Low cost	High cost Hazardous chemical which requires recycling network
Availability of necessary equipment	High	High	Low
Experiment time	Short	Long	Long
Analysis time	Long	Long	Short
Sample conservation (long- term)	Not possible[a]	Possible	Possible
Adipose tissue architecture analysis	Not possible	Possible	Not possible
Number of cells counted	Up to ~250-300	~50- 250	Between 6,000 and 20,000
Small cell fraction	Detected in some samples	Not detected	Detected
Standardization (between laboratories)	Difficult	Possible	Easy to achieve
Deferred measurement	Not possible[a]	Possible	Possible
Possible biases	Limited floatation of small cells due to low lipid content Cell breaking	Assumption that cells show their largest diameter Cell shrinkage Defining cells, especially small cells	Multilocular cells could be ruptured during fixation process Possibility of residual fragments Possibility of staining dead cells
Advantages	First step to functional experiments Isolation of stromal vascular cell fraction	Possibilities for further analyses such as immunohistochemistry immunofluorescence, etc.	Allows analysis of cell subpopulations

CD, collagenase digestion; HIS, histological analysis; OS, osmium tetroxide fixation.
a: Collagenase-digested cells can be fixed in formaldehyde or osmium tetroxide to postpone analysis and preserve the cells (6).
b: addition of adenosine may limit the cell breakage (4).

Figure Headings

Figure 1: Techniques used to assess adipocyte diameter all showed a Gaussian curve. Gaussian adipocyte distribution from formalin-fixed paraffin-embedded H&E colored slides (HIS) (**A**). Bimodal distribution of mature adipocytes isolated after collagenase digestion (CD) (**C**) or osmium tetroxide fixation (OS) and Multisizer analysis (**E**). Depot-specific differences were observed in the three techniques (B, D, F). Subcutaneous (SC) median adipocyte diameter was larger than omental (OM) median adipocyte diameter, n=46 (**B**), n=54 (**D**) n=52 (**F**), Student paired t-test, ***p<0.0001.

Figure 2: Correlations between collagenase digestion median adipocyte diameter, histological analysis median adipocyte diameter or osmium tetroxide fixation median adipocyte diameter in omental (OM) (**A, B, C**) or subcutaneous (SC) (**D, E, F**). Collagenase digestion and histology analysis in OM (r=0.60, p<0.0001) (**A**) and SC adipose tissue (r=0.43, p<0.0018) (**D**). Osmium fixation and histology analysis in OM (r=0.83, p<0.0001) (**B**) and SC adipose tissue (r=0.49, p<0.0003) (**E**). Osmium fixation and collagenase digestion in OM (r=0.71, p<0.0001) (**C**) and SC adipose tissue (r=0.80, p<0.0001) (**F**).

Figure 3: Omental (OM) (**A**) and subcutaneous (SC) (**B**) median adipocyte diameter in each BMI category (Lean<25 kg/m^2, Overweight ≤25-<30 kg/m^2, Obese ≥30 kg/m^2); linear mixed-model, ***p<0.0001. Statistically different from the lean subgroup (a) and/or the overweight subgroup (b) from the same technique. P <0.05 were considered significant.

Figure 4: Omental (OM) (**A**) and subcutaneous (SC) (**B**) median adipocyte diameter in each BMI category (Lean<25 kg/m^2, Overweight ≤25-<30 kg/m^2, Obese ≥30 kg/m^2); linear mixed-model, ***p<0.0001. HIS median diameters were corrected accordingly to Weibel et al. (2, 3). Statistically different from the lean subgroup (a) and/or the overweight subgroup (b) from the same technique. P<0.05 were considered significant.

Figure 1.

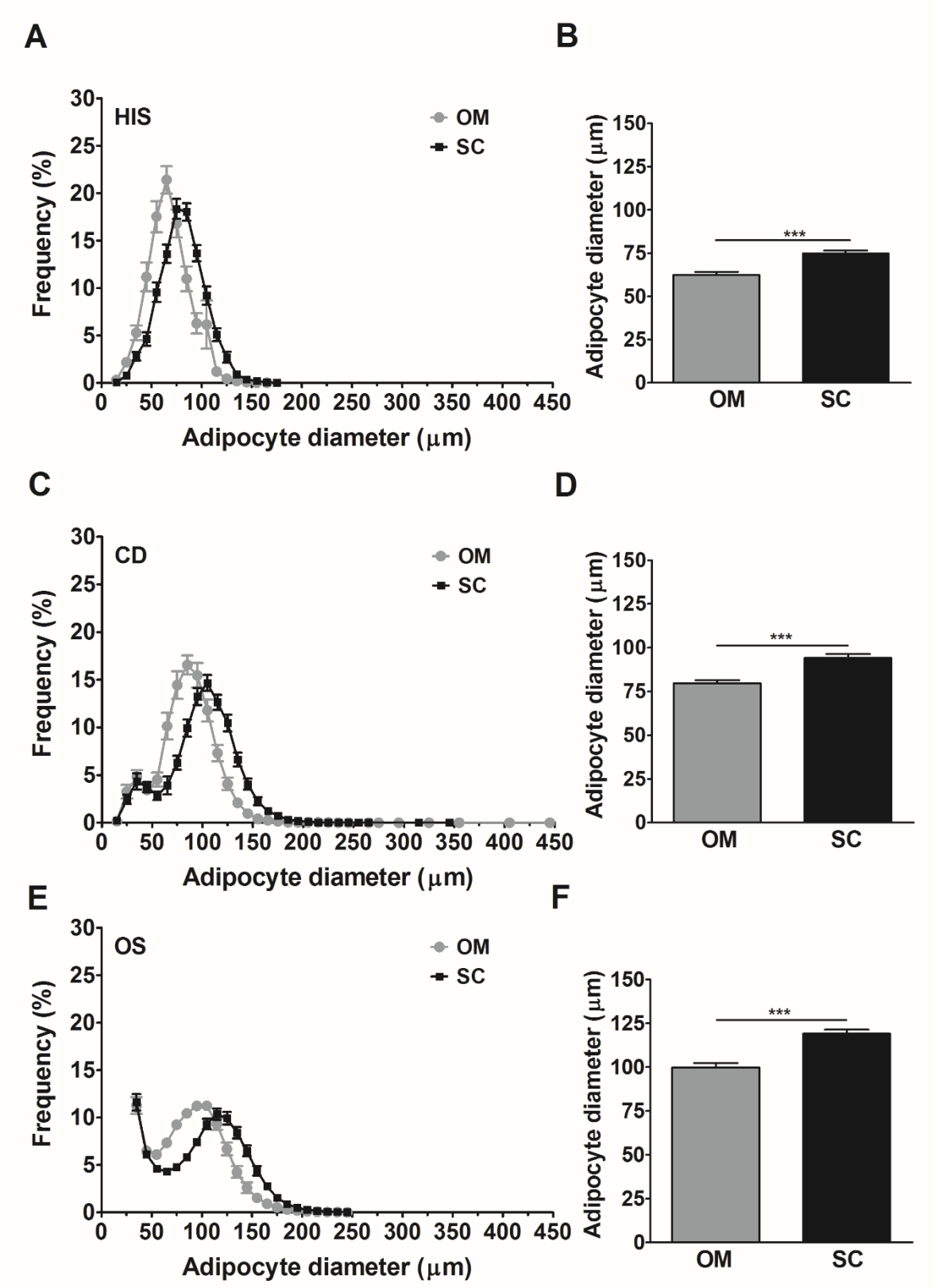

A
HIS
OM
SC
Frequency (%)
Adipocyte diameter (μm)
B
Adipocyte diameter (μm)
***
OM
SC
C
CD
OM
SC
Frequency (%)
Adipocyte diameter (μm)
D
Adipocyte diameter (μm)
***
OM
SC
E
OS
OM
SC
Frequency (%)
Adipocyte diameter (μm)
F
Adipocyte diameter (μm)
***
OM
SC

Figure 2.

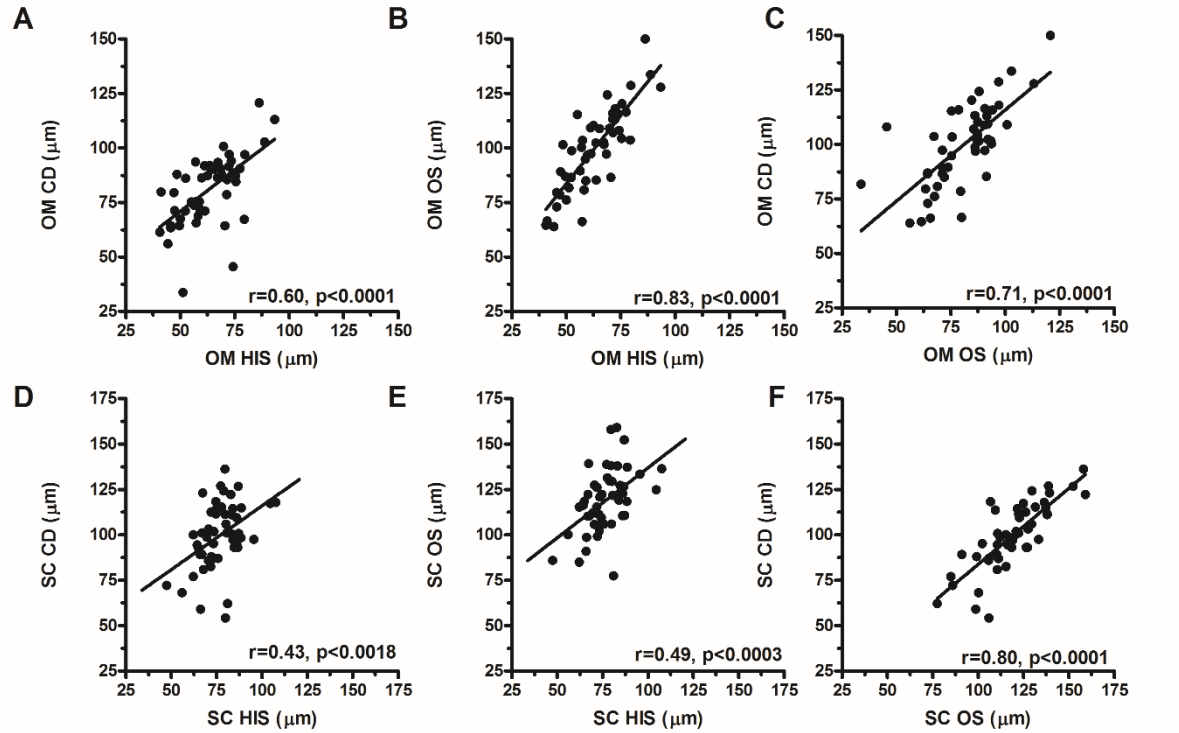

A
OM CD (μm)
OM HIS (μm)
r=0.60, p<0.0001
B
OM OS (μm)
OM HIS (μm)
r=0.83, p<0.0001
C
OM CD (μm)
OM OS (μm)
r=0.71, p<0.0001
D
SC CD (μm)
SC HIS (μm)
r=0.43, p<0.0018
E
SC OS (μm)
SC HIS (μm)
r=0.49, p<0.0003
F
SC CD (μm)
SC OS (μm)
r=0.80, p<0.0001

Figure 3.

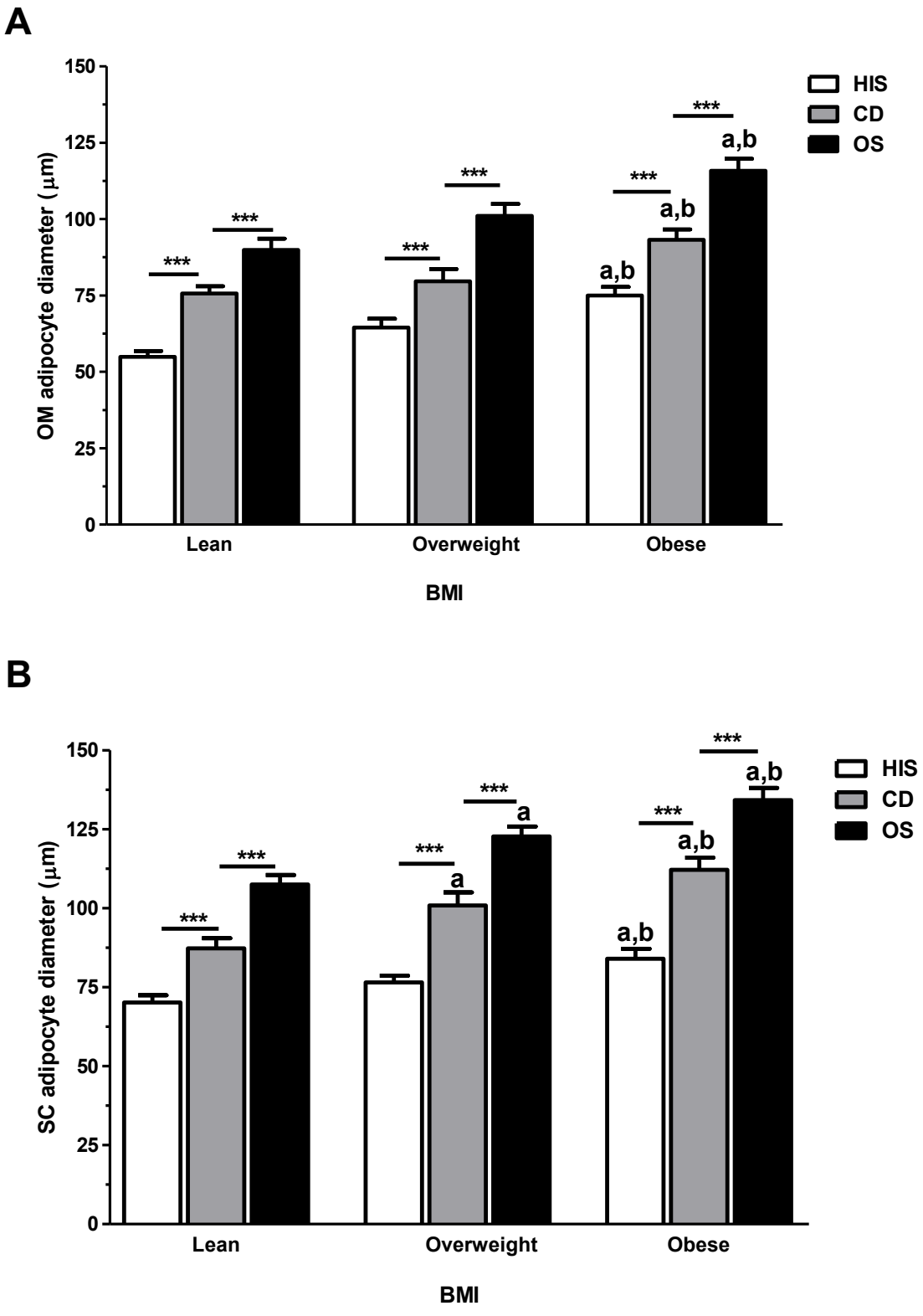
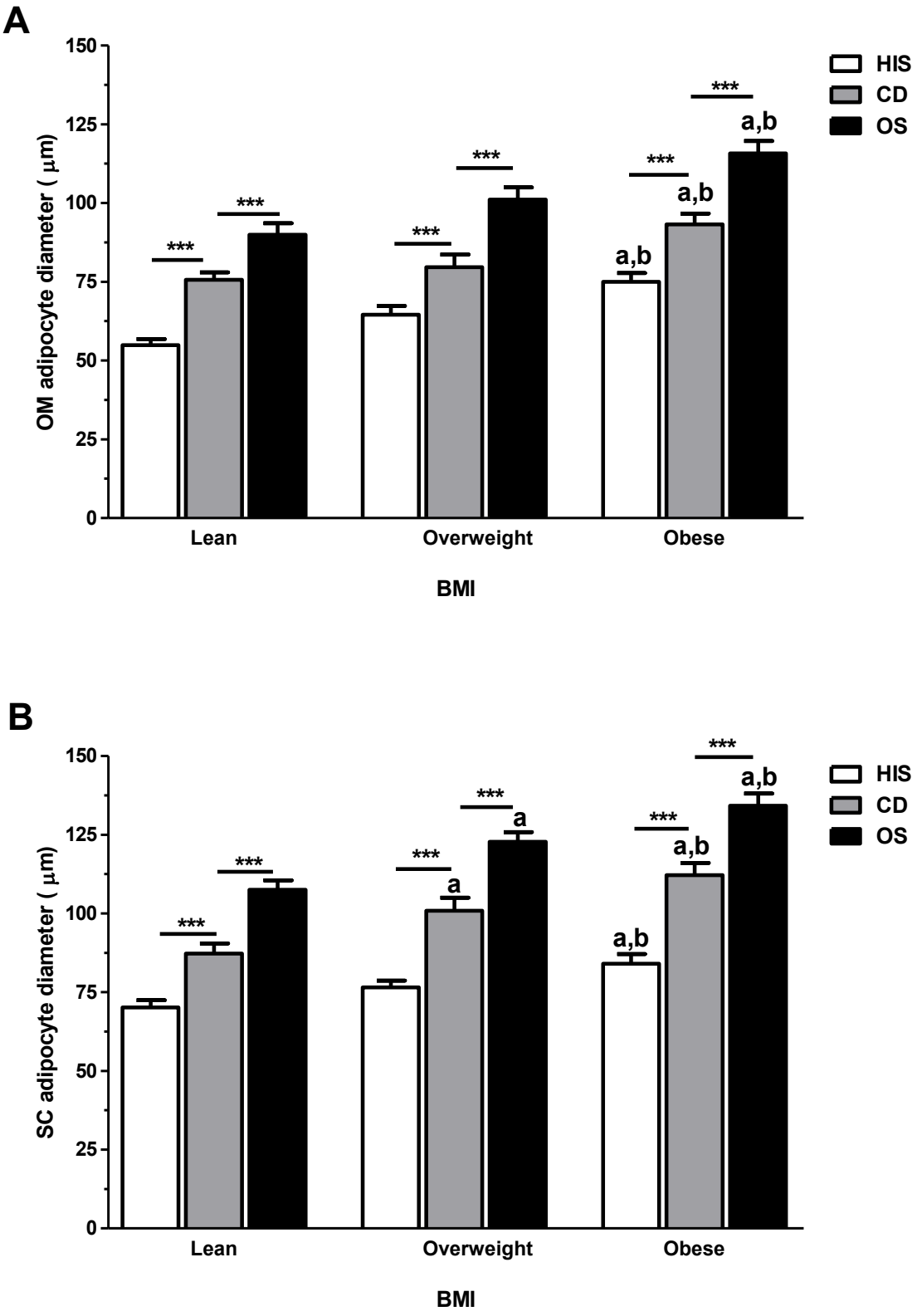

A
OM adipocyte diameter (μm)
150
125
100
75
50
25
0
HIS
CD
OS
***
***
***
***
***
***
a,b
a,b
a,b
Lean
Overweight
Obese
BMI
B
SC adipocyte diameter (μm)
150
125
100
75
50
25
0
HIS
CD
OS
***
***
***
***
***
***
a
a
a,b
a,b
a,b
Lean
Overweight
Obese
BMI

Figure 4.


A
OM adipocyte diameter (μm)
150
125
100
75
50
25
0
***
***
***
***
***
***
a,b
a,b
a,b
Lean
Overweight
Obese
BMI
HIS
CD
OS
B
SC adipocyte diameter (μm)
150
125
100
75
50
25
0
***
***
***
***
***
***
a
a
a,b
a,b
a,b
Lean
Overweight
Obese
BMI
HIS
CD
OS

Conclusion

Malgré une stabilisation de l'incidence d'obésité ces dernières années au Canada, celle-ci demeure un problème de santé majeur entraînant des coûts astronomiques au pays. Plusieurs équipes de recherche tentent depuis bon nombre d'années de déterminer et de contrôler les facteurs impliqués dans l'hétérogénéité de cette maladie. Pour notre part, nous nous sommes intéressés aux différents profils de distribution des graisses qui sont liés de façon étroite aux comorbidités associés à l'obésité.

Tout d'abord, nous avons évalué le potentiel de l'alimentation en général à moduler la distribution des graisses et particulièrement, l'accumulation de gras au niveau viscéral. Notre analyse critique de la littérature a révélé que l'effet des nutriments sur la composition corporelle était expliqué en grande partie par son effet sur la masse grasse totale.

Nous avons aussi examiné la possibilité que la taille adipocytaire représente un marqueur important des altérations métaboliques associées à l'obésité. Notre revue critique de la littérature ainsi que nos résultats originaux suggèrent qu'en effet, la taille adipocytaire moyenne ou médiane est un fort corrélat des altérations du profil lipidique plasmatique et de l'homéostasie de l'axe glucose-insuline, et ce, indépendamment d'autres mesures d'adiposité. De plus, la majorité des associations observées étaient plus fortes pour la taille adipocytaire viscérale, démontrant encore une fois l'importance de l'obésité viscérale en tant que marqueur de la physiopathologie de l'obésité. De fait, l'hypertrophie des adipocytes dans les deux dépôts principaux, ainsi que l'excès de masse grasse viscérale sont des marqueurs potentiels d'une capacité limitée d'expansion du tissu sous-cutané, ce qui entraîne l'apparition des altérations métaboliques déjà discutées.

Tel que rapporté dans notre revue de littérature, les associations répertoriées ne font pas toutes consensus dans la littérature. Nous nous sommes questionnés sur l'impact de la technique de mesure de la taille adipocytaire sur la force de ses associations. Nos résultats originaux montrent que bien que les valeurs médianes

de la taille adipocytaire varient selon la technique utilisée, ces variations sont semblables entre les catégories d'IMC. De plus, les tailles médianes des trois techniques étaient fortement corrélées entre elles. De surcroît, les associations avec les variables d'adiposité régionales et totales étaient peu affectées par la technique utilisée. Les associations avec les facteurs de risque cardiométabolique, quant à elles étaient somme toute peu affectées par la technique, quoique les associations étaient légèrement plus fortes avec la taille adipocytaire provenant de la fixation à l'acide osmique, reflétant probablement sa précision due au très grand nombre de cellules mesurées.

Forces et limites

Les forces de notre étude sont tout d'abord la caractérisation de deux dépôts adipeux (sous-cutané et omental) chez l'humain. La quantité de tissu était suffisante pour effectuer un très grand nombre de mesures ainsi que la mesure de la taille des adipocytes par les trois méthodes couramment utilisées dans la littérature. En outre, les participantes à l'étude ont été étudiées en détail, que ce soit par des questionnaires de fréquence alimentaire, des journaux alimentaires, par leur bilan sanguin total (profil lipidique, glycémie et HOMA-IR). Leur niveau d'adiposité, c'est-à-dire leur composition corporelle ainsi que la distribution des graisses ont été mesurées par tomographie axiale et par absorptiométrie aux rayons X, ainsi que les mesures anthropométriques utilisées en clinique, soit l'IMC, le tour de taille et le ratio taille-hanche.

Cependant, puisque notre étude est de type transversal, nous ne pouvons pas conclure à un lien de cause à effet. D'autre part, notre échantillon n'incluait que des femmes, nous ne pouvons donc pas extrapoler ces résultats pour les hommes, qui présentent comme on le sait des différences par rapport aux femmes en ce qui a trait à la distribution des graisses. En effet, étant donné que le dépôt omental n'est accessible que pendant une chirurgie sous anesthésie générale, il demeure un défi de recruter des hommes en bonne santé générale nécessitant une intervention chirurgicale. Une autre limite de notre étude concerne le choix des participantes à l'étude qui ne représentent pas la population générale. En effet, il s'agissait de

femmes ayant une condition gynécologique nécessitant une intervention. Nous avons collecté l'historique médical, la prise de médicaments, dont les dérivés hormonaux, ainsi que le statut de ménopause. Il est important de spécifier que ces dernières données ont été prises en compte et que cela n'a pas influencé nos résultats. Par contre, plusieurs patientes ont été exclues car leur tissu adipeux (sous-cutané ou omental) post-fixation à la formaline était trop endommagé pour effectuer une analyse histomorphométrique, tel que discuté dans le **Chapitre 3**. Finalement, nos associations avec la taille adipocytaire sont peut-être légèrement surestimées, car notre échantillon de femmes contenait des personnes avec des valeurs d'adiposité variant de minces à modérément obèses. Tel qu'illustré au **Chapitre 2**, la relation entre la taille des adipocytes et l'adiposité atteint un plateau à des valeurs d'IMC plus élevées. Il est aussi connu que les associations avec la graisse viscérale sont diminuées chez les obèses morbides, chez qui la pathologie est rendue à un autre stade. Le fait d'étudier une population qui couvre la partie linéaire de la relation entre le diamètre adipocytaire et l'adiposité maximise notre capacité à détecter des associations.

Implications cliniques

Notre chapitre de livre soulève des questions importantes pour la pratique clinique des nutritionnistes. En effet, à l'exception de quelques nutriments, où les résultats restent à valider dans des études randomisées contrôlées et dans des études prospectives longitudinales, les données probantes disponibles nous indiquent que l'alimentation ne joue pas le rôle de premier plan dans l'accumulation de gras viscéral, contrairement à ce que bon nombre de scientifiques et de cliniciens auraient pu anticiper. En effet, le bilan énergétique positif est lié à l'accumulation de gras, particulièrement au niveau sous-cutané, le principal site d'entreposage des lipides chez l'humain. Dans ce contexte, il est légitime de se questionner sur l'impact de nutriments isolés dans la résolution des comorbidités associées à l'obésité ou encore sur leur importance d'un point de vue préventif. Deux distinctions doivent toutefois être faites. Une réduction de l'apport alimentaire amène des bienfaits via une perte de poids qui, en pourcentage, est plus importante dans les dépôts

viscéraux et ectopiques que dans les dépôts sous-cutanés (260-263). D'ailleurs, même une légère perte de gras viscéral pourrait amener des bénéfices au niveau cardiovasculaire, et ce, en absence de perte de poids, dû à une augmentation de la masse musculaire dans la plupart des cas (40, 264, 265). Donc, la réduction calorique et la perte de poids qui en résulte a bien sûr des bénéfices sur la santé. Ensuite, les bénéfices de la perte de poids ne se limitent pas à la réduction des facteurs de risque cardiovasculaires et métaboliques. En effet, ceux-ci comprennent une amélioration de la santé osseuse et articulaire ainsi que de la santé mentale. Pour conclure sur le potentiel de l'alimentation sur la santé cardiométabolique, bon nombre d'études sont en cours pour déterminer le potentiel de certains aliments à améliorer la glycémie ou encore le profil lipidique, sans effet sur le poids, ou sur la masse grasse. Le rôle de l'alimentation et ses mécanismes associés pourraient donc passer pas des sentiers métaboliques ne touchant peu ou pas le métabolisme du tissu adipeux.

La mesure de la taille adipocytaire fournit bon nombre d'informations, autant sur la dysfonction du tissu adipeux que sur les facteurs de risque cardiométabolique, particulièrement celle provenant des adipocytes viscéraux. Cependant, sa mesure en clinique pose plusieurs problèmes en raison de son caractère invasif. Il est donc peu réaliste d'instaurer la mesure de la taille des adipocytes pour cibler les individus, obèses ou non, à plus haut risque cardiométabolique. Caroline Fox a récemment proposé que la «qualité» de la masse grasse, mesurée par l'atténuation radiologique, prédit de façon aussi importante le risque de maladies cardiovasculaires et métaboliques que l'aire de tissu adipeux mesurée par tomographie axiale (266). Dans cette étude, il a été démontré que la «qualité» et la «quantité» de masse grasse étaient des prédicteurs indépendant du risque de maladies cardiovasculaires (266). Notre groupe a obtenu des résultats similaires et a également analysé l'association entre le poids des adipocytes, un dérivé de la taille des adipocytes, et l'aire de tissu adipeux ainsi que son atténuation radiologique (267). De manière frappante, les deux paramètres (la surface adipeuse ou la quantité et l'atténuation ou la qualité) étaient des très forts corrélats du poids moyen des adipocytes, particulièrement dans le dépôt viscéral. La taille adipocytaire,

particulièrement au niveau viscéral, se positionne donc comme un intégrateur de toutes les mesures reliées à la physiopathologie de l'obésité tant en quantité qu'en qualité. Une diminution de la capacité d'entreposage des lipides dans son site de prédilection- le tissu sous-cutané- est associé à une augmentation de la taille adipocytaire sous-cutanée et particulièrement viscérale, qui à son tour est fortement associée à une déposition accrue des lipides dans les sites ectopiques et donc parallèlement aux complications métaboliques de l'obésité, la stéatose hépatique et la résistance à l'insuline dans le muscle, et ce, indépendamment de l'adiposité totale (40). L'importance de la taille adipocytaire est donc primordiale en recherche afin de mieux comprendre la physiopathologie de l'obésité. Une meilleure compréhension des mécanismes impliqués permettra le développement de nouvelles solutions thérapeutiques.

Perspectives futures de recherche

Dans une perspective future, il serait intéressant d'étudier l'effet des aliments de manière prospective sur une grande cohorte avec des mesures d'adiposité régionales précises telles que la tomographie axiale et l'imagerie par résonance magnétique en contrôlant pour les principaux facteurs confondants tels que l'âge, le sexe, l'activité physique et l'apport calorique. En effet, jusqu'à maintenant, seulement quatre études prospectives ont été publiées avec ce type de données, avec des populations très disparates (268-271). Leurs résultats ont une forte plausibilité biologique. En effet, un apport en fibre augmenté diminuerait l'aire de tissu adipeux viscéral (deux études sur quatre) (269, 271). Les fibres augmenteraient la satiété et diminueraient donc l'apport calorique. Chez les femmes, l'apport en calcium est négativement associé au changement sur un an de l'aire de tissu adipeux viscéral (268), ce qui pourrait être expliqué en partie par la capacité du calcium à augmenter la lipolyse (272-275). Il n'y a pas de consensus clair sur les effets délétères des acides gras saturés dans la littérature. La provenance et la longueur de la chaîne de carbone semblent en partie expliquer les différences qui apparaissent dans la littérature. Néanmoins, l'apport total en acides gras saturés est négativement associé avec le changement sur cinq ans de l'aire de

tissu adipeux viscéral, indépendamment de l'IMC (270). Tous ces résultats démontrent que d'autres études épidémiologiques doivent être réalisées ainsi que des études *in vitro* pour valider davantage les associations identifiées.

Dans le cadre de ce mémoire, nous avons principalement étudié la taille médiane de la population gaussienne adipocytaire i.e. des gros adipocytes. En effet, cette mesure est similaire à la moyenne pour ce qui est de la technique de la digestion à la collagénase et de l'analyse histomorphométrique. Il a déjà été démontré que la moyenne générée à la suite de la fixation à l'acide osmique et de l'analyse par le Compteur Coulter Multisizer n'offrait pas d'information d'ordre biologique i.e. aucune corrélation n'a été rapportée dans la littérature. Cette mesure est donc imparfaite et ne représente pas toutes les variations de taille et donc de fonctions pour l'ensemble des cellules d'un individu. L'analyse de la distribution complète et non d'une mesure substitutive serait particulièrement intéressante. Une étude réalisée par McLaughlin et collaborateurs a déjà étudié l'effet d'une diète hypercalorique sur différents paramètres de la distribution des adipocytes chez des individus diabétiques et non-diabétiques, déjà en surpoids ou obèses (203). Une analyse réalisée lors d'une perte de poids, par exemple après une chirurgie bariatrique ou à la suite d'une diète hypocalorique ou d'activité physique accrue, pourrait donner des informations complémentaires. De plus, la population de très petites cellules vues en majorité à la suite de la fixation à l'acide osmique et dans une moindre mesure à la suite de la digestion à la collagénase se doit d'être caractérisée plus en détail, étant donné les associations qu'elle génère avec les principaux facteurs de risque cardiométabolique et l'adiposité (231, 232). Limiter le temps de digestion à la collagénase pourrait augmenter le taux de survie des cellules et possiblement de ces petites cellules, ce qui pourrait rendre leur étude *in vitro* possible (212).

De plus, notre analyse porte sur les deux dépôts principaux chez l'humain (omental et sous-cutané) et ne peut donc pas être extrapolée pour l'ensemble des tissus adipeux humains. De la même manière, nous n'avons pas pu étudier plus en détail le dimorphisme sexuel de la taille des adipocytes, notre échantillon ne comportant que des femmes. Il serait donc intéressant dans le futur de s'intéresser à d'autres

dépôts adipeux (mésentérique, fémoral et mammaire) afin de mieux caractériser ces tissus chez l'humain. En effet, la morphologie des adipocytes en condition pathologique, par exemple dans un cas de cancer, peut être grandement modifiée et affecter la croissance et la progression tumorale (276, 277). Il est déjà connu que les adipocytes près des tumeurs subissent une délipidation majeure, entre autres pour nourrir la cellule et ses besoins accrus en composantes des membranes cellulaires (278). Le tissu adipeux local a donc un effet favorable pour la tumeur. Le tissu adipeux, comme organe endocrinien peut aussi contribuer à la transformation et la progression tumorale. Nous savons déjà que l'adipocyte hypertrophié présente un profil de sécrétion altéré des adipokines (182, 279, 280). Les facteurs sécrétés par les adipocytes et les cellules immunitaires recrutées dans l'obésité créent un microenvironnement propice à une tumeur, particulièrement au niveau mammaire, chez la femme (277, 281-286). Les métastases de certains cancers sont souvent situées près de tissu adipeux, par exemple celles du cancer des ovaires (287, 288). Comme le tissu adipeux viscéral présente généralement un profil inflammatoire plus important que les autres dépôts adipeux, l'effet de l'inflammation du tissu adipeux pourrait passer par un effet systémique des cytokines pro-inflammatoires provenant du tissu adipeux viscéral, faisant le lien entre l'obésité viscérale et le risque de plusieurs cancers.

En résumé, nous avons démontré l'importance de la graisse viscérale et de la taille adipocytaire comme prédicteur de la dysfonction du tissu adipeux et des complications métaboliques de l'obésité. D'autres études restent à faire pour mieux comprendre la dysfonction du tissu adipeux et son rôle dans la physiopathologie de l'obésité

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