PASCAL IMBEAULT

RÉGULATION DU MÉTABOLISME DU TISSU ADIPEUX SOUS-CUTANÉ CHEZ L'HUMAIN SOUS DIVERS ÉTATS PHYSIOLOGIQUES

Thèse
présentée
à la Faculté des études supérieures
de l'Université Laval
pour l'obtention
du grade de Philosophiae Doctor (Ph.D.)

Département de médecine sociale et préventive
Division de kinésiologie
FACULTÉ DE MÉDECINE
UNIVERSITÉ LAVAL

AVRIL 2000

RÉSUMÉ COURT

Le tissu adipeux ne présente pas les mêmes facultés lipomobilisatrices et lipogéniques selon la localisation anatomique du dépôt et le sexe des individus. Nos travaux ont permis de documenter dans un premier temps l'hétérogénéité fonctionnelle du tissu adipeux sous-cutané en réponse à une perte pondérale modérée chez des hommes et des femmes préménopausées obèses. En second lieu, nous avons caractérisé le métabolisme des adipocytes sous-cutanés d'hommes jeunes et d'âge moyen ainsi que de femmes pré et postménopausées en considérant leur distribution régionale. Finalement, nous avons montré l'importance de la taille et du métabolisme de l'adipocyte dans la détermination du profil métabolique, de la lipémie postprandiale et de l'oxydation des substrats chez l'être humain. Dans l'ensemble, nos travaux relatent les effets de la restriction calorique et de l'âge sur les capacités lipolytiques et lipogéniques de l'adipocyte et renforcent également le rôle que joue la cellule adipeuse dans la régulation du profil métabolique et de l'équilibre énergétique chez l'Homme.

RÉSUMÉ LONG

Le but de nos travaux consistait à vérifier les effets de la restriction calorique et de l'âge sur la capacité lipolytique et/ou lipogénique de l'adipocyte ainsi qu'à examiner le rôle que joue la cellule adipeuse dans la régulation du profil métabolique et de l'équilibre énergétique chez l'être humain. Nous avons montré que des hommes caractérisés par une hypertrophie des adipocytes sous-cutanés (sc) du site abdominal présentent une détérioration accrue de leur profil métabolique par rapport à des hommes présentant de petits adipocytes. Par ailleurs, nous avons rapporté un dimorphisme sexuel quant aux variations de l'activité de la LPL en réponse à une perte de poids modérée. Une augmentation de la sensibilité β-adrénergique (AR) des adipocytes des régions sc d'hommes et de femmes préménopausées obèses après une perte pondérale fut aussi observée. Dans un second volet, nous avons montré que la capacité lipolytique des adipocytes d'individus moyennement âgés était moins élevée que celle de sujets jeunes de masse grasse identique. Cette altération ne semble toutefois pas se répercuter au niveau de l'expression du gène de l'enzyme clé responsable de l'hydrolyse des triglycérides intraadipocytaires. Nos travaux ont également mis en évidence que des femmes pré et postménopausées caractérisées par une distribution régionale du tissu adipeux similaire présentent une balance lipolytique/lipogénique comparable. Par ailleurs, nous avons documenté l'impact de la sensibilité antilipolytique α_2-AR des adipocytes sc abdominaux sur la lipémie postprandiale. De plus, nous avons rapporté que la réponse lipolytique maximale d'adipocytes isolés à un agoniste β-AR s'avérait un déterminant de l'oxydation lipidique chez des sujets obèses. Enfin, nous avons observé une diminution de l'expression du facteur transcriptionnel impliqué dans la différenciation adipocytaire, PPARγ, au niveau du site sc abdominal après une perte de poids chez des individus obèses. Dans l'ensemble, nous avons montré que la restriction calorique et l'âge affectent la balance lipolytique/lipogénique de l'adipocyte. Nos travaux renforcent également le rôle que joue la cellule adipeuse dans la régulation du profil métabolique et de l'équilibre énergétique chez l'Homme.

AVANT-PROPOS

Tout d'abord, j'aimerais souligner le support financier accordé au cours de ma formation par les Fonds pour la Formation de Chercheurs et l'Aide à la Recherche.

Je profite de l'occasion pour remercier sincèrement tous les collaborateurs ayant participé de près ou de loin à la bonne réalisation des travaux présentés dans cette thèse. Un merci particulier à M. Hubert Vidal et sa joyeuse équipe de l'Unité 449 INSERM à Lyon pour leur accueil chaleureux et leur contribution au cours de mon initiation à la RT-PCR compétitive. Un merci bien spécial également à un M. Jean-Pierre Després et sa dynamique équipe sans qui nous utiliserions probablement encore aujourd'hui l'indice de masse corporelle pour caractériser la distribution régionale du tissu adipeux. Merci donc à toi Jean-Pierre pour ton bon support et les collaborations fructueuses dont j'ai pu bénéficier avec ton équipe au cours de mon périple doctoral.

Je ne peux passer sous silence ma seconde famille, dirais-je "adoptive", du laboratoire des sciences de l'activité physique!! D'un point de vue statistique, nous pouvons dire que le temps consacré au travail entre nos quatre murs de béton atteint des niveaux significatifs au cours d'une année, d'où l'importance d'être entouré de gens fort intéressants. Je salue donc bien bas tous mes amis du laboratoire qui sont selon moi les rayons de soleil de cette belle famille souterraine!! Un clin d'œil particulier à des camarades précieux (Éric Doucet, Chantale Gagnon, Rémy Thériault et Natalie Alméras) avec qui j'ai l'occasion de défaire et refaire le monde lors de nos "pauses-travail" ou lors de nos loisirs.

Un merci tout spécial à mes deux mentors et amis Pascale et Angelo qui ont contribué par leurs paroles et gestes sincères ainsi que leur confiance et bons conseils au déploiement de mes ailes pour favoriser mon envol dans ce vaste monde scientifique.

Je tiens aussi à dédier des remerciements particuliers à ma tendre famille composée de Marc, Nicole, Jérôme et Marie-Andrée dont le soutien moral est toujours source d'énergie intense. Je ne peux passer sous silence ma belle famille formée de Claude André, Juliette et Martin qui sont aussi résurgence de bons conseils et de soutien.

J'aimerais finalement remercier ma meilleure amie et amoureuse Sylvie, celle qui partage quotidiennement les aléas de l'adolescent scientifique que je suis et qui catalyse ma recette magique du bonheur.

Pascal

TABLE DES MATIÈRES

INTRODUCTION

Au cours des dernières années, l'essor de la restauration rapide et de l'industrialisation a contribué sans aucun doute à une modification de nos comportements alimentaires et d'activités physiques favorisant ainsi l'emmagasinage de réserves corporelles sous forme de graisse. À ce titre, il est bien connu qu'une accumulation excessive de tissu adipeux, notamment au niveau abdominal, est fortement associée à des complications métaboliques néfastes pour la santé telles que l'hypertension artérielle, les maladies cardiovasculaires et le diabète noninsulinodépendant (Björntorp, 1991b; Després, 1991; Kissebah & Krakower, 1994).

L'afflux des connaissances ainsi que l'importance accordée au tissu adipeux lors de la dernière décennie nous ont donc permis de considérer ce dernier autrement qu'un simple isolant thermique et mécanique. En effet, le tissu adipeux blanc, constitué d'adipocytes et de nombreux autres types cellulaires (préadipocytes, fibroblastes, cellules endothéliales et sanguines, etc.) s'est révélé ultérieurement capable de répondre à diverses hormones qui modulent l'accumulation et la mobilisation des réserves de triglycérides intraadipocytaires, pouvant ainsi contribuer au développement de la masse grasse ainsi qu'à sa distribution régionale.

Dans une première partie (Chapitre 1), nous abordons les principaux facteurs contrôlant le développement du tissu adipeux. Cette thématique est au cœur de la problématique actuelle qu'est la pandémie de l'obésité et favorise la découverte de cibles moléculaires potentielles

pour la conception de nouvelles stratégies thérapeutiques des déséquilibres métaboliques et/ou nutritionnels. L'accent est mis ensuite sur l'équipement enzymatique du tissu adipeux blanc qui assure un ajustement permanent des apports (Chapitre 2) et des pertes (Chapitre 3) des métabolites lipidiques nécessaires à l'organisme. Ces sections forment la base constitutive de nos travaux. En effet, 8 des 9 études composant cette thèse s'intéressent à la capacité lipolytique et/ou lipogénique de l'adipocyte lors de diverses situations physiologiques (perte de poids, vieillissement et lipémie postprandiale). Finalement, au cours du Chapitre 4, nous passerons en revue certaines complications métaboliques associées à l'accumulation excessive du tissu adipeux.

La première étude de cette thèse (Chapitre 5) documente la contribution de l'hypertrophie des adipocytes sous-cutanés abdominal et fémoral dans la détérioration du profil métabolique d'hommes et de femmes. Cette dernière s'intègre bien au débat actuel qui règne quant à l'effet néfaste du tissu sous-cutané abdominal sur le profil de santé métabolique d'un individu. Dans un autre ordre d'idées, l'impact d'une perte pondérale sur les aspects lipogénique (Chapitre 6) et lipolytique (Chapitre 7) d'adipocytes isolés des sites sous-cutanés d'hommes et femmes obèses est étudié. Ces travaux documentent les adaptations métaboliques intrinsèques à l'adipocyte suite à une restriction calorique et leurs variations en fonction du sexe de l'individu.

Outre l'impact d'une restriction calorique, l'influence du vieillissement sur le métabolisme de la cellule adipeuse est à la base des Chapitres 8, 9 et 10 de cette thèse. Il est bien connu que le vieillissement est associé à un gain de poids corporel chez l'humain sans toutefois qu'il y ait d'explication claire à ce phénomène (Silver et al., 1993). À ce titre, une étude récente réalisée au sein de notre laboratoire rapporte une augmentation du poids corporel chez des hommes et des femmes après un suivi de 12 ans, malgré le fait que ces derniers présentent une diminution du contenu lipidique dans leur alimentation et une augmentation de leur participation à des activités physiques moyennement intenses (Tremblay et al., 1998). Ces résultats suggèrent l'existence d'effets du vieillissement dans le contrôle de la balance lipidique et semblent prédominer sur les effets de changements de style de vie qui auraient dû entraîner une perte de poids corporel. Ainsi, nous abordons dans un premier volet le métabolisme du tissu adipeux

des sites sous-cutanés abdominal et fémoral d'hommes jeunes et moyennement âgés. Un second volet documente également l'impact du vieillissement au niveau de l'expression de protéines clé impliquées dans la mobilisation et l'accumulation des triglycérides du tissu adipeux de l'homme. Enfin, les capacités lipolytique et lipogénique des cellules adipeuses sous-cutanées de femmes pré et postménopausées sont comparées.

Sur la base du fait que les capacités lipolytique et lipogénique de l'adipocyte agissent de façon coordonnée et qu'elles influencent de façon importante la régulation du métabolisme des acides gras et des lipoprotéines plasmatiques, nous nous sommes intéressés (Chapitre 11) à l'impact de la sensibilité antilipolytique des adipocytes sous-cutanés abdominaux sur le devenir postprandial des lipoprotéines riches en triglycérides en situation postprandiale. Dans le même ordre d'idées, le Chapitre 12 traite de l'influence de la capacité lipolytique des adipocytes sous-cutanés au niveau de l'oxydation lipidique déterminée par calorimétrie indirecte chez des sujets obèses. Finalement, le Chapitre 13 présente les effets d'une perte pondérale sur l'expression d'un facteur transcriptionnel de la différenciation adipocytaire, PPARγ (*peroxisome proliferator activated receptor gamma*). Ces travaux documentent également les changements de concentrations d'organochlorés plasmatiques et du tissu adipeux en relation avec les variations des niveaux d'expression de PPARγ suite à une perte pondérale.

L'ensemble de nos travaux consiste donc à examiner les effets de la restriction calorique et de l'âge sur la capacité lipolytique et/ou lipogénique de l'adipocyte ainsi qu'à étudier le rôle que joue la cellule adipeuse dans la régulation du profil métabolique et de l'équilibre énergétique chez l'être humain.

CHAPITRE 1

LE DÉVELOPPEMENT DU TISSU ADIPEUX

Le développement excessif de la masse adipeuse fait à la fois intervenir une hypertrophie et une hyperplasie cellulaires. Le développement du tissu adipeux blanc a lieu essentiellement en période postnatale, mais tout au long de la vie, il peut aussi se produire une augmentation de la population adipocytaire à partir de précurseurs cellulaires présents dans la fraction vasculaire du stroma de ce tissu. À cet égard, certains travaux ont montré que de nouvelles cellules adipeuses apparaissent quand le poids moyen des cellules existantes excède 0.6 μg de lipides/cellule (Björntorp, 1991a).

La compréhension des mécanismes contrôlant la croissance des précurseurs et la différenciation adipocytaire, aussi appelée adipogenèse, se trouve ainsi au coeur de la problématique du développement adipeux. Dans ce chapitre, l'accent sera mis sur les facteurs transcriptionnels jouant un rôle propre et coopératif dans le développement de la différenciation adipocytaire qui ont été identifiés récemment. Nous nous attarderons également à la capacité sécrétoire de l'adipocyte mature qui lui confère un statut de cellule endocrine capable de communiquer avec le système nerveux central. Finalement, nous aborderons sommairement la capacité qu'a le tissu adipeux d'accumuler des substances toxiques et les conséquences des ces dernières sur le métabolisme de la cellule adipeuse.

1.1 Facteurs transcriptionnels de l'adipogenèse

Plusieurs facteurs transcriptionnels contrôlent l'engagement et/ou le maintien de la différenciation terminale des adipocytes (Figure 1.1). Tel que mentionné précédemment, ces facteurs peuvent agir de façon propre ou coopérative. Pour l'instant, trois familles de facteurs de transcription participant à différentes étapes de l'adipogenèse sont identifiées. Mentionnons notamment la famille des C/EBP (CCAAT/*enhancer binding proteins*), des PPAR (*peroxisome proliferator-activated receptors*) et des protéines à motif hélice-boucle-hélice.

1.1.1 La famille des C/EBP

Trois membres de cette famille, C/EBPα, C/EBPβ et C/EBPδ, sont impliqués dans l'induction de la différenciation adipocytaire (Darlington et al., 1998). Brièvement, ces facteurs de transcription possèdent d'une part, un domaine basique de liaison à des séquences CCAAT présentes dans les régions régulatrices des gènes cibles et, d'autre part, un domaine à glissière de leucines (*leucine-zipper*) qui leur permet de réaliser des homodimérisations ou des hétérodimérisations avec d'autres membres de la famille des C/EBP.

Au cours du processus de différenciation, C/EBPβ et C/EBPδ émergent précocement (Darlington et al., 1998) (Figure 1.1). Les activateurs de la voie de l'AMP cyclique et les glucocorticoïdes activent respectivement l'expression de la C/EBPβ et de la C/EBPδ (Yeh et al., 1995). La surexpression de la protéine C/EBPβ dans les fibroblastes NIH-3T3 est suffisante pour induire le phénotype adipocytaire en présence de glucocorticoïdes et d'insuline (Yeh et al., 1995). Cet effet de la C/EBPβ sur la différenciation adipocytaire est optimisé par sa co-expression avec la C/EBPδ (Wu et al., 1996). La double invalidation (*knock-out*) des C/EBPβ et δ, lorsqu'elle n'est pas létale, provoque une altération substantielle du développement des tissus adipeux blanc et brun chez la souris (Tanaka et al., 1997).

Plusieurs arguments plaident en faveur du rôle inducteur de la C/EBPα sur l'adipogenèse (Umek et al., 1991). La protéine est exprimée avant le démarrage de la transcription de nombreux gènes spécifiques de l'adipocyte. Elle transactive nombre de ces gènes par l'intermédiaire des sites CCAAT et s'avère capable d'assurer sa propre transactivation (Loftus & Lane, 1997). L'expression de l'ARN messager antisens de la C/EBPα inhibe la différenciation adipocytaire de la lignée cellulaire 3T3-L1 (Samuelsson et al., 1991), alors que la surexpression du gène est suffisante pour induire la différenciation adipocytaire (Lin & Lane, 1994; Umek et al., 1991). Enfin, mentionnons que l'invalidation du gène chez la souris engendre des animaux qui ne présentent pas d'accumulation de graisse (Wang et al., 1995).

Il convient finalement de souligner que l'effet de ces facteurs C/EBPpeut être inhibé par une protéine de la même famille, CHOP-10 (C/EBP *homologous protein*-10) (Ron & Habener, 1992). CHOP-10 est exprimée tardivement au cours de la différenciation. Sa surexpression inhibe l'adipogenèse et s'accompagne d'une diminution de l'expression des C/EBPα et C/EBPβ.

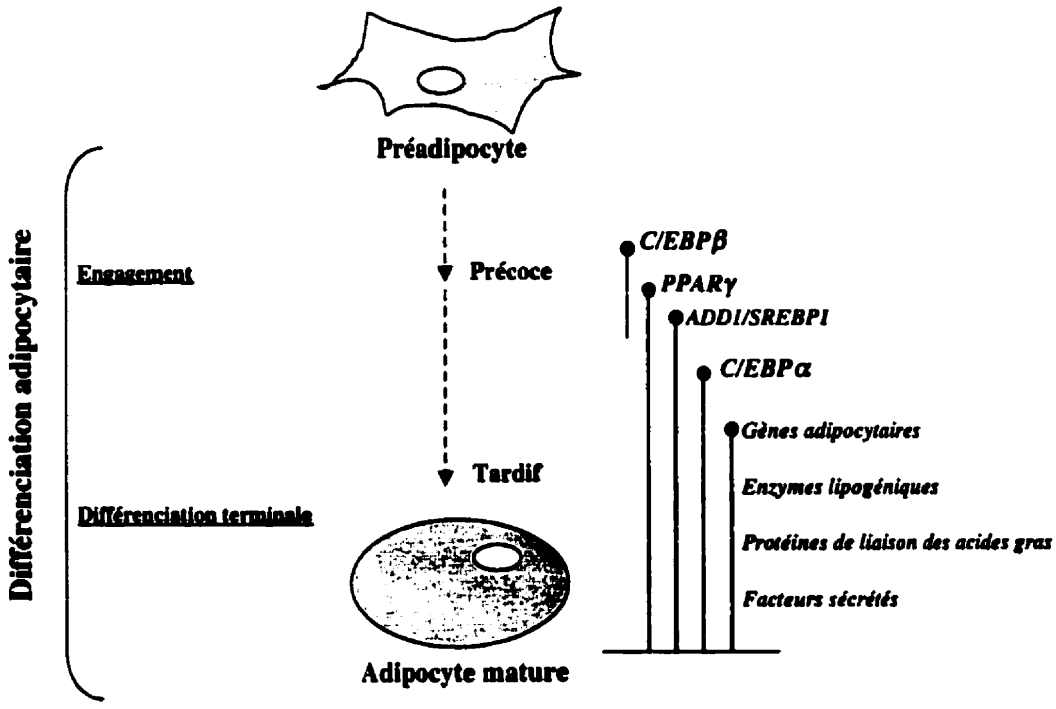


Figure 1.1 Facteurs transcriptionnels impliqués dans la différenciation adipocytaire. L'expression temporelle des principaux facteurs de l'adipogenèse est indiquée par les lignes. C/EBP: CCAAT/*enhancer binding proteins*; PPARγ: *peroxisome proliferator-activated receptors gamma*; ADD1/SREBP1: *adipocyte determination- and differentiation-dependent factor1/sterol regulatory element-binding protein 1*. Adapté de Grégoire et al. (1998).

1.1.2 La famille des PPAR

Les PPAR appartiennent à la superfamille des récepteurs nucléaires hormonaux de type stéroïdien. Ils forment des hétérodimères avec les récepteurs de l'acide 9-cis rétinoïque (*retinoïd X receptor* ou RXR) et modulent l'expression des gènes contenant des éléments de réponse aux PPAR, les PPRE (peroxysome proliferator response elements) (Auwerx, 1999; Desvergne & Wahli, 1999; Spiegelman, 1998).

Chez l'homme, trois sous-types de PPAR ont été décrits: α, β et γ. Le PPARγ est le sous-type le plus exprimé dans le tissu adipeux (Auboeuf et al., 1997; Mukherjee et al., 1997). Deux isoformes ont été caractérisés, PPARγ1 et PPARγ2 et sont issus d'un même gène par épissage alternatif et par l'utilisation de sites de mise en route de transcription distincts. En règle générale chez l'homme, la proportion d'ARNm de PPARγ2 représente environ 17% des niveaux d'ARNm totaux de PPARγ (Auboeuf et al., 1997). L'expression de PPARγ précède celle de la C/EBPα au cours de la différenciation (Figure 1.1). Par ailleurs, la surexpression de PPARγ est suffisante pour assurer l'induction du phénotype adipocytaire dans les fibroblastes NIH-3T3 (Tontonoz et al., 1994).

Certaines prostaglandines, en particulier la PGJ2, seraient des ligands endogènes de ce facteur de transcription (Forman et al., 1995; Kliewer et al., 1995). Il est intéressant de noter qu'une nouvelle classe d'antidiabétiques, les thiazolidinediones, et certains acides gras sont des puissants inducteurs de la différenciation adipocytaire agissant par l'intermédiaire de PPARγ (Forman et al., 1995; Kliewer et al., 1995; Krey et al., 1997).

Comparativement au rôle important de PPARγ, les deux autres isoformes semblent avoir des influences plus limitées dans le développement adipocytaire (Brun et al., 1996). PPARα est peu exprimé dans le tissu adipeux de l'homme alors qu'il y est de façon plus abondante au niveau du foie, du muscle squelettique et des reins (Auboeuf et al., 1997). L'invalidation du

gène PPARα chez la souris a démontré que ce sous-type contribue à la régulation de la β-oxydation peroxysomale hépatique (Lee et al., 1995).

Enfin, le PPARβ, aussi appelé PPARδ, est exprimé de façon ubiquitaire et serait impliqué dans le contrôle de certains gènes de la différenciation adipocytaire tel que celui de l'aP2 (transporteur intracellulaire des acides gras) (Amri et al., 1995). De plus, PPARβ est induit au cours de la différenciation et est également activé par les thiazolidinediones et les acides gras saturés ou insaturés (Amri et al., 1995; Forman et al., 1997).

1.1.3 La famille des facteurs à motif hélice-boucle-hélice (HLH)

Il est intéressant de noter que le domaine HLH permet à ces facteurs de former des homodimères ou des hétérodimères avec les protéines de la même famille. Après dimérisation, le complexe se lie par le domaine basique (b) à des séquences spécifiques dites *E-box*, situées au niveau des promoteurs de gènes régulés par ces facteurs.

ADD1/SREBP1 (*adipocyte determination- and differentiation-dependent factor1/sterol regulatory element-binding protein 1*) est un facteur à motif bHLH dont l'expression est induite tôt au cours de la différenciation (Tontonoz et al., 1993) (Figure 1.1). Ce facteur a une expression étroitement dépendante du processus de différenciation et exerce également un effet adipogénique (Kim & Spiegelman, 1996). En effet, l'expression ectopique du facteur ADD1 dans les fibroblastes NIH-3T3 entraîne une induction de l'expression de gènes impliqués dans le métabolisme des acides gras, (i.e. la synthase des acides gras qui favorise la synthèse des acides gras à partir du glucose et la lipoprotéine lipase qui favorise l'approvisionnement des acides gras suite à l'hydrolyse des TG plasmatiques), sans entraîner la différenciation adipocytaire. Il a également été montré que des fibroblastes surexprimant ADD1 se différencient grâce à une augmentation transactivatrice de PPARγ2 induite par ADD1 (Kim et al., 1996).

À l'inverse de ces facteurs dits dominants positifs ou activateurs de la différenciation, il existe d'autres membres de la classe HLH ne possédant pas le domaine basique. Ces derniers agissent comme dominants négatifs en séquestrant les facteurs positifs par hétérodimérisation et en empêchant leur activité transactivatrice. Les *inhibitors of DNA binding* (Id) font partie de cette catégorie de facteurs composée de quatre membres (Kadesch, 1993). L'activité transcriptionnelle des gènes Id2 et Id3 est diminuée très tôt au cours de la différenciation adipocytaire des cellules 3T3-F442A (Moldes et al., 1997). La surexpression ectopique de Id3 au niveau des préadipocytes 3T3-F442A entraîne notamment une inhibition de la différenciation adipocytaire (Moldes et al., 1997).

L'émergence du phénotype terminal adipocytaire est donc contrôlée par des protéines de la famille des C/EBP, des PPAR et des protéines à motif HLH. Il convient de souligner que le processus de différenciation implique une interaction séquentielle et coopérative des membres à l'heure actuelle découverts des familles de facteurs transcriptionnels (Loftus et al., 1997; Spiegelman & Flier, 1996). À ce titre, ces partenaires transcriptionnels de l'adipogenèse constituent des cibles moléculaires potentielles pour la conception de nouvelles stratégies thérapeutiques des déséquilibres métaboliques et nutritionnels.

1.2 L'adipocyte, cellule sécrétrice et endocrine

Au cours de la dernière décennie, nos connaissances et l'importance accordée au tissu adipeux blanc se sont accrues de façon importante. Le tissu adipeux blanc, antérieurement perçu comme un simple isolant thermique et mécanique, est maintenant considéré comme un organe présentant le potentiel d'influencer la régulation de l'équilibre lipidique et énergétique via les nombreux facteurs peptidiques et non peptidiques qu'il sécrète. Dans cette section, nous aborderons les principales protéines sécrétées par l'adipocyte modulant le métabolisme des lipides. Par ailleurs, nous discuterons du peptide ayant conféré à la cellule adipeuse le statut de cellule endocrine, la leptine. L'accent sera également mis sur un facteur à activité autocrine/paracrine réglant la cellularité du tissu adipeux qu'est le *tumor necrosis factor alpha*

(TNF-α). Finalement, une brève vision des facteurs angiogéniques également libérés par l'adipocyte sera brossée.

1.2.1 Protéines sécrétées et métabolisme des lipides

Parmi les protéines sécrétées par l'adipocyte qui possèdent une activité enzymatique, la lipoprotéine lipase (LPL) se retrouve après divers processus de maturation à la surface luminale des cellules endothéliales. La LPL hydrolyse alors les triglycérides des particules VLDL (*very low density lipoproteins*) et des chylomicrons en libérant des acides gras pouvant être captés par la cellule adipeuse (Eckel, 1989). La régulation de cet enzyme sera discutée en détail dans le prochain chapitre (Chapitre 2).

Parallèlement à la LPL, la protéine *acylation stimulating protein* (ASP), qui a pour origine l'adipocyte, contrôle l'accumulation de triglycérides dans la cellule adipeuse (Baldo et al., 1993). En fait, l'adipocyte sécrète les trois protéines du système alterne du complément: facteur C_3, D (adipsine) et B (Choy et al., 1992). Sommairement, l'adipsine, qui est une protéase à sérine, engendre à partir des facteurs C_3 et B le facteur C3a qui, après attaque du résidu carboxy-terminal d'arginine, donne naissance à l'ASP. Plus fortement et indépendamment de l'insuline, l'ASP est capable de stimuler la synthèse de triglycérides dans les adipocytes (Baldo et al., 1993). Dans le tissu adipeux de l'homme, spécialement au cours de la période postprandiale, la sécrétion d'ASP favorise l'hydrolyse des triglycérides circulants, renforçant ainsi l'hypothèse selon laquelle l'ASP jouerait parallèlement à la LPL un rôle paracrine autorégulateur (Saleh et al., 1998).

Outre sa participation au contrôle de son contenu en triglycérides, l'adipocyte intervient aussi dans le métabolisme du cholestérol et des rétinoïdes. En effet, le tissu adipeux de l'homme est riche en ARNm codant pour la protéine de transfert des esters de cholestérol (*CETP*) (Martin et al., 1993; Radeau et al., 1995). De plus, de récents travaux montrent que la contribution du tissu adipeux après synthèse et sécrétion de la CETP est étroitement reliée aux concentrations

plasmatiques de cette protéine (Radeau et al., 1998). Le rôle de la CETP est de catalyser le transfert des esters de cholestérol des particules de la fraction HDL (*high density lipoproteins*) vers celles de la fraction VLDL (*very low density lipoproteins*) et le transfert des triglycérides de cette dernière vers la première (Tall, 1995). L'incapacité de l'adipocyte à synthétiser du cholestérol suggère ainsi que la CETP participe vraisemblablement à l'homéostase du cholestérol de la cellule en favorisant la captation des esters de cholestérol dérivés des particules de la fraction HDL (Benoist et al., 1997).

Dans un autre ordre d'idées, la protéine de liaison du rétinol (RBP) est également synthétisée et sécrétée par l'adipocyte (Tsutsumi et al., 1992). Cette synthèse étant évaluée au quart du niveau observé dans l'hépatocyte, la contribution du tissu adipeux s'avère importante si l'on considère l'ensemble de la masse adipeuse et donc le nombre total d'adipocytes de l'organisme. Dans la mesure où l'adipocyte concentre du rétinol (vitamine A) et des esters de rétinol associés aux triglycérides et au cholestérol, la mobilisation de l'ensemble de ces diverses molécules lipophiles au cours du jeûne ou lors d'un déficit vitaminique fait apparaître le rôle clé de réservoir joué par l'adipocyte sur le plan physiologique. Cependant, si la régulation hormonale de la mobilisation des triglycérides est bien connue (Chapitre 3), celle de la mobilisation du rétinol et du cholestérol demeure encore vague.

1.2.2 Facteurs à activité autocrine, paracrine et endocrine

Dans un premier temps, il convient de définir ce que l'on entend par activités autocrine, paracrine et endocrine. Tel qu'illustré à la Figure 1.2, une activité endocrine réfère à la capacité par une glande spécialisée de sécréter des hormones qui sont relarguées dans la circulation pour agir sur des cellules cibles distantes de leur lieu de synthèse. Par ailleurs, les hormones sécrétées par une cellule pouvant influencer des cellules avoisinantes sont dites à activité paracrine. Parallèlement, les hormones sécrétées par une cellule dans le compartiment péricellulaire et dont l'action négative ou positive est exercée sur cette même cellule réfèrent à une activité autocrine. Bien que ceci ne soit pas illustré, il est intéressant de noter que les

hormones peuvent aussi exercer leur action sur la même cellule sans toutefois passer par le milieu péricellulaire. On définit cette activité comme étant intracrine (Labrie, 1991).

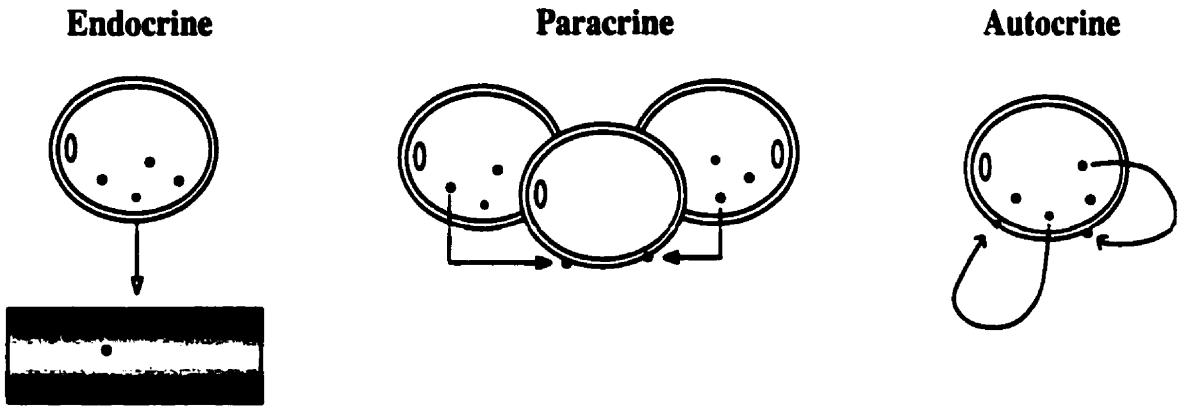


Figure 1.2 Représentation schématique d'une sécrétion hormonale à activité endocrine, paracrine ou autocrine. Adapté de Labrie F (1991).

Le concept selon lequel la masse adipeuse peut être réglée via des facteurs circulants sécrétés par le tissu adipeux résulte d'expériences de parabiose entre rats normopondéraux et rats devenus obèses après lésion hypothalamique ainsi qu'entre souris obèses de génotypes ob/ob et db/db (Coleman, 1978). Le clonage du gène ob chez la souris et l'homme et dont le produit est la leptine a validé cette hypothèse (Zhang et al., 1994). La leptine exerce son action à partir du noyau ventro-médian de l'hypothalamus en inhibant la production du neuropeptide Y qui par conséquent entraîne une diminution de la prise alimentaire chez l'animal (Stephens et al., 1995; Wang et al., 1997). La leptine joue également un rôle au niveau du système nerveux central en augmentant la dépense d'énergie (Pelleymounter et al., 1995).

Contrairement au modèle de souris ob, l'obésité tributaire d'un défaut dans le gène ob conduisant à une absence totale de leptine s'avère très rare chez l'homme. Il existe toutefois quelques rares cas d'humains déficients en leptine caractérisés tel que prévu par une hyperphagie chronique et conséquemment une obésité considérable. De récents travaux cliniques ont révélé que l'administration quotidienne de leptine durant une année complète chez une jeune fille incapable de produire l'hormone a conduit à une perte de masse corporelle

de 16 kg, majoritairement expliquée par la perte de masse grasse (Farooqi et al., 1999). Il faut toutefois garder à l'esprit que le nombre de patients dont l'obésité résulte d'une absence de leptine en circulation ou d'un défaut dans son récepteur est infime (Yeo et al., 1998). Ceci explique notamment la raison pour laquelle le traitement de l'obésité dans la population par l'administration de la leptine est peu fructueux jusqu'à ce jour (Heymsfield et al., 1999).

Le fait que les adipocytes atteignent une taille maximale au-delà de laquelle de nouvelles cellules sont recrutées suggère également l'existence de facteurs locaux provenant des adipocytes hypertrophiés. Dans le tissu adipeux de l'homme, le TNF-α est absent du préadipocyte et n'est synthétisé que dans l'adipocyte (Hauner et al., 1995). L'expression de l'ARNm codant pour le TNF-α est proportionnelle à l'indice de masse corporelle (Kern et al., 1995). À l'inverse, et tel qu'attendu, une restriction calorique entraîne une diminution du TNF-α mesuré dans le tissu adipeux (Kern et al., 1995). Chez l'homme, une relation inverse entre l'expression de TNF-α et l'activité de la LPL du tissu adipeux et son transcrit (Kern et al., 1995) ainsi que d'autres gènes de la lipogenèse (i.e. les transporteurs de glucose (Glut-4) (Hotamisligil et al., 1993) et l'adipsine (Torti et al., 1985)) suggère une action locale du TNF-α limitant l'hypertrophie des adipocytes. De plus, il a été démontré que le TNF-α stimule l'activité lipolytique d'adipocytes humains différenciés (Hauner et al., 1995). Par ailleurs, l'inhibition de l'action de l'insuline et de son récepteur par le TNF-α a récemment été rapportée au niveau d'adipocytes humains en culture suggérant ainsi un rôle potentiel de cette cytokine dans le développement de l'insulinorésistance (Liu et al., 1998). Enfin, mentionnons que les mécanismes par lesquels cette cytokine est sécrétée par le tissu adipeux demeurent encore imprécis (Mohamed-Ali et al., 1998).

Les adipocytes humains sécrètent également l'interleukine-6 (IL-6) (Hotamisligil et al., 1995). La concentration plasmatique en IL-6 étant proportionnelle à l'indice de masse corporelle (Hotamisligil et al., 1995), le tissu adipeux pourrait se révéler une source importante de cette cytokine. L'IL-6 diminue comme le TNF-α l'expression de la LPL (Fried et al., 1998) et active la lipolyse de la lignée cellulaire 3T3-L1 (Berg et al., 1994), suggérant à nouveau un rôle local

de cette cytokine dans la régulation de l'entrée et de la sortie des acides gras dans le tissu adipeux.

Le tissu adipeux constitue aussi une source importante d'angiotensinogène après le foie (Harp & DiGirolamo, 1995) et on ne peut exclure qu'une sécrétion accrue d'angiotensinogène puisse conduire chez l'homme, via l'angiotensine II, à une augmentation de la tension artérielle (Frederich et al., 1992). Par ailleurs, la sécrétion et l'expression de l'inhibiteur de l'activateur du plasminogène, PAI-1 (*plasminogen activated inhibitor receptor-1*), ont été rapportées au niveau d'explants de tissu adipeux humain (Alessi et al., 1997; Eriksson et al., 1998) ainsi que de préadipocytes humains en culture (Crandall et al., 1999). L'individu obèse présente vraisemblablement une sécrétion ainsi qu'un niveau d'expression de PAI-1 augmentés dans le tissu sous-cutané abdominal comparativement à celui d'une personne de poids normal (Eriksson et al., 1998). De récents travaux ont aussi rapporté une augmentation de son expression au niveau du dépôt intraabdominal comparativement au site sous-cutané (Alessi et al., 1997). En se basant sur le fait que PAI-1 diminue la fibrinolyse et donc augmente le risque de thrombose (Reilly et al., 1991; Vaughan et al., 1992), la sécrétion accrue de PAI-1 au niveau des dépôts adipeux profonds est à la base de l'hypothèse suggérant qu'il pourrait contribuer à l'augmentation des problèmes vasculaires associés à ce type d'obésité (Alessi et al., 1997). Cependant, le mécanisme responsable des forts taux d'expression de ce facteur angiogénique associés à l'obésité s'avère encore inconnu.

En résumé, l'adipocyte se révèle capable d'interagir dans son propre environnement et avec d'autres organes et systèmes. Les différentes protéines (LPL, ASP), hormones (leptine) et cytokines (TNF-α) sécrétées par l'adipocyte (Figure 1.3) mettent fin à l'ère où le tissu adipeux était perçu comme un simple réservoir métaboliquement inactif. En effet, son statut d'organe sécréteur, synthétisant et libérant un grand nombre de molécules de nature peptidique et non peptidique lui confère un rôle de pilier offensif pouvant intervenir dans la régulation de l'équilibre énergétique.

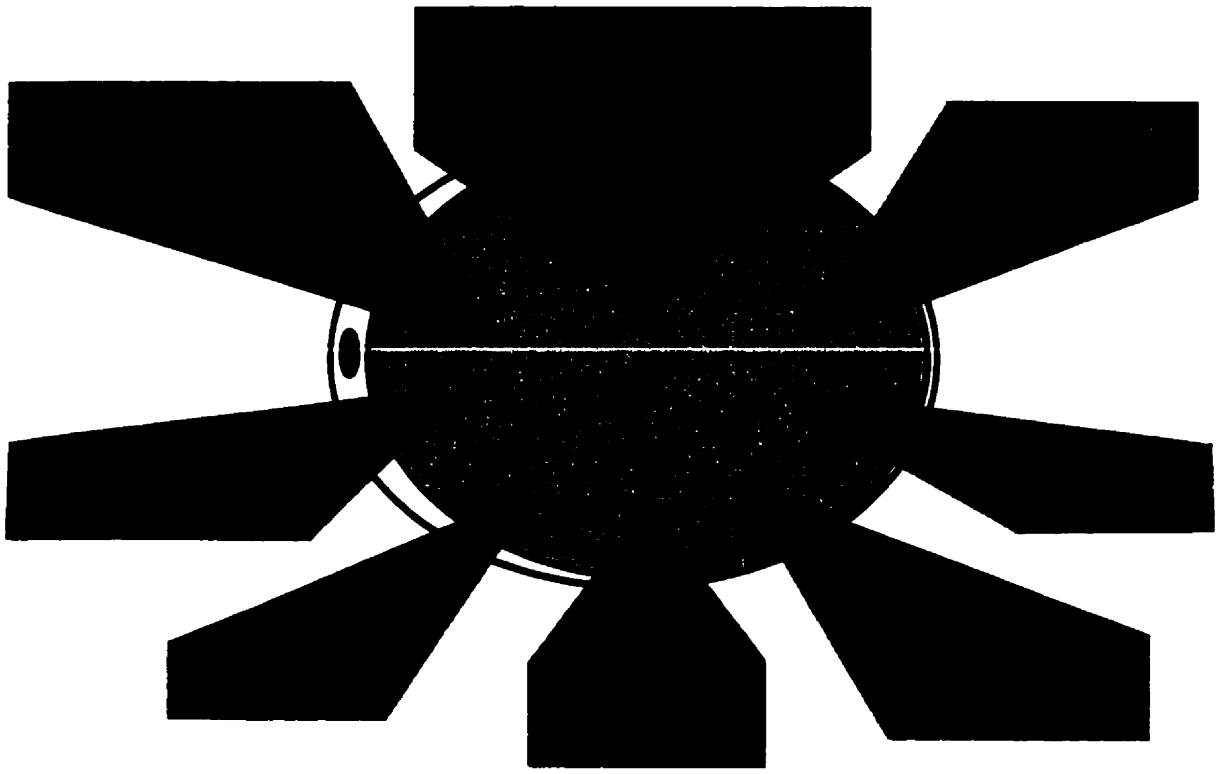


Figure 1.3 Facteurs peptidiques et non peptidiques sécrétés par l'adipocyte et leur action respective. TNF-α: *tumor necrosis factor alpha*; LPL: lipoprotéine lipase; Glut4: transporteur de glucose 4; TG: triglycérides; ASP: *acylation stimulating protein*; IL-6: interleukine-6; PAI-1: *plasminogen activated receptor-1*; CETP: protéine de transfert des esters de cholestérol; RBP: protéine de liaison du rétinol. Adapté de Mohamed-Ali et al. (1998).

1.3 L'adipocyte, cellule tampon de notre environnement

Notre qualité de vie et notre confort se sont considérablement améliorés au cours de l'ère industrielle. Cependant, cette période a aussi été caractérisée par la mise en marché de produits synthétiques de tout usage présentant une haute résistance à la dégradation, les organochlorés. Ces produits furent et sont pour certains encore utilisés à titre d'insecticides, de fongicides, d'herbicides, d'agents de contrôle du feu, de diélectriques et de constituants plastiques. En plus

de présenter une résistance à la dégradation, les organochlorés s'avèrent solubles dans les graisses et peuvent donc s'accumuler au niveau de l'adipocyte. À cet égard, la bioconcentration des organochlorés s'accentue au fur et à mesure que l'on progresse vers le sommet de la chaîne alimentaire (McFarland & Clarke, 1989). Cette bioaccumulation d'organochlorés dans l'environnement et chez l'homme fait l'objet depuis plusieurs années de préoccupations par la communauté scientifique en raison des effets potentiellement néfastes connus de ces derniers sur les systèmes neurologique (Keifer & Mahurin, 1997; Rogan & Gladen, 1992), endocrinien (Brouwer et al., 1998) et reproducteur (Carlsen et al., 1992). Sur la base du fait que les organochlorés s'accumulent notamment au niveau des réserves sous-cutanées et profondes de tissu adipeux de l'homme (Chevrier et al., 2000; Dewailly et al., 1999), il n'est pas exclu que ces derniers puissent exercer leurs effets sur différents enzymes, récepteurs ou facteurs transcriptionnels influençant l'équilibre lipidique de l'adipocyte. Les prochaines lignes mettent l'accent sur quelques études ayant évalué l'impact de certains organochlorés sur le métabolisme du tissu adipeux.

1.3.1 Organochlorés et métabolisme de la cellule adipeuse

Les effets sur la régulation du métabolisme lipidique de l'adipocyte ont principalement été étudiés avec le 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD), l'un des membres les plus toxiques de la grande famille des hydrocarbures polyaromatisés que forment les dioxines, les furanes et les biphényles (Poland & Knutson, 1982). L'une des caractéristiques proéminentes de l'action toxique du TCDD est l'induction de la perte de masse grasse chez les animaux traités en raison de son effet hypophagiant (Kelling et al., 1985). La malabsorption des nutriments (Neal et al., 1979) et la hausse du métabolisme énergétique (Neal et al., 1979) ne sont pas à l'origine de la perte de poids des animaux contaminés. Au cours des dernières années, un rôle direct de ce composé a également été observée au niveau du métabolisme adipocytaire. En effet, certains travaux ont rapporté une diminution notable de l'activité enzymatique des molécules impliquées dans la lipogenèse du tissu adipeux telles que le transporteur de glucose de type 4 (Glut 4) (Enan et al., 1992; Liu & Matsumura, 1995), la LPL (Brewster & Matsumura, 1988) et la synthase des acides gras (FAS) (Lakshman et al., 1989)

chez des animaux traités au TCDD. L'altération de la capacité de différenciation de préadipocytes de la lignée cellulaire 3T3-L1 traités au TCDD est également mise en cause dans la perte de masse grasse induite par ce polluant et s'explique notamment par l'inhibition par le TCDD d'un facteur transcriptionnel important impliqué dans la différenciation adipocytaire, le C/EBPα (Liu et al., 1996).

L'ensemble de ces observations suggère que le TCDD entraîne des effets marqués sur le poids corporel d'animaux traités, ce qui s'explique par des modifications notables au niveau d'enzymes à caractère lipogénique (Glut 4, LPL, FAS) et de facteurs transcriptionnels (C/EBPα) modulant le métabolisme de l'adipocyte. D'un point de vue global, il n'est pas impossible que d'autres contaminants puissent entraîner des effets similaires ou encore contraires à ceux décrits ci-dessus. De plus, étant donné le nombre important de polluants pouvant s'accumuler dans le tissu adipeux, il se peut que certains effets résultant de la présence d'un organochloré quelconque soient contrecarrés par une autre substance donnée. L'impact des organochlorés sur le métabolisme du tissu adipeux de l'homme demeure totalement à être exploré.

CHAPITRE 2

RÉGULATION DE L'ACTIVITÉ DE LA LIPOPROTÉINE LIPASE DU TISSU ADIPEUX HUMAIN

Tel que mentionné précédemment, la lipoprotéine lipase (LPL) est un enzyme responsable de l'hydrolyse des triglycérides transportés par les chylomicrons et les lipoprotéines de très basse densité (VLDL) du plasma sanguin. Dans le tissu adipeux, cet enzyme joue un rôle régulateur clé au niveau de l'entrée des lipides dans l'adipocyte. À cet égard, il a été rapporté que l'activité de la LPL était fortement reliée à la grosseur des cellules adipeuses à la fois chez l'homme et la femme (Fried & Kral, 1987; Rebuffé-Scrive et al., 1985; Rebuffé-Scrive et al., 1987). L'existence d'associations positives entre la masse adipocytaire sous-cutanée, l'indice de masse corporelle et l'activité de la LPL est aussi à la base de l'hypothèse suggérant un rôle physiopathologique éventuel de l'activité de cet enzyme dans le développement de l'obésité chez l'homme (Eckel, 1989). Au cours de la prochaine section, nous aborderons le mécanisme d'action de la LPL et nous nous intéresserons également aux divers facteurs modulant l'activité de cet enzyme.

2.1 Biosynthèse de la LPL

Les travaux portant sur la synthèse de la LPL ont été le plus fréquemment effectués sur le tissu adipeux. Cependant, le schéma de synthèse proposé ci-dessous (Figure 2.1) s'applique également à d'autres tissus tels que le coeur, le muscle squelettique ou la glande mammaire.

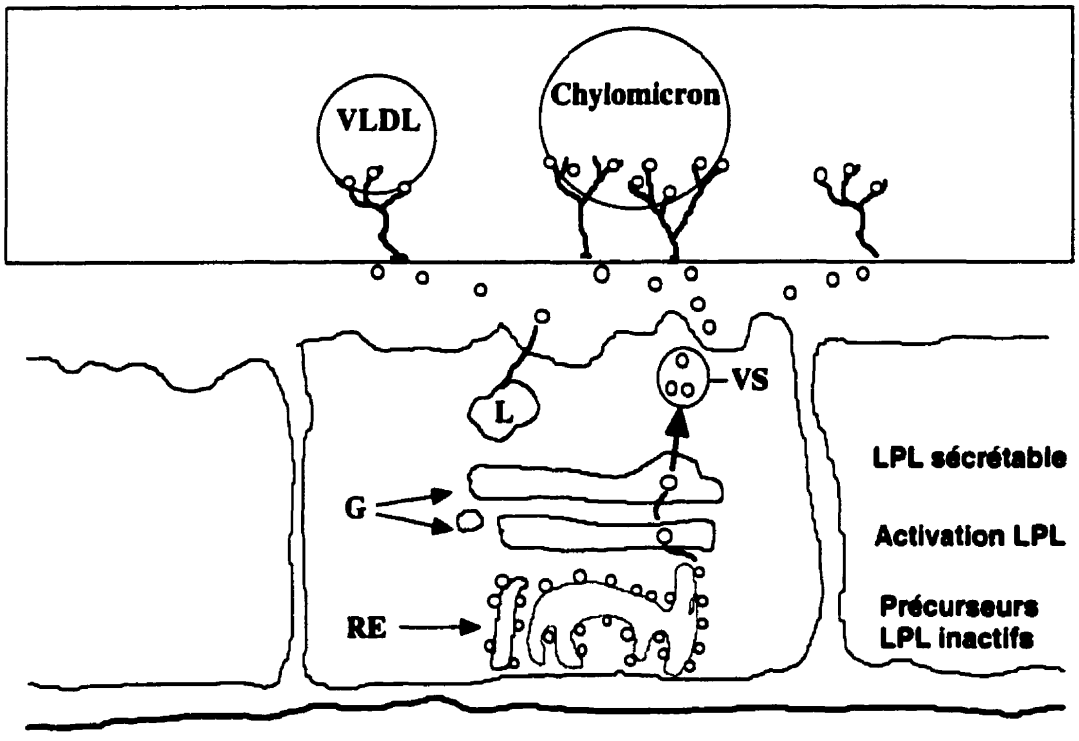


Figure 2.1 Biologie cellulaire de la lipoprotéine lipase. RE: réticulum endoplasmique; G: appareil de Golgi; VS: vésicules sécrétrices; L: lysosomes; VLDL: lipoprotéine de très faible densité. Adapté de Eckel RH (1989).

En résumé, la LPL est synthétisée sous une forme inactive monomérique au niveau du réticulum endoplasmique. Ce précurseur est ensuite transporté vers l'appareil de Golgi où il acquiert les caractéristiques de l'enzyme actif, glycosylé et dimérique (Ailhaud, 1990). La LPL mature s'accumulerait ensuite à l'intérieur de vésicules sécrétrices et en présence de facteurs tels que l'héparine ou d'autres facteurs hormonaux (i.e. insuline, cortisol), l'enzyme actif serait sécrété et transféré vers la paroi de l'endothélium vasculaire des capillaires

adjacents pour aller se fixer au niveau de protéoglycans liés à la membrane de leur face intraluminale. Lorsqu'il n'atteint pas la paroi de l'endothélium vasculaire, l'enzyme peut être recapté par les vésicules sécrétrices ou encore être dégradé par les lysosomes.

L'activité de la LPL dépend de certains facteurs. En effet, l'activité optimale de cette dernière s'avère être en pH alcalin (Bengtsson & Olivecrona, 1982). De plus, pour atteindre une activité maximale, la LPL nécessite la présence d'une apolipoprotéine CII (apo CII) que l'on retrouve dans les lipoprotéines de très faible densité (VLDL) et les chylomicrons. Ainsi, les substrats physiologiques de la LPL que sont les VLDL et les chylomicrons apportent à la fois triglycérides (TG) et cofacteurs (Chung & Scanu, 1977).

2.2 Mécanismes d'action de la LPL

La LPL, après synthèse et transfert au niveau de la membrane externe des cellules endothéliales, se trouve donc en contact avec la circulation sanguine. C'est à ce niveau qu'elle hydrolyse les TG inclus dans les VLDL provenant du foie et les chylomicrons formés dans l'intestin en situation postprandiale. Au cours de l'hydrolyse des lipoprotéines riches en TG, la lipoprotéine sera temporairement immobilisée contre la paroi endothéliale. Comme nous l'avons mentionné précédemment, les VLDL et chylomicrons vont se lier à la LPL essentiellement par l'intermédiaire de l'apo CII qu'ils contiennent à leur surface. Il semble qu'une VLDL (ou un chylomicron) subisse dans le courant sanguin plusieurs hydrolyses successives (Higgins & Fielding, 1975). Après plusieurs cycles d'attachement et de détachement, la VLDL sera ainsi métabolisée en résidus de VLDL (Figure 2.2). À ce stade, non seulement une grande partie des TG inclus dans la lipoprotéine est déjà hydrolysée, mais on note également un transfert d'une partie des constituants de surface de la lipoprotéine (i.e. apo CII, phospholipides et cholestérol) aux lipoprotéines de haute densité (HDL) en circulation (Felts et al., 1975).

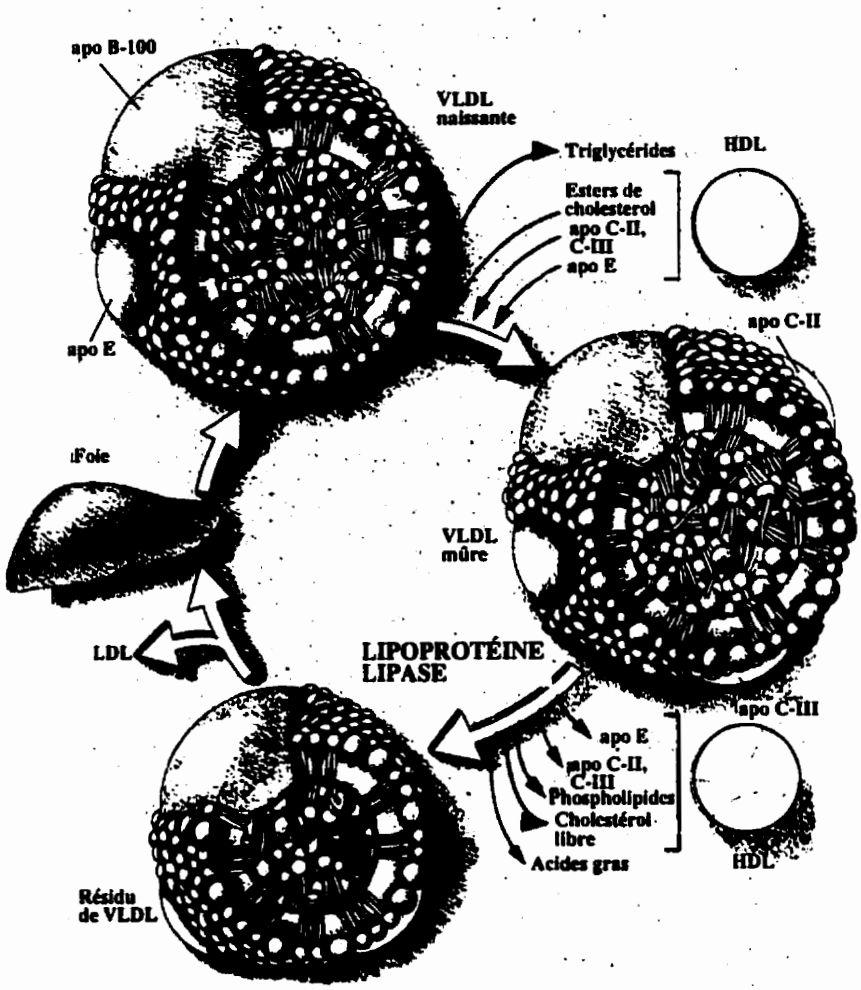


Figure 2.2 Métabolisme des VLDL: implication de la lipoprotéine lipase. VLDL: lipoprotéine de très faible densité; Apo: apolipoprotéine; HDL; lipoprotéine de haute densité; LDL: lipoprotéine de faible densité. Adapté de Grundy SM (1990).

Le transfert de l'apo E des résidus de VLDL aux HDL fait également partie de cette voie métabolique qui donnera naissance aux métabolites intermédiaires des VLDL, les IDL. Progressivement, les IDL redonneront le restant d'apo C de types II et III aux HDL et deviendront alors des lipoprotéines de faible densité (LDL). L'avenir métabolique des LDL est fonction d'un récepteur spécifique situé à la surface des cellules du foie, le récepteur B/E. Ce dernier reconnaîtra la protéine B présente à la surface des LDL et dégradera cette dernière en

ses différentes composantes. Il est important de noter que la perte de lipides (TG et phospholipides) qui survient lors des échanges d'apolipoprotéines est catalysée par la lipase hépatique. Tout au cours de cette cascade de transformations des VLDL en LDL, les HDL transfèrent du cholestérol estérifié à toutes ces classes de particules en échange de molécules de TG. Ceci modifie graduellement la composition du noyau lipidique des lipoprotéines circulantes (Figure 2.2).

L'hydrolyse des TG à l'intérieur des chylomicrons s'effectue selon un processus comparable à celui des VLDL. Toutefois, en ce qui concerne le transfert des constituants de surface, il faut se rappeler que les chylomicrons contiennent en plus de l'apo AI (Glickman & Green, 1977). Similairement aux particules de LDL, les résidus de chylomicrons, grâce à leur apo E, seront reconnus par le récepteur LRP (*LDL receptor related protein*) situé à la surface des cellules hépatiques. Le résidu se fixera ainsi au récepteur et pénètrera dans la cellule hépatique où il sera dégradé en ses différents constituants. Ces derniers seront recyclés dans la synthèse de lipoprotéines (VLDL) ou éliminés. Enfin, lors de l'hydrolyse des TG par la LPL, les acides gras libérés traversent la paroi des capillaires sanguins et pénètrent dans les cellules où ils vont être, selon les cas, oxydés (apport énergétique pour les cellules musculaires et cardiaques) ou bien réestérifiés (formation de dépôts de TG dans les cellules adipeuses).

En résumé, la LPL joue un rôle essentiel dans l'assimilation des acides gras au niveau des tissus. De plus, par son action sur le catabolisme des VLDL et des chylomicrons, elle participe de manière très active à la modulation de la concentration en lipoprotéines circulantes.

2.3 Facteurs modulant l'activité de la LPL

2.3.1 Effet du sexe, de l'âge, de la territorialité du tissu adipeux et de l'obésité

Il est bien connu que la femme préménopausée présente une activité de la LPL supérieure à celle de l'homme au niveau des sites adipeux sous-cutanés abdominal et fémoral (Arner et al., 1991; Lithell et al., 1978; Pedersen et al., 1994; St-Amand et al., 1995). Par ailleurs, peu d'études se sont penchées sur les effets de l'âge au niveau de l'activité de la LPL adipeuse. En effet, l'activité de la LPL des sites adipeux abdominal, mammaire et fémoral a été mesurée uniquement chez des femmes pré et postménopausées (Rebuffé-Scrive et al., 1986). À ce titre, aucune différence régionale au niveau de l'activité enzymatique de la LPL de ces trois sites n'a été rapportée. Les résultats composant le Chapitre 10 ont également démontré l'absence de différence entre l'activité de la LPL des sites sous-cutanés abdominal et fémoral de femmes pré et postménopausées présentant des niveaux comparables d'obésité. De plus, tel que rapporté dans le Chapitre 8, l'activité de la LPL des sites adipeux sous-cutanés abdominal et fémoral d'hommes jeunes et moyennement âgés s'avère comparable.

Quant à l'effet de la territorialité, il convient de noter que l'activité de la LPL du site glutéo fémoral est plus marquée que celle du site abdominal chez la femme préménopausée (Arner et al., 1991; Lithell et al., 1978; Pedersen et al., 1994; Pouliot et al., 1991; Rebuffé-Scrive et al., 1985; St-Amand et al., 1995; Yost & Eckel, 1992). Chez l'homme, l'activité de cet enzyme apparaît plus élevée au niveau sous-cutané abdominal comparativement au site fémoral (Arner et al., 1991), bien que certaines études aient aussi rapporté l'absence de différence régionale (Rebuffé-Scrive et al., 1987; St-Amand et al., 1995). Contrairement aux données relatives aux sites sous-cutanés, les études ayant mesuré l'activité de la LPL des sites adipeux profonds sont moins nombreuses. Fried et Kral (1987) ont rapporté une activité de la LPL supérieure au niveau de la région sous-cutanée abdominale comparativement à l'omentum chez la femme atteinte d'obésité massive. Par ailleurs, d'autres études n'ont relevé aucune différence significative entre l'activité enzymatique de la LPL de l'omentum et celle de la région sous-

cutanée abdominale d'hommes et de femmes de poids normal ou sévèrement obèses (Mauriège et al., 1995a; Rebuffé-Scrive et al., 1989; Rebuffé-Scrive et al., 1990).

2.3.2 Effet des hormones

Parmi les hormones modulant l'activité de la LPL, l'insuline est l'une des plus importantes (Garfinkel et al., 1976). En effet, il a été montré que l'insuline augmentait l'activité de la LPL adipocytaire en accentuant la transcription du gène et le taux de synthèse de cet enzyme chez le rat (Ong et al., 1988). Ces résultats ont également été confirmés ultérieurement chez l'humain par l'étude d'échantillons de tissu adipeux (sous-cutané de la région abdominale) incubés dans un milieu de culture où la concentration d'insuline variait (Burton & Fried, 1992; Fried et al., 1993). Chez l'homme, l'augmentation de l'activité de la LPL adipocytaire est positivement corrélée à la dose d'insuline administrée (Eckel, 1989). Ces données confirment la contribution importante de l'insuline aux changements d'activité de la LPL observés lors des différentes conditions nutritionnelles mentionnées précédemment.

D'autres hormones exercent également un contrôle sur l'activité de la LPL du tissu adipeux. À cet égard, il a déjà été rapporté que des femmes atteintes du syndrome de Cushing, qui résulte notamment en de fortes concentrations plasmatiques de glucocorticoïdes, présentent une augmentation de l'accumulation de tissu adipeux sous-cutané abdominal et de l'activité de la LPL des adipocytes de cette région (Rebuffé-Scrive et al., 1988). De plus, il a été démontré qu'en présence de dexaméthasone, un glucocorticoïde synthétique, l'activité et le transcrit de la LPL sont légèrement augmentés au niveau des tissus sous-cutanés abdominal et omental à la fois chez l'homme et la femme (Fried et al., 1993). Au niveau de ces mêmes sites adipeux, il a aussi été démontré qu'en présence d'insuline et de dexaméthasone, l'activité et la transcription de la LPL sont stimulées de façon synergique. Des résultats similaires ont été rapportés alors que les échantillons de tissus adipeux (sous-cutanés abdominal et fémoral, omental) étaient incubés dans un milieu contenant cette fois de l'insuline et du cortisol (Ottosson et al., 1994).

La progestérone semble aussi promouvoir le stockage des graisses en stimulant l'activité de la LPL des adipocytes de la région glutéo fémorale (Lindberg et al., 1990; Lindberg et al., 1991; Xu & Björntorp, 1990). Par ailleurs, il a été démontré que l'administration de testostérone chez l'homme inhibe l'activité de la LPL des adipocytes sous-cutanés abdominaux alors qu'aucun effet n'a été observé au niveau des cellules adipeuses fémorales (Rebuffé-Scrive et al., 1991).

Finalement, mentionnons l'implication de l'hormone de croissance au niveau de la régulation de l'activité de la LPL adipocytaire. En effet, une étude menée chez des femmes obèses traitées à l'hormone de croissance pour une durée de 5 semaines a révélé une baisse d'environ 50% de l'activité de la LPL des tissus sous-cutanés abdominal et glutéal (Richelsen et al., 1994). Pour leur part, Ottosson et al. (1995) ont démontré en incubant des échantillons de tissu adipeux sous-cutané abdominal que l'hormone de croissance contrerégule les effets stimulants du cortisol sur l'activité de la LPL et ce, sans toutefois entraîner une diminution de la transcription simultanée de l'enzyme. Ces dernières observations suggèrent ainsi que l'inhibition de l'activité de la LPL par l'hormone de croissance pourrait survenir au niveau des mécanismes traductionnels et/ou posttraductionnels de l'enzyme.

En résumé, la LPL peut être soumise à de nombreuses actions hormonales qui ne sont pas encore toutes bien connues. Rappelons que l'action de ces hormones s'exerce essentiellement pendant la phase où la "particule" de LPL se trouve dans la cellule. Une fois secrétée hors de la cellule, la LPL échappe au contrôle hormonal potentiel.

2.3.3 Effet du jeûne ou d'un régime hypocalorique

L'activité de la LPL du tissu adipeux sous-cutané varie en fonction de l'état métabolique d'un individu. En effet, cette dernière augmente en situation postprandiale (Lithell et al., 1978) ainsi qu'après une surcharge glucidique (Taskinen & Nikkilä, 1981), alors qu'elle diminue en situation de jeûne (Lithell et al., 1978; Taskinen et al., 1981). L'existence de relations

positives entre l'activité de la LPL du tissu adipeux, la masse des adipocytes et l'indice de masse corporelle renforce l'hypothèse d'un rôle physiopathologique de la LPL dans le développement de l'obésité (Eckel, 1989). À cet égard, plusieurs groupes de recherche ont étudié l'impact d'une perte de poids sur l'activité de cet enzyme qui contribue en bonne partie au stockage des graisses du tissu adipeux.

Schwartz et Brunzell (1978) furent les premiers à observer que des individus caractérisés par une obésité réduite présentent une activité de la LPL du tissu adipeux sous-cutané supérieure à celle de sujets contrôles pairés pour une même proportion de masse adipeuse. Plus tard, ces derniers auteurs confirmèrent leurs résultats en rapportant qu'après perte de poids, l'activité de la LPL du tissu adipeux glutéal de sujets caractérisés par une obésité réduite est en moyenne 4 fois supérieure à son niveau basal (Schwartz & Brunzell, 1981). Au cours de ce protocole, 8 hommes obèses avaient subi une perte de poids moyenne de 16 kg via une diète liquide (600 kcal/jour) d'une durée variable. Plus précisément, le traitement prenait fin au moment où le sujet considérait qu'il ne serait plus en mesure de maigrir ou bien lorsqu'il présentait le désir d'arrêter la diète. Au cours de cette étude, il a été démontré que l'augmentation de l'activité de la LPL est inversement associée à la fraction de poids perdu au cours du traitement, suggérant ainsi que la LPL du tissu adipeux joue un rôle important au niveau du maintien de la masse adipeuse.

À la lumière de ces données, les auteurs ont proposé que la présence d'une activité basale élevée de la LPL chez les individus obèses et son augmentation après une perte pondérale peut constituer une partie des mécanismes favorisant le maintien d'une masse adipeuse élevée de l'individu obèse et peut également expliquer la prédisposition au regain de poids à laquelle les individus "post-obèses" sont confrontés. Ces mêmes hypothèses ont été étayées par l'intermédiaire d'une étude montrant qu'une perte de poids considérable (en moyenne 43 kg) d'individus massivement obèses entraîne une élévation à la fois de l'activité et de l'expression de la LPL adipeuse de la région sous-cutanée abdominale (Kern et al., 1990).

À l'inverse, d'autres études ont démontré un maintien (Sörbris et al., 1981) ou une baisse (Eckel & Yost, 1987; Rebuffé-Scrive et al., 1983; Reitman et al., 1982; Taskinen & Nikkilä,

1987) de l'activité de la LPL suite à une perte pondérale. Plusieurs hypothèses ont été avancées dans le but d'expliquer cette controverse. La valeur initiale de l'activité de la LPL semble notamment importante à considérer. En effet, il a été démontré que le changement d'activité de la LPL entre son niveau basal et suite à une perte de poids est inversement associé à l'activité basale de l'enzyme. Ainsi, un individu ayant une faible activité de la LPL avant la diète présenterait une forte activité enzymatique en situation d'obésité réduite et vice-versa (Eckel et al., 1987). Il semble pertinent de rappeler que l'activité de la LPL ainsi que son expression dans les régions adipeuses fémorale et glutéale sont généralement plus élevées qu'au niveau du dépôt sous-cutané abdominal chez les femmes préménopausées alors que l'inverse est observé chez l'homme (Arner et al., 1991; Rebuffé-Scrive et al., 1985).

La période de restriction énergétique et la quantité de poids perdu au cours de cette période peuvent aussi jouer un rôle non négligeable dans la variation d'activité de la LPL du tissu adipeux. En effet, les deux principales études (Kern et al., 1990; Schwartz et al., 1981) ayant entraîné des pertes de poids moyennes considérables (16 et 43 kg, respectivement) suite à une restriction énergétique font état d'une augmentation de l'activité de la LPL. Par ailleurs, les études ayant rapporté une diminution de l'activité de la LPL adipeuse soulignent des pertes de poids moyennes se situant entre 4 et 12 kg (Eckel et al., 1987; Rebuffé-Scrive et al., 1983; Taskinen et al., 1987). Ces dernières observations concordent avec les résultats présentés au Chapitre 6 qui traite de l'impact d'une perte pondérale modérée sur l'activité de la LPL des sites sous-cutanés abdominal et fémoral d'hommes et de femmes obèses.

2.3.4 Effet de l'exercice

Les études ayant traité des effets à court terme de l'exercice sur l'activité de la LPL rapportent une hausse de cette dernière. En effet, Taskinen et al. (1980) ont relevé une augmentation de l'activité de la LPL du site abdominal chez des sujets entraînés ayant parcouru 20 km à la course. Dans le même ordre d'idées, une augmentation significative de l'activité de l'enzyme du site abdominal chez des individus sédentaires suite à un exercice de 90 minutes (ergocycle,

88% de la fréquence cardiaque de réserve des sujets) a été observée (Savard et al., 1987). Le mécanisme responsable de cette hausse de l'activité de la LPL demeure toutefois inconnu.

En ce qui a trait aux effets à long terme de l'exercice, les résultats issus de la littérature actuelle sont un peu plus controversés. Une augmentation de l'activité de la LPL adipeuse a été rapportée chez des hommes de poids normal ayant effectué un programme d'entraînement d'une durée de 15 semaines à une intensité modérée (Peltonen et al., 1981). Ces résultats ont été confirmés par Stubbe et al. (1983) qui ont soumis 18 sujets à un entraînement de 6 semaines à une intensité de moyenne à forte. À l'inverse, un entraînement aérobique d'une durée de 24 semaines de faible intensité a entraîné une diminution de l'activité de la LPL des sites sous-cutanés abdominal et fémoral de femmes préménopausées obèses (Lamarche et al., 1993). Par ailleurs, la pratique d'exercices de nature aérobique d'une durée de 60 minutes réalisés à 60-75% de la VO_2max sur une période de 2 semaines n'a été associée à aucune variation de l'activité, de la masse et de l'ARN messager de la LPL du site sous-cutanée abdominal d'hommes sédentaires et de poids normal (Seip et al., 1995). Finalement, il convient de souligner qu'une période de désentraînement de 2 semaines chez des athlètes d'élite suffit pour entraîner une augmentation de l'ordre de 86% de l'activité de la LPL du site sous-cutané abdominal (Simsolo et al., 1993).

CHAPITRE 3

RÉGULATION DE LA LIPOLYSE DU TISSU ADIPEUX HUMAIN

Il est bien connu que la plupart des réserves d'énergie de l'organisme sont emmagasinées sous forme de triglycérides au niveau du tissu adipeux. La libération des acides gras libres (AGL) non estérifiés issus de la dégradation des triglycérides intraadipocytaires et libérés dans la circulation sanguine permet de subvenir aux besoins énergétiques de l'organisme et constitue une fonction très spécifique du tissu adipeux, la lipolyse. De façon plus précise, les AGL plasmatiques peuvent être captés rapidement par les hépatocytes où ils seront estérifiés et emmagasinés sous forme de triglycérides. Au niveau du foie, les AGL peuvent également servir de constituants pour la formation de lipoprotéines de très faible densité (*very low density lipoproteins*, VLDL) qui seront relarguées en circulation (Lewis, 1997). Finalement, les AGL peuvent être captés par les tissus périphériques, notamment les muscles, dans le but de répondre aux besoins énergétiques de l'organisme.

Au-delà de leur contribution essentielle à l'équilibre énergétique de l'organisme, les AGL peuvent avoir un rôle métabolique particulièrement délétère lorsqu'ils demeurent longtemps et en excès dans la circulation. En effet, certaines évidences suggèrent que les AGL peuvent être associés au développement de l'insulinorésistance et à l'apparition du diabète noninsulinodépendant chez des individus présentant une surcharge pondérale (Boden, 1997; Frayn et al., 1996; Randle et al., 1963).

En règle générale, les voies lipolytiques sont activées dans des conditions diverses telles que le jeûne et le stress de différentes origines (activité physique, exposition au froid, infection). La

mobilisation des lipides est notamment provoquée à très court terme par divers agents libérés au cours d'un stress (amines physiologiques, polypeptides lipolytiques), par des molécules à effet autocrine/paracrine (adénosine, prostaglandines), mais également à plus long terme par une baisse ou un déficit en insuline. Par ailleurs, il convient de souligner que l'hydrolyse des triglycérides est assurée par un ensemble d'enzymes et de récepteurs caractérisant l'adipocyte (Figure 3.1). Mentionnons notamment la lipase hormono-sensible (LHS) qui est responsable de l'hydrolyse des triglycérides emmagasinés dans l'adipocyte (Holm et al., 1989; Langin et al., 1996) dont l'activité est principalement régulée par l'activation des récepteurs β- et α_2-adrénergiques qui sont couplés à l'adénylyl cyclase par le biais de protéines activatrices (Gs) ou inhibitrices (Gi), contrôlant ainsi les niveaux intracellulaires d'AMPc. L'adénylyl cyclase catalyse l'hydrolyse de l'ATP en AMPc qui induit une protéine kinase (PKA) responsable de l'activation de la LHS. Par ailleurs, l'insuline agit sur un récepteur spécifique (Insulin-R) couplé à l'*insulin receptor substrate 1* (IRS-1), protéine cytosolique phosphorylable dont l'activation via les *mitogen activating protein* (MAP)-kinases se traduit par la stimulation d'une phosphodiestérase (PDE) qui dégrade l'AMPc en 5'-AMP (Lönnroth & Smith, 1986). Finalement, l'adénosine exerce un effet antilipolytique via le récepteur A1 (A1-R) qui est couplé également à une protéine régulatrice de type Gi (Ohisalo et al., 1984). Concernant la lipogenèse, les acides gras provenant de l'hydrolyse des TG contenus dans les lipoprotéines (VLDL, chylomicrons) sous l'action de la lipoprotéine lipase (LPL) sont transportés dans la cellule adipeuse. Le glucose pénètre dans l'adipocyte grâce à un transporteur (Glut 4) pour être transformé en glycérol-3-phosphate (G3P) qui formera les TG avec les AGL.

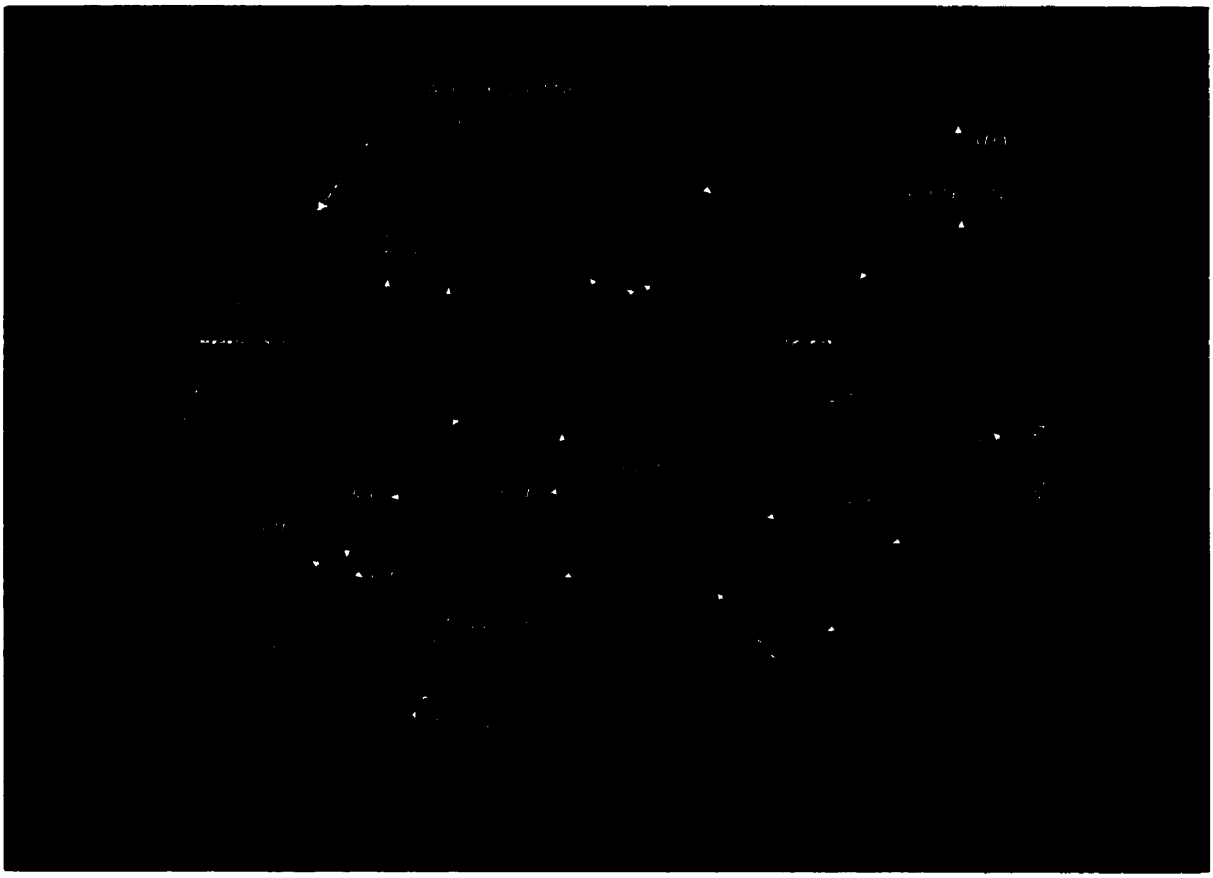


Figure 3.1 Caractéristiques des éléments de la membrane plasmique et des systèmes de transduction du signal de l'adipocyte impliqués dans le contrôle de la lipolyse et de la lipogenèse. AGL: acides gras libres; AR; récepteur adrénergique; Gs: protéines activatrices; Gi: protéines inhibitrices; LHS: lipase hormono-sensible; Insulin-R: récepteur à l'insuline; IRS-1: *insulin receptor substrate-1*; MAP: *mitogen activated protein*; PDE: phosphodiestérase; A1-R: récepteurs A1 de l'adénosine; ADA: adénosine désaminase; LPL: lipoprotéine lipase (LPL); Glut 4: transporteur de glucose 4; G3P: glycérol-3-phosphate. Adapté de Mauriège P (1996).

3.1 Les voies de stimulation de la lipolyse

Tel que mentionné précédemment, les concentrations d'AMPc intracellulaire modulées par l'activité de l'adénylyl cyclase participent activement au contrôle de la lipolyse. Plusieurs agents régulateurs de l'adénylyl cyclase peuvent exercer des effets soit stimulants ou inhibiteurs selon le niveau d'expression des récepteurs et le type de récepteurs recrutés. Le Tableau 3.1 résume les différents agents ou hormones qui stimulent la lipolyse au niveau de l'adipocyte humain. Tout d'abord, mentionnons les catécholamines qui sont des agents lipolytiques majeurs chez l'homme adulte. Ces hormones peuvent atteindre le tissu adipeux via la circulation sanguine (principalement l'adrénaline) ou via l'innervation sympathique (noradrénaline) (Lafontan & Berlan, 1993). Leur action lipolytique est médiée par le recrutement des récepteurs β-adrénergiques couplés à une protéine Gs qui enclenche la cascade lipolytique discutée précédemment. Il convient de souligner que trois types de récepteurs β-adrénergiques sont impliqués dans le contrôle positif de l'adénylyl cyclase de la cellule adipeuse (β1, β2, β3). Les sous-types β1 et β2 (Mauriège et al., 1988), majoritairement exprimés au niveau de l'adipocyte humain, ont plus d'affinité pour les amines physiologiques que le sous-type β3 (Lönnqvist et al., 1993). De plus, ils se désensibilisent plus facilement que le sous-type β3 lors d'une stimulation prolongée par un agoniste β-adrénergique (Lafontan et al., 1993). Quant aux récepteurs β3-adrénergiques, il semble que le rôle de ces derniers soit beaucoup plus limité au niveau de la cellule adipeuse de l'homme comparativement aux adipocytes bruns ou blancs de rongeurs (Tavernier et al., 1996).

D'autres hormones ont le potentiel d'exercer un effet lipolytique au niveau de l'adipocyte humain. Toutefois, leur effet respectif est moindre que celui des catécholamines et leur rôle physiologique ou pathophysiologique demeure incompris. Ces hormones incluent la *thyroid-stimulating hormone* (TSH) (Marcus et al., 1988) et l'hormone parathyroïdienne (Taniguchi et al., 1987). Certaines hormones telles que les hormones thyroïdiennes (Hellström et al., 1997) et l'hormone de croissance (Marcus et al., 1994) exercent des effets positifs également appelés effets permissifs à court terme sur la lipolyse induite par les catécholamines, notamment par

une augmentation des récepteurs β-adrénergiques de l'adipocyte. De plus, les stéroïdes sexuels entraînent certains effets sur la lipolyse induite par les catécholamines chez la femme (Heiling & Jensen, 1992). À ce titre, il a été montré qu'une administration orale d'oestradiol entraînait une diminution de la réponse lipolytique des adipocytes sous-cutanés du site abdominal chez des femmes postménopausées (Lindberg et al., 1990). Il a aussi été suggéré que les niveaux circulants de testostérone pouvaient moduler la lipolyse au niveau sous-cutané abdominal (Björntorp, 1994), bien que la normalisation des concentrations de testostérone chez des femmes affectées de polykystose ovarienne ne semble pas avoir d'influence sur la lipolyse (Ek et al., 1997). Chez l'homme adulte toutefois, l'administration orale de testostérone induit une augmentation de la réponse lipolytique adipocytaire au niveau sous-cutané abdominal mais non fémoral (Rebuffé-Scrive et al., 1991). Mentionnons également le facteur de nécrose tumorale alpha (TNF-α), une cytokine sécrétée par l'adipocyte humain qui a été reconnue pour son rôle activateur de la lipolyse (Hauner et al., 1995; Starnes et al., 1988) par son action inhibitrice des protéines Gi (Gasic et al., 1999). Finalement, des agents pharmacologiques peuvent aussi exercer des effets lipolytiques sans nécessairement activer un récepteur membranaire. En effet, la forskoline et certains de ses dérivés stimulent directement la sous-unité catalytique de l'adénylyl cyclase et induisent donc la production d'AMPc. Des dérivés de l'AMPc (AMPc dibutyrylé, chlorodiphényl-thio-AMPc, 8-bromo-AMPc) capables de franchir la membrane plasmatique et non hydrolysables par la phosphodiestérase-3B activent également la lipolyse par stimulation directe de la PKA (Weishaar 1995).

Tableau 3.1. Hormones ou agents qui stimulent la lipolyse au niveau de l'adipocyte humain et leurs principaux mécanismes d'action.

Hormones ou agents	**Mécanismes d'action**
Catécholamines	• Stimulation de la lipolyse via les récepteurs β-adrénergiques couplés aux protéines Gs
TSH	• Stimulation de la lipolyse via un récepteur couplé aux protéines Gs
Hormones parathyroïdiennes	• Stimulation sur la lipolyse via un récepteur couplé aux protéines Gs
Hormones thyroïdiennes	• Effet permissif sur la lipolyse induite par les catécholamines • "Uprégulation" des récepteurs β2-adrénergiques • Inhibition de la PDE-3
Hormone de croissance	• Effet permissif sur la lipolyse induite par les catécholamines
Facteur de nécrose tumorale α	• Stimulation de la lipolyse via une inhibition des protéines Gi

3.2 Les voies d'inhibition de la lipolyse

Au niveau de l'adipocyte humain, outre l'insuline, hormone antilipolytique majeure qui agit via l'activation de la phosphodiestérase-3, il existe quatre familles de récepteurs couplés négativement à l'adénylyl cyclase et donc capables d'engendrer une inhibition de la lipolyse lorsqu'ils sont stimulés par leurs agonistes spécifiques. Ce sont les récepteurs α_2-adrénergiques (Lafontan & Berlan, 1982), les récepteurs de l'adénosine de type A1 (Green et al., 1989; Larrouy et al., 1991), les récepteurs de prostaglandines E et plus particulièrement du type E2 (Richelsen et al., 1984) et les récepteurs du neuropeptideY (Valet et al., 1990)/peptide YY, de type PYY2 (Castan et al., 1993; Valet et al., 1990). Les mécanismes moléculaires conduisant à l'activation de Gi, à l'inhibition de l'adénylyl cyclase et aux effets antilipolytiques sont relativement bien compris et semblent identiques pour les quatre grandes familles de récepteurs inhibiteurs. On peut distinguer deux grandes composantes au niveau de l'inhibition de la lipolyse de l'adipocyte humain: 1) l'une constitutive, mettant en jeu des effecteurs autocrines/paracrines aux effets antilipolytiques puissants et peu modulables tels que l'adénosine et les prostaglandines (Arner, 1993); 2) l'autre, régulatrice, beaucoup plus modulable, qui implique des agents neuroendocrines tels que les catécholamines (Lafontan & Berlan, 1995), le neuropeptide Y et le peptide YY (Castan et al., 1993; Valet et al., 1990). Comparativement aux cellules adipeuses de rongeurs, l'efficacité de ces systèmes inhibiteurs est importante au niveau de l'adipocyte humain. Un résumé des principales hormones ou substances qui inhibent la lipolyse au niveau de l'adipocyte humain est présenté ci-dessous (Tableau 3.2).

Tableau 3.2. Hormones ou agents qui inhibent la lipolyse au niveau de l'adipocyte humain et leurs principaux mécanismes d'action.

Hormones ou agents	**Mécanismes d'action**
Insuline	• Inhibition de la lipolyse via la stimulation de la phosphodiesterase-3
Catécholamines	• Inhibition de la lipolyse via les récepteurs α_2-adrénergiques couplés aux protéines Gi
Adénosine	• Inhibition de la lipolyse via les protéines Gi • Agent autocrine et paracrine
Prostaglandines E1 et E2	• Inhibition de la lipolyse via les protéines Gi • Agent autocrine et paracrine
Neuropeptide Y	• Inhibition de la lipolyse via les protéines Gi
Peptide YY	• Inhibition de la lipolyse via les protéines Gi

À la lumière de la diversité des systèmes stimulateurs et inhibiteurs de la lipolyse, il est pertinent de se demander si et comment ces derniers systèmes peuvent être influencés selon l'âge et le sexe d'un individu. Il est également intéressant de s'attarder à la prédominance des systèmes stimulateurs et inhibiteurs selon la territorialité du tissu adipeux et le degré d'obésité d'un individu considéré ainsi qu'à la réponse à divers stress tels que la perte de poids et l'exercice. Ces volets sont considérés au cours des lignes suivantes.

3.3 Facteurs modulant les voies de stimulation ou d'inhibition de la lipolyse

3.3.1 Effet du sexe

En règle générale, la réponse lipolytique aux catécholamines des adipocytes de la région sous-cutanée abdominale des femmes préménopausées est supérieure à celle des adipocytes de l'homme (Wahrenberg et al., 1989). Cette différence sexuelle s'explique par une densité de récepteurs α_2-adrénergiques plus prononcée au niveau des adipocytes sous-cutanés abdominaux de l'homme (Wahrenberg et al., 1989). À l'inverse, les adipocytes glutéo fémoraux de la femme préménopausée présentent une densité de récepteurs α_2-adrénergiques plus marquée que ceux de l'homme, ce qui explique notamment la réponse lipolytique moindre aux catécholamines des ces adipocytes par rapport à ceux de l'homme (Richelsen, 1986; Wahrenberg et al., 1989). Il semble important de noter que les différences sexuelles mentionnées précédemment sont issues d'échantillons de sujets non-obèses et qu'aucune étude n'a pour le moment vérifié les différences sexuelles possibles au niveau des sites adipeux profonds de cette population.

Peu d'études ont également vérifié l'existence de différences sexuelles au niveau lipolytique des divers dépôts adipeux de sujets obèses. À ce titre, le Chapitre 6 de cette thèse réfère à l'étude du métabolisme des tissus adipeux sous-cutanés abdominal et fémoral d'hommes et de femmes présentant une surcharge corporelle. Brièvement, nous n'avons rapporté aucune différence sexuelle de la capacité lipolytique induite par les catécholamines ainsi que de la réponse antilipolytique à un agoniste α_2-adrénergique au niveau des deux dépôts étudiés. Par ailleurs, Lönnqvist et al. (1997) ont rapporté une capacité lipolytique à la noradrénaline significativement plus marquée au niveau d'adipocytes viscéraux d'hommes en comparaison à ceux de femmes obèses. Cette dernière observation semble toutefois être expliquée par la présence de cellules adipeuses viscérales hypertrophiées chez l'homme obèse.

L'ensemble de ces résultats suggère qu'il existe certes des différences sexuelles au niveau de la capacité lipolytique de l'adipocyte notamment chez l'individu non obèse. Toutefois, à la lumière des résultats observés chez la personne présentant une surcharge pondérale, il semble pertinent de croire que ces différences sexuelles soient moins marquées.

3.3.2 Effet de l'âge

Les études *in vitro* suggèrent que la mobilisation des triglycérides du tissu adipeux induite par les catécholamines varie en fonction de l'âge d'un individu. En effet, peu après la naissance, l'activité lipolytique est relativement faible et augmente graduellement pour atteindre sa capacité maximale vers l'âge de 2 ans (Marcus et al., 1987). La faible réponse lipolytique des adipocytes d'enfants s'explique en partie par le fait qu'ils possèdent de petites cellules adipeuses caractérisées par une forte réponse α_2-adrénergique (Marcus et al., 1987; Marcus et al., 1989; Rosenbaum et al., 1991). La principale hormone régulant la lipolyse du tissu adipeux lors de l'enfance est la TSH (*thyroid stimulating hormone*) (Marcus et al., 1988). Bien que les concentrations plasmatiques thyroïdiennes diminuent progressivement avec l'âge pour laisser place aux effets lipolytiques des catécholamines, une faible induction de la lipolyse par les hormones thyroïdiennes persiste à l'âge adulte. Par ailleurs, quelques études ont rapporté une relation inverse entre l'activation de la lipolyse par les catécholamines et l'âge des individus, suggérant ainsi une altération de la réponse lipolytique des cellules adipeuses lors du vieillissement (James et al., 1971; Ostman et al., 1969). Lönnqvist et al. (1990) rapportaient plus récemment que l'altération de la réponse lipolytique aux catécholamines des cellules adipeuses sous-cutanées abdominales d'hommes âgés résultait possiblement d'un défaut du complexe protéine kinase A-lipase hormono-sensible. Ces affirmations étaient étayées par le fait que les agents lipolytiques utilisés entraînaient une réponse lipolytique de l'ordre de 50% inférieure chez les individus âgés en comparaison à des sujets jeunes malgré que la densité et l'affinité des récepteurs α_2- et β-adrénergiques étaient similaires entre les deux groupes étudiés. Ceci concorde avec les résultats présentés dans le Chapitre 8 montrant une diminution significative de la capacité lipolytique des adipocytes sous-cutanés abdominaux et fémoraux

d'individus âgés comparativement à ceux de sujets jeunes et ce, bien que les deux groupes présentent une masse grasse identique. Pour leur part, les effets du vieillisement sur la lipolyse du tissu adipeux de la femme ont notamment été étudiés en comparant des femmes pré- vs postménopausées. À ce titre, la réponse lipolytique à la noradrénaline d'adipocytes du site sous-cutané abdominal est plus faible chez la femme postménopausée que chez la femme jeune (Rebuffé-Scrive et al., 1986). Curieusement, la réponse lipolytique d'adipocytes du site sous-cutané abdominal de femmes postménopausées présentant une accumulation importante de tissu adipeux au niveau abdominal apparaît augmentée en comparaison à celle des femmes postménauposées ayant une accumulation adipeuse plutôt de type glutéo fémoral (Nicklas et al., 1996). Ces observations tendent à suggérer que la distribution régionale du tissu adipeux module la réponse lipolytique de l'adipocyte sous-cutané. À la lumière de ces résultats, nous avons étudié la lipolyse des tissus adipeux sous-cutanés abdominal et fémoral de femmes pré et postménopausées présentant une distribution régionale similaire de tissu adipeux. Nos résultats ont montré que, malgré un état endocrinien différent, des femmes pré ou postménopausées caractérisées par une distribution régionale similaire du tissu adipeux présentent un profil lipolytique adipocytaire comparable (Chapitre 10).

En résumé, il convient de dire qu'immédiatement après la naissance, l'activité lipolytique de l'enfant est faible et augmente pour atteindre une activité maximale aux environs de 2 ans. Du stade enfant à adulte, l'activité lipolytique demeure stable et décline au gré des années, notamment chez l'homme. En ce qui a trait aux données chez la femme, il semble qu'il y ait aussi une altération de l'activité lipolytique reliée avec l'âge, bien que nos travaux suggèrent qu'après avoir considéré la distribution régionale du tissu adipeux, cette altération de l'activité lipolytique du tissu adipeux ne persiste plus.

3.3.3 Effet de la territorialité du tissu adipeux et de l'obésité

Les mécanismes mis en jeu dans le contrôle de la lipolyse varient grandement selon la localisation anatomique considérée, confèrant donc au tissu adipeux une activité métabolique hétérogène. Chez l'homme et la femme, il convient de dire que l'activité lipolytique en réponse aux catécholamines décroît selon l'ordre suivant: adipocytes viscéraux, adipocytes du site sous-cutané abdominal et finalement ceux du site fémoral (Mauriège et al., 1987). La première explication pour ces différences réfère à la "balance fonctionnelle" α_2/β adrénergique. Tel que présenté à la Figure 3.2, les cellules adipeuses bien équipées en récepteurs β-adrénergiques et plus particulièrement de type β3 (tissu viscéral) possèdent un rapport α_2/β faible conduisant ainsi à une réponse à l'adrénaline strictement lipolytique (Lönnqvist et al., 1993; Lönnqvist et al., 1997; Mauriège et al., 1987; Ostman et al., 1979). À l'opposé, les tissus richement équipés en récepteurs α_2-adrénergiques (tissus sous-cutanés) présentent une réponse à l'adrénaline majoritairement antilipolytique (tissu glutéo fémoral) ou biphasique: antilipolytique à faibles concentrations et lipolytique à fortes concentrations (tissu abdominal) (Mauriège et al., 1987; Ostman et al., 1979). L'effet antilipolytique moindre de l'insuline observé au niveau des adipocytes du site viscéral en comparaison à ceux du site sous-cutané abdominal est une autre explication de la capacité lipolytique marquée des adipocytes viscéraux (Bolinder et al., 1983; Mauriège et al., 1995a; Zierath et al., 1998).

Il convient de souligner l'influence du degré d'obésité des sujets sur les variations régionales de l'activité lipolytique et ce, tant chez l'homme que chez la femme préménopausée. Chez l'homme, l'adrénaline se révèle strictement lipolytique sur les adipocytes sous-cutanés abdominaux d'individus non obèses alors qu'elle provoque une inhibition puis une stimulation de la lipolyse chez des sujets caractérisés par un surplus de poids (Mauriège et al., 1991). De plus, l'individu non obèse ne présente aucune différence régionale au niveau de la réponse lipolytique à la noradrénaline des adipocytes sous-cutanés abdominaux et viscéraux, alors que la réponse lipolytique à la noradrénaline des adipocytes viscéraux est augmentée comparativement à celle des adipocytes de la région sous-cutanée abdominale chez l'homme obèse (Hoffstedt et al., 1997). Par ailleurs, les différences dans le contrôle de la

lipomobilisation en réponse aux catécholamines des adipocytes des sites sous-cutanés abdominal et fémoral entre femmes préménopausées obèses et non obèses impliquent des changements de la composante α_2-adrénergique ainsi que des variations relatives aux activités enzymatiques de l'adénylyl cyclase, de la PDE et/ou de la lipase hormono-sensible (Mauriège et al., 1995b). En effet, les femmes dont la masse adipeuse totale est la plus élevée montrent une inhibition α_2-adrénergique de la lipolyse plus prononcée. De plus, la réponse lipolytique aux agents agissant par des voies indépendantes des récepteurs (AMPc dibutyrylé, forskoline et théophylline) est plus faible au niveau des adipocytes abdominaux et fémoraux de la femme obèse. Ces obervations confirment l'hypothèse selon laquelle une déficience dans l'activité de la lipase hormono-sensible serait responsable de la diminution de la réponse lipolytique du tissu adipeux aux hormones physiologiques observée chez des femmes préménopausées caractérisées par une obésité abdominale (Reynisdottir et al., 1994).

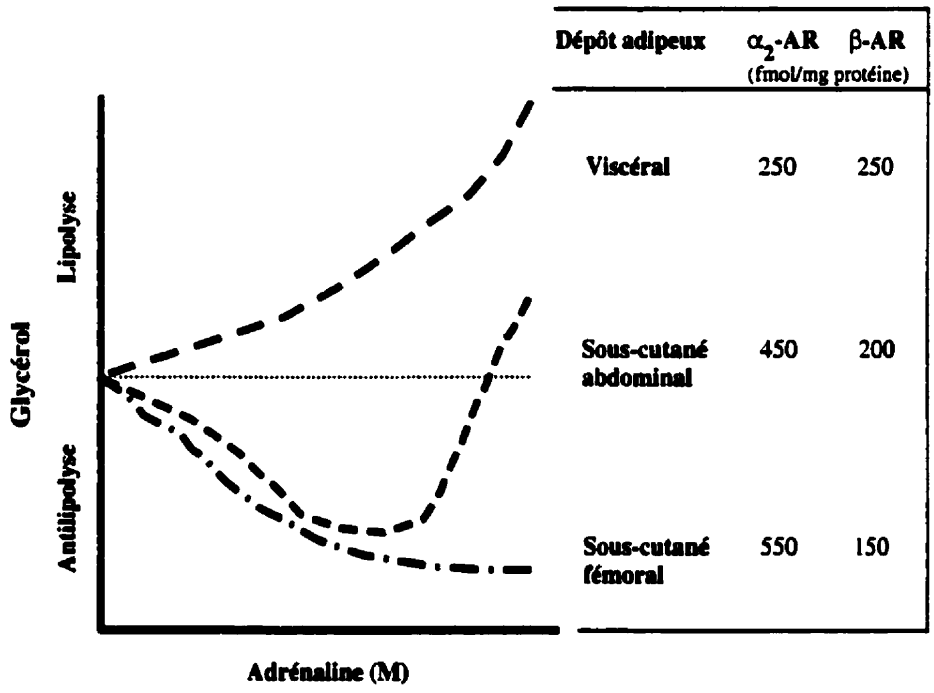


Figure 3.2 Effets métaboliques de l'adrénaline sur l'adipocyte et répartition des récepteurs adrénergiques (AR) en fonction de la localisation anatomique du tissu adipeux blanc. Adapté de Mauriège et al. (1987).

3.3.4 Effet du jeûne ou d'un régime hypocalorique

Le tissu adipeux constitue une réserve d'énergie importante pour l'organisme. Lors d'une privation énergétique à court ou à long terme telle qu'engendrée par des périodes de jeûne ou des régimes à faible contenu énergétique, certains processus métaboliques favorables à l'épargne du glucose et des acides aminés et à l'utilisation des lipides surviennent. Mentionnons notamment une diminution des concentrations plasmatiques d'insuline et une augmentation des niveaux de catécholamines circulantes (Coppack et al., 1994). La mobilisation des triglycérides du tissu adipeux s'avère ainsi une réponse adaptative au jeûne ou à la restriction calorique puisqu'elle résulte des effets antilipolytique de l'insuline et lipolytique des catécholamines sur le tissu adipeux. Bien que ces observations aient été confirmées par des études *in vivo* du tissu adipeux par la technique de différence artério-veineuse (Samra et al., 1996), elles semblent être en contradiction avec des études *in vitro* menées sur des cellules adipeuses isolées. En effet, suite à une période de jeûne de 7 jours, l'action lipolytique d'agents stimulateurs β-adrénergiques sur les adipocytes sous-cutanés de sujets obèses n'apparaît pas modifiée au niveau du site abdominal, voire diminuée au niveau du tissu fémoral (Ostman et al., 1984).

Certaines controverses sont aussi observées en ce qui a trait aux études qui ont vérifié les effets d'une restriction calorique sur la régulation de la lipolyse d'adipocytes isolés. Plusieurs auteurs ont rapporté une augmentation de la lipolyse basale en réponse à un programme de perte de poids (Berlan et al., 1981; Crampes et al., 1989; Hellström et al., 1996b; Kather et al., 1985b; Stich et al., 1997) alors que d'autres ont observé une diminution marquée de cette dernière (Reynisdottir et al., 1995). Autant de divergences existent quant à l'effet d'une perte de poids induite par une restriction calorique au niveau de la lipolyse stimulée par les catécholamines. En effet, certains relatent une diminution de la réponse lipolytique aux catécholamines suite à une restriction énergétique sévère (300 kcal/jour) d'une durée de quatre semaines (Kather et al., 1985b), alors que d'autres n'ont relevé aucun effet en réponse à une restriction calorique quasi-similaire (Crampes et al., 1989; Hellström et al., 1996a). Cette divergence de résultats dans la littérature peut être expliquée en partie par 1) la sévérité et la durée du régime; 2) le degré d'obésité des sujets étudiés; 3) la quantité de poids perdu lors de

la restriction calorique; 4) le moment auquel les échantillons de tissu adipeux ont été prélevés; 5) et le site de prélèvement. Le Tableau 3.3 qui suit résume la majorité des études ayant vérifié l'effet d'une perte pondérale sur la lipolyse de cellules adipeuses isolées en tenant compte des points précédents.

Tableau 3.3 Bilan des études ayant vérifié l'effet d'une restriction calorique sur la lipolyse de cellules adipeuses isolés.

Auteurs	Sujets	Traitement	Résultats
Berlan et al. (1981)	7 femmes obèses	• Diète (800-1000 kcal/j), 2 sem. • Perte de poids moyenne = 4.9 kg • Site sc abd avant et après diète	⇑ lipolyse basale ⇑ effet antilipolytique adrénaline = effet lipolytique isoprénaline
Rozen et al. (1984)	19 femmes obèses	•Diète (220 kcal/j), 3 sem. • Perte de poids moyenne = 7.1 kg • Site sc abd avant et après diète	⇑ lipolyse basale ⇑ effet antilipolytique clonidine = effet lipolytique isoprénaline
Kather et al. (1985b)	15 obèses (9 F, 6 H)	•Diète (300 kcal/j), 4 sem. • Perte de poids moyenne = 10 kg • Site sc abd avant et après diète	⇑ lipolyse basale = effet antilipolytique clonidine = effet lipolytique isoprénaline
Kather et al. (1985a)	17 obèses (9 F, 8 H)	• Diète (300 kcal/j), 4 sem. • Perte de poids moyenne = 10 kg • Site sc abd avant et après diète	⇑ lipolyse basale = sensibilité N6-phenylisopropyladenosine et prostaglandine E2
Crampes et al. (1989)	11 femmes obèses	• Diète (1000-1200 kcal/j), 3 sem. • Perte de poids moyenne = 7 kg • Site sc abd avant et après diète	⇑ lipolyse basale = effet antilipolytique adrénaline = effet lipolytique isoprénaline ⇑ récepteurs α_2-adrénergiques
Presta et al. (1990)	18 femmes obèses	• Diète (840 kcal/j), jusqu'à ce que 15% de perte de poids initial • Perte de poids moyenne = 4 kg/m^2 • Site sc abd avant et après diète	⇑ lipolyse basale = effet lipolytique isoprénaline

Légende: ⇑: augmentation; ⇓: diminution; =: aucune variation; sem.: semaines; sc: sous-cutané; F: femme; H: homme; IMC: indice de masse corporelle; LHS; lipase hormono-sensible; Abd: abdominal; Fem: fémoral; Glut: glutéal.

Tableau 3.3 (suite)

Auteurs	Sujets	Traitement	Résultats
Reynisdottir et al. (1995)	14 femmes obèses (profil androïde)	• Diète (400 kcal/j), 8-12 sem. • Perte de l'IMC moyen = 6 kg/m^2 • Site sc abd avant et après diète	⇓ lipolyse basale ⇓ LHS = effet antilipolytique clonidine = effet lipolytique isoprénaline ⇑ sensibilité lipolytique noradrénaline
Hellström et al. (1996b)	9 femmes obèses	• Diète (400 kcal/j), 4 sem. • Perte de l'IMC moyen = 4 kg/m2 • Site sc abd avant et après diète	⇑ lipolyse basale = LHS = effet antilipolytique UK-14304 = effet lipolytique isoprénaline, dobutamine, terbutaline, forskoline, DcAMP ⇑ sensibilité noradrénaline ⇑ effet antilipolytique et sensibilité insuline
Stich et al. (1997)	8 femmes obèses	• Diète (383 kcal/j), 4 sem. • Perte de poids moyenne = 9 kg • Site sc abd avant et après diète	⇑ lipolyse basale ⇑ activité et protéine LHS ⇑ effet lipolytique isoprénaline
Mauriège et al. (1999b)	14 hommes et 18 femmes obèses	• Diète (restriction 500-800 kcal/j de la diète normale), 15 sem. • Perte de poids moyenne = 10 kg • Sites sc abd et fem avant et 4-6 sem. après diète	= lipolyse basale = activité et protéine LHS = effet antilipolytique UK-14304 et adrénaline ⇑ sensibilité UK-14304 (H abd; F abd + fem) = effet lipolytique isoprénaline ⇑ sensibilité isoprénaline

3.3.5 Effet de l'exercice

Certaines adaptations propres aux tissus adipeux et musculaire squelettique sont à l'origine d'une meilleure prédisposition à utiliser les lipides comme substrat énergétique chez la personne entraînée. Au début d'un effort physique de longue durée, le muscle squelettique utilise préférentiellement ses réserves de glycogène afin de répondre à la demande énergétique. Puisque cette réserve de substrat intramusculaire est limitée, il devient nécessaire que la mobilisation des triglycérides contenus dans les adipocytes augmente afin de procurer au muscle l'énergie nécessaire à la réalisation d'un effort de longue durée. À cet égard, un certain nombre de changements hormonaux surviennent lors d'un exercice de nature aérobique et contribuent de façon importante à la régulation de la mobilisation des triglycérides emmagasinés au niveau du tissu adipeux. En effet, un effort physique s'accompagne généralement d'une diminution des concentrations plasmatiques d'insuline et d'une augmentation des niveaux sanguins de noradrénaline, de cortisol et d'hormone de croissance, favorisant ainsi la stimulation de la lipolyse du tissu adipeux (Hodgetts et al., 1991).

Les effets de l'exercice sur le métabolisme du tissu adipeux ont majoritairement été vérifiés par l'intermédiaire d'études *in vitro*. Après avoir effectué une biopsie adipeuse au niveau fémoral de sujets sains avant et après que ces derniers aient réalisé un effort sur ergocycle d'une durée de 30 minutes à 60% de leur capacité maximale de consommation d'oxygène (VO_2max), Wahrenberg et al. (1987) ont observé une augmentation de la réponse lipolytique à la noradrénaline des adipocytes fémoraux. Ces observations concordent bien avec celles de Savard et al. (1987) qui ont rapporté une augmentation de la réponse lipolytique à l'adrénaline d'adipocytes prélevés dans la région sous-cutanée abdominale chez des sujets ayant effectué un effort de 90 minutes sur ergocycle à 88% de leur fréquence cardiaque (Fc) maximale. Il a également été démontré dans notre laboratoire que la pratique régulière d'exercices (20 semaines, 4-5 fois/semaine à raison de 40 min/séance à une intensité de 80% de la Fc de réserve) entraînait une réponse lipolytique accrue à l'adrénaline au niveau d'adipocytes des dépôts adipeux sous-cutanés abdominal et fémoral d'hommes et de femmes (Després et al.,

1984a; Després et al., 1984b). Récemment, les effets d'un programme d'exercices d'endurance (12 semaines, 4 fois/semaine à raison de 40 min/séance à une intensité de 60% de la Fc de réserve) au niveau de la réponse lipolytique d'adipocytes du site sous-cutané abdominal d'individus obèses furent rapportés (de Glisezinski et al., 1998). En réponse à l'entraînement et ce, malgré l'absence de variation du poids corporel lors du programme, les effets lipolytiques de l'adrénaline, de l'isoprénaline et de la dobutamine furent augmentés. Par ailleurs, une diminution des effets antilipolytiques de l'insuline a été rapportée. Ce même groupe confirmait récemment que la réponse lipolytique à l'isoprénaline mesurée par microdialyse au niveau du site sous-cutané abdominal était augmentée suite à l'entraînement (Stich et al., 1999).

Enfin, l'ensemble de ces observations suggère qu'à court ou à long terme, l'exercice entraîne une augmentation de la réponse lipolytique aux catécholamines favorisant ainsi une élévation des concentrations d'acides gras libres en circulation, lesquels pourront être oxydés par le muscle squelettique.

CHAPITRE 4

L'OBÉSITÉ ET SES EFFETS MÉTABOLIQUES

Dans le chapitre précédent, nous avons vu que le tissu adipeux ne présentait pas les mêmes facultés lipomobilisatrices dépendamment de la localisation anatomique du dépôt et du sexe des individus. Au cours de la dernière décennie, de nombreuses études ont également confirmé l'hétérogénéité de l'obésité quant à son incidence au niveau des complications métaboliques (Björntorp, 1991b; Després, 1991; Kissebah et al., 1994), confirmant ainsi les observations cliniques du Professeur Jean Vague (1956) qui soulignait, à l'époque, que les individus qui accumulent la graisse préférentiellement au niveau abdominal sont plus susceptibles de développer des pathologies coronariennes et un diabète de type II que ceux dont le tissu adipeux se localise dans les régions glutéo fémorales (Figure 4.1).

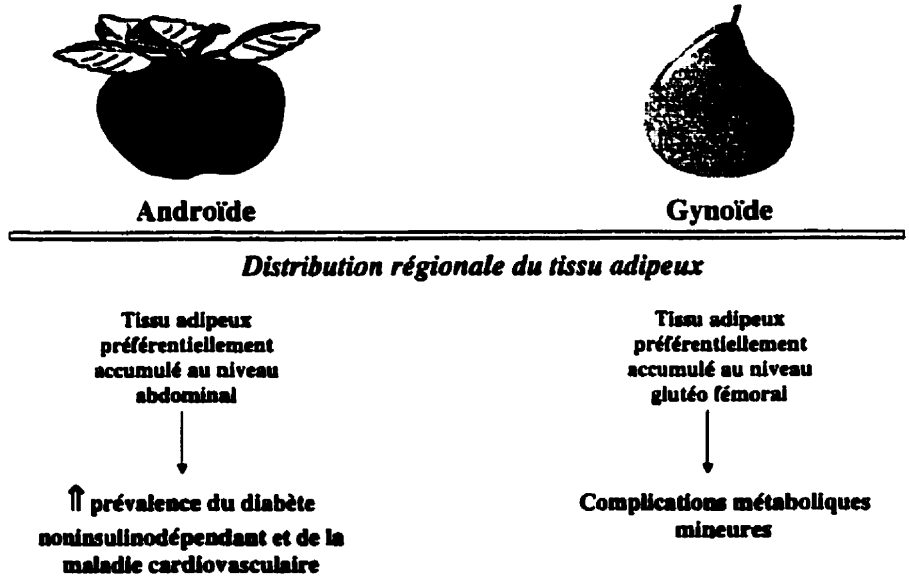


Figure 4.1 Risque de complications métaboliques relatif au profil de distribution du tissu adipeux d'un individu. Selon Vague J (1956).

Avec le développement récent des techniques d'imagerie, nous pouvons maintenant mesurer d'une façon bien précise la quantité de tissu adipeux au niveau abdominal, et, plus particulièrement, distinguer le dépôt adipeux intraabdominal (ou viscéral) du tissu graisseux sous-cutané. À cet égard, plusieurs études issues de notre laboratoire ont rapporté que les concentrations élevées de triglycérides (TG) et d'apolipoprotéine B, de faibles taux de cholestérol lié aux lipoprotéines de haute densité (HDL-C) ainsi que l'intolérance au glucose et l'hyperinsulinémie à jeun de même que suite à une surcharge orale glucidique de 75g de glucose caractérisaient les individus présentant une accumulation élevée de tissu adipeux viscéral (Després et al., 1989a; Després et al., 1990; Pouliot et al., 1992). Des analyses multivariées ont aussi révélé que la quantité de tissu adipeux viscéral constituait la variable la plus étroitement reliée à différents rapports de lipoprotéines utilisés dans la prédiction du risque de maladies cardiovasculaires (Després et al., 1989a). Afin de départager les contributions indépendantes de l'obésité en soi de l'excédent de tissu adipeux viscéral, deux groupes de sujets obèses de même âge et ayant une adiposité globale similaire (déterminée par la technique de pesée hydrostatique) mais caractérisés par une quantité élevée ou faible de tissu adipeux viscéral ont été comparés (Després et al., 1990; Pouliot et al., 1992). Les

résultats de ces travaux ont démontré qu'en l'absence d'une accumulation importante de graisse viscérale, l'obésité en soi ne s'avérait pas associée à un profil dyslipidémique. À l'inverse, l'obésité combinée à une accumulation importante de tissu adipeux viscéral était reliée à de nombreuses complications métaboliques. Par ailleurs, il convient de souligner que de récentes études ont rapporté que l'accumulation de tissu sous-cutané abdominal contribue aussi à la détérioration du profil métabolique des individus, notamment en affectant la sensibilité à l'insuline évaluée par clamp euglycémique hyperinsulinémique (Abate et al., 1995; Goodpaster et al., 1997; Misra et al., 1997). À la lumière de ces observations, nous avons évalué (Chapitre 5) la contribution respective de l'accumulation du tissu adipeux viscéral et de l'hypertrophie adipocytaire des sites sous-cutanés abdominal et fémoral sur le profil métabolique d'hommes et de femmes. Les résultats de ces travaux suggèrent qu'une accumulation importante de tissu adipeux au niveau viscéral ainsi que l'hypertrophie des adipocytes sous-cutanés abdominaux chez l'homme s'avèrent des variables importantes à considérer dans l'étiologie du syndrome dyslipidémique et/ou d'insulinorésistance.

Jusqu'à ce jour, le principal mécanisme impliqué dans la relation entre l'obésité abdominale et les complications métaboliques qui en découlent réfère à l'activité lipolytique élevée des cellules adipeuses intraabdominales. En effet, cette activité lipolytique marquée pourrait contribuer, via la circulation porte, à exposer le foie à des concentrations élevées d'acides gras libres (AGL) (Hellmér et al., 1992; Hoffstedt et al., 1997; Mauriège et al., 1987; Mauriège et al., 1995a), diminuant ainsi l'extraction hépatique d'insuline et conduisant au développement de l'hyperinsulinémie systémique (Hennes et al., 1990; Svedberg et al., 1990). L'élévation des AGL en circulation favoriserait également une production hépatique du glucose et d'apolipoprotéine B (Sniderman & Cianflone, 1995) ainsi qu'une augmentation de la synthèse des TG au niveau du foie (Byrne et al., 1991). Mauriège et al. (1999a) ont également montré qu'une augmentation de la sensibilité lipolytique β2-adrénergique des adipocytes sous-cutanés abdominaux est associée à une diminution de la sensibilité à l'insuline chez des hommes, suggérant ainsi un rôle potentiel de l'activité lipolytique du tissu adipeux sous-cutané abdominal dans la détérioration du profil métabolique chez l'humain. Il convient de souligner que certaines altérations du profil métabolique apparaissent indépendantes du métabolisme des AGL. À ce titre, les modifications au niveau des concentrations de stéroïdes sexuels qui ont

été rapportées chez des individus présentant une obésité abdominale pourraient être mis en cause dans le développement des désordres du profil métabolique (Björntorp, 1995; Tchernof et al., 1996). Plus précisément, les femmes caractérisées par une accumulation excessive de tissu adipeux abdominal montrent des concentrations élevées d'androgènes libres et de faible taux de SHBG (*sex hormone binding globulin*) (Evans et al., 1983; Peiris et al., 1987; Tchernof et al., 1999). À l'inverse, l'obésité abdominale chez l'homme est plutôt associée à de faibles taux de testostérone et de SHBG (Seidell, 1997; Tchernof et al., 1997). Il a d'ailleurs été rapporté que ces changements dans les taux de stéroïdes circulants agissent à titre de prédicteurs indépendants des dyslipoprotéinémies et de l'état d'insulinorésistance caractérisant l'obésité abdominale (Peiris et al., 1987; Tchernof et al., 1997). Ces observations suggèrent donc que les changements des taux de stéroïdes sexuels pourraient contribuer au développement des désordres métaboliques reliés à l'obésité viscérale.

L'insulinorésistance pourrait également être impliquée dans les changements du métabolisme des lipides et des lipoprotéines notés chez les patients caractérisés par un excès de tissu adipeux viscéral. En effet, l'insulinorésistance mesurée *in vivo* (i.e. surcharge orale glucidique) a été associée à une faible activité de la lipoprotéine lipase plasmatique et à une activité élevée de la lipase hépatique (Després et al., 1989b). Ces changements dans l'activité des lipases pourraient contribuer à diminuer le catabolisme des lipoprotéines riches en TG et à augmenter la conversion des HDL_2 en HDL_3, expliquant ainsi les concentrations élevées de TG et les faibles taux d'HDL_2-C observés dans l'obésité abdominale (Després et al., 1989b). L'insulinorésistance apparaît ainsi comme une composante importante des altérations métaboliques de l'individu caractérisé par un excès de tissu adipeux viscéral.

Outre les altérations du profil métabolique en situation de jeûne qui sont associées à l'augmentation du risque de la maladie coronarienne chez l'individu obèse, les anomalies au niveau du métabolisme des lipoprotéines en situation postprandiale peuvent aussi être à l'origine de l'athérosclérose (Zilversmit, 1979). Selon un processus normal après l'ingestion d'un repas, l'activité de la LHS du tissu adipeux est rapidement supprimée par l'action de l'insuline alors que le processus d'estérification des AGL à l'intérieur du tissu adipeux est favorisé par l'action de la LPL (Frayn et al., 1994). Ainsi, la capacité des tissus à réestérifier

les AGL provenant de la circulation et la capacité de l'insuline à inhiber la lipolyse au niveau du tissu adipeux sont des déterminants importants de la "clairance" des AGL plasmatiques en situation postprandiale. Ces deux caractéristiques sont altérées chez l'individu obèse et expliquent notamment leurs concentrations élevées d'AGL et de lipoprotéines riches en TG après un repas (Coppack et al., 1992; Couillard et al., 1998; Roust & Jensen, 1993).

Par ailleurs, plusieurs études suggèrent que l'obésité peut être associée à une moins bonne capacité à oxyder les graisses, contribuant ainsi au maintien et/ou au développement des réserves corporelles (Campbell et al., 1994; Colberg et al., 1995; Zurlo et al., 1990b). Il convient de noter que ces évidences sont à la base des travaux de Flatt (1987) qui offrent une base conceptuelle voulant que l'équilibre lipidique représente la composante du système de régulation du bilan d'énergie qui est maintenue avec le moins de précision. Cette observation est en accord avec le fait qu'un régime alimentaire riche en lipides est associé à une augmentation de l'apport énergétique spontané (Lissner et al., 1987; Tremblay et al., 1991; Tremblay et al., 1989) pouvant être expliquée par la haute densité énergétique des lipides (Stubbs et al., 1995) et/ou par le faible niveau de satiété généré par la prise d'aliments riches en lipides (Blundell et al., 1993; Lawton et al., 1993). Chez l'individu obèse (Thomas et al., 1992) ou "post-obèse" (Astrup et al., 1994), l'augmentation de l'oxydation lipidique s'avère faible en réponse à une diète riche en lipides et rend ce dernier vulnérable à la prise de poids corporel à long terme (Zurlo et al., 1990b). Étant donné le rôle important que joue le muscle squelettique dans l'oxydation des substrats énergétiques (Zurlo et al., 1990a), certains travaux ont porté leur attention sur le métabolisme du muscle squelettique chez la personne caractérisée par un excès de poids corporel. Ferraro et al. (1993) ont notamment montré qu'une faible activité de la LPL du muscle squelettique est associée à une diminution de l'oxydation des graisses. De plus, certains travaux ont mis en évidence que l'augmentation du pourcentage de graisse est associée à une diminution de la capacité oxydative musculaire squelettique (Simoneau & Bouchard, 1995a; Simoneau et al., 1995b). Certaines observations ont également montré que les individus obèses et "post-obèses" présentent une altération de leur capacité oxydative lipidique en réponse à une stimulation β-adrénergique (Blaak et al., 1994a; Blaak et al., 1994b; Tremblay et al., 1984). Ces études confirment bien que l'altération de l'oxydation lipidique pouvait être causée par une diminution de la capacité du muscle squelettique à capter

les AGL et/ou être expliquée par une mobilisation réduite des TG au niveau du tissu adipeux. Ce n'est que récemment que la capacité du tissu adipeux à mobiliser les graisses (mesurée à l'aide de la technique de microdialyse) a été identifiée comme étant un déterminant de l'oxydation lipidique systémique (Snitker et al., 1998).

En résumé, il convient de souligner qu'un excès de tissu adipeux, notamment au niveau abdominal, est associé à des concentrations plasmatiques à jeun élevées de glucose, d'insuline et de lipoprotéines. Ces altérations métaboliques sont également observées en situation postprandiale et s'expliquent notamment par une difficulté des tissus à réestérifier les AGL ainsi que par une moins bonne capacité de l'insuline à inhiber la lipolyse au niveau du tissu adipeux.

CHAPITRE 5

RELATION ENTRE L'ACCUMULATION DE TISSU ADIPEUX VISCÉRAL ET LES FACTEURS DE RISQUE DE LA MALADIE CORONARIENNE: EXISTE T-IL UNE CONTRIBUTION DE L'HYPERTROPHIE DES CELLULES ADIPEUSES SOUS-CUTANÉES?

L'article composant ce chapitre est intitulé:

"Relationship of visceral adipose tissue to metabolic risk factors for coronary heart disease: is there a contribution of subcutaneous fat cell hypertrophy?"

(*Metabolism* 48; 355-362, 1999)

Résumé

Le but de cette étude consistait à évaluer la contribution respective de l'accumulation de tissu adipeux viscéral (TAV) et de l'hypertrophie d'adipocytes sous-cutanés abdominaux et fémoraux au niveau du profil métabolique de 69 hommes et de 65 femmes préménopausées. L'accumulation de TAV était positivement reliée aux concentrations plasmatiques à jeun d'insuline, de triglycérides (TG) et d'apolipoprotéine B de la fraction des lipoprotéines de faible densité (LDL-apoB) ainsi qu'au le rapport cholestérol (CHOL)/lipoprotéine de haute densité (HDL)-CHOL ($0.24 < r < 0.71$, $P < 0.05$). Parallèlement, des relations positives entre la grosseur des cellules adipeuses sous-cutanées abdominales et le profil métabolique ont été observées chez les deux sexes ($0.33 < r < 0.60$, $P < 0.01$). Des associations positives ont aussi été relevées chez les femmes entre la grosseur des cellules adipeuses fémorales et les concentrations plasmatiques à jeun d'insuline, de TG, de CHOL et du rapport CHOL/HDL-CHOL ($0.29 < r < 0.42$, $P < 0.05$). Cependant, seuls la concentration plasmatique de TG et le rapport CHOL/HDL-CHOL étaient positivement corrélés à la taille des cellules adipeuses fémorales chez l'homme ($r = 0.30$, $P < 0.05$). Dans le but de mieux cerner la relation entre le profil métabolique de risque et l'hypertrophie adipocytaire, des individus ayant de petits ou de gros adipocytes ont été pairés sur la base de leur accumulation de TAV. Les hommes présentant des adipocytes sous-cutanés abdominaux hypertrophiés étaient caractérisés par des concentrations de TG et de LDL-apoB supérieures aux hommes ayant de petits adipocytes ($P < 0.05$). Aucune différence au niveau du profil métabolique n'a été observée entre les sujets présentant de petits vs de gros adipocytes fémoraux. Ces résultats suggèrent que, pour une accumulation donnée de tissu adipeux viscéral, les hommes caractérisés par une hypertrophie des adipocytes sous-cutanés abdominaux présentent une détérioration accrue de leur profil métabolique comparativement à des individus présentant de plus petits adipocytes au niveau du même site. À l'inverse, l'hypertrophie des adipocytes fémoraux ne semble pas aggraver les complications métaboliques généralement reliées à l'obésité chez l'homme et la femme.

Relationship of visceral adipose tissue to metabolic risk factors for coronary heart disease: Is there a contribution of subcutaneous fat cell hypertrophy?

P. Imbeault[1,3], S. Lemieux[1], D. Prud'homme[3], A. Tremblay[3], A. Nadeau[2], J.-P. Després[1] and P. Mauriège[1,3]

[1] Lipid Research Center, [2] Diabetes Research Unit, Laval University Medical Research Center, and [3] Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec, Canada.

Supported by the Medical Research Council of Canada and by the Fonds pour la Formation de Chercheurs et l'aide à la Recherche.

Address correspondence: P. Mauriège Ph.D.
Physical Activity Sciences Laboratory, PEPS,
Laval University, Ste-Foy, Québec, Canada, G1K 7P4
Tel: (418) 656-2131 ext 6067; Fax (418) 656-2441;
e-mail: pascale.mauriege@kin.msp.ulaval.ca

Running title: Adipose tissue and CHD risk.

Keywords: obesity, body fat distribution, lipid-lipoprotein profile, glucose-insulin homeostasis, adipose cell size.

Abstract

Visceral adipose tissue (VAT) accumulation is an important correlate of the metabolic complications found in obese patients. The aim of this study was to evaluate the respective contribution of VAT deposition vs subcutaneous abdominal or femoral fat cell hypertrophy as correlates of the metabolic risk profile in 69 men and 65 premenopausal women (both 35 ± 5 yrs old) covering a wide range of fatness values (body mass indices ranging from 18 to 57 kg/m^2). In both genders, VAT accumulation was positively correlated with fasting plasma insulin, triglyceride (TG), LDL-apo B levels and with the cholesterol (CHOL)/HDL-CHOL ratio ($0.24 \leq r \leq 0.71$, $P < 0.05$). A similar pattern of positive relationships was found between subcutaneous abdominal fat cell weight and metabolic risk variables in men and women ($0.33 \leq r \leq 0.60$, $P < 0.01$). Positive associations were also observed in women between femoral fat cell weight and fasting plasma insulin, TG, CHOL levels and the CHOL/HDL-CHOL ratio ($0.29 \leq r \leq 0.42$, $P < 0.05$). However, only plasma TG concentrations and the CHOL/HDL-CHOL ratio were positively correlated with femoral fat cell weight in men ($r = 0.30$, $P < 0.05$). To better investigate the relationships between the subjects' metabolic risk profile and hypertrophic subcutaneous obesity, individuals with small vs large subcutaneous abdominal adipocytes were matched according to VAT accumulation. Men with large abdominal fat cells displayed higher plasma TG and LDL-apo B levels, compared to men characterized by small abdominal adipocytes ($P < 0.05$). Stepwise multiple regression analyses showed that subcutaneous abdominal fat cell weight was the best independent variable predicting plasma TG and LDL-apo B levels in men. No significant difference was found in the metabolic profile of subjects displaying small vs large femoral adipocytes. Taken together, these results suggest that, for a given VAT deposition, the presence of hypertrophied subcutaneous abdominal adipocytes in men appears to be associated with further deterioration in the metabolic risk profile. On the other hand, the hypertrophy of femoral adipocytes does not further alter the metabolic complications generally related to obesity in both men and women.

Introduction

Several epidemiological and experimental studies have confirmed the pioneering observations of Jean Vague [1] emphasizing the importance of body fat distribution. Indeed, it is now commonly accepted that a preferential accumulation of fat in the abdominal region is associated with an increased risk of non insulin-dependent diabetes mellitus (NIDDM) and coronary heart disease (CHD) [2-7]. Moreover, the development of new imaging techniques has allowed several groups of investigators to propose that visceral adipose tissue deposition was a critical correlate of the metabolic complications found among obese patients [4, 7, 8]. However, some recent studies have reported that variation in subcutaneous abdominal fat was also an important determinant of individual differences in insulin sensitivity [9-11].

The apparent heterogeneity of human obesity has led clinicians to propose several classifications of this condition [7]. One of these is based on the cellular characteristics of adipose tissue and identifies two main subtypes of obesity: "hypertrophic obesity" resulting from an enlargement of adipocytes vs "hyperplastic obesity", which is related to an increased number of adipose cells. Krotkiewski et al [12] have examined this issue in a comprehensive study of adipose cellularity. They concluded that body fat accretion was mainly due to fat cell weight enlargement, this latter being subsequently followed by an increase in adipose cell number. In this regard, Björntorp et al [13] have already suggested that adipose tissue hyperplasia occurred when fat cell weight approached 0.6 µg lipid/ cell.

The impact of individual variation in adipose tissue cellularity on the metabolic abnormalities of obesity has also been examined. Björntorp et al [14] have reported that fasting plasma insulin was positively related to fat cell size of adipocytes derived from subcutaneous abdominal and gluteal fat depots in middle-aged men. Similarly, subcutaneous epigastric fat cell weight has also been associated with fasting plasma insulin, glucose and TG levels in women, whereas epigastric fat cell weight has been related to plasma insulin and TG levels in men [12]. Kissebah et al [15] have also shown that upper-body obese premenopausal women also displayed enlarged subcutaneous abdominal fat cells and elevated plasma glucose and insulin levels following an

oral glucose load, whereas lower-body obese women had smaller subcutaneous abdominal adipocytes and lower glycemic and insulinemic responses. Taken together, these previous observations suggested that an excessive abdominal fat deposition and the presence of enlarged subcutaneous adipose cells could synergistically act in the etiology of the metabolic complications associated with obesity. However, to the best of our knowledge, no study has attempted to investigate the independent contribution of visceral adipose tissue vs subcutaneous adipose cell hypertrophy to the metabolic risk profile of men and women.

The aim of the present investigation was therefore to evaluate the respective contribution of visceral adipose tissue as well as that of subcutaneous abdominal and femoral fat cell hypertrophy to variation in the metabolic profile predictive of CHD risk in a sample of 69 men and 65 premenopausal women.

Material and Methods

Subjects

One hundred and thirthy-four healthy volunteers (69 men and 65 women), aged 35 ± 6 (mean ± SD) years, were recruited through the media and gave their written informed consent to participate in a study which examined the potential relationship of obesity and body fat distribution indices to metabolic risk variables [16, 17]. This study has been approved by the Laval University Medical Ethics Committee. All individuals were subjected to a medical evaluation by a physician, which included medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication that could have influenced carbohydrate or lipid metabolism (ß-blockers, antihypertensive drugs, etc) were excluded from the study. All subjects were sedentary, non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight loss program, and their body weight had been stable for at least six months prior to the experiment. Women had regular menstrual cycles and none was using oral contraceptives or lactating at the time of the study. All measurements were performed while women were in the early follicular phase of their menstrual cycle.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique [18] and percent body fat was derived from body density [19]. Pulmonary residual volume was measured using the helium dilution method [20]. Fat mass was calculated as total body weight minus fat-free mass. Waist girth was measured according to the procedures recommended at the Airlie Conference [21]. Computed tomography (CT) was performed on a Siemens Somaton DRH scanner (Erlangen, Germany), according to the methodology of Sjöström et al [22]. Briefly, the subjects were examined in the supine position with both arms stretched above their head. CT scans were performed at both the abdominal (between L4 and L5 vertebrae) and femoral (midthigh) levels, using a radiograph of the skeleton as a reference to establish the position of the scans to the nearest millimeter as previously described [23]. Total adipose tissue (AT) areas were calculated by delineating these areas with a graph pen and then computing the AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) [22], as previously described [23].

Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Adipose tissue biopsy procedure

After an overnight fast, participants were subjected to biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the midthigh level (femoral site). A small cutaneous incision (1 cm) was performed at both sites and about 100 mg of subcutaneous adipose tissue were surgically removed from the two depots. Adipocytes were isolated according to the method of Rodbell [24] in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin (KRBA) and 5 mM glucose, plus 1 mg/ml collagenase, as previously described [25]. Digestion took place in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2, for 40 min at 37°C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml of KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 µl. Fat cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ, USA). Mean adipose cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described [25].

Oral glucose tolerance test (OGTT)

A 75 g OGTT was performed in the morning after an overnight fast. Blood samples were collected in tubes containing EDTA and Trasylol (Miles Pharmaceutics, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein at -15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. Plasma glucose was measured enzymatically [26], whereas plasma insulin concentration was determined by radioimmunoassay with polyethylene glycol separation [27]. Plasma free-fatty acid (FFA) levels were determined at -15, 0, 30, 60, 120, and 180 min, with a colorimetric method [28]. The total glucose and insulin areas under the curve measured during the OGTT were calculated using the trapezoid method.

Plasma lipids and lipoproteins

Blood samples were obtained in the morning after a 12-h fast from an antecubital vein into vacutainer tubes containing EDTA. Plasma cholesterol (CHOL) and triglyceride (TG) levels were measured enzymatically in plasma and lipoprotein fractions on a RA-1000 Autoanalyzer (Technicon Instruments Corporation, Tarrytown, NY, USA) referenced to Centers for Disease Control (Atlanta, USA). Plasma very low density lipoproteins (VLDL, d < 1.006 g/ml) were isolated by ultra-centrifugation [29], and the high density lipoprotein (HDL) fraction was obtained after precipitation of low density lipoprotein (LDL) in the infranatant (d > 1.006 g/ml) with heparin and $MnCl_2$ [30]. Plasma LDL-apolipoprotein (apo) B levels were measured by the rocket immunoelectrophoretic method of Laurell, as previously described [31].

Drugs and chemicals

Collagenase and bovine serum albumin were obtained from Boehringer Mannheim (Canada). All other chemicals and organic solvents were of the highest purity grade commercially available. The same batches of collagenase and albumin were used in all experiments.

Statistical methods

Data reported in tables are expressed as means ± standard deviation (SD) whereas values shown in figures are means ± standard error (SE). Associations between two variables were quantified by using the Pearson's product-moment correlation coefficients. Partial correlational analyses were also performed to estimate the independent contribution of visceral adipose tissue deposition, abdominal and femoral fat cell weights to the variance of several metabolic variables. As results obtained from partial correlational analyses were essentially similar to data resulting from the matching procedure, only results on matched subjects are reported in the present paper. The normality of the distribution for all variables was studied with a Shapiro-Wilk W test. Concerning variables which were not normally distributed, the Mann-Whitney test for nonparametric variables was used instead of the Student's t test. All analyses were performed using JMP version 3.1.5 for Macintosh (SAS, Cary, NC).

Results

Subjects' characteristics

The subjects' physical and metabolic characteristics are presented in Tables 1 and 2, respectively. The wide range of percent body fat values (from 10 to 39% and from 16 to 59% for men and women, respectively) indicated that our sample included lean to obese individuals. Gender comparisons revealed that men displayed both higher body weight and waist girth, compared to women (P values ranging from 0.0001 to 0.001). Women were however characterized by a greater fat mass and a higher percent body fat than men (P values ranging from 0.0001 to 0.05). Both subcutaneous abdominal and midthigh adipose tissue areas determined by computed tomography were larger in women than in men (P values ranging from 0.0001 to 0.001). On the other hand, men were characterized by a greater visceral adipose tissue accumulation, compared to women ($P < 0.001$). Femoral fat cells were also significantly larger than subcutaneous abdominal adipocytes in women ($P < 0.05$), although no regional variation was observed in men. Finally, women displayed larger subcutaneous femoral adipose cells than men ($P < 0.001$), whereas no gender difference was found in subcutaneous abdominal fat cell weight. On the other hand, men had higher fasting plasma glucose and insulin levels as well as both lower HDL-CHOL levels and a greater CHOL/HDL-CHOL ratio than women (P values ranging from 0.0001 to 0.01). Moreover, the insulin response to an oral glucose load was also higher in men than in women ($P < 0.0001$).

Insert Tables 1 and 2.

In an attempt to investigate the contribution of visceral adipose tissue accumulation and regional subcutaneous fat cell size to the metabolic complications associated with obesity, relationships between visceral adipose tissue as well as between subcutaneous abdominal and femoral fat cell weights with the metabolic risk profile were examined in both men and women (Tables 3 and 4, respectively).

Correlational analyses

In both genders, fasting plasma glucose, insulin, FFA, TG and LDL-apo B levels as well as the CHOL/HDL-CHOL ratio were positively related to VAT accumulation ($0.24 \leq r \leq 0.71$, P values ranging from 0.0001 to 0.05). In addition, VAT deposition was positively associated with both glucose and insulin areas measured during the oral glucose load ($0.42 \leq r \leq 0.65$, $P < 0.001$). A similar pattern of significant associations was found between subcutaneous abdominal fat cell weight and both the plasma lipid-lipoprotein profile and indices of glucose-insulin homeostasis in both genders ($0.26 \leq r \leq 0.60$, P values ranging from 0.0001 to 0.05). On the other hand, positive associations were observed between femoral fat cell weight and fasting plasma glucose, insulin, TG and CHOL levels as well as with the CHOL/HDL-CHOL ratio in women ($0.29 \leq r \leq 0.52$, P values ranging from 0.0001 to 0.05), whereas plasma TG levels and the CHOL/HDL-CHOL ratio were the only variables correlated with femoral fat cell weight in men ($r = 0.30$, P values ranging from 0.01 to 0.05). However, correlation coefficients observed between the latter metabolic variables and femoral fat cell weight were generally lower than those reported when subcutaneous abdominal fat cell weight was plotted against these metabolic indices. Finally, the subjects' metabolic profile was correlated with the subcutaneous abdominal fat deposition measured by CT ($0.25 \leq r \leq 0.48$ and $0.28 \leq r \leq 0.68$ in men and women, respectively; P values ranging from 0.0001 to 0.05) (not shown) and these correlation coefficients were of similar magnitude to those previously observed with subcutaneous fat cell weight.

Insert Tables 3 and 4.

As an approach to further quantify the metabolic complications associated with hypertrophic subcutaneous obesity, two subgroups of subjects matched for a similar VAT accumulation but with either small vs large subcutaneous abdominal or femoral fat cells were compared (Figs 1 and 2). In both genders, subgroups with either small or large subcutaneous abdominal fat cells did not differ for age, total body fatness and subcutaneous abdominal fat deposition. However, men characterized by large subcutaneous abdominal fat cells had higher plasma TG and LDL-apo B levels ($P < 0.05$). In contrast, there was no difference in the metabolic profile of women with either small or large subcutaneous abdominal fat cells (Fig. 1). Moreover, no significant

difference was found in the metabolic risk profile of subgroups displaying small or large subcutaneous femoral adipocytes, in both genders (Fig. 2).

Insert Figures 1 and 2.

Stepwise multiple regression analyses

To estimate the respective contributions of regional and total adiposity in accounting for variance in TG, LDL-apo B, HDL-CHOL and insulin levels, stepwise multiple regression analysis was performed. Our model included fat mass, femoral fat, visceral and subcutaneous abdominal adipose tissue accumulation measured by CT as well as subcutaneous abdominal and femoral fat cell weights. In men, 35% of the variance in fasting insulin was best predicted by visceral adipose tissue (Table 5). Moreover, 31% of the variance in TG was predicted by subcutaneous abdominal fat cell weight, femoral and abdominal subcutaneous adipose tissue accumulation. On the other hand, subcutaneous abdominal fat cell weight was the only variable retained as a significant predictor of LDL-apoB levels (15% of variance), while femoral fat cell weight was the best predictor of the variance observed in HDL-CHOL concentrations (10%). With the exception of fat mass and femoral fat cell weight, all variables included in our stepwise regression model accounted for 55% of variance in insulin area. However, visceral adipose tissue was the best predictor of the variance in insulin area (43%) determined during the oral glucose load, in men.

In women, 65% of variance in fasting insulin was accounted for by fat mass, femoral and subcutaneous abdominal adipose tissue accumulation and femoral fat cell weight (Table 6). In addition, visceral adipose tissue was the only variable which accounted for 51% and 25% of the variance in TG and HDL-CHOL levels, respectively. Moreover, 23% of variance in LDL-apo B levels was best predicted by subcutaneous abdominal adipose tissue accumulation and fat mass. Finally, subcutaneous abdominal fat accounted for 21% of the variance in insulin area.

Insert Tables 5 and 6.

Discussion

The present study was designed as an attempt to verify whether variations in either subcutaneous abdominal and femoral adipose cell size could influence the metabolic risk profile of both men and women after control for individual differences in visceral adipose tissue accumulation.

It is now well recognized that there are gender differences in body fat distribution, women being characterized by a greater accumulation of subcutaneous fat than men [1, 7, 13, 32]. Furthermore, women display a preferential accumulation of gluteal-femoral adipose tissue, a finding concordant with the fact that they show enlarged gluteo-femoral adipocytes as compared to subcutaneous abdominal adipose cells [33-37]. On the other hand, men have a larger proportion of intra-abdominal fat and they do not show a marked regional variation in subcutaneous adipose cell size [37, 38].

The significant correlations observed in the present study between visceral adipose tissue measured by computed tomography and fasting plasma glucose, insulin and free fatty acid levels in both genders (Tables 3 and 4) are in good accordance with the positive associations previously reported between intra-abdominal fat deposition and alterations in both insulin-glucose homeostasis and plasma lipoprotein-lipid levels [6, 7, 16, 17, 39]. Although the mechanism(s) responsible for the deleterious metabolic impact of excess intra-abdominal fat is (are) not fully understood, it has been hypothesized that adipose tissue lipolysis could play a non negligible role in the metabolic complications related to abdominal obesity [3, 7, 40, 41]. Indeed, it is now well established that due to its important lipolytic rate, visceral adipose tissue, which is drained by the portal vein, could expose the liver to an enhanced FFA flux which could in turn alter glucose-insulin homeostasis by promoting a reduction in hepatic insulin degradation and an inhibition of peripheral glucose utilization [4, 6, 7] which could lead to systemic hyperinsulinemia and in vivo insulin resistance.

In the present study, significant relationships were also observed between subcutaneous abdominal fat cell weight and most of the metabolic indices measured in both genders (Tables

3 and 4). These results are in good accordance with the previous associations reported between large subcutaneous adipocytes and metabolic aberrations such as hyperinsulinemia [42], hypertriglyceridemia [14] as well as with non insulin-dependent diabetes mellitus [43]. Thus, the present results re-emphasize the notion that hypertrophy of subcutaneous abdominal adipose cell is a significant correlate of the metabolic disturbances associated with abdominal obesity in both genders [38, 44]. However, although these previous studies have provided interesting results, they did not take into account the concomitant variation in visceral adipose tissue accumulation which may have affected the conclusions reached relating hypertrophic obesity to the metabolic deteriorations that generally accompany abdominal fat deposition [14, 38, 42-44].

To the best of our knowledge, our study is the first to investigate the independent contribution of an hypertrophic subcutaneous obesity versus visceral adipose tissue accumulation to the variation observed in the metabolic risk profile of both men and women. Our results showed that for similar levels of visceral adipose tissue, men with enlarged subcutaneous abdominal adipocytes displayed further deterioration in their metabolic risk profile than individuals with small subcutaneous abdominal adipose cells (Fig 1). Considering the fact that large adipocytes are generally characterized by a high lipolytic rate [15, 25, 35, 45], the hypertrophy of subcutaneous abdominal adipocytes could lead to an increased adipocyte-hepatocyte fatty acid flux which, in turn, may partly explain metabolic disturbances such as hypertriglyceridemia [46] or hyperapobetalipoproteinemia [47]. This hypothesis does not appear to be justified in women since no significant difference was found in the metabolic profile of subjects displaying small vs large subcutaneous abdominal adipose cells. The latter observation is confirmed by the stepwise regression analysis which revealed that women's metabolic profile seems to be more influenced by both visceral and subcutaneous abdominal adipose tissue deposition as well as by fat mass than by hypertrophied subcutaneous abdominal fat cells.

However, the fact that a high lipolytic rate of enlarged subcutaneous abdominal fat cells could contribute to the observed hypertriglyceridemia and hyperapobetalipoproteinemia is based exclusively on in vitro measurements. Some discrepancies exist between in vitro and in vivo findings regarding adipose cell lipolysis. There is now increasing evidence that such discordant observations are probably due, in part, to blood flow effects [48]. In this regard, it has

already been shown that, when expressed par kilogram of fat mass, whole body lipolytic rates determined during infusions of stable isotopically labelled glycerol were lower in obese than in lean individuals [49]. However, the main problem concerning the assessment of in vivo lipolysis in obesity relates to the expression of FFA and glycerol release (R_a) in terms of lean body mass or adipose tissue mass. These different modes of expression may have considerable influence on data interpretation. Indeed, it has previously been reported that obese subjects display increased basal rates of lipolysis when FFA R_a is expressed per units of lean body mass [50]. On the other hand, an in vivo study using microdialysis reported higher glycerol concentrations in obese than in lean men [51], a difference which was also ascribed to an increased lipolytic rate in larger fat cells. In addition, interstitial glycerol levels were higher in abdominal than femoral subcutaneous adipose tissues [52], a finding which attested for an increased lipolytic rate from the abdominal depot. Based upon these observations, although in vivo lipolysis measurements might explain the associations reported between the subjects' metabolic profile and fat cell hypertrophy, in vitro fat cell metabolic data should also be considered.

On the other hand, results of the present study also suggest that plasma insulin levels are best accounted for by visceral adipose tissue accumulation in men, a finding which re-emphasizes the important role of "portal" adipose tissue as a correlate of insulin sensitivity [4, 6, 8]. However, subcutaneous abdominal fat has recently been shown to also be an important correlate of insulin resistance [10, 11]. One potential explanation for this finding could be due to the method used to study in vivo insulin action. Indeed, insulin sensitivity was measured by using a euglycemic clamp in these studies [10, 11], whereas we used an oral glucose tolerance test. During the euglycemic clamp, the glucose-insulin infusion bypasses the gut and it is possible that under those conditions, subcutaneous fat may be more critical than visceral adipose tissue in modulating in vivo insulin action. However, during the oral glucose load, it is possible that hormonal or metabolic stimuli originating from the gut may play a more important role in explaining the greater contribution of visceral adipose tissue when the body is challenged by oral glucose. This hypothesis will require further studies.

Finally, previous studies have already proposed that a preferential femoral fat accumulation could even be associated with a "protective" metabolic risk profile in obese men [17] and women [53]. Results of the present study indicate that femoral fat cell hypertrophy did not have, per se, deleterious consequences on the metabolic risk profile in both genders (Fig. 2), a finding which has already been observed in men [38]. On the other hand, fat cell hypertrophy has been shown to be related to increased lipolysis [45]. However, femoral adipocytes of obese subjects display similar basal lipolytic rates compared to those of lean individuals, a finding which is probably due to the strong α_2-adrenoceptor antilipolytic component found in these cells [33, 34]. Such observations suggest that large femoral adipocytes do not significantly contribute to increase FFA flux into the circulation and are unlikely to be deleterious to the metabolic risk profile of both men and women.

Conclusion

In summary, results of the present study support the notion that the amount of visceral adipose tissue is an important correlate of both indices of plasma glucose-insulin homeostasis and lipoprotein levels. However, this study documents for the first time that, for a similar visceral adipose tissue accumulation, only men with hypertrophied subcutaneous abdominal adipocytes are characterized by further alterations in plasma lipoprotein levels, compared to individuals with smaller subcutaneous abdominal adipose cells. Finally, our results suggest that the hypertrophy of subcutaneous femoral adipocytes does not contribute to exacerbate the metabolic risk profile of both men and women.

Acknowledgments

The authors want to express their gratitude to Judith Maheux, Jacinthe Hovington, France Levasseur, Martine Marcotte, Henri Bessette and Germain Thériault for their excellent collaboration at various stages of the study. We also like to thank Yolande Montreuil, Marie Martin and Rachel Duchesne of the Diabetes Research Unit for their assistance in data collection. Thanks are also expressed to Suzanne Brulotte from the Department of Radiology (University Hospital) for her excellent work with the tomograph. The subjects of the studies as well as the staff of the Physical Activity Sciences Laboratory and of the Lipid Research Center are also gratefully acknowledged.

References

1. Vague J: The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. Am. J. Clin. Nutr. 4:20-34, 1956
2. Kissebah AH: Insulin resistance in visceral obesity. Int. J. Obesity 15:109-115, 1991
3. Björntorp P: Metabolic implications of body fat distribution. Diabetes Care 14:1132-1143, 1991
4. Björntorp P: Visceral obesity: a "civilization syndrome". Obesity Res. 1:206-222, 1993
5. Després JP: Obesity and lipid metabolism: relevance of body fat distribution. Curr. Opin. Lipidol. 2:5-15, 1991
6. Després JP: Dyslipidaemia and obesity. Baillière's Clin. Endocrinol. Metabol. 8:629-660, 1994
7. Kissebah AH and Krakower GR: Regional adiposity and morbidity. Physiol. Rev. 74:761-811, 1994
8. Kissebah AH: Intra-abdominal fat: is it a major factor in developing diabetes and coronary artery disease? Diab. Res. Clin. Pract. 30:S25-S30, 1996
9. Misra A, Gard A, Abate N, et al: Relationship of anterior and posterior subcutaneous abdominal fat to insulin sensitivity in nondiabetic men. Obesity Res. 5:93-99, 1997
10. Goodpaster BH, Thaete FL, Simoneau J-A, et al: Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes 46:1579-1585, 1997
11. Abate N, Garg A, Peshock RM, et al: Relationships of generalized and regional adiposity to insulin sensitivity in men. J. Clin. Invest. 96:88-98, 1995
12. Krotkiewski M, Björntorp P, Sjöström L, et al: Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. J. Clin. Invest. 72:1150-1162, 1983
13. Björntorp P: Adipose tissue distribution and function. Int. J. Obesity 15:67-81, 1991
14. Björntorp P, Bengtsson C, Blohmé G, et al: Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women. Metabolism 20:927-935, 1971

15. Kissebah AH, Vydelingum N, Murray R, et al: Relation of body fat distribution to metabolic complications of obesity. J. Clin. Endocrinol. Metab. 54:254-260, 1982

16. Pouliot MC, Després JP, Nadeau A, et al: Associations between regional body fat distribution, fasting plasma free fatty acid levels and glucose tolerance in premenopausal women. Int. J. Obesity 14:293-302, 1990

17. Pouliot MC, Després JP, Nadeau A, et al: Visceral obesity in men: associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 41:826-834, 1992

18. Behnke AR and Wilmore JH: Evaluation and regulation of body build and composition, in (eds): Englewood Cliffs, NJ, Prentice-Hall, 1974, pp 20-37

19. Siri WE: The gross composition of body fat. Adv. Biol. Med. Physiol. 4:239-280, 1956

20. Meneely GR and Kaltreider NL: Volume of the lung determined by helium dilution. J. Clin. Invest. 28:129-139, 1949

21. Lohman TG, Roche AF and Martorell R: The Airlie (VA) consensus conference, in (eds): Anthropometric Standardisation Reference Manual. Champaign (Illinois), Human Kinetics Publishers, 1988, pp 39-80

22. Sjöström L, Kvist H, Cederblad A, et al: Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. Am. J. Physiol. 250:E736-E786, 1986

23. Ferland M, Després JP, Tremblay A, et al: Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br. J. Nutr 61:139-148, 1989

24. Rodbell M: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 239:375-380, 1964

25. Mauriège P, Després JP, Prud'homme D, et al: Regional variation in adipose tissue lipolysis in lean and obese men. J. Lipid Res. 32:1625-1633, 1991

26. Richterich R and Dauwwalder H: Zur bestimmung der plasmaglukose-konzentration mit der hexokinase-glucose-6-phosphat-deshydrogenase-methode. Schweiz Med Wochenschr 101:615-618, 1971

27. Desbuquois B and Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J. Clin. Endocrinol. Metab. 37:732-738, 1971

28. Noma A, Okabe H and Kita M: A new colorimetric microdetermination of free fatty acids in serum. Clin Chim Acta. 43:317-320, 1973

29. Havel RJ, Eder H and Bragdon HF: The distribution and chemical composition of utracentrifugally separated lipoproteins in human serum. J. Clin. Invest. 34:1345-1353, 1955

30. Moorjani S, Gagné C, Lupien PJ, et al: Plasma triglycerides related decrease in high density lipoprotein cholesterol and its association with myocardial infarction in heterozygous familial hypercholesterolemia. Metabolism 35:311-316, 1986

31. Avogaro P, Bittolo Bon G, Cazzolato G, et al: Are apolipoprotein better discriminators than lipids for atherosclerosis? Lancet 1:901-903, 1979

32. Lemieux S, Prud'homme D, Bouchard C, et al: Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. Am. J. Clin. Nutr. 58:463-467, 1993

33. Leibel RL and Hirsch J: Site- and sex-related differences in adrenoceptor status of human adipose tissue. J. Clin. Endocrinol. Metab. 64:1205-1210, 1987

34. Mauriège P, Galitzky J, Berlan M, et al: Heterogeneous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. Eur. J. Clin. Invest. 17:156-165, 1987

35. Mauriège P, Prud'homme D, Lemieux S, et al: Regional differences in adipose tissue lipolysis from lean and obese women: existence of post-receptor alterations. Am. J. Physiol. 269:341-350, 1995

36. Rebuffé-Scrive M, Enk L, Crona N, et al: Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy and lactation. J. Clin. Invest. 75:1973-1976, 1985

37. Wahrenberg H, Lönnqvist F and Arner P: Mechanisms underlying regional differences in lipolysis in human adipose tissue. J. Clin. Invest. 84:458-467, 1989

38. Mauriège P, Després JP, Moorjani S, et al: Abdominal and femoral adipose tissue lipolysis and cardiovascular disease risk factors in men. Eur. J. Clin. Invest. 23:729-740, 1993

39. Després JP, Lemieux S, Lamarche B, et al: The insulin resistance-dyslipidemic syndrome: contribution of visceral obesity and therapeutic implications. Int. J. Obesity 19:S76-S86, 1995

40. Bouchard C, Després JP and Mauriège P: Genetic and nongenetic determinants of regional fat distribution. Endocr. Rev. 14:72-93, 1993

41. Arner P: Regulation of adipose tissue lipolysis, importance for the metabolic syndrome. Adv. Biol. Med. Physiol. 334:259-267, 1993

42. Björntorp P, Berchtold P and Tibblin G: Insulin secretion in relation to adipose tissue in man. Diabetes 20:65-70, 1971

43. Krotkiewski M, Sjöström L, Björntorp P, et al: Regional adipose tissue cellularity in relation to metabolism in young and middle-aged women. Metabolism 24:703-710, 1975

44. Mauriège P, Després JP, Marcotte M, et al: Abdominal fat cell lipolysis, body fat distribution, and metabolic variables in premenopausal women. J. Clin. Endocrinol. Metab. 71:1028-1035, 1990

45. Arner P: Control of lipolysis and its relevance to the development of obesity in man. Diabetes Metab. Rev. 4:507-515, 1988

46. Byrne CD, Brindle NPJ, Wang TWM, et al: Interaction of non-esterified fatty acid and insulin in control of triacylglycerol secretion by Hep G2 cells. Biochem. J. 280:99-104, 1991

47. Sniderman AD and Cianflone K: Metabolic disruptions in the adipocyte-hepatocyte fatty acid axis as the causes of hyperapoB. Int. J. Obesity 19:S27-S33, 1995

48. Coppack SW, Jensen MD and Miles JM: In vivo regulation of lipolysis in humans. J. Lipid Res. 35:177-193, 1994

49. Lillioja S, Foley J, Bogardus C, et al: Free fatty acid metabolism and obesity in man: in vivo in vitro comparisons. Metabolism 35:505-514, 1986

50. Björntorp P, Bergman H and Varnauskas E: Plasma free fatty acid turnover in obesity. Acta Medica Scandinavia 185:351-356, 1969

51. Jansson P-A, Larsson A, Smith U, et al: Glycerol production in subcutaneous adipose tissue in lean and obese humans. J. Clin. Invest. 89:1610-1617, 1992

52. Jansson P-A, Smith U and Lönnroth P: Interstitial glycerol concentration measured by microdialysis in two subcutaneous regions in humans. Am. J. Physiol. 258:E918-E922, 1990

53. Terry RB, Stefanick ML, Haskell WL, et al: Contributions of regional adipose tissue depots to plasma lipoprotein concentrations in overweight men and women: possible protective effects of thigh fat. Metabolism 40:733-740, 1991

Legends to figures

Figure 1. Comparison of selected metabolic indices of 8 pairs of men with a low (0.44 ± 0.05 μg/lipid) (open columns) or a high (0.71 ± 0.04 μg/lipid) (filled columns) subcutaneous (sc) abdominal fat cell weight (FCW) to those of 9 pairs of women displaying a low (0.43 ± 0.07 μg/lipid) (open columns) or a high (0.85 ± 0.09 μg/lipid) (filled columns) sc abdominal FCW. Subjects were matched for a similar visceral adipose tissue accumulation (137 ± 21 vs 141 ± 22 cm^2 and 102 ± 17 vs 104 ± 18 cm^2 in men and women, respectively). Statistical significance at * $P < 0.05$.

Figure 2. Comparison of selected metabolic indices of 9 pairs of men with a low (0.44 ± 0.02 μg/lipid) (open columns) or a high (0.74 ± 0.04 μg/lipid) (filled columns) femoral fat cell weight (FCW) to those of 9 pairs of women displaying a low (0.56 ± 0.04 μg/lipid) (open columns) or a high (0.99 ± 0.05 μg/lipid) (filled columns) femoral FCW. Subjects were matched for a similar visceral adipose tissue accumulation (136 ± 18 vs 139 ± 19 cm^2 and 108 ± 16 vs 111 ± 17 cm^2 in men and women, respectively).

Table 1. Physical characteristics of subjects.

	Women (n = 65)	Men (n = 69)
Age (years)	35 ± 5	36 ± 4
Anthropometric variables		
Body weight (kg) [l]	73 ± 21	81 ± 12 ‡
BMI (kg/m^2) [l]	28 ± 9	27 ± 4
Fat mass (kg) [l]	29 ± 16	22 ± 8 *
% Fat	37 ± 11	26 ± 6 §
Waist girth (cm) [l]	84 ± 17	94 ± 11 §
Adipose tissue areas measured by CT (cm^2)		
Abdomen (L4-L5)		
Subcutaneous [l]	361 ± 202	242 ± 101 ‡
Visceral [l]	90 ± 50	121 ± 48 ‡
Midthigh subcutaneous [l]	176 ± 68	97 ± 35 §
Regional fat cell weight (µg lipid /cell)		
Abdominal	0.57 ± 0.25	0.51 ± 0.13
Femoral	0.67 ± 0.22 [a]	0.55 ± 0.12 ‡

Value are means ± standard deviation (SD).

BMI = body mass index; CT = computed tomography.

Significant gender difference at • P< 0.05, ‡ P< 0.001 and § P< 0.0001.

[a] indicates a regional variation in adipose cell size of women at P< 0.05.

[l] Mann-Whitney test for nonparametric variables was performed because this variable was not normally distributed

Table 2. Metabolic profile of subjects.

	Women (n = 65)	Men (n = 69)
Glucose (mmol/l) [1]	4.8 ± 0.5	5.2 ± 0.6 §
Insulin (pmol/l) [1]	66.5 ± 47.9	78.7 ± 35.1 †
FFA (mmol/l)	0.5 ± 0.2	0.6 ± 0.2
TG (mmol/l) [1]	1.3 ± 0.6	1.6 ± 0.1
CHOL (mmol/l)	4.9 ± 0.9	4.9 ± 0.7
LDL-CHOL (mmol/l)	3.3 ± 0.9	3.3 ± 0.7
HDL-CHOL (mmol/l)	1.2 ± 0.3	1.0 ± 0.2 ‡
LDL-apo B (mg/dl)	79.3 ± 21.4	81.7 ± 18.2
CHOL/HDL-CHOL	4.3 ± 1.2	5.0 ± 1.3 †
Glucose area	1.12 ± 0.23	1.17 ± 0.28
Insulin area [1]	46.0 ± 34.8	71.9 ± 34.2 §

Value are means ± standard deviation (SD).

TG = triglycerides; CHOL = cholesterol; FFA = free fatty acids;

APO = apolipoprotein.

Significant gender difference at • $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ and

§ $P < 0.0001$.

Glucose and insulin areas represent integrated plasma concentrations measured for 3h after an oral glucose load (75g/OGTT). Glucose area is expressed in (mmol/l/min) x 10^{-3} whereas insulin area is expressed in (pmol/l/min) x 10^{-3}.

[1] Mann-Whitney test for nonparametric variables was performed because this variable was not normally distributed

Table 3. Pearson correlation coefficients between subcutaneous abdominal and femoral fat cell weights, visceral adipose tissue (VAT) accumulation versus metabolic variables in the sample of 69 men.

	VAT	Fat cell weight	
		Abdominal	Femoral
Glucose	.43§	.26*	.02
Insulin	.55§	.35†	.06
FFA	.28*	.33†	.13
TG	.28*	.34†	.30†
CHOL	.16	.36†	.12
LDL-CHOL	.13	.27*	.09
HDL-CHOL	-.22	-.21	-.32†
LDL-apo B	.28*	.40‡	.14
CHOL/HDL-CHOL	.24*	.33†	.29*
Glucose area	.56§	.35†	.08
Insulin area	.65§	.56§	.20

* P< 0.05, † P< 0.01, ‡ P< 0.001 and § P< 0.0001.

For abbreviations, see footnotes to Table 2.

Table 4. Pearson correlation coefficients for the associations between subcutaneous abdominal and femoral fat cell weights, visceral adipose tissue (VAT) accumulation versus metabolic variables in the sample of 65 women.

	VAT	Fat cell weight	
		Abdominal	Femoral
Glucose	.57§	.52§	.38‡
Insulin	.62§	.60§	.52§
FFA	.52§	.42‡	-.13
TG	.71§	.56§	.45‡
CHOL	.27*	.31†	.29*
LDL-CHOL	.24*	.32†	.29*
HDL-CHOL	-.51§	-.46§	-.33†
LDL-apo B	.33†	.36†	.20
CHOL/HDL-CHOL	.54§	.52§	.42‡
Glucose area	.42‡	.27*	.06
Insulin area	.44‡	.42‡	.31*

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ and § $P < 0.0001$.

For abbreviations, see footnotes to Table 2.

Table 5. Stepwise multiple regression analysis showing the independent contributions of some anthropometric variables to the variation in the metabolic risk profile of the 69 men.

Dependent Variable	Independent Variable	Partial (R^2 x 100)	Total (R^2 x 100)	P
Model 1				
TG	Abdominal fat cell weight (+)	16.5	30.7	0.0009
	Femoral fat (+)	5.0		0.05
	Subcutaneous abdominal fat (+)	9.2		0.007
Model 1				
LDL-apo B	Abdominal fat cell weight (+)	15.1	15.1	0.002
Model 1				
HDL-CHOL	Femoral fat cell weight (-)	9.5	9.5	0.01
Model 1				
Insulin	Visceral fat (+)	35.4	35.4	0.0001
Model 1				
Insulin area	Visceral fat (+)	43.0	54.4	0.0001
	Abdominal fat cell weight (+)	5.4		0.01
	Femoral fat (+)	2.7		0.05
	Subcutaneous abdominal fat (+)	3.3		0.04

Model 1: Included fat mass, femoral fat, visceral and subcutaneous abdominal adipose tissue measured by CT as well as subcutaneous abdominal and femoral fat cell weights.

For abbreviations, see footnotes to Table 2.

Positive (+) or negative (-) relationships between the independent and the dependent variables.

Table 6. Stepwise multiple regression analysis showing the independent contributions of some anthropometric variables to the variation in the metabolic risk profile of the 65 women.

Dependent Variable	Independent Variable	Partial (R^2 x 100)	Total (R^2 x 100)	P
Model 1				
TG	Visceral fat (+)	50.7	50.7	0.0001
Model 1				
LDL-apo B	Subcutaneous abdominal fat (+)	18.0	23.2	0.01
	Fat mass (+)	5.2		0.05
Model 1				
HDL-CHOL	Visceral fat (-)	25.5	25.5	0.0001
Model 1				
Insulin	Fat mass (+)	46.4	65.0	0.0001
	Femoral fat (+)	6.8		0.005
	Subcutaneous abdominal fat (+)	7.6		0.002
	Femoral fat cell weight (+)	4.2		0.01
Model 1				
Insulin area	Subcutaneous abdominal fat (+)	21.0	21.0	0.0002

Model 1: Included fat mass, femoral fat, visceral and subcutaneous abdominal adipose tissue measured by CT as well as subcutaneous abdominal and femoral fat cell weights.

For abbreviations, see footnotes to Table 2.

Positive (+) or negative (-) relationships between the independent and the dependent variables.

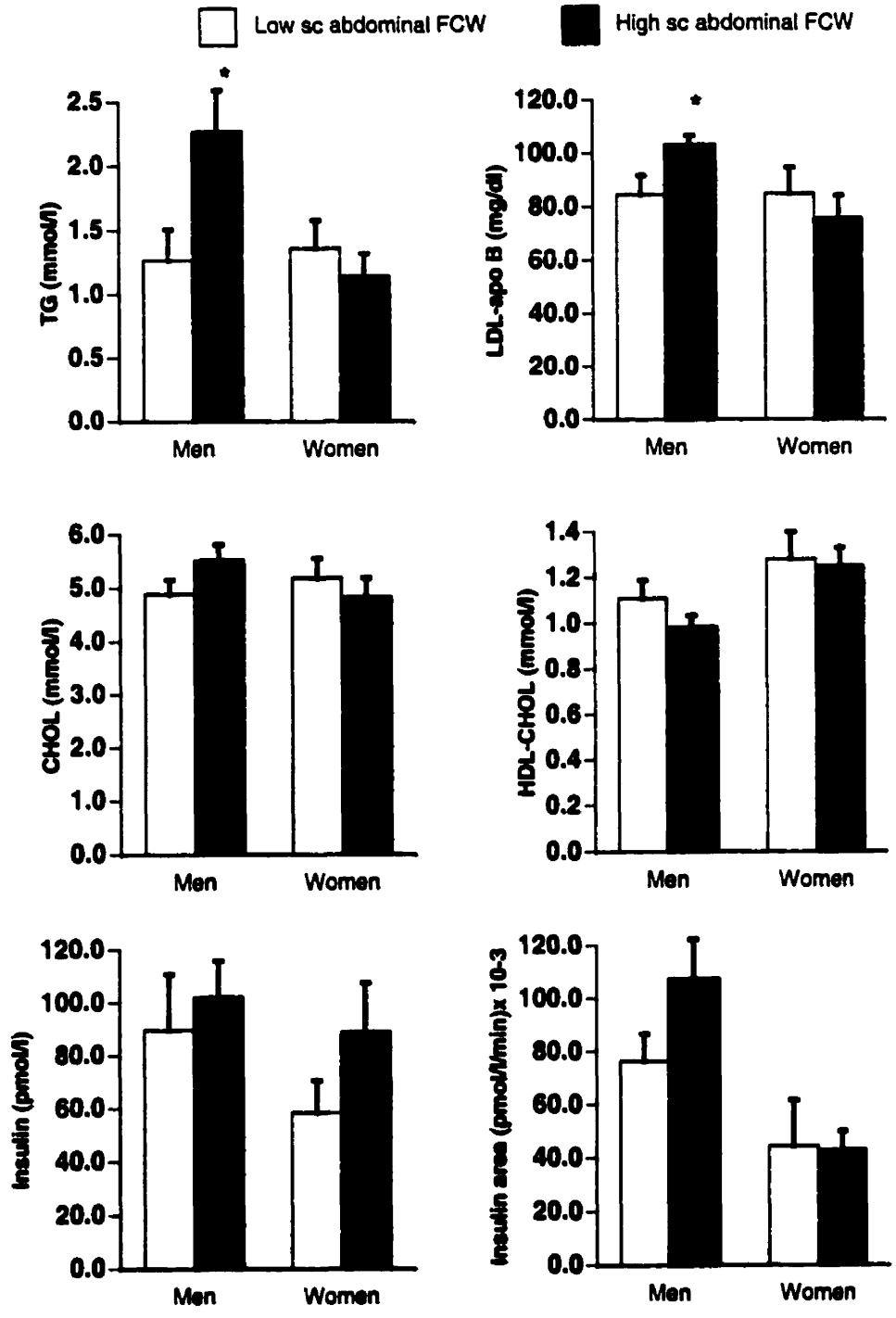

Low sc abdominal FCW
High sc abdominal FCW
TG (mmol/l)
2.5
2.0
1.5
1.0
0.5
0.0
*
Men
Women
LDL-apo B (mg/dl)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
*
Men
Women
CHOL (mmol/l)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Men
Women
HDL-CHOL (mmol/l)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Men
Women
Insulin (pmol/l)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Men
Women
Insulin area (pmol/l/min)x 10-3
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Men
Women

Figure 1

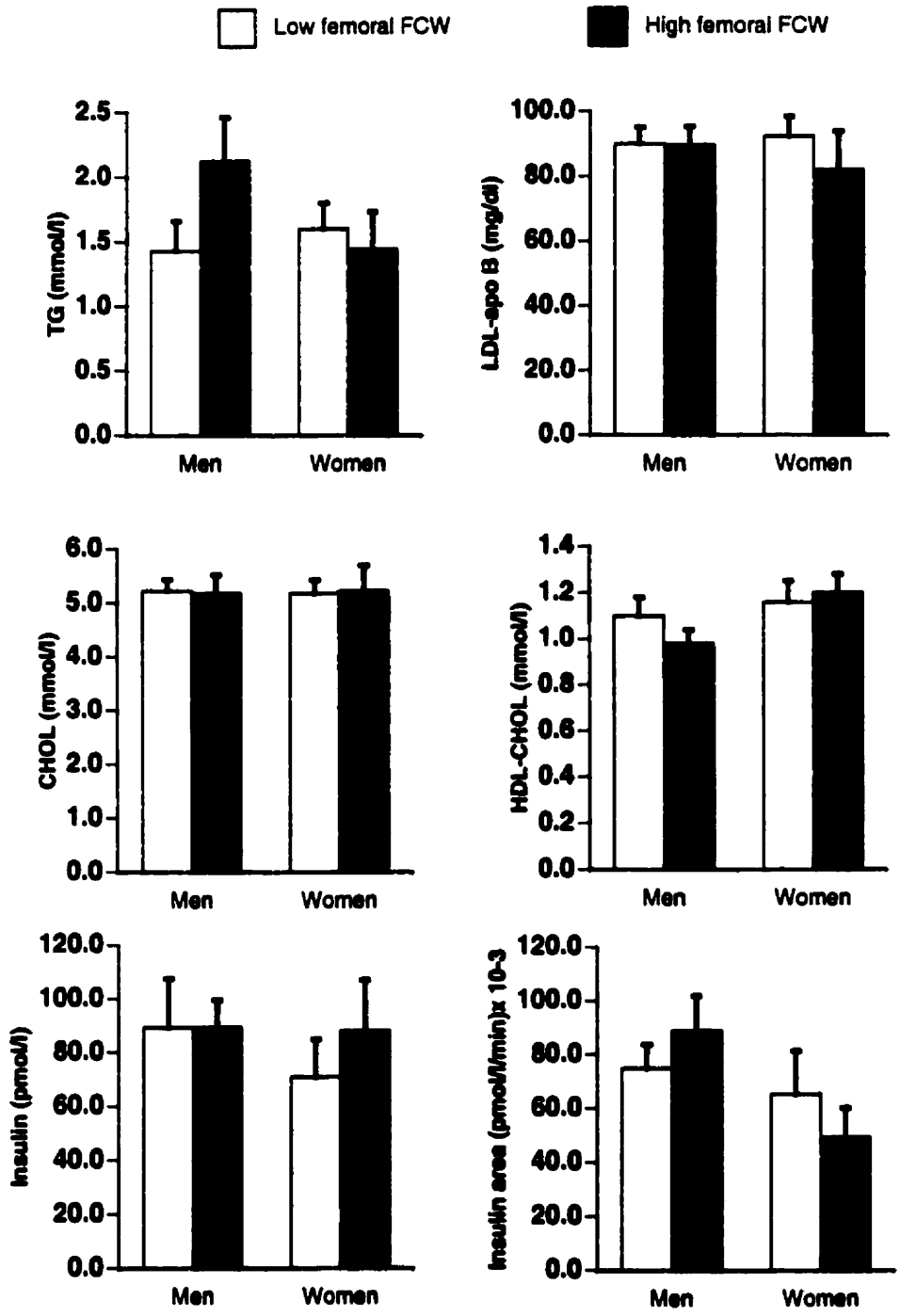

Low femoral FCW
High femoral FCW
TG (mmol/l)
2.5
2.0
1.5
1.0
0.5
0.0
Men
Women
LDL-apo B (mg/dl)
100.0
80.0
60.0
40.0
20.0
0.0
Men
Women
CHOL (mmol/l)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Men
Women
HDL-CHOL (mmol/l)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Men
Women
Insulin (pmol/l)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Men
Women
Insulin area (pmol/l/min)x 10-3
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Men
Women

Figure 2

CHAPITRE 6

EFFET D'UNE PERTE PONDÉRALE MODÉRÉE SUR L'ACTIVITÉ ET L'EXPRESSION DE LA LIPOPROTÉINE LIPASE DU TISSU ADIPEUX: EXISTENCE DE VARIATION SEXUELLE ET DE DIFFÉRENCES RÉGIONALES

L'article composant ce chapitre est intitulé:

"Effect of a moderate weight loss on adipose tissue lipoprotein lipase activity and expression: existence of sexual variation and regional differences"

(*International Journal of Obesity* 23; 957-965, 1999)

Résumé

Cette étude avait pour but de vérifier l'impact d'une perte de poids modérée sur l'activité et l'expression de la lipoprotéine lipase du tissu adipeux (TA-LPL) et le lien entre les variations de l'activité de la TA-LPL et celles des concentrations plasmatiques de certaines lipoprotéines. À cet égard, 32 sujets caractérisés par une surcharge corporelle (14 hommes et 18 femmes préménopausées; âge variant de 36 à 50 ans) ont subi une restriction calorique d'une durée de 15 semaines et dont le déficit énergétique était de 500-800 kcal/jour en-dessous de leur dépense énergétique estimée. Des biopsies au niveau des sites sous-cutané abdominal et fémoral ainsi que des mesures de lipoprotéines plasmatiques à jeun ont été effectuées avant et 4-6 semaines après la restriction calorique alors que les sujets présentaient un poids stable. En réponse à la perte de poids, l'activité de la TA-LPL des deux régions étudiées a diminué chez la femme alors qu'aucun changement n'a été observé chez l'homme. Par ailleurs, aucun changement au niveau de l'expression de la TA-LPL n'a été relevé suite au traitement chez les deux sexes. La perte de poids a entraîné une diminution significative des concentrations plasmatiques de triglycérides (TG), d'insuline et de la fraction cholestérol (C) des lipoprotéines de faible densité (LDL-C) chez l'homme alors que seules les concentrations de TG et de LDL-C étaient diminuées chez la femme ($P < 0.05$). Chez la femme, une diminution de l'activité de la TA-LPL du site fémoral a été associée à une réduction des concentrations plasmatiques de la fraction C des lipoprotéines de haute densité (HDL-C) en réponse à la perte de poids ($r = 0.50$, $P < 0.05$). Une relation inverse à celle décrite précédemment a été rapportée chez l'homme entre l'activité de la TA-LPL du site fémoral et les concentrations plasmatiques de HDL-C ($r = -0.64$, $P < 0.05$). En conclusion, ces résultats démontrent qu'en réponse à une perte de poids 1) les changements de l'activité de la TA-LPL diffèrent en fonction du sexe de l'individu alors que les changements des niveaux d'expression de l'enzyme demeurent stables; 2) les changements de l'activité de la TA-LPL du site fémoral semblent être reliés aux variations des concentrations plasmatiques de HDL-C, chez les deux sexes.

Effect of a moderate weight loss on adipose tissue lipoprotein lipase activity and expression: existence of sexual variation and regional differences

P. Imbeault [1], N. Alméras [3], D. Richard [2], J-P. Després [3],
A. Tremblay [1] and P. Mauriège [1,3]

[1] Physical Activity Sciences Laboratory, Department of Social & Preventive Medicine,
[2] Department of Physiology, Laval University, [3] Lipid Research Center, CHUQ Medical Research Center, Québec, Canada.

Supported by the Medical Research Council of Canada, the Fonds FCAR-Québec and by Servier Canada.

Address correspondence: P. Mauriège Ph.D., Physical Activity Sciences Laboratory, PEPS, Laval University, Ste-Foy, Québec, Canada, G1K 7P4
Tel: (418) 656-3851; Fax (418) 656-2441.

Running title: Adipose tissue lipoprotein lipase and weight loss

Keywords: obesity, regional fat distribution, lipid-lipoprotein profile

Abstract

Objective: To evaluate the impact of a moderate body weight loss on both adipose tissue lipoprotein lipase (AT-LPL) activity and expression and to verify whether variation in AT-LPL could be related to changes in the lipid-lipoprotein metabolism.

Design: Intervention study of a 15-week weight reducing program (energy deficit: 500-800 kcal/day under subjects' estimated sedentary energy expenditure).

Subjects: Thirthy two obese subjects (14 men and 18 premenopausal women; aged 36-50 years) whose body fatness ranged from 34 to 54% fat.

Measurements: Adipose tissue biopsies from the abdominal and femoral depots, various fatness and fat distribution parameters (computed tomography and anthropometry), fasting plasma concentrations of high- and low-density lipoprotein cholesterol (HDL-C, LDL-C), and triglycerides at baseline and 4-6 weeks after the 15-week weight reducing program, when subjects were weight stable.

Results: In response to weight loss, AT-LPL activity of both regions did not change in men, but decreased in women. Regarding AT-LPL expression, no interaction between time and sex was observed in response to the treatment. In both genders, the higher the basal AT-LPL activity, the greater the reduction in enzyme activity was in response to weight loss, in both the abdominal and femoral depots ($-0.53 < r < -0.84$, P values ranging from 0.0001 to 0.05). Body weight loss promoted a significant reduction in plasma triglyceride (TG), insulin and LDL-cholesterol (C) concentrations in men whereas only plasma TG and LDL-C levels were decreased in women ($P < 0.05$). Although the average reduction in HDL-C levels in response to weight loss was not significant, the higher the decrease in femoral AT-LPL activity, the greater was the reduction in plasma HDL-C levels ($r = 0.50$, $P < 0.05$) in response to weight loss, in women. An inverse relationship was observed between changes in femoral AT-LPL activity and HDL-C level variations in men ($r = -0.64$, $P < 0.05$).

Conclusion: These results suggest that 1) variation in AT-LPL activity differs between men and women in response to a moderate body weight loss, although the corresponding enzyme mRNA levels remain unchanged; 2) changes in femoral AT-LPL activity are related to weight loss induced variation in plasma HDL-C levels.

Introduction

Since the pioneering clinical observations of Jean Vague (1), numerous studies have reemphasized the notion that men tend to accumulate excess fat predominantly in the abdominal region which represents a health hazard whereas women display a preferential fat deposition in the gluteo-femoral areas which may, at least to a certain extent, be cardioprotective. It is also well known that lipoprotein lipase is involved in the regulation of adipose cell triglyceride storage and therefore seems to be a key regulator of fat accumulation in adipose tissue (2-5). In this regard, regional variation in adipose tissue lipoprotein lipase (AT-LPL) activity has already been hypothesized to account for the gender difference observed in body fat distribution since the enzyme activity is higher in subcutaneous abdominal than in gluteal fat cells in men, whereas the opposite has been observed in premenopausal women (6-9). However, lipoprotein lipase messenger RNA (mRNA) levels have been reported to be higher in abdominal than in gluteal adipose tissue in both genders (9).

It is also well known that variations in AT-LPL activity occur with changes in body energy balance since the enzyme activity has been reported to increase in the postprandial state (10, 11), or after a glucose load (10, 12) and to decrease during fasting (10, 12). Dietary restriction is a strategy commonly used to alter body energy balance and to treat obesity (13). Previous studies which have assessed the impact of hypocaloric diet-induced weight loss on AT-LPL activity have reported either a decrease (14-16) or a lack of change (17) in AT-LPL activity, whereas others have observed an increase in enzyme activity (18, 19). However, to the best of our knowledge, no study has attempted to compare the potential gender difference in AT-LPL activity as well as in its mRNA level in response to a weight-reducing program.

Thus, the aims of the present investigation were 1) to evaluate the impact of a moderate weight loss on both AT-LPL activity and on corresponding mRNA levels in the subcutaneous abdominal and femoral regions in a sample of obese men and premenopausal women, and 2) to verify whether variation in AT-LPL activity and expression could be related to changes in the lipid-lipoprotein metabolism.

Material and Methods

Subjects

Thirthy-two obese subjects (14 men and 18 premenopausal women), aged 43 ± 5 years (yrs) (36-50 yrs), were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. All individuals were subjected to a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication which could have influenced lipid metabolism (ß-blockers, antihypertensive drugs, etc) were excluded from the study. All subjects were sedentary, non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight reducing program, and their body weight had been stable during the last six months prior to the study. Women had regular menstrual cycles and none was using oral contraceptives or lactating at the time of the study. All measurements were performed while women were in the early follicular phase of their menstrual cycle.

All subjects participated in a 15-week weight loss program induced by a moderate caloric restriction which took into account the individual macronutrient composition of subjects, as reported in their three-day dietary record before experiment (18-19% protein, 38-39% fat, 41-46% carbohydrate and 1-2% alcohol of total energy intake). With the exception of carbohydrate intake which was higher in women than in men ($P < 0.05$), the macronutrient composition was similar in both genders. The energy deficit determined by the protocol averaged 500-800 kcal/day under subjects' estimated sedentary energy expenditure. This energy deficit was established according to indirect calorimetry measurements as well as from an estimation of a daily energy intake which has been evaluated by a three-day dietary record. Dietary restriction was accompanied by the daily oral intake of 60 mg of fenfluramine, a serotonin reuptake inhibitor which has been shown to facilitate body weight loss by a hypocaloric diet (20).

Adipose tissue biopsy procedure and adipose tissue lipoprotein lipase (AT-LPL) activity

After an overnight fast, participants were subjected to biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the anterior midthigh level (femoral site). Local anesthesia (1% xylocaine, without epinephrine) was performed in such a way that it did not influence the metabolic activity of excised AT (21). Biopsies were performed before and 4-6 weeks after the end of the treatment when all subjects were weight stable. A small cutaneous incision (1 cm) was performed in both sites and approximately 150 mg of adipose tissue from each region were immediately frozen in liquid nitrogen for later measurement of heparin-releasable LPL activity, according to Savard et al (22). Briefly, duplicates of 20-30 mg adipose tissue were incubated for 40 min at 28°C with 0.5 ml of Krebs-Ringer-0.1 M Tris-HCL (pH 8.4) containing bovine serum albumin (1 %) and heparin (2.5 IU) to achieve LPL release from the tissue. The tissue was then removed and 0.5 ml of substrate was added to the released LPL solution which was therefore incubated 2h at 28°C under gentle shaking. The substrate consisted of a mixture containing [^{14}C]triolein (1.3 μCi/ml) and cold triolein (11.1 mg/ml) sonicated in gum arabic (5 %), NaCl (2 %), fatty acid free bovine serum albumin (10 %) and fasted human serum. AT-LPL activity was expressed as micromoles of free-fatty acids (FFA) released per hour per 10^6 cells. Since AT-LPL activity is associated with fat cell size (3, 22), AT-LPL activity was also expressed per cell surface area (nanomoles FFA per hour per micrometer squared times 10^8). AT-LPL activity experiments were performed in all subjects (14 men and 18 women). The same batches of collagenase and bovine serum albumin (Boehringer Mannheim, Canada) were used in all experiments. [^{14}C]-triolein was obtained from Dupont NEN (Canada) whereas cold triolein came from Sigma (St-Louis, MO, USA). All other chemicals and organic solvents were of the highest purity grade commercially available.

Another sample of 200 mg adipose tissue was used for the measurement of fat cell size. Adipocytes were isolated according to the method of Rodbell (23), in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin (KRBA) and 5 mM glucose, plus 1 mg/ml collagenase, as previously described (24). Mean adipose cell diameter was assessed from the measurement of at least 500 cells on a microscope equipped with a

graduated ocular (Rockleight, NJ), and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described (24).

RNA preparation

Adipose tissue samples of approximately 50 mg were put in liquid nitrogen and total RNA was isolated using guanidinium thiocyanate phenol chloroform extraction and alcohol precipitation (25). Due to the limited amount of fat obtained in some subjects, AT-LPL mRNA assays could be performed only in 9-11 men as well as in 11-13 women for the abdominal and femoral adipose regions, respectively. The average yield of total RNA was 38.1 ± 28.1 and 40.5 ± 26.4 μg/g adipose tissue (wet weight) (not significantly different) for biopsies performed before and after the treatment, respectively. The absorption ratio (260:280 nm) ranged between 1.6 and 1.9 for all preparations. Total RNA was stored at -80^{0}C until quantification of the target mRNA.

Quantification of mRNA

AT-LPL mRNA was quantified by RT-PCR in the presence of an internal standard, i.e., rat adipose tissue, as previously described (26). Briefly, this method is based on the fact that rat and human cDNA segments are homologous to each other, except for 31 bases dispersed throughout the region (27). There exists a unique BsaJI restriction site in both the rat and human sequences, but they occur in different positions in the two molecules. Therefore, when digested with the BsaJI enzyme (New England Biolabs, Beverly, MA, USA), the human cDNA yields two fragments of equal size for the human cDNA (147 bp), while the rat cDNA yields two fragments of unequal size (136 and 158 bp). The two rat fragments can then be easily separated from each other and from the human fragment when one of the cDNAs is used as an internal standard in the PCR amplification of the other. cDNA was synthesized from 500 ng of total RNA in 20 μl of reverse transcription mixture that had the following final composition: 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 1 mM each of deoxribonucleotides (dNTPs) and 150 pmol of dowstream primer (5'-CTTCACTAGCTGGTCCACAT-3'). The tubes were heated at 95^{0}C for 5 min and cooled immediately in ice. One microliter (200 U) of Moloney murine leukemia virus (M-MLV)

reverse transcriptase was added, and the reaction mixture was overlaid with 30 µl of mineral oil. The tubes were incubated at 42^{0}C for 1 h and 95^{0}C for 10 min. The following solutions were added to the reaction tubes on top of the mineral oil: 5 µl of PCR buffer (300 mM Tris-HCl, pH 8.3, 200 mM KCl, 13 mM MgCl2, 1 mg/ml bovine serum albumine, 150 pmol of upstream primer (5'-GCAGGAAGTCTGACCAATAAG-3'). 3 µCi of [α-^{32}P]dCTP, 2 units of *Taq* DNA Polymerase (Boehringer, Mannheim, Canada) and water to a final volume of 50 µl. The samples were heated at 95^{0}C for 3 min and amplified for 30 cycles in a DNA thermal cycler (GeneAmp PCR System 9600, Perkin-Elmer, Norwalk, CT, USA) using the following parameters: 1 min at 94^{0}C, 1 min at 56^{0}C and 1 min at 72^{0}C. Aliquots (30 µl) of PCR products were digested with 2.25 units of the restriction enzyme BsaJI for 2 h at 60^{0}C. The digestion products were separated on a 13.5% polyacrylamide gel, and the bands were revealed by ethidium bromide staining. Radioactivity in the bands were determined by Cherenkov counting after excision from the gel.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique and percent body fat was derived from body density (28). Pulmonary residual volume was measured using the helium dilution method (29). Fat mass was calculated as total body weight minus fat-free mass. Waist girth was measured according to the procedures recommended at the Airlie Conference (30). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology of Sjöström et al (31). Briefly, subjects were examined in the supine position with both arms stretched above their head. CT scans were performed at both the abdominal (between L4 and L5 vertebrae) and femoral (midthigh) levels, using a radiograph of the skeleton as a reference to establish the position of the scans to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating these areas with a graph pen and then computing the AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) (31), as previously described (32). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Plasma determinations

Blood samples were obtained in the morning after a 12-h fast from an antecubital vein. Fasting plasma glucose and free-fatty acid (FFA) levels were measured enzymatically (33, 34), whereas plasma insulin concentration was determined by radioimmunoassay with polyethylene glycol separation (35). Cholesterol (C) and triglyceride (TG) levels in plasma and lipoprotein fractions, were measured enzymatically on a RA-1000 automated analyzer (Technicon Instruments Corporation, Tarrytown, NY, USA). After an ultracentrifugation, plasma high density lipoprotein (HDL) fraction was obtained after precipitation of low density lipoprotein (LDL) in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$ (36). Plasma LDL-apolipoprotein (apo) B levels were measured by the rocket immunoelectrophoretic method of Laurell, as previously described (37).

Statistical analyses

Gender variations were tested for significance with the Student's t-test. The difference between values obtained before and after weight loss was analyzed with a paired t-test. A two-way analysis of variance was also performed to verify whether significant sex and site differences for AT-LPL activity exist before and after weight loss and post hoc comparisons were tested with a paired t-test. Finally, univariate associations between variables were quantified using Pearson's product moment correlation coefficients. All analyses were performed using the Jump program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers.

Results

Subjects' characteristics

Subjects' physical characteristics before and after the weight-reducing program are presented in Table 1. Before weight loss, gender comparisons revealed that subcutaneous adipose tissue areas were higher in women than in men (P values ranging from 0.0001 to 0.05), whereas visceral fat accumulation was greater in the latter than in the former subjects ($P < 0.001$). On the other hand, women had larger femoral adipose cells than men ($P < 0.001$), whereas no sex difference was found in subcutaneous abdominal fat cell weight.

As expected, total adiposity was reduced after weight loss, in both genders (P values ranging from 0.0001 to 0.001). Weight reduction was also accompanied by a significant decrease in all the adipose tissue areas and subcutaneous fat cell weights (P values ranging from 0.0001 to 0.05).

The mean weight loss achieved 10 ± 4 kg (range -4 to -17 kg) vs 9 ± 6 kg (range 0 to -23 kg) in men vs women, respectively. However, men displayed a greater decrease in fat mass, percent fat and waist girth (P values ranging from 0.001 to 0.05), although they showed a lower reduction in their fat-free mass as compared to women ($P < 0.001$). Finally, subcutaneous abdominal and visceral adipose tissue areas as well as regional fat cell weight decreased similarly in response to weight loss, in both genders.

Insert Table 1

Subjects' metabolic profile

Table 2 shows the metabolic profile of both men and women, before and after weight loss. At baseline, gender comparisons revealed that men displayed lower plasma HDL-C concentrations compared to women ($P < 0.05$), whereas no sex difference was observed in indices of plasma glucose and insulin levels. Plasma TG, LDL-C and LDL-apo B concentrations and fasting insulin levels were reduced in men after weight loss (P values ranging from 0.01 to 0.05). Plasma HDL-C levels tended to be lower in men after weight

reduction, although this difference did not reach statistical significance (P = 0.06). In women, fasting plasma TG and LDL-C concentrations decreased in response to weight loss (P values ranging from 0.01 to 0.05). No significant gender difference was observed in the response of metabolic variables to weight reduction.

Insert Table 2

AT-LPL activity and expression

Figure 1 illustrates the effect of weight reduction on AT-LPL activity expressed either per adipose tissue mass (A), per cell number (B) or corrected for variation in adipocyte surface area (C), in subcutaneous abdominal and femoral adipose regions of both men and women. Before weight loss, no regional variation was found in AT-LPL activity in men, whereas the enzyme activity was higher in the femoral than in the subcutaneous abdominal fat depot in women ($P < 0.05$). Moreover, women displayed a greater pre-weight loss AT-LPL activity in both depots than men ($P < 0.001$). In response to the treatment, a significant time and sex interaction was observed for AT-LPL activity, regardless of the mode of expression of the data and the region studied (F values ranging from 5.4 to 6.7; P values ranging from 0.01 to 0.05). This observation was probably due to the lack of variation in AT-LPL activity in men and to the decrease of the enzyme activity in women. Femoral AT-LPL activity was significantly higher in women than in men, after weight loss ($P < 0.01$).

Insert Figure 1

Figure 2 shows the effect of weight loss on AT-LPL expression. No regional variation was observed in AT-LPL mRNA levels before and after weight loss in both genders. On the other hand, women presented higher pre-weight loss subcutaneous abdominal AT-LPL mRNA levels as compared to men ($P < 0.05$). AT-LPL mRNA levels of both subcutaneous abdominal and femoral depots did not change in response to weight loss, in men and women. However, women displayed greater femoral AT-LPL mRNA levels than men at the end of the treatment ($P = 0.05$).

Insert Figure 2

Relationships between AT-LPL activity and mRNA levels

The extent of the fall in LPL activity observed after weight reduction in either the subcutaneous abdominal or the femoral fat depot was associated with the initial level of enzyme activity. Indeed, the higher the pre-weight loss AT-LPL activity measured in both adipose regions, the greater the reduction of the enzyme activity was when expressed per cell surface area in both men ($-0.53 < r < -0.71$, P values ranging from 0.01 to 0.05) and women ($-0.84 < r < -0.87$, P values ranging from 0.0001 to 0.001) (Fig. 3). Similar results were observed regardless of the mode of expression of AT-LPL activity, i.e., results being expressed per cell number or per adipose tissue mass ($-0.53 < r < -0.84$, P values ranging from 0.0001 to 0.05) (not shown). Moreover, mRNA level was not significantly correlated with the enzyme activity, irrespective of the gender or the adipose depot ($-0.14 < r < 0.29$; not shown).

Insert Figure 3

Furthermore, to verify whether the changes observed in the subjects' metabolic profile could be associated with variation in AT-LPL activity or expression, correlational analyses have also been performed.

Relationships between AT-LPL activity or expression vs metabolic variables

Plasma LDL-C and HDL-C levels were the only variables out of the subjects' metabolic profile which were associated with AT-LPL activity variation. Indeed, a decrease in subcutaneous abdominal AT-LPL activity was related to a lower reduction of plasma LDL-C levels, in men ($r = -0.55$, $P < 0.05$), whereas no significant association was observed between the latter variables in women (not shown). However, men who were characterized by a decreased femoral AT-LPL activity after weight loss displayed a lower reduction of plasma HDL-C levels ($r = -0.64$, $P < 0.05$) (Fig. 4). In contrast, the higher the decrease in femoral AT-LPL activity, the greater the reduction in plasma HDL-C levels was in women ($r = 0.50$, $P < 0.05$) (Fig. 4).

Insert Figure 4

Discussion

The present investigation was conducted i) to examine the effects of a 15 week- caloric restriction period on both AT-LPL activity and expression in subcutaneous abdominal and femoral fat depots of men and women and ii) to verify whether variation in AT-LPL function could be associated with a more favorable or deleterious metabolic profile. To the best of our knowledge, our study is the first to investigate the gender variation in the impact of a moderate weight loss on LPL activity and its gene expression. Our results show that the enzyme activity does not respond similarly in both genders after weight loss. Indeed, AT-LPL activity of both regions remained unchanged in men, whereas the enzyme activity decreased in both adipose sites from women. This marked gender difference was, however, not observed for AT-LPL mRNA levels in response to the weight-reducing program.

The fact that premenopausal women displayed a higher AT-LPL activity in the femoral than in the subcutaneous abdominal region (Fig. 1) has already been reported by us (38, 39) and others (6, 8, 9). The finding that men had a similar AT-LPL activity in both adipose depots (Fig. 1) is also in agreement with previous studies (7-9, 39). Moreover, our data support the notion that both subcutaneous abdominal and femoral AT-LPL activities are higher in women than in men, as previously reported (8, 9, 39-41). This sex related difference in AT-LPL activity could be potentially influenced by variation in steroid hormones (4, 42). Indeed, progesterone has already been reported to promote the filling of gluteo-femoral adipocytes by stimulating LPL activity in these cells (43), whereas testosterone administered to men has been shown to inhibit LPL in the subcutaneous abdominal but not in the femoral adipose tissue (44).

We also found a significant gender effect on AT-LPL activity in response to weight reduction since AT-LPL activity did not change in men, whereas it decreased in women. Our findings in women, but not in men, are concordant with previous observations (7, 15, 16). Indeed, one study exclusively composed of men (18) and another that included 6 men and 3 women (19) have already reported an increase in AT-LPL activity after weight loss. It is possible that such discrepancy could be due to the substantial mean weight loss (16 and 43 kg) reported in the

two previous studies, as already hypothesized (18, 19). Rebuffé-Scrive et al (14) have also proposed several hypotheses to explain this discrepancy such as differences in the gender and in the subjects' fatness as well as the nature and duration of the restrictive diet used to promote weight loss. Up to now, the gender difference observed in AT-LPL activity following weight reduction in the present study cannot be easily explainable by available literature, although steroid hormones have been shown to vary between men and women who were subjected to weight loss (45). Although all these factors may have potentially affected the predicted AT-LPL response to weight loss, the positive relationship observed between the fall in LPL activity and the pre-weight loss enzyme activity (Fig 3) is commonly observed among studies reporting a decreased AT-LPL activity (14-16). However, further studies are clearly warranted to clarify this issue.

A previous study showed that AT-LPL mRNA levels were higher in the abdominal than in the gluteal site of both lean men and women (9). However, the fact that we did not observe any regional variation in LPL mRNA abundance in both sexes could be partly explained by our subjects' fatness. Arner et al. (9) also reported that in both regions, AT-LPL mRNA levels were significantly higher in women than in men. Our results revealed that pre-weight loss AT-LPL mRNA content was higher in the abdominal region of women as compared to men. On the other hand, Kern et al. (19) have already reported an increase in the AT-LPL mRNA levels of the abdominal region following a substantial weight loss in massively obese individuals. In our study, no significant variation in AT-LPL expression was observed following the treatment, in both regions and genders. This discrepancy could, once again, be explained by the difference in the magnitude of weight lost before subjects stabilized their body weight or by the subjects' level of fatness itself. In the present study, both AT-LPL activity and expression did not change in men whereas the enzyme activity, but not its corresponding mRNA level decreased following weight reduction in women. Even though we did not measure the lipoprotein lipase mass, it is likely that weight loss induced a decrease AT-LPL activity of women through postranslational mechanisms, as already documented (9, 11, 46).

Finally, it is well known that a substantial part of HDL is derived from surface constituents of chylomicrons and VLDL during breakdown of their triglycerides by lipoprotein lipase (47,

48). In this regard, Magill et al (49) have already observed that a high gluteal AT-LPL activity increased the fractional rate of catabolism of VLDL, which, in turn, raised HDL-C concentrations. Our results show that a reduced femoral AT-LPL activity is associated with decreased plasma HDL-C levels in women. Since a high femoral AT-LPL activity has been reported to be a significant correlate of the more favorable plasma lipoprotein-lipid profile (i.e., HDL-C) observed in premenopausal women (39), a reduced enzyme activity could have an undesirable impact on HDL-C levels. St-Amand et al (39) have recently reported that plasma LDL-C levels were negatively related to plasma postheparin LPL activity in men, but not to subcutaneous abdominal AT-LPL activity. Although we did not observe any relationship between plasma LDL-C levels and subcutaneous abdominal AT-LPL activity before weight loss, changes in subcutaneous abdominal AT-LPL activity were significantly associated with LDL-C variation following weight loss, in men. These results could be partly explained by the role of LPL in the retention of LDL in the subendothelial matrix (50).

Conclusion

In summary, this study documents for the first time that variation in AT-LPL activity of both subcutaneous abdominal and femoral regions differs according to gender, even though the magnitude of weight loss is similar in both men and women. However, this sexual dimorphism was not observed at the mRNA level. Variation in AT-LPL activity is not a good predictor of changes occurring in adipose cell size and regional fat during dietary weight reducing therapy. Finally, a reduction in femoral AT-LPL activity following a moderate weight loss through caloric restriction does not appear to preserve the favorable plasma lipoprotein profile against the risk of developing coronary heart disease observed in premenopausal women.

Acknowledgments

The authors wish to express their gratitude to Sylvie St-Pierre, Éric Doucet, Vicky Drapeau, France Levasseur, Henri Bessette and Antoine Labrie for their excellent collaboration at various stages of the study. Drs Gilles Lortie and Sylvie Leclerc are also gratefully acknowledged for their excellent medical supervision. Thanks are also expressed to Suzanne Brulotte of the Department of Radiology (Laval University Hospital, Québec, Canada) for their remarkable work with the computed tomograph. The subjects and the staff of the Lipid Research Center are also gratefully acknowledged.

References

1 Vague J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am J Clin Nutr* 1956; **4**: 20-34.

2 Golberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res* 1996; **37**: 693-707.

3 Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989; **320**: 1060-1068.

4 Leibel RL, Edens NK, Fried SK. Physiologic basis for the control of body fat distribution in humans. *Annu Rev Nutr* 1989; **9**: 417-443.

5 Bouchard C, Després JP, Mauriège P. Genetic and nongenetic determinants of regional fat distribution. *Endocr Rev* 1993; **14**: 72-93.

6 Rebuffé-Scrive M, Enk L, Crona N, Lönnroth P, Abrahamsson L, Smith U, Björntorp P. Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy and lactation. *J Clin Invest* 1985; **75**: 1973-1976.

7 Rebuffé-Scrive M, Lönnroth P, Marin P, Wesslau C, Björntorp P, Smith U. Regional adipose tissue metabolism in men and postmenopausal women. *Int J Obesity* 1987; **11**: 347-355.

8 Fried SK, Kral JG. Sex differences in regional distribution of fat cell size and lipoprotein lipase activity in morbidly obese patients. *Int J Obesity* 1987; **11**: 129-140.

9 Arner P, Lithell H, Wahrenberg H, Brönnegard M. Expression of lipoprotein lipase in different subcutaneous adipose tissue regions. *J Lipid Res* 1991; **32**: 423-429.

10 Olivecrona T, Bergö M, Hultin M, Olivecrona G. Nutritional regulation of lipoprotein lipase. *Can J Cardiol* 1995; **11**: 73-78.

11 Ong JM, Kern PA. Effect of feeding and obesity on lipoprotein lipase activity, immunoreactive protein, and messenger RNA levels in human adipose tissue. *J Clin Invest* 1989; **84**: 305-311.

12 Taskinen MR, Nikkilä EA. Lipoprotein lipase of adipose tissue and skeletal muscle in human obesity: Response to glucose and to semistarvation. *Metabolism* 1981; **30**: 810-817.

13 Van Gaal LF. Dietary treatment of obesity. In: Bray GA, Bouchard C and James WPT (eds). *Handbook of obesity*. Marcel Dekker, Inc.: New York, 1998, 875-890.

14 Rebuffé-Scrive M, Basdevant A, Guy-Grand B. Nutritional induction of adipose tissue lipoprotein lipase in obese subjects. *Am J Clin Nutr* 1983; **37**: 974-980.

15 Taskinen MR, Nikkilä EA. Basal and postprandial lipoprotein lipase activity in adipose tissue during caloric restriction and refeeding. *Metabolism* 1987; **36**: 625-630.

16 Eckel RH, Yost TJ. Weight reduction increases adipose tissue lipoprotein lipase responsiveness in obese women. *J Clin Invest* 1987; **80**: 992-997.

17 Sörbris R, Petersson B-G, Nilsson-Ehle P. Effects of weight reduction on plasma lipoproteins and adipose tissue metabolism in obese subjects. *Eur J Clin Invest* 1981; **11**: 491-498.

18 Schwartz R, Brunzell J. Increase of adipose tissue LPL activity with weight loss. *J Clin Invest* 1981; **67**: 1425-1430.

19 Kern PA, Ong JM, Saffari B, Carty J. The effects of weight loss on the activity and expression of adipose-tissue lipoprotein lipase in very obese humans. *N Engl J Med* 1990; **322**: 1053-1059.

20 Bray GA. Drug treatment of obesity. *Am J Clin Nutr* 1992; **55**: 538S-544S.

21 Mauriège P, Després JP, Prud'homme D, Pouliot MC, Marcotte M, Tremblay A, Bouchard C. Regional variation in adipose tissue lipolysis in lean and obese men. *J Lipid Res* 1991; **32**: 1625-1633.

22 Savard R, Deshaies Y, Després JP, Marcotte M, Bukowiecki LJ, Allard C, Bouchard C. Lipogenesis and lipoprotein lipase activity in human adipose tissue: reproducibility of measurements and relationship with fat cell size. *Can J Physiol Pharmacol* 1984; **62**: 1448-1452.

23 Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* 1964; **239**: 375-380.

24 Mauriège P, Marette A, Atgié C, Bouchard C, Thériault G, Bukowiecki LJ, Marceau P, Biron S, Nadeau A, Després JP. Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J Lipid Res* 1995; **36**: 672-684.

25 Chomezynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thyocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; **162**: 156-159.

26 Lévesque G, Lamarche B, Murthy MRV, Julien P, Després JP. Determination of changes in specific gene expression by reverse transcription PCR using interspecies mRNA as internal standards. *Biotechniques* 1994; **17**: 738-741.

27 Brault D, Noé L, Étienne J, Hammelin A, Raisonnier A, Soulou A, Chuat JC, Dugail I, Quignard-Boulangé A, Lavau M, Gallibert F. Sequence of rat lipoprotein lipase encoding cDNA. *Genes* 1992; **121**: 237-246.

28 Siri WE. The gross composition of body fat. *Adv Biol Med Physiol* 1956; **4**: 239-280.

29 Meneely GR, Kaltreider NL. Volume of the lung determined by helium dilution. *J Clin Invest* 1949; **28**: 129-139.

30 Lohman TG, Roche AF, Martorell R. The Airlie (VA) consensus conference. In: (eds). *Anthropometric Standardisation Reference Manual.* Human Kinetics Publishers: Champaign (Illinois), 1988, 39-80.

31 Sjöström L, Kvist H, Cederblad A, Tylen U. Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. *Am J Physiol* 1986; **250**: E736-E786.

32 Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, Lupien PJ, Thériault G, Bouchard C. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br J Nutr* 1989; **61**: 139-148.

33 Richterich R, Dauwwalder H. Zur bestimmung der plasmaglukose-konzentration mit der hexokinase-glucose-6-phosphat-deshydrogenase-methode. *Schweiz Med Wochenschr* 1971; **101**: 615-618.

34 Dole VP, Meinertz H. Microdetermination of long chain fatty acids in plasma and tissues. *J Lipid Res* 1960; **235**: 2595-2599.

35 Desbuquois B, Aurbach GD. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 1971; **37**: 732-738.

36 Moorjani S, Gagné C, Lupien PJ, Brun D. Plasma triglycerides related decrease in high density lipoprotein cholesterol and its association with myocardial infarction in heterozygous familial hypercholesterolemia. *Metabolism* 1986; **35**: 311-316.

37 Avogaro P, Bittolo Bon G, Cazzolato G, Quinci GB. Are apolipoprotein better discriminators than lipids for atherosclerosis? *Lancet* 1979; **1**: 901-903.

38 Pouliot M-C, Després JP, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C. Regional variation in adipose tissue lipoprotein lipase activity: association with plasma high density lipoprotein levels. *Eur J Clin Invest* 1991; **21**: 398-405.

39 St-Amand J, Després JP, Lemieux S, Lamarche B, Moorjani S, Prud'homme D, Bouchard C, Lupien PJ. Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women? *Metabolism* 1995; **44**: 491-498.

40 Lithell H, Boberg J, Hellsing K, Lundqvist G, Vessby B. Lipoprotein lipase activity in human skeletal and adipose tissue in the fasting and the fed states. *Atherosclerosis* 1978; **30**: 89-94.

41 Pedersen SB, Jonler M, Richelsen B. Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 1994; **78**: 1354-1359.

42 Kissebah AH, Krakower GR. Regional adiposity and morbidity. *Physiol Rev* 1994; **74**: 761-811.

43 Rebuffé-Scrive M, Basdevant A, Guy-Grand B. Effect of local application of progesterone on human adipose tissue lipoprotein lipase. *Horm Metabol Res* 1983; **15**: 566.

44 Rebuffé-Scrive M, Marin P, Björntorp P. Effect of testosterone on abdominal adipose tissue in men. *Int J Obesity* 1991; **15**: 791-795.

45 Zumoff B, Strain GW. A perspective on the hormonal abnormalities of obesity: are they cause or effect? *Obesity Res* 1994; **2**: 56-67.

46 Maheux P, Azhar S, Kern PA, Chen Y-DI, Reaven GM. Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. *Diabetologia* 1997; **40**: 850-858.

47 Nikkilä EA. Metabolic and endocrine control of plasma high density lipoprotein concentration. In: Gotto AM, Jr. MNE and Oliver MF (eds). *High density lipoproteins and atherosclerosis*. Elsevier/North-Holland Biomedical Press: Amsterdam, 1978, 177-192.

48 Redgrave TG, Small DM. Quantification of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J Clin Invest* 1979; **64**: 162-171.

49 Magill P, Rao SN, Miller NE, Nicoll A, Brunzell J, St-Hilaire J, Lewis B. Relationships between the metabolism of high-density and very-low-density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur J Clin Invest* 1982; **12**: 113-120.

50 Rutledge JC, Woo MM, Rezai AA, Curtiss LK, Goldberg IJ. Lipoprotein lipase increases lipoprotein binding to the artery wall and increases endothelial layer permeability by formation of lipolysis products. *Circ Res* 1997; **80**: 819-28.

Legends to figures

Figure 1

Adipose tissue lipoprotein lipase (AT-LPL) activity expressed either per g of adipose tissue mass (A), per cell number (B) or corrected for variation in adipocyte surface area (C) in subcutaneous abdominal and femoral regions of men and women, before and after weight loss. Values are means ± SE. Significant gender variation at † $P < 0.01$ and ‡ $P < 0.001$. [a] indicates a significant difference within each sex, before and after weight loss at $P < 0.05$.

Figure 2

Adipose tissue lipoprotein lipase expression (AT-LPL mRNA level) in subcutaneous abdominal and femoral regions of men and women, before and after weight loss. Values are means ± SE. Significant gender variation at * $P < 0.05$.

Figure 3

Relationships between the changes in AT-LPL activity induced by weight loss and the pre-weight loss enzyme activity in subcutaneous abdominal and femoral regions of men and women.

Figure 4

Relationships between the changes in plasma HDL-C levels and the AT-LPL activity variation following weight loss in subcutaneous abdominal and femoral regions of men and women.

Table 1. Subjects' physical characteristics before and after weight loss.

	Men (n =14)		Women (n = 18)	
	Before	**After**	**Before**	**After**
Body weight (kg)	104 ± 11 [b]	94 ± 10 [&]	92 ± 14	84 ± 14 [&]
BMI (kg/m^2)	34 ± 3	31 ± 3 [&]	36 ± 4	33 ± 5 [&]
Fat-free mass (kg)	64 ± 7 [d]	64 ± 8	47 ± 6	44± 5
Fat mass (kg)	40 ± 6	30 ± 5 [&]	45 ± 10	40 ± 11 [‡]
% Fat	38 ± 4 [d]	32 ± 5 [&]	49 ± 5	47 ± 6 [&]
Waist girth (cm)	110 ± 8	101 ± 6 [&]	99 ± 11	93 ± 11 [&]
Adipose tissue areas mesured by CT (cm^2)				
Abdomen (L4-L5)				
Subcutaneous	404 ± 71 [a]	328 ± 70 [&]	559 ± 144	499 ± 152 [‡]
Visceral	192 ± 55 [c]	140 ± 52 [&]	153 ± 47	121 ± 42 [‡]
Midthigh subcutaneous	76 ± 11 [d]	60 ± 12 [&]	181 ± 41	159 ± 40 [‡]
Regional fat cell weight (µg lipid/cell)				
Abdominal	0.64 ± 0.12	0.54 ± 0.14 [*]	0.71 ± 0.15	0.61 ± 0.13 [*]
Femoral	0.58 ± 0.10 [c]	0.48 ± 0.10 [†]	0.81 ± 0.18	0.62 ± 0.16 [†]

Values are means ± standard deviation (SD)

BMI = body mass index; CT = computed tomography.

Significant difference before and after weight loss at * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, & $P < 0.0001$.

Significant gender variation before weight loss at [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, [d] $P < 0.0001$.

Table 2. Subjects' metabolic profile before and after weight loss.

	Men (n =14)		Women (n =18)	
	Before	After	Before	After
Insulin (pmol/l)	122 ± 46	90 ± 52 †	101 ± 71	89 ± 68
Glucose (mmol/l)	5.0 ± 0.7	5.0 ± 0.9	4.9 ± 0.5	4.9 ± 0.5
FFA (mmol/l)	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
TG (mmol/l)	2.0 ± 0.9	1.5 ± 0.6 *	1.6 ± 0.7	1.3 ± 0.3 *
HDL-C (mmol/l)	0.88 ± 0.12 [a]	0.83 ± 0.12	1.00 ± 0.18	0.96 ± 0.20
LDL-C (mmol/l)	3.26 ± 0.64	3.13 ± 0.60 †	3.34 ± 0.66	3.02 ± 0.58 †
LDL-apo B (mg/dl)	0.91 ± 0.18	0.85 ± 0.17 †	0.86 ± 0.20	0.80 ± 0.19

Values are means ± standard deviation (SD).

TG = triglycerides; C = cholesterol; FFA = free fatty acids; apo = apolipoprotein

Significant difference before and after weight loss at * $P < 0.05$, † $P < 0.01$.

Significant gender variation before weight loss at [a] $P < 0.05$.

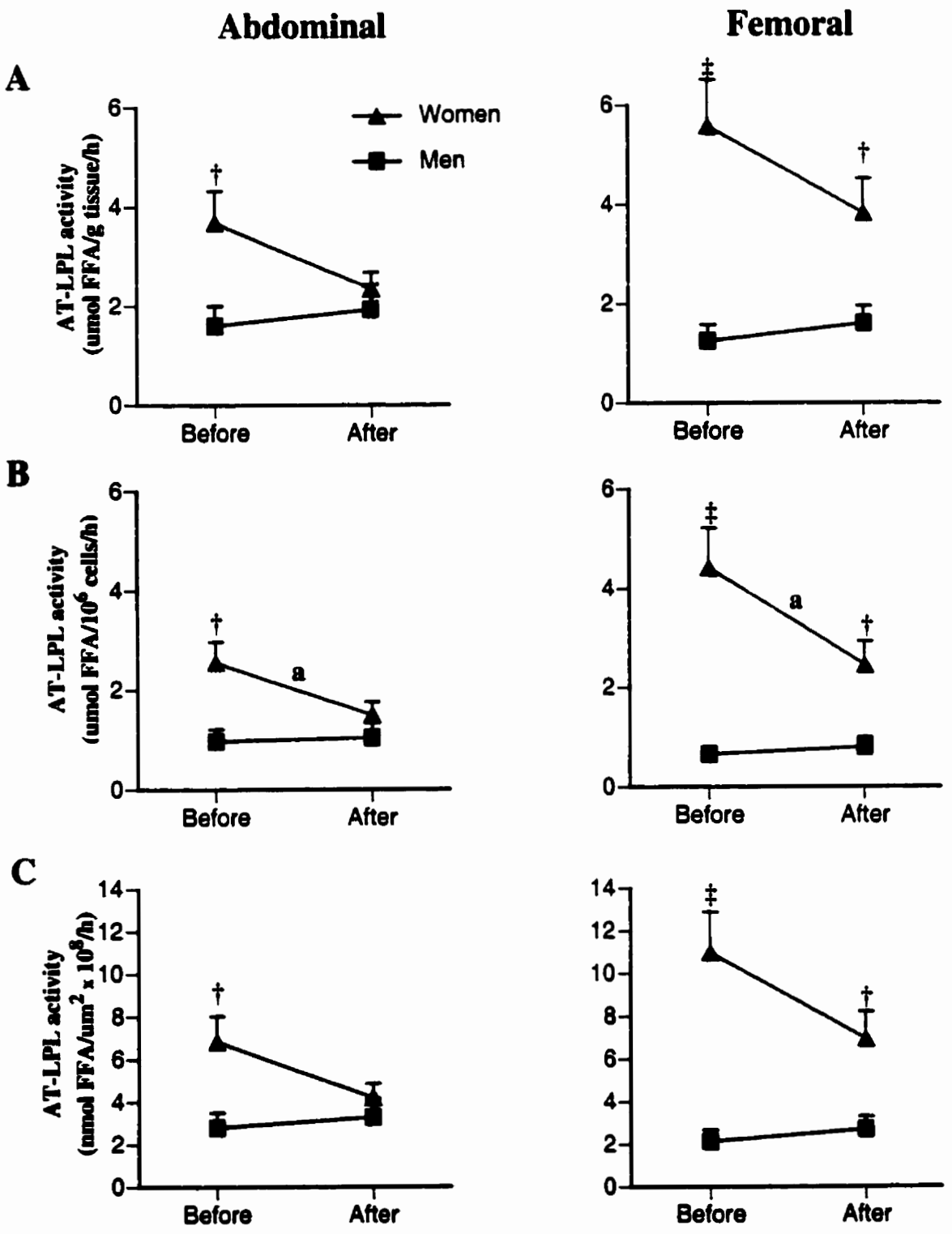

Abdominal
Femoral
A
B
C
Women
Men
AT-LPL activity (umol FFA/g tissue/h)
AT-LPL activity (umol FFA/10^6 cells/h)
AT-LPL activity (nmol FFA/um^2 x 10^6/h)
Before
After
†
‡
a

Figure 1

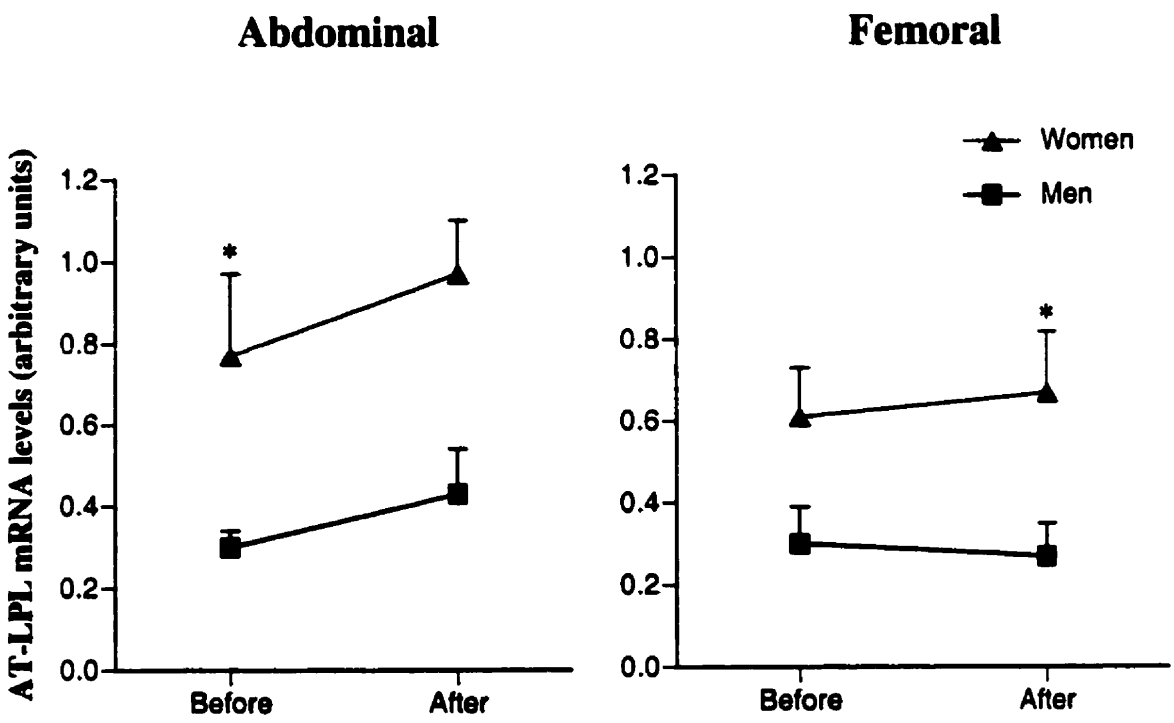

Abdominal
Femoral
Women
Men
AT-LPL mRNA levels (arbitrary units)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Before
After
Before
After

Figure 2

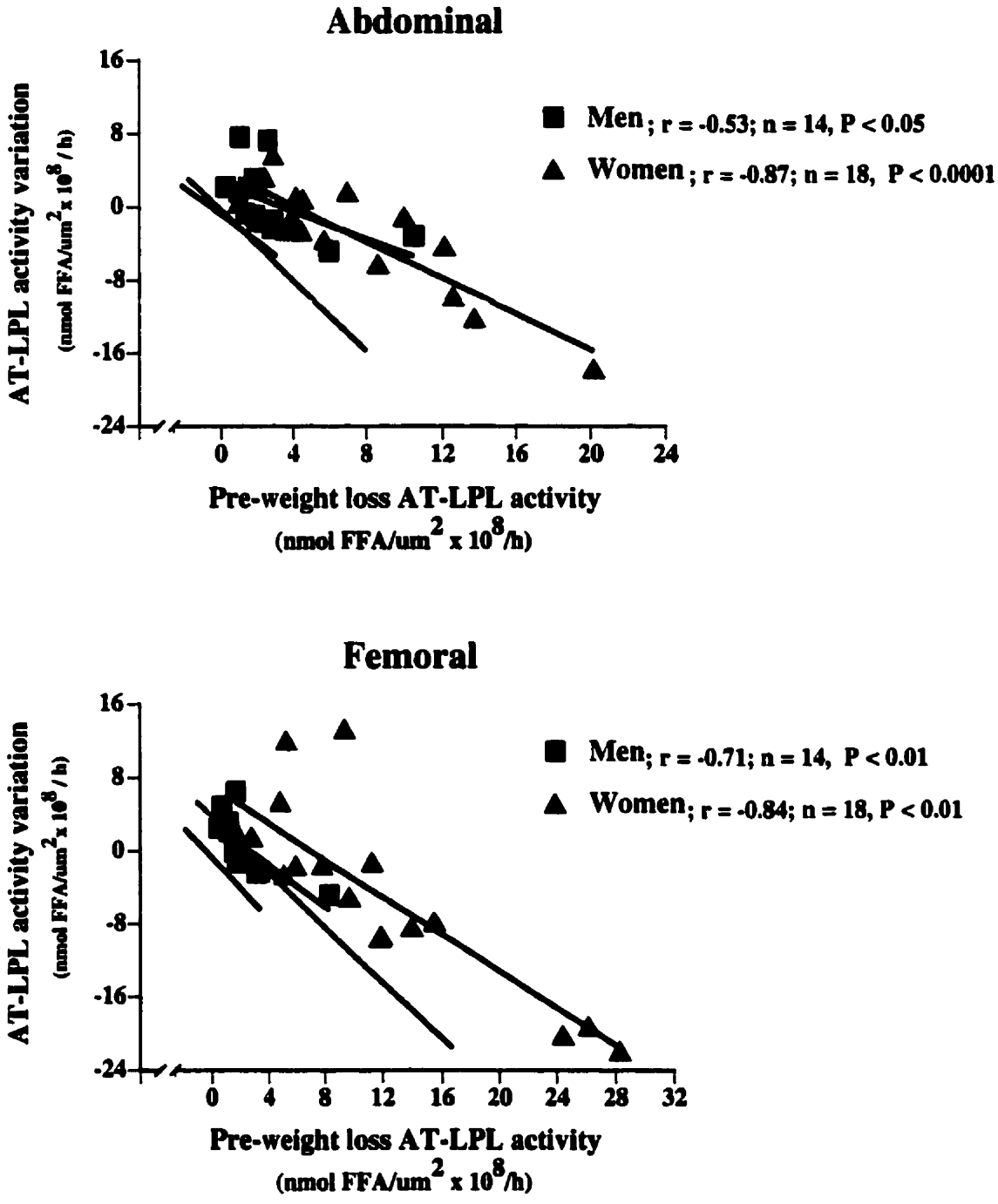
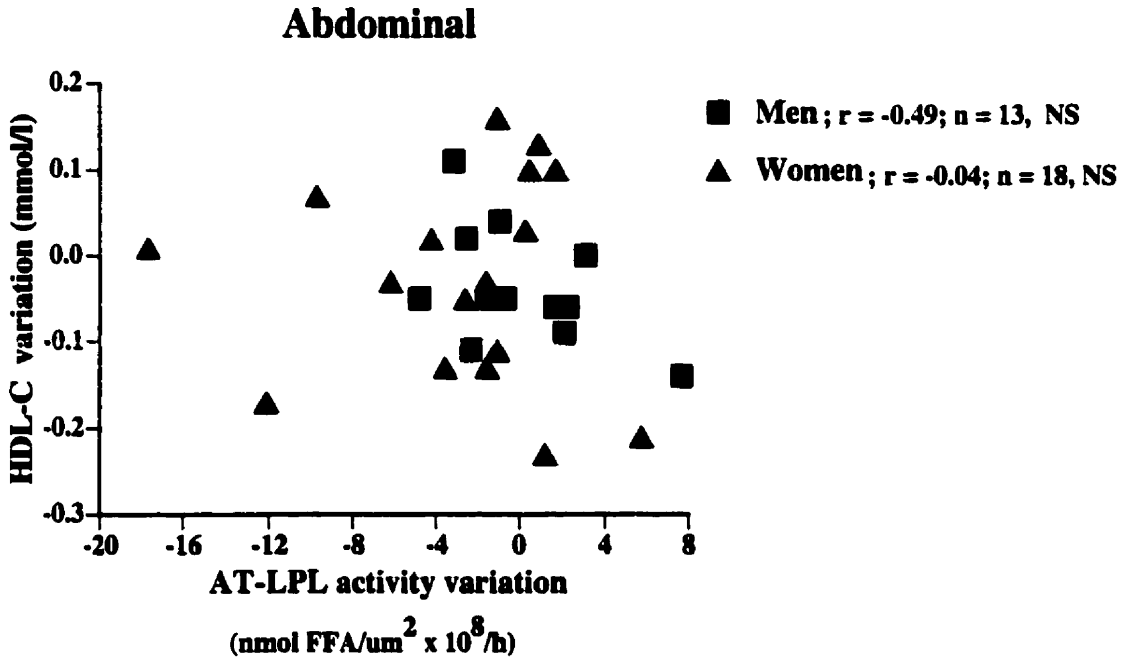

Abdominal
AT-LPL activity variation
(nmol FFA/um2 x 10^8 / h)
16
8
0
-8
-16
-24
0
4
8
12
16
20
24
Pre-weight loss AT-LPL activity
(nmol FFA/um2 x 10^8/h)
Men ; r = -0.53; n = 14, P < 0.05
Women ; r = -0.87; n = 18, P < 0.0001
Femoral
AT-LPL activity variation
(nmol FFA/um2 x 10^8 / h)
16
8
0
-8
-16
-24
0
4
8
12
16
20
24
28
32
Pre-weight loss AT-LPL activity
(nmol FFA/um2 x 10^8/h)
Men; r = -0.71; n = 14, P < 0.01
Women; r = -0.84; n = 18, P < 0.01

Figure 3


Abdominal
HDL-C variation (mmol/l)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-20
-16
-12
-8
-4
0
4
8
AT-LPL activity variation
(nmol FFA/um2 x 10^8/h)
Men ; r = -0.49; n = 13, NS
Women ; r = -0.04; n = 18, NS

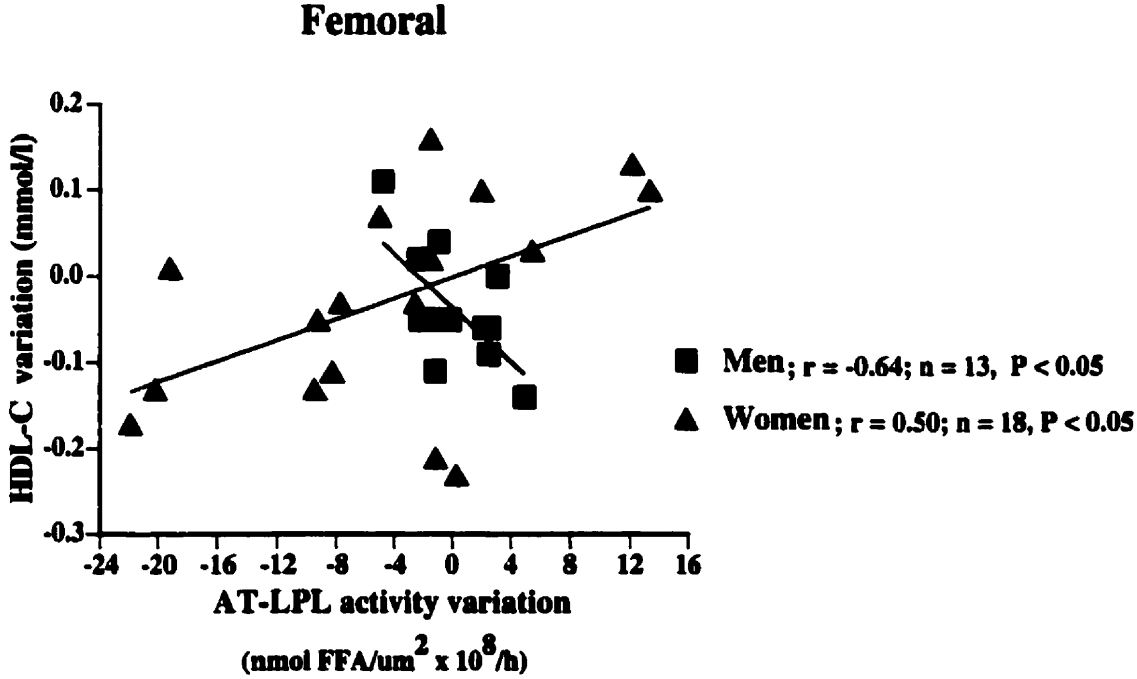

Femoral
HDL-C variation (mmol/l)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-24
-20
-16
-12
-8
-4
0
4
8
12
16
AT-LPL activity variation
(nmol FFA/um2 x 10^8/h)
Men; r = -0.64; n = 13, P < 0.05
Women ; r = 0.50; n = 18, P < 0.05

Figure 4

CHAPITRE 7

VARIATIONS RÉGIONALES ET SEXUELLES DE LA LIPOLYSE DU TISSU ADIPEUX EN RÉPONSE À UNE PERTE DE POIDS

L'article composant ce chapitre est intitulé:

"Regional and gender variations in adipose tissue lipolysis in response to weight loss"

(*Journal of Lipid Research* 40; 1559-1571, 1999)

Résumé

La réponse lipolytique d'adipocytes isolés aux catécholamines a été mesurée chez 32 sujets obèses (14 hommes et 18 femmes préménopausées) âgés entre 36 et 50 ans et dont l'indice de masse corporelle variait de 30 à 42 kg/m^2. La lipolyse des cellules adipeuses sous-cutanées abdominales et fémorales a été étudiée avant et après un programme de restriction calorique de 15 semaines au cours duquel la perte moyenne de poids respective fut de 9 et 10 kg chez les femmes et les hommes ($P < 0.0001$). La taille des cellules adipeuses a diminué d'environ 15-20% au niveau des deux dépôts suite à la restriction énergétique (P variant entre 0.01 et 0.05). Aux faibles concentrations, l'adrénaline (agoniste mixte α_2/β-adrénergique (AR)) a entraîné un effet antilipolytique alors qu'à fortes concentrations un effet lipolytique de l'hormone fut observé, indépendamment du traitement et du dépôt adipeux. La lipolyse basale, la réponse lipolytique maximale à l'isoprénaline (agoniste β-AR), à la dobutamine (agoniste β1-AR) et au procatérol (agoniste β2-AR) ainsi que l'effet antilipolytique de l'UK-14304 (agoniste α_2-AR) étaient similaires après la perte de poids. Cependant, la sensibilité β- et plus particulièrement β2-AR et la densité des récepteurs β-AR ont été augmentées en réponse à la restriction calorique chez les deux sexes, cet effet étant plus marqué au niveau des adipocytes abdominaux que fémoraux (P variant entre 0.001 et 0.05). Une diminution de la sensibilité α_2-AR des adipocytes fut observée au niveau des deux régions étudiées chez la femme, mais seulement au niveau sous-cutané abdominal chez l'homme ($P < 0.05$) après la perte de poids, sans qu'il n'y ait eu toutefois de changements dans la densité en récepteurs α_2-AR. En conclusion, cette étude montre qu'une perte de poids modérée entraîne une meilleure efficacité lipolytique de l'adipocyte s'expliquant par une augmentation de la sensibilité β2-AR et une diminution de la sensibilité α_2-AR.

Regional and gender variations in adipose tissue lipolysis in response to weight loss

P. Mauriège [1,2], P. Imbeault [1,2], D. Langin [3], M. Lacaille [2], N. Alméras [1,2], A. Tremblay [2] and J.P. Després [1].

[1]Lipid Research Center, Laval University Medical Research Center and [2]Physical Activity Sciences Laboratory, Laval University, Ste-Foy, Québec, Canada; [3]INSERM Unit 317, Faculty of Medicine, Toulouse, France.

Supported by the Medical Research Council of Canada, the Fonds FCAR-Québec and Servier-Amérique.

Running title: Weight loss and adipose cell lipolysis in humans.

Address correspondence to: Pascale Mauriège, Ph.D.,
Physical Activity Sciences Laboratory,
Division of Kinesiology,
P.E.P.S., Laval University,
Ste-Foy, Québec, CANADA G1K 7P4
Tel: 418-656-2131 ext 6067
Fax: 418-656-2441

Abstract

Catecholamine-induced lipolysis was investigated in 32 obese subjects (14 men and 18 premenopausal women), aged 36-50 years, whose body mass index ranged from 30 to 42 kg/m^2. Isolated subcutaneous (subc) abdominal and femoral adipocytes were studied before and after a 15 week-weight reducing program, during which mean body weight loss averaged 9 vs 10 kg in women and men, respectively ($P < 0.0001$). Participants were re-examined when they were weight stable. Fat cell weight decreased by about 15-20 % in both depots (P values ranging from 0.01 to 0.05). Epinephrine (mixed α2-/ β-adrenoceptor (AR) agonist) induced antilipolysis at low concentrations and a net lipolytic response at higher doses, irrespective of subjects fatness and anatomic location of fat. Basal lipolysis, maximal lipolytic responses to isoprenaline (β-AR agonist), dobutamine and procaterol (β1- and β2-AR agonists, respectively) as well as maximal antilipolytic effects of epinephrine or UK-14304 (α2-AR agonist) were similar before and after weight reduction. However, both β- and β2-AR lipolytic sensitivities and the β-AR density were increased in both genders after weight reduction, this effect being more marked in subc abdominal than in femoral adipocytes (P values ranging from 0.001 to 0.05). The α2-AR antilipolytic sensitivity was reduced in adipose cells from both regions in women, but only in subc abdominal adipocytes in men ($P < 0.05$), although the α2-AR density remained unchanged following weight reduction. In conclusion, a moderate weight loss leads to a higher adipose cell lipolytic efficiency which is associated with changes at receptor levels (mainly an increased β2- and a decreased α2-AR sensitivity), in both genders.

Key words: adipocyte, lipolysis, catecholamines, adrenoceptors, hormone-sensitive lipase, regional variation, weight reduction.

Introduction

Since the pioneering clinical observations of Vague (1), numerous studies have re-emphasized the notion that premenopausal women who tend to accumulate fat preferentially in the gluteal and femoral regions are at lower risk of complications than men who are generally characterized by a high relative accumulation of abdominal adipose tissue (2-4). Human adipose tissue is also well established as being heterogeneous in its metabolic activity, and regional variations in rates of lipid storage and/or mobilization in adipose cells have already been suggested as contributing to local differences in adiposity (5-9). Among the various hormones which control lipid mobilization, catecholamines and insulin appear to be powerful regulators of *in vitro* adipose cell lipolysis in adult humans (5,6,10,11). The final response of subcutaneous adipocytes to catecholamines (antilipolysis or lipolysis) depends upon the functional balance between inhibitory α2- and stimulatory β-adrenoceptors (6,9-11) whose activation regulates adenylyl cyclase activity and thereby cAMP production, which in turn modulates hormone-sensitive lipase (12,13). Moreover, marked sex- and site-differences for the *in vitro* adipose cell lipolytic response to catecholamines have been extensively described in both normal and obese individuals (14-18). The *in vitro* lipolytic resistance of subcutaneous abdominal adipose cells to catecholamines in obese patients could be mainly attributed to a reduced β2- and/or an increased α2-adrenoceptor component and to an impaired activation of hormone-sensitive lipase, compared to non obese controls (13,17,19-21).

Among the various forms of treatment of obesity, dietary interventions such as fasting, low or very low-calorie diets (LCD or VLCD) remain widely used therapies to promote weight reduction in obese patients (22). As body weight loss and the related decrease in adipose tissue mass are generally accompanied by a concomitant reduction in adipose cell size, changes in the adipocyte lipolytic potencies could contribute to explain variation in lipid mobilization (5,8,9). However, to the best of our knowledge, with the exception of two studies which examined the effect of caloric restriction on both subcutaneous abdominal and gluteal adipose tissues of obese women (23,24), there has been no attempt to clarify the mechanisms underlying the changes in regional adipose cell lipolysis occurring during weight reduction, in both genders. Indeed, prior experiments addressing this issue have been generally conducted on women who underwent LCD or VLCD therapy (25-31). Furthermore, these studies

generally did not consider the well known regional differences in fat cell lipolysis, since subcutaneous abdominal adipose tissue was the only depot investigated.

Therefore, the aims of the present study were **1)** to examine the lipolysis regulation of subcutaneous abdominal and femoral adipocytes in both obese men and premenopausal women who were subjected to a weight loss therapy through dietary energy restriction, participants being re-examined after weight stabilization, **2)** to verify the putative existence of regional and gender variations in these responses, and **3)** to identify the cellular mechanisms (located at receptor and/or post-receptor levels) underlying such differences.

Material and Methods

Subjects and experimental design

Thirty-two healthy obese subjects (14 men and 18 premenopausal women) (all Caucasian), aged 43 ± 5 years (mean ± SD) (range: 36-50 yr) were recruited through the media and gave their written informed consent to participate in this intervention protocol aiming at weight reduction. All individuals were subjected to a physical examination by a physician, which included a medical history. Subjects with metabolic (cardiovascular disease or non-insulin dependent diabetes mellitus) or endocrine disorders such as hypogonadism or hirsutism, or those on medication potentially affecting lipid metabolism (β-blockers, anti-hypertensive drugs, etc) were excluded from the study. All participants were sedentary, non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight reducing program, and their body weight had been stable for at least six months prior to the study. Women had regular menstrual cycles and none was using oral contraceptives or lactating at the time of the study. All measurements were performed while they were in the early follicular phase of their menstrual cycle.

Subjects were first examined 2-4 weeks before entering the study which was approved by the Laval University Medical Ethics Committee. They were then subjected to a 15-week dietary restriction which took into account their individual macronutrient composition evaluated by a three-day dietary record prior to the experiment (15-18% protein, 38-39% fat, 41-46% carbohydrate and 1-2% alcohol). With the exception of carbohydrate being higher in women than in men ($P < 0.05$), the macronutrient composition did not differ between genders. The energy deficit which was established according to measurements of daily energy expenditure estimated by indirect calorimetry and from the estimated daily energy intake obtained by a three-day dietary record, corresponded approximately to 500-800 kcal/day. This dietary restriction was accompanied by the daily oral intake of 60 mg fenfluramine, a serotonin reuptake inhibitor which has been shown to facilitate body weight loss (32). Participants were then re-examined 4-6 weeks after the end of the treatment when they were weight stable. As managing treatment of obesity is highly dependent of the subject's attendance to the weight-reducing program, participants underwent weight control and a 24h-dietary recall interview

with a dietetician, twice a month, to verify compliance to the experimental procedure. Subjects whose body weight has varied ± 2 kg from the end of the protocol to the day of the adipose tissue biopsy were excluded from the study.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique (33) and percent body fat was derived from body density (34). Pulmonary residual volume was measured using the helium dilution method (35). Fat mass was calculated as total body weight minus fat-free mass. Waist girth was measured according to the procedures recommended at the Airlie Conference (36).

Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, West Germany), according to the methodology previously described (37). Briefly, the subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) and at the femoral (mid-distance between the knee joint and the iliac crest) levels with a radiograph of the skeleton as a reference to establish the position of the scan to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing the AT surfaces using an attenuation range of -190 to -30 HU (37). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Adipocyte isolation and lipolysis

After an overnight fast, participants were subjected to biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the midthigh level (femoral site). A small cutaneous incision (1 cm) was performed in both sites and about 400-500 mg of subcutaneous adipose tissue were surgically removed from the two fat depots. Samples of approximately 100 mg of adipose tissue from each region were immediately frozen in liquid nitrogen and stored at -80°C for later measurement of the hormone-sensitive lipase (HSL) activity as well as of the α2- and β-adrenoceptor density.

Samples of 250 mg of adipose tissue from each site were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (38) in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus 1 mg/ml collagenase, as previously described (17). Digestion took place in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2, for 40 min at 37°C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml of KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 µl.

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. 50 µl aliquots of the continuously stirred cell suspension were placed in 1.5 ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 10 µl KRB were immediately placed on ice and provided an evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation or inhibition were added just before starting the incubation in 10-µl portions in order to obtain the desired final concentration. After a 2h incubation at 37°C in a shaking water bath, under 95 % O_2 and 5 % CO_2 gas phase, 50 µl HCl (1N) were added to all tubes to stop the reaction, then 50 µl NaOH (1N) were added to neutralize the medium. All tubes were stoppered and stored at -20° C until glycerol determination and NADH concentration was measured by bioluminescence with a luciferase solution, using an automated 2250 Dynatech luminometer (17,39). For each concentration of stimulator or inhibitor, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Glycerol measurement by bioluminescence is very sensitive and especially well adapted when only small amounts of adipose tissue are available (17,39). Briefly, a 50 µl-aliquot of adipose cell suspension was taken and dropped into 25 µl of saline (NaCl 0.9%) containing 0.4% of trypan blue. A 14 µl-aliquot of this final suspension was taken and the average fat cell diameter was assessed using a Leitz microscope equipped with a graduated ocular, at a magnification of 100 X (Rockleigh, NJ, USA). Adipocyte size was measured with a precision of 1 µm and fat cell diameters were individually computer

recorded in 5-μm classes from 0 to 260 μm. Mean adipose cell diameter was assessed from the measurement of at least 500 cells per site and per subject. Because of the spherical shape and high lipid content of the adipocytes (95%), both the adipose cell volume and surface area can be calculated from the mean adipocyte diameter and the density of triolein (0.915 g/ml) was used to transform adipose cell volume into fat cell weight, as previously described (17,19,39,40).

The lipolytic activity of the isolated fat cells was tested with epinephrine which is a mixed agonist (α2/β) with a higher affinity for α 2- than for β -adrenoceptor (AR) sites (17), UK-14304 (selective α2-AR agonist), isoproterenol (non selective β -AR agonist) (17), procaterol (β 2-AR agonist) and dobutamine (β 1-AR agonist) (19,41). Ascorbic acid (0.1 mmol/l) was included in the incubation medium in order to prevent catecholamine degradation. Some experiments were conducted with forskolin (direct activator of adenylate cyclase), dibutyryl-cyclicAMP (stimulator of the protein kinase hormone-sensitive lipase complex and phosphodiesterase-resistant cyclic AMP analogue), theophylline (mainly inhibitor of cyclicGMP-inhibited phosphodiesterase, cGI-PDE) (39), and cilostamide (selective inhibitor of cGI-PDE) (42). When antilipolytic effects were investigated, the incubation buffer was supplemented with 5 μg/ml adenosine deaminase (ADA) to remove adenosine released into the incubation medium by the isolated fat cells, this procedure allowing better investigations of α2-AR mediated antilipolytic effects (16,17,39). Lipolysis was expressed either per cell number (ie, in μmol of glycerol/10^6 cells x 2 h) or per unit of cell surface area (ie, in nmol of glycerol/μm^2 x 10^8 x 2 h) in order to compensate for regional and gender variations in fat cell size (17,39) as well as for the putative differences due to weight reduction. In cases where complete concentration-response curves were obtained, they were compared for both responsiveness and sensitivity. The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-5} M various β-adrenoceptor agonists, forskolin or cilostamide, 10^{-3} M dibutyryl-cyclicAMP or theophylline). Maximal inhibition of lipolysis noted either at 10^{-7} M epinephrine, or at 10^{-6} M UK-14304 was calculated as the following ratio: (ADA - epinephrine

or UK-14304/ADA - basal) where ADA represents ADA-stimulated lipolysis. The β-adrenergic sensitivity was considered as the β-AR agonist concentration giving half-maximal stimulation of lipolysis (EC_{50}), whereas the α2-adrenergic sensitivity was calculated as the concentration of UK-14304 which produced half-maximal inhibition of lipolysis (IC_{50}). Both were evaluated by logarithmic conversion of each concentration-response curve. The higher the EC_{50} (various β-AR agonists) or the IC_{50} (UK-14304) value, the lower was the β- or the α2-adrenergic sensitivity, respectively.

Hormone-sensitive lipase (HSL) assays

This assay was performed as previously described by Fredrikson et al. (43) with some modifications for the handling of small samples (44). Briefly, small pieces of adipose tissue (about 100 mg) were homogenized at 4°C in 0.8 ml of a buffer containing 0.25 M sucrose, 1mM EDTA, 1mM dithiothreitol and the protease inhibitors leupeptin and antipain, both at 20 μg/ml (pH 7.4). Samples were then centrifuged at 100000 g for 45 min at 4°C in a Beckman ultracentrifuge, and the fat cake removed. Pellets containing crude adipose tissue membranes obtained after centrifugation were used for radioligand binding studies. The fat-free infranatant was recovered for analysis of maximal enzymatic activity, using 1(3)-mono-[^{3}H]oleoyl-2-oleylglycerol as substrate (43,44). All samples were incubated in duplicate for 30 min at 37°C and were analyzed on the same occasion. Since this substrate has only one hydrolysable ester bond at the 1(3)-position, neither the substrate itself nor its hydrolysis products can be hydrolyzed by monoacyl-glycerol lipase which is abundant in adipose tissue. Furthermore, under our incubation conditions (pH 7.0 and no apo CII present), lipoprotein lipase activity is negligible (12,43). As the phosphorylated and dephosphorylated forms of the enzyme have similar activities towards the substrate, the total amount of activable enzyme in the sample is measured. Moreover, the sensitivity of the assay is enhanced by the use of a diacylglycerol analogue as substrate, since HSL has a 10-fold higher activity towards diacylglycerol than triacylglycerol (12,43). One unit of enzyme activity is defined as 1 μmol of fatty acid released per minute at 37°C. Lipase activity was related to both fat cell number and adipocyte surface area which were estimated from *in vitro* lipolysis assays.

Radioligand binding studies

α2- as well as β-ARs were quantified with radioligands selective for each adrenergic receptor subtype, ie, [^{3}H]-RX 821002 (a more selective α2-AR antagonist than [^{3}H]-Yohimbine currently used) and [^{125}I]-Cyanopindolol (CYP), a non-selective β-AR antagonist (19,41). Previous experiments have shown that radioligand binding assays were unaffected by the composition of the buffer (0.25 M sucrose, 1mM EDTA, 1mM dithiothreitol) used for adipose tissue membrane preparations (Mauriège P, unpublished observations). Briefly, membranes were incubated either with 5 nM of [^{3}H]-RX 821002 or with 300 pM of [^{125}I]-CYP for α2- or the β-AR tracer experiments, respectively. As both radioligands bind to a single class of homogenous noninteracting binding sites that give straight lines on Scatchard analysis leading to Hill coefficients close to 1, the use of only one ligand concentration to determine maximal antagonist binding is therefore justified under such conditions (16,18,19,41). In addition, the latter concentrations corresponding to almost twice the affinity of each radioligand label the totality of both ARs (19). Thawed crude adipose tissue membranes were homogenized further with four pestle strokes in a Potter apparatus and washed once in 50 mM Tris-HCl, 1 mM $MgCl_2$, pH 7.5 (Tris-Mg buffer). The pellet was then adjusted to a final concentration of approximately 0.5 mg protein/ml. The protein content was determined according to the method of Lowry et al. (45), using bovine serum albumin as standard.

Total binding was determined by incubating 50 µl-aliquots of the resuspended membrane adipose tissue preparation with a fixed concentration of [^{125}I]-CYP (300 pM) in a total volume of 200 µl Tris-Mg buffer. Under these conditions, it is believed that [^{125}I]-CYP binds mainly to the high affinity binding sites which correspond to β1-/β2-ARs rather than to the low affinity binding sites which can be ascribed to β3-ARs (10). Specific binding was defined as the difference between total binding and binding in the presence of 10 µM unlabeled (-) propranolol (non selective β-adrenergic antagonist). A similar radioligand binding technique was used to identify α2-ARs with a fixed concentration of [^{3}H]-RX 821002 (5 nM) in a total volume of 200 µl Tris-Mg buffer. Specific binding was defined as the difference between total

and non-specific binding determined in the presence of 10 μM unlabeled (-) phentolamine (non selective α-adrenergic antagonist). Incubations were carried out in a water bath for 25-30 min at 37°C, under constant shaking at around 120 cycles/min and the reaction was stopped by the addition of 4 ml of ice-cold binding buffer followed by rapid filtration, using a Cell Harvester Micromate C-96 (Packard, Canada). The tubes and filters were then washed twice with 10 ml-portions of ice-cold binding buffer. For [^{125}I]-CYP binding, the radioactivity retained on the filters was directly counted in a Clini Gamma counter (at an efficiency of 85%), whereas for RX binding, filters were placed in minivials containing 2 ml of liquid scintillation cocktail and counted in a LKB scintillation counter (at an efficiency of 35%). Both radioligands, [^{3}H]-RX and [^{125}I]-CYP, displayed saturable specific binding to crude fat cell membranes prepared from the different tissues, and non-specific binding did not exceed 20-30% of total binding (19). The maximum number of α2- and β-AR binding sites was expressed per cell number or corrected for variation in adipocyte surface area which were both estimated from *in vitro* lipolysis assays.

Drugs and chemicals

Collagenase, bovine serum albumin, adenosine deaminase, and enzymes for glycerol assays were obtained from Boehringer (Mannheim, Canada). Ascorbic acid, leupeptin, antipain, (-) isoproterenol bitartrate, (-) epinephrine bitartrate, (-) propranolol hydrochloride, theophylline, forskolin and dibutyryl-cyclic AMP were purchased from Sigma Chemical Co (St Louis, MO). UK 14304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) was generously provided from Dr D.A. Faulkner (Pfizer, Sandwich, England) whereas phentolamine mesylate came from Ciba Geigy (Canada). Procaterol (OPC-2009) (5-(1-hydroxy-2-isopropylaminobutyl)-8-hydroxycarbostyril hydrochloride hemihydrate) and cilostamide were generous gifts from Otsuka Pharmaceuticals (Tokushima, Japan), whereas dobutamine (Dobutrex) came from Eli Lilly (Indianapolis, IN). (-)[^{3}H]-RX821002 (RX) (1,4-[6,7(n)-^{3}H]benzodioxan-2-methoxy-2-yl)-2-imidazoline hydrochloride) (specific activity, 53 Ci/mmol) and (-)[^{125}I]-Cyanopindolol (CYP) (specific activity, 2200 Ci/mmol) were obtained from Amersham International (Canada) and Mandel Scientific (Canada), respectively. 1(3)-mono-[^{3}H]oleoyl-2-oleylglycerol was generously provided by Sevicon AB (Lund, Sweden). All other chemicals and organic

solvents were of the highest purity grade commercially available. The same batches of hormones, pharmacological agents, collagenase and albumin were used in all experiments.

Statistical analysis

Values presented in figures are means ± standard error (SE). Lipolysis and HSL experiments were performed on all subjects (ie, 14 men and 18 women). However, as radioligand binding assays required large amounts of adipose tissue, these assays could only be performed on 12 men and 16 women. A two-way analysis of variance was also performed to verify whether significant sex and site differences for lipolysis, HSL activity or $\alpha 2$-vs β-adrenoceptor density existed before and after weight loss and post hoc comparisons were tested with a paired t-test. All statistical analyses were performed using the Jump Program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers.

Results

Subjects'characteristics

Subjects'physical characteristics are presented in Table 1. Visceral abdominal fat accumulation, estimated by CT, was more pronounced in men, whereas higher levels of subcutaneous abdominal adipose tissue were observed in women (P values ranging from 0.001 to 0.05). Women also showed a greater femoral adipose depot and larger femoral adipocytes than men (P values ranging from 0.0001 to 0.001), although no sex difference was found for subcutaneous abdominal fat cell weight. Both men and women achieved a moderate weight loss (9-10 kg) in response to the 15 week-weight reducing program ($P < 0.0001$). However, all subjects were weight stable when re-examined 4-6 weeks after the end of the treatment. Changes in selected body fatness indices were significantly more pronounced in men than in women (P values ranging from 0.001 to 0.05). In both genders, subcutaneous and visceral abdominal adipose tissue areas as well as the cross-sectional area of femoral fat determined by CT were reduced to a similar extent in response to weight loss (P values ranging from 0.0001 to 0.001). Fat cell weight also decreased by about 15-20 %, regardless of the depot and the sex (P values ranging from 0.01 to 0.05).

Adipocyte function

Basal lipolytic rate and ADA-stimulated lipolysis

Basal lipolysis measured in subcutaneous abdominal or femoral adipocytes did not differ among genders, in response to weight reduction, whatever the mode of expression of data (Table 2). On the other hand, when the incubation buffer was supplemented with adenosine deaminase (ADA) at 5 μg/ml, the basal lipolytic rate was increased by about 1.5 to 2.5-times in all cell types. Neither sex nor site differences were observed in the glycerol release achieved in the presence of this enzyme, results being expressed per cell number or per unit of cell surface area (Table 2). Indeed, the difference noted in basal and ADA-stimulated lipolysis (when corrected for the adipocyte surface area) of male subcutaneous abdominal fat cells, after weight loss, was below the level of statistical significance ($P = 0.065$).

In order to control for the variation in fat cell weight observed during the weight-reducing program, all lipolysis measurements were further expressed per unit of cell surface area which may be physiologically more relevant, as previously suggested (5,19,21,30,46). Such a mode of expression of the data allows an adequate comparison of lipolysis in large vs small adipocytes, as differences in adipose cell size were found in both genders, in response to weight loss (Table 1).

Epinephrine responsiveness

In the presence of ADA, epinephrine (EPI) which is known for its mixed (α2/β- adrenergic) agonist properties on lipolysis, initiated a biphasic responsiveness in subcutaneous abdominal and femoral adipocytes (Fig 1). The catecholamine promoted an inhibition of lipolysis at low concentrations (from 10^{-9} to 10^{-7} M), but exerted a net lipolytic response at higher doses (10^{-6}-10^{-5} M), indicating thus a preferential recruitment of α2-, followed by β-adrenoceptor sites. Significant differences were observed after weight reduction in epinephrine-induced antilipolysis at 10^{-8} M (concentration at which the α2-adrenoceptor component is predominant) in subcutaneous abdominal adipose cells of men ($P < 0.01$) and women ($P < 0.05$). However, maximal antilipolysis promoted by the hormone at 10^{-7} M, did not show any site or sex difference, subjects being examined at both occasions. On the other hand, the maximal adipose cell lipolytic response to the catecholamine (at 10^{-5} M) did not differ in response to weight reduction, although subcutaneous abdominal adipocytes appeared to be more responsive to hormonal stimulation than femoral adipose cells, regardless of the gender or of the treatment-period (P values ranging from 0.01 to 0.05).

Insert Tables 1, 2 and Figure 1.

Selective α2- and β-adrenergic effects

As epinephrine responsiveness results from both α2- and β-adrenoceptor stimulation, selective adrenergic agonists were used to discriminate between these two antagonistic effects.

To study the influence of the α2-adrenoceptor component, the effect of the selective α2-agonist UK-14304 was tested on ADA-stimulated lipolysis (Fig 2). UK-14304 inhibited lipolysis in a dose-dependent manner in all adipocytes, and the maximal antilipolytic response noted at 10^{-6} M did not strikingly differ among genders and adipose sites. However, at 10^{-8} M, UK-14304 promoted a less potent antilipolysis in subcutaneous abdominal adipocytes of men and in adipose cells of women, after weight reduction (P values ranging from 0.01 to 0.05). In addition, the α2-adrenergic sensitivity estimated as the half-maximal antilipolysis induced by UK-14304 was almost 2 times lower in subcutaneous abdominal adipocytes of men after weight loss compared to baseline ($P < 0.05$) (Table 3). The concentration-response curves slightly shifted to the right for UK-14304 after weight reduction, which revealed a 1.8 to 2 times decrease in adipose cell α2-adrenergic sensitivity in women, irrespective of the anatomic site investigated ($P < 0.05$) (Table 3).

In order to characterize the β-adrenoceptor component, the effect of the β-agonist isoproterenol on basal lipolysis was examined (Fig 3). Maximal lipolytic responses to isoproterenol at 10^{-5} M, when expressed as absolute rates, were similar regardless of the anatomical location of fat or of the gender (Fig 3). Isoproterenol-stimulated maximal lipolysis, when expressed on a relative basis (ie, after subtraction of basal lipolytic rate) also did not differ in response to weight loss, whatever the adipose site or the gender considered [values clustering 9 to 11 ± 2 vs 10 to 12 ± 3 nmol glycerol/(μm^2 x 10^8 x 2 h), before vs after weight loss]. However, the shift to the left of the concentration-response curves in all adipose cells after weight reduction attested for a 3 to 5 times increased β-adrenergic sensitivity in both genders (P values ranging from 0.005 to 0.05). The concentration of β-agonist required for half-maximal lipolysis was also higher in subcutaneous abdominal than in femoral adipocytes of men ($P < 0.05$) and women (P values ranging from 0.005 to 0.01), before and after weight loss (Table 3).

Selective β-adrenergic receptor subtype effects

As site differences in the catecholamine response profile appeared to be partly explained by the β-adrenoceptor function, additional experiments were conducted using procaterol (β2-agonist) and dobutamine (β1-agonist) (Figs 4 and 5, respectively). Concentration-response curves for these agonists were examined to evaluate the relative importance of each adrenoceptor subtype for explaining the changes observed in isoproterenol sensitivity. In men, the concentration-response curves for procaterol shifted to the left in adipocytes from both regions, attested for a 2 to 3.5 times increased β2-adrenoceptor sensitivity after weight loss (P values ranging from 0.01 to 0.05) (Table 3). However, the fact that the curves for dobutamine were practically superimposed, suggested an unchanged β1-adrenoceptor sensitivity (Table 3). In contrast, the shift to the left of concentration-response curves for both agonists in response to weight reduction indicated an increase in the adipose cell sensitivity to the β1- and β2-adrenergic agonists used (1.8 to 1.9 and 3 to 3.5 times, respectively; P values ranging from 0.001 to 0.05), in women (Table 3). The β2-adrenergic sensitivity was also higher in subcutaneous abdominal than in femoral adipocytes (P values ranging from 0.01 to 0.05), from both genders, before and after weight loss. However, sensitivity of subcutaneous abdominal adipocytes to dobutamine was greater in men than in women, irrespective of the treatment-period ($P < 0.05$) (Table 3). Despite the lack of site or sex difference in the maximal lipolytic response to the selective β-agonist tested (Figs 4 and 5), neither procaterol nor dobutamine was as potent as isoproterenol in stimulating lipolysis, before and after weight loss. Indeed, both agents (used at 10^{-5} M) only induced a partial activation of lipolysis (values clustering at 65 to 80 % of the maximal effect promoted by the non selective β-agonist). When expressed on a relative basis, responsiveness to either the β1- or β2-agonist was also not significantly different, irrespective of the gender or of the adipose depot [values clustering 5 to 7 ± 2 vs 6 to 7 ± 2 nmol glycerol/(μm^2 x 10^8 x 2 h), before vs after weight loss]. On the other hand, similar results were obtained for epinephrine-, UK14304-, isoproterenol-, procaterol- and dobutamine-stimulated lipolysis when expressed on a per cell basis (not shown).

Insert Figures 2, 3, 4 and 5.

Radioligand binding assays

To verify whether differences observed in catecholamine-induced lipolysis could be explained at the receptor level, β- and α2-adrenoceptor sites were also studied in adipose tissue membranes, in response to the weight-reducing program. Since it was not possible to perform complete saturation experiments because of the limitations in the amount of tissue available, only one maximal concentration of each radioligand (5 nM of [^{3}H]-RX 821002 or 300 pM of [^{125}I]-CYP) was used to evaluate the number of α2- or β-adrenergic binding sites on crude adipose tissue membranes (Table 4). As both subcutaneous abdominal and femoral adipocytes were larger before than after weight loss (Table 1), binding results were corrected for variation in adipose cell size. At 300 pM, [^{125}I]-CYP binding was 1.7 to 2 times higher in adipose tissue membranes of both genders (P values ranging from 0.01 to 0.05), whereas at 5 nM, [^{3}H]-RX binding did not change significantly in response to weight reduction. However, the β-adrenoceptor density was higher in subcutaneous abdominal than in femoral adipose tissue membrane preparations of both genders (P values ranging from 0.001 to 0.05). A greater number of α2-adrenoceptors was also observed in femoral than in subcutaneous abdominal adipose tissue membranes of women, before and after weight loss ($P < 0.01$). The study of potential gender differences revealed a greater estimated number of α2-adrenergic receptors in femoral adipose tissue membranes of women, compared to men, irrespective of the treatment period ($P < 0.05$). Similar results were obtained when binding data were expressed per cell number (not shown). Since the evaluation of the functional balance between α2- and β-adrenoceptors appears physiologically relevant (17,40), the ratio of [^{3}H]-RX to [^{125}I]-CYP binding sites was also calculated for each adipose tissue membrane and averaged for the different sites and by gender. The mean ratio of α2- to β-adrenoceptors was always higher in femoral than in subcutaneous abdominal adipose tissue membranes, before and after weight reduction (P values ranging from 0.005 to 0.05) (Table 4).

Lipolytic responses to post-receptor agents and hormone-sensitive lipase assays

As any step in the lipolytic cascade could be responsible for the site differences observed in catecholamine responsiveness, the effects of agents acting at well defined post-adrenoceptor

sites were also investigated (Fig 6). There was no regional variation, nor any gender difference when lipolysis was stimulated at maximum concentrations of dibutyryl-cyclic AMP (10^{-3} M), forskolin (10^{-5} M), theophylline (10^{-3} M) or cilostamide (10^{-3} M), at baseline. In addition, the rates of glycerol release reached in the presence of these compounds were similar before and after weight reduction, when expressed either on an absolute (not shown) or on a relative basis (Fig 6). Finally, hormone-sensitive lipase activity was not affected by weight reduction in men, although it was significantly increased in response to weight loss in femoral adipocytes of women ($P < 0.005$). The lipase activity was also higher in femoral than in subcutaneous abdominal adipose cells of women, after weight loss ($P < 0.01$) (Table 4). Similar results were obtained when enzyme activity was expressed either per cell number or on the basis of adipocyte triglyceride content (not shown).

Insert Tables 3, 4 and Figure 6.

Discussion

Until recently, few studies had attempted to elucidate the mechanisms responsible for the regional and gender variation observed in adipose tissue lipolysis of obese individuals subjected to diet-induced weight loss (23,24). Indeed, most experiments published so far have dealt exclusively with obese women and did not consider site differences as subcutaneous abdominal adipose tissue was the only depot investigated (25-31). On the other hand, controversial findings resulting from *in vitro* experiments have pointed out either an unchanged (24,29) or even reduced (28) catecholamine-induced lipolysis as well as an enhanced (25-27,29,31) or a decreased (30) basal lipolytic rate in subcutaneous abdominal fat cells of reduced-obese individuals. Therefore, to the best of our knowledge, the present study is the first to examine the cellular mechanisms underlying regional variation in adipose cell lipolysis of both overweight men and premenopausal women who underwent dietary treatment for obesity.

The fact that adipose cell weight did not differ according to the anatomic location of fat was probably due to our subject's high level of total body fat which is commonly associated with hypertrophy of adipose cells. This finding is concordant with previous observations from our group and other investigators (15,17,18,39). Although femoral adipose cells were larger in women than in men (14,15,18), this gender variation was no longer significant after weight loss, as adipose cell size reduction was of the same order of magnitude in both genders (Table 1).

The similar basal lipolytic rate, regardless of the adipose depot or the gender, is in agreement with the unchanged ADA-induced lipolysis observed in response to weight loss. This is in clear contrast to findings resulting from short- or long-term VLCD studies (25,27,30,31,46). Assuming that an increased basal lipolysis may represent a physiological adaptation to negative energy balance, the discrepancy observed between our results and earlier data was probably due to the fact that our subjects were examined 4-6 weeks after the end of the treatment when they were weight stable. Moreover, differences in basal lipolysis among various fat depots or after weight reduction have already been suggested to be partly due to

variations in HSL activity (12,13). In this regard, conflicting results have reported either a decreased (30), an increased (31) or a similar enzyme activity ((29); the present study) in subcutaneous abdominal adipocytes of obese women subjected to weight reduction, despite a marked reduction in adipose cell size ((29-31); the present study). Once again, the fact that subjects were examined several weeks after the completion of the hypocaloric diet program, could explain, at least to a certain extent, the unchanged basal lipolysis and lipase activity that we observed in response to weight loss. In addition, the unchanged adipose cell lipolytic capacity clearly indicated an unaltered hormone responsiveness. Indeed, the similar maximal lipolytic rates promoted by β-agonists or by agents acting selectively at the adenylate cyclase, the lipase-protein kinase A complex or the phosphodiesterase level, is concordant with unaltered post-adrenoceptor pathways after weight reduction, as previously reported (28,29,47). The lack of post-receptor changes is further strengthened by the similar maximal lipase activity, with the exception of the increased enzyme activity found in femoral adipocytes of women after weight loss. As the present assay used for the measurement of lipase activity does not differentiate between the phosphorylated active and the dephosphorylated inactive forms of the enzyme (13,44), the discrepancy observed between lipolysis and enzyme data in femoral adipocytes of women could be explained either by an increased phosphorylation capacity of HSL (via the cAMP-dependent protein kinase A) or by the regulation of cellular components involved in the translocation of the lipase to the lipid droplet (48,49). The similar lipolytic rates promoted by a PDE-resistant (dibutyryl-cAMP) and a PDE-sensitive (theophylline) cAMP-analogue, indicated an unchanged phosphodiesterase activity after weight reduction, in contrast to its marked increase during short-term fasting (50).

Marked effects of body weight reduction on the regulation of catecholamine-induced lipolysis were observed in the present study. However, the greater lipid mobilization in subcutaneous abdominal than in femoral fat depots of both genders re-emphasizes the notion that the typical "female" fat storage depot is highly resistant to slimming (14-16,23-25,51). The greater sensitivity of subcutaneous abdominal than femoral adipose cells to catecholamine stimulation, which could be attributed to the higher number of β-adrenergic receptors in the former adipocytes, is consistent with previous observations in obese (15,17,39) and healthy

normal-weight subjects (14,18,20). However, our results demonstrate a similar biphasic epinephrine response profile in all cell types that probably reflects the interaction of the hormone with both types of adrenoceptors. This finding supports the notion of the differential recruitment of α2- then β-binding sites (16,17,39). The functional α2-/β-adrenoceptor balance seems to be of importance in explaining the different adipose cell lipolytic responses to catecholamines. As subcutaneous adipocytes possess a higher α2- than β-adrenoceptor density, the greater ratio of α2- to β-sites could explain the predominant α2-adrenergic component of epinephrine responsiveness observed irrespective of the gender or of the fat depot. The strong α2-antilipolytic effect noted at the lowest concentrations of epinephrine could not entirely be compensated by the β-adrenergic activity of the hormone, although both enhanced β-adrenoceptor sensitivity and density were observed in men and women, after weight reduction.

Our results clearly indicate that the cellular mechanisms responsible for the increased ability of obese individuals to mobilize lipids during dietary energy restriction are mainly localized at the β- (and more particularly β2-) and α2-adrenoceptor levels in both adipose regions and genders. As only a few available α2- and β-adrenoceptors need to be occupied to obtain a maximal effect in human fat cells (52), changes in sensitivity may reflect alterations in hormone action which are located at or near the receptor level whereas alterations in responsiveness are usually linked to changes in hormone action at further intracellular steps in the pathway of the signal. However, whether the higher β-adrenergic lipolytic sensitivity noted in response to weight loss may result from an increased β1/β2-adrenoceptor density and/or an improved coupling efficiency of β-adrenoceptors to the stimulatory GTP-sensitive Gs proteins remains to be established. The increased β-adrenergic sensitivity of adipose tissue that we have noted after weight loss is concordant with the enhanced β-adrenoceptor density determined by radioligand binding assays. This up-regulation of β-adrenergic receptors has already been attributed to a reduced basal activity of the sympathetic nervous system which is frequently observed after energy restriction (9). Indeed, reduced-weight obese subjects have

been shown to be characterized by a lower basal sympathetic activity and a higher lipolytic response to catecholamine infusion than obese or lean individuals (53). Although our findings are concordant with the increased β2-adrenergic lipolytic sensitivity of subcutaneous abdominal adipocytes of obese women after weight loss (30), they contrast with other observations resulting from *in vitro* and *in vivo* studies which have shown an unchanged sensitivity to isoproterenol (29,31). The specific improvement of the β1-adrenergic lipolytic pathway of female adipose cells after weight loss may also appear at variance with previous studies which did not detect any change in this component following weight reduction (29-31). However, Barbe et al. (54) have recently shown an increased β1-adrenergic lipolytic response of the subcutaneous abdominal fat depot, in obese women subjected to hypocaloric diet, using the *in situ* microdialysis technique. Further studies are clearly warranted to address this issue. On the other hand, the decreased adipose cell α2-adrenergic antilipolytic sensitivity observed in subcutaneous abdominal adipocytes of men and in adipose cells of women, after weight loss, could not be explained by a reduction in the α2-adrenoceptor density as the estimated number of α2-adrenoceptors was similar, irrespective of the treatment-period (Table 4). It is thus possible that the lower α2-adrenergic sensitivity could be related to a decreased coupling efficiency of these receptors to inhibitory GTP-sensitive Gi proteins, as a minor role of G proteins as modulators of clonidine sensitivity has already been suggested (18). Although it would have been interesting to measure the relative amounts of Gi and Gs proteins, this was beyond the scope of the present study since these methods required large amounts of adipose tissue which cannot be removed for obvious ethical reasons.

In conclusion, a moderate weight loss resulting from 15 weeks of dietary energy restriction leads to an increased lipolytic catecholamine sensitivity, despite a preserved lipolytic hormonal capacity which is mainly reflected by both similar basal lipolysis and hormone-sensitive lipase activity. The resulting higher efficiency in the regulation of subcutaneous adipocyte lipolysis after weight loss is partly mediated by increased β- and β2-adrenergic (combined with a higher β-adrenoceptor density) and decreased α2-adrenergic sensitivities (without any change in the α2-adrenoceptor number), in both men and women. These changes

are more pronounced in the subcutaneous abdominal than in the femoral fat depot and suggest that a preferential fat mobilization from the major energy depot storage (ie, the subcutaneous abdominal adipose region) occurs during dietary caloric restriction, in both genders.

Acknowledgments

The authors wish to express their gratitude to Sylvie St-Pierre, Vicky Drapeau, Eric Doucet, and Henri Bessette for their excellent collaboration at various stages of the study. Drs Sylvie Leclerc and Gilles Lortie are also gratefully acknowledged for their excellent medical supervision. Thanks are also expressed to Suzanne Brulotte and Line Bargone from the Department of Radiology of the University Hospital (Québec, Canada) for their excellent work with the computed tomograph and to Michèle Dauzats from INSERM Unit 317 (Toulouse, France) for her technical assistance related to hormone-sensitive lipase assays. The subjects and the Physical Activity Sciences Laboratory staff are also gratefully acknowledged.

References

1. Vague, J. 1956. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am. J. Clin. Nutr.* **4:** 20-34.
2. Després, J. P. 1994. Dyslipidaemia and obesity. *Baillière's Clin. Endocrinol. Metabol.* **8:** 629-660.
3. Björntorp, P. 1996. The regulation of adipose tissue distribution in humans. *Int. J. Obesity.* **20:** 291-302.
4. Kissebah, A. H., and G. R. Krakower. 1994. Regional adiposity and morbidity. *Physiol. Rev.* **74:** 761-811.
5. Arner, P. 1988. Control of lipolysis and its relevance to the development of obesity in man. *Diabetes Metab. Rev.* **4:** 507-515.
6. Arner, P. 1996. Regulation of lipolysis in fat cells. *Diabetes Rev.* **4:** 1-13.
7. Bouchard, C., J. P. Després, and P. Mauriège. 1993. Genetic and nongenetic determinants of regional fat distribution. *Endocr. Rev.* **14:** 72-93.
8. Leibel, R. L., N. K. Edens, and S. K. Fried. 1989. Physiologic basis for the control of body fat distribution in humans. *Annu. Rev. Nutr.* **9:** 417-443.
9. Coppack, S. W., M. D. Jensen, and J. M. Miles. 1994. In vivo regulation of lipolysis in humans. *J. Lipid Res.* **35:** 177-193.
10. Lafontan, M., and M. Berlan. 1993. Fat cell adrenergic receptors and the control of white and brown fat cell function. *J. Lipid Res.* **34:** 1057-1091.
11. Lafontan, M., and M. Berlan. 1995. Fat cell alpha 2-adrenoceptors: the regulation of fat cell function and lipolysis. *Endocr. Rev.* **16:** 716-738.
12. Holm, C., D. Langin, V. Manganiello, P. Belfrage, and E. Degerman. 1997. Regulation of hormone-sensitive lipase activity in adipose tissue. *Methods Enzymol.* **286:** 45-67.
13. Langin, D., C. Holm, and M. Lafontan. 1996. Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc. Nutr. Soc.* **55:** 93-109.
14. Richelsen, B. 1986. Increased alpha$_2$- but similar beta-adrenergic receptor activities in subcutaneous gluteal adipocytes from females compared with males. *Eur. J. Clin. Invest.* **16:** 302-309.

15. Leibel, R. L., and J. Hirsch. 1987. Site- and sex-related differences in adrenoceptor status of human adipose tissue. *J. Clin. Endocrinol. Metab.* **64:** 1205-1210.

16. Mauriège, P., J. Galitzky, M. Berlan, and M. Lafontan. 1987. Heterogeneous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. *Eur. J. Clin. Invest.* **17:** 156-165.

17. Mauriège, P., J. P. Després, D. Prud'homme, M. C. Pouliot, M. Marcotte, A. Tremblay, and C. Bouchard. 1991. Regional variation in adipose tissue lipolysis in lean and obese men. *J. Lipid Res.* **32:** 1625-1633.

18. Wahrenberg, H., F. Lönnqvist, and P. Arner. 1989. Mechanisms underlying regional differences in lipolysis in human adipose tissue. *J. Clin. Invest.* **84:** 458-467.

19. Mauriège, P., A. Marette, C. Atgié, C. Bouchard, G. Thériault, L. J. Bukowiecki, P. Marceau, S. Biron, A. Nadeau, and J. P. Després. 1995. Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J. Lipid Res.* **36:** 672-684.

20. Lönnqvist, F., H. Wahrenberg, L. Hellstrom, S. Reynisdottir, and P. Arner. 1992. Lipolytic catecholamine resistance due to decreased beta 2-adrenoceptor expression in fat cells. *J. Clin. Invest.* **90:** 2175-2186.

21. Reynisdottir, S., K. Ellerfeldt, H. Wahrenberg, H. Lithell, and P. Arner. 1994. Multiple lipolysis defects in the insulin resistant (metabolic) syndrome. *J. Clin. Invest.* **93:** 2590-2599.

22. van Gaal, L. F. 1998. Dietary treatment of obesity. *In* Handbook of obesity. Bray, G. A., Bouchard, C. and James, W. P. T. Marcel Dekker, Inc., New York. 875-890.

23. Smith, U., J. Hammersten, P. Björntorp, and J. G. Kral. 1979. Regional differences and effect of weight reduction on human fat cell metabolism. *Eur. J. Clin. Invest.* **9:** 327-332.

24. Presta, E., R. L. Leibel, and J. Hirsch. 1990. Regional changes in adrenergic receptor status during hypocaloric intake do not predict changes in adipocyte size or body shape. *Metabolism.* **39:** 307-315.

25. Berlan, M., L. Dang-Tran, M. Lafontan, and Y. Denard. 1981. Influence of hypocaloric diet on alpha-adrenergic responsiveness of obese human subcutaneous adipocytes. *Int. J. Obesity.* **5:** 145-153.

26. Rozen, R., E. Banegas, M. Davilla, and M. Apfelbaum. 1984. Effects of a very-low-calorie diet on adrenergic responsiveness in human adipose tissue. *Int. J. Obesity.* **8:** 141-149.

27. Kather, H., E. Wieland, B. Fischer, A. Wirth, and G. Schlierf. 1985. Adrenergic regulation of lipolysis in abdominal adipocytes of obese subjects during caloric restriction: reversal of catecholamine action caused by relief of endogenous inhibition. *Eur. J. Clin. Invest.* **15:** 30-37.

28. Crampes, F., D. Riviere, M. Beauville, M. Marceron, and M. Garrigues. 1989. Lipolytic response of adipocytes to epinephrine in sedentary and exercise-trained subjects: sex-related differences. *Eur. J. Appl. Physiol.* **59:** 249-255.

29. Hellström, L., S. Reynisdottir, D. Langin, S. Rossner, and P. Arner. 1996. Regulation of lipolysis in fat cells of obese women during long-term hypocaloric diet. *Int. J. Obesity.* **20:** 745-752.

30. Reynisdottir, S., D. Langin, K. Carlstrom, C. Holm, S. Rossner, and P. Arner. 1995. Effects of weight reduction on the regulation of lipolysis in adipocytes of women with upper-body obesity. *Clin. Sci.* **89:** 421-429.

31. Stich, V., I. Harant, I. De Glisezinski, F. Crampes, M. Berlan, M. Kunesova, V. Hainer, M. Dauzats, D. Rivière, M. Garrigues, C. Holm, M. Lafontan, and D. Langin. 1997. Adipose tissue lipolysis and hormone-sensitive lipase expression during very-low-calorie diet in obese female identical twins. *J. Clin. Endocrinol. Metab.* **82:** 739-744.

32. Bray, G. A., and D. H. Ryan. 1997. Drugs used in the treatment of obesity. *Diabetes Metab. Rev.* **5:** 83-103.

33. Behnke, A. R., and J. H. Wilmore. 1974. Evaluation and regulation of body build and composition. *In* Prentice-Hall, Englewood Cliffs, NJ. 20-37.

34. Siri, W. E. 1956. The gross composition of body fat. *Adv. Biol. Med. Physiol.* **4:** 239-280.

35. Meneely, G. R., and N. L. Kaltreider. 1949. Volume of the lung determined by helium dilution. *J. Clin. Invest.* **28:** 129-139.

36. Lohman, T. G., A. F. Roche, and R. Martorell. 1988. The Airlie (VA) consensus conference. *In* Anthropometric Standardisation Reference Manual. Lohman, T. G., Roche, A. F. and Martorell, R. Human Kinetics Publishers, Champaign (Illinois). 39-80.

37. Ferland, M., J. P. Després, A. Tremblay, S. Pinault, A. Nadeau, S. Moorjani, P. J. Lupien, G. Thériault, and C. Bouchard. 1989. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br. J. Nutr.* **61:** 139-148.

38. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375-380.

39. Mauriège, P., D. Prud'homme, S. Lemieux, A. Tremblay, and J. P. Després. 1995. Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. *Am. J. Physiol.* **269:** 341-350.

40. Mauriège, P., J. P. Després, M. Marcotte, M. Ferland, A. Tremblay, A. Nadeau, S. Moorjani, P. J. Lupien, G. Thériault, and C. Bouchard. 1990. Abdominal fat cell lipolysis, body fat distribution, and metabolic variables in premenopausal women. *J. Clin. Endocrinol. Metab.* **71:** 1028-1035.

41. Mauriège, P., G. de Pergola, M. Berlan, and M. Lafontan. 1988. Human fat cell beta-adrenergic receptors: beta-agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta 1-antagonists. *J. Lipid Res.* **29:** 587-601.

42. Manganiello, V. C., M. Taira, E. Degerman, and P. Belfrage. 1995. Type III cGMP-inhibited cyclic nucleotide phosphodiesterases (PDE3 gene family). *Cell Signal.* **7:** 445-455.

43. Fredrikson, G., P. Stralfors, N. O. Nilsson, and P. Belfrage. 1981. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J. Biol. Chem.* **256:** 6311-6320.

44. Frayn, K. N., D. Langin, C. Holm, and P. Belfrage. 1993. Hormone-sensitive lipase: quantitation of enzyme activity and mRNA level in small biopsies of human adipose tissue. *Clin. Chim. Acta.* **216:** 183-189.

45. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and N. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.

46. Hellström, L., D. Langin, S. Reynisdottir, M. Dauzats, and P. Arner. 1996. Adipocyte lipolysis in normal weight subjects with obesity among first- degree relatives. *Diabetologia.* **39:** 921-928.

47. Reynisdottir, S., M. Eriksson, B. Angelin, and P. Arner. 1995. Impaired activation of adipocyte lipolysis in familial combined hyperlipidemia. *J. Clin. Invest.* **95:** 2161-2169.

48. Egan, J. J., A. S. Greenberg, M. K. Chang, S. A. Wek, M. C. Moos, Jr., and C. Londos. 1992. Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. *Proc. Natl. Acad. Sci.* **89:** 8537-8541.

49. Anthonsen, M. W., L. Ronnstrand, C. Wernstedt, E. Degerman, and C. Holm. 1998. Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *J. Biol. Chem.* **273:** 215-221.

50. Engfeldt, P., P. Arner, and J. Ostman. 1982. Changes in phosphodiesterase activity of human subcutaneous adipose tissue during starvation. *Metabolism.* **31:** 910-916.

51. Lafontan, M., A. Bousquet-Melou, J. Galitzky, P. Barbe, C. Carpene, D. Langin, M. Berlan, P. Valet, I. Castan, A. Bouloumie, and et al. 1995. Adrenergic receptors and fat cells: differential recruitment by physiological amines and homologous regulation. *Obesity Res.* **3 Suppl 4:** 507S-514S.

52. Arner, P., J. Hellmér, A. Wennlund, J. Ostman, and P. Engfeldt. 1988. Adrenoceptor occupancy in isolated human fat cells and its relationship with lipolysis rate. *Eur. J. Pharmacol.* **146:** 45-56.

53. Leibel, R. L., E. M. Berry, and J. Hirsch. 1991. Metabolic and hemodynamic responses to endogenous and exogenous catecholamines in formerly obese subjects. *Am. J. Physiol.* **260:** R785-791.

54. Barbe, P., V. Stich, J. Galitzky, M. Kunesova, V. Hainer, M. Lafontan, and M. Berlan. 1997. In vivo increase in beta-adrenergic lipolytic response in subcutaneous adipose tissue of obese subjects submitted to a hypocaloric diet. *J. Clin. Endocrinol. Metab.* **82:** 63-69.

Legends to figures

Figure 1. Effect of epinephrine (EPI) on adenosine deaminase (ADA)-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 14; upper panels) and women (n = 18; lower panels) before (open symbols) and after (filled symbols) weight reduction. Values are means ± SE of (n) experiments performed in duplicate. Glycerol release was expressed as the difference between stimulated (with epinephrine) and basal values determined in the presence of 5 μg/ml of adenosine deaminase (ADA). Negative values reflect inhibition of lipolysis. Significant effect of treatment at *P < 0.01 and **P < 0.05. ^{a,b} indicate regional variation at P < 0.05 and P < 0.01.

Figure 2. UK14304-induced inhibition of ADA-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 14; upper panels) and women (n = 18; lower panels) before (open symbols) and after (filled symbols) weight reduction. Values are means ± SE of (n) experiments performed in duplicate. Fat cells were incubated in the presence of ADA (5 μg/ml). Antilipolysis is given as the difference between values in the presence of UK and basal values (with ADA alone). Significant effect of treatment *P < 0.01 and **P < 0.05. Agonist concentrations required for half-maximal inhibition of lipolysis (IC_{50}) were determined from these concentration-response curves.

Figure 3. Isoproterenol (ISO)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 14; upper panels) and women (n = 18; lower panels) before (open symbols) and after (filled symbols) weight reduction. Fat cells were incubated without ADA (ie, in standard conditions) and values are means ± SE of (n) experiments performed in duplicate. Significant effect of treatment at P < 0.005, *P < 0.01 and **P < 0.05. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these concentration-response curves.

Figure 4. Procaterol (PROCAT)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 12; upper panels) and women (n = 14; lower panels) before (open symbols) and after (filled symbols) weight reduction. Fat cells were incubated without ADA (ie, in standard conditions) and values are means ± SE of (n) experiments performed in duplicate. Significant effect of treatment at *P < 0.01 and **P < 0.05. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these concentration-response curves.

Figure 5. Dobutamine (DOBUT)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal (left) and femoral (right) regions of men (n = 10; upper panels) and women (n = 14; lower panels) before (open symbols) and after (filled symbols) weight reduction. Fat cells were incubated without ADA (ie, in standard conditions) and values are means ± SE of (n) experiments performed in duplicate. Significant effect of treatment at *P < 0.01 and **P < 0.05, in women, exclusively. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these concentration-response curves.

Figure 6. Lipolytic responsiveness to post-adrenoceptor agents of isolated adipocytes from the abdominal (left) and femoral (right) regions in men (upper panels) (n values ranging from 8 to 14, depending on the agent used) and women (lower panels) (n values ranging from 9 to 18, depending on the agent used) before (open columns) and after (filled columns) weight reduction. Fat cells were incubated without ADA, in the presence of either dibutyryl-cyclicAMP (DcAMP) (10^{-3} M), forskolin (FK) (10^{-5} M), theophylline (THEO) (10^{-3} M) or cilostamide (CILO) (10^{-5} M). Previous experiments revealed that the concentrations of the different drugs used were maximally effective doses. Values are means ± SE of (n) experiments performed in duplicate and basal glycerol release has already been subtracted.

Table 1. Physical characteristics of men and women before and after weight loss.

	Men (n = 14)		Women (n = 18)	
	Before	After	Before	After
Weight (kg)	104 ± 11 c	94 ± 10 *	92 ± 14	83 ± 14 *
BMI (kg/m^2)	34 ± 3	30 ± 3 *	36 ± 4	33 ± 5 *
Body fat (%)	38 ± 4 a	32 ± 5 *, b	49 ± 5	47 ± 7 *
Fat mass (kg)	40 ± 6	30 ± 5 *, c	45 ± 10	40 ± 11 **
Fat-free mass (kg)	64 ± 7 a	64 ± 8 b	47 ± 6	44 ± 5
Waist girth (cm)	110 ± 8	101 ± 6 *, d	99 ± 11	93 ± 11 *
Adipose tissue areas measured by CT (cm^2)				
Abdomen (L4-L5)				
Subcutaneous	404 ± 71 d	328 ± 70 *	559 ± 144	498 ± 152 **
Visceral	192 ± 55 b	140 ± 52 *	153 ± 47	121 ± 42 **
Midthigh	76 ± 11 a	60 ± 12 *, b	181 ± 41	159 ± 40 **
Regional fat cell weight (ug lipid/cell)				
Abdominal	0.64 ± 0.12	0.54 ± 0.14 &	0.71 ± 0.15	0.61 ± 0.13&
Femoral	0.58 ± 0.10 b	0.48 ± 0.10 ***	0.81 ± 0.18	0.62 ± 0.16 ***

Values are means ± standard deviation (SD).

BMI = body mass index; CT = computed tomography.

Significant difference before and after weight loss at $^{*}P < 0.0001$, $^{**}P < 0.001$, $^{***}P < 0.01$, $^{\&}P < 0.05$. Significant gender variation at $^{a}P < 0.0001$, $^{b}P < 0.001$, $^{c}P < 0.01$, $^{d}P < 0.05$.

Table 2. Basal lipolytic rate and adenosine deaminase (ADA)-stimulated lipolysis in subcutaneous abdominal and femoral adipocytes of men and women, before and after weight loss.

	Abdominal		Femoral	
	Before	After	Before	After
Men (n = 14)				
Basal (/cell)	0.7 ± 0.1	1.2 ± 0.3	0.7 ± 0.2	0.9 ± 0.2
ADA (/cell)	1.3 ± 0.3	2.1 ± 0.5	1.0 ± 0.2	1.8 ± 0.4
Basal (/surface area)	1.7 ± 0.4	3.5 ± 0.9 &	1.8 ± 0.4	3.1 ± 0.6
ADA (/surface area)	3.7 ± 0.7	6.8 ± 1.8 &	3.1 ± 0.7	5.3 ± 1.2
Women (n = 18)				
Basal (/cell)	1.0 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
ADA (/cell)	1.9 ± 0.3	1.9 ± 0.4	1.1 ± 0.2	1.1 ± 0.2
Basal (/surface area)	2.0 ± 0.4	2.7 ± 0.5	1.4 ± 0.3	1.6 ± 0.3
ADA (/surface area)	4.9 ± 1.0	5.8 ± 1.6	2.9 ± 0.6	3.3 ± 0.6

Values are means ± SE of (n) separate experiments performed in duplicate.

&P = 0.065. Lipolysis is expressed per cell number (μmol glycerol/10^6 cells x 2 h) or corrected for variation in cell surface area (nmol glycerol/μm^2 x 10^8 x 2 h).

Table 3. Sensitivity for α2- and β-adrenoceptor agonists estimated from *in vitro* lipolysis studies on subcutaneous abdominal and femoral adipocytes of men and women, before and after weight loss.

	Subcutaneous adipose cell sensitivity			
	Abdominal		Femoral	
	Before	After	Before	After
Men				
EC_{50} (isoproterenol) (14)	27 ± 4^{a}	$5 \pm 1^{*,a}$	44 ± 7	$13 \pm 3^{**}$
EC_{50} (procaterol) (12)	12 ± 3^{b}	$6 \pm 1^{\&,a}$	62 ± 17	$18 \pm 5^{\&}$
EC_{50} (dobutamine) (10)	$97 \pm 30^{\$}$	$61 \pm 13^{\$\$}$	140 ± 26	131 ± 46
EC_{50} (UK-14304) (14)	2.2 ± 0.4	$4.3 \pm 1.0^{\&}$	3.0 ± 0.9	4.9 ± 1.1
Women				
EC_{50} (isoproterenol) (18)	26 ± 3^{b}	$6 \pm 1^{*,c}$	56 ± 7	$17 \pm 3^{*}$
EC_{50} (procaterol) (14)	26 ± 7^{b}	$9 \pm 2^{***,a}$	56 ± 11	$16 \pm 3^{***}$
EC_{50} (dobutamine) (12)	306 ± 62	$169 \pm 40^{\&}$	218 ± 46	$114 \pm 29^{\&}$
EC_{50} (UK-14304) (18)	2.8 ± 0.5	$5.8 \pm 1.6^{\&}$	3.3 ± 0.7	$6.1 \pm 1.6^{\&}$

Values are means ± SE of (n) separate experiments performed in duplicate.

β-adrenergic sensitivity estimated by the concentration of either isoproterenol, procaterol or dobutamine required for half-maximal stimulation of lipolysis (EC_{50}) (nM) was calculated from each concentration-response curve (10^{-9} to 10^{-5} M of the β-AR agonists tested).

α2-adrenergic sensitivity estimated by the concentration of UK-14304 required for half-maximal inhibition of lipolysis (IC_{50}) (nM) was calculated from each concentration-response curve (10^{-10} to 10^{-6} M).

Significant difference before and after weight loss at $^{*}P < 0.0001$, $^{**}P < 0.005$, $^{***}P < 0.01$, $^{\&}P < 0.05$.

Significant regional variation at $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

Significant gender difference at $^{\$}P < 0.01$, $^{\$\$}P < 0.05$.

Table 4. α2- vs β-adrenoceptor density and hormone-sensitive lipase activity in subcutaneous abdominal and femoral adipose depots of men and women, before and after weight loss.

	Subcutaneous adipose tissue			
	Abdominal		Femoral	
	Before	After	Before	After
Men				
HSL activity (14)	29 ± 6	38 ± 6	43 ± 8	47 ± 5
α2-AR sites (12)	16 ± 4	23 ± 5 †	18 ± 4	22 ± 3
β-AR sites (12)	1.0 ± 0.1 [a]	2.1 ± 0.2 [b]	0.6 ± 0.1	1.2 ± 0.2 *
α2/β-AR ratio (12)	16 ± 3 [a]	13 ± 3 [b]	30 ± 6	26 ± 3
Women				
HSL activity (18)	27 ± 6	31 ± 4	34 ± 7	55 ± 8 †,[b]
α2-AR sites (16)	18 ± 2 [b]	21 ± 2 [b]	26 ± 2 &	30 ± 2 &
β-AR sites (16)	1.0 ± 0.1 [a]	1.9 ± 0.2 †,[c]	0.7 ± 0.1	1.3 ± 0.2 **
α2/β-AR ratio (16)	24 ± 7 [a]	14 ± 3 [c]	41 ± 9	36 ± 6

AR: adrenoceptor; HSL: hormone-sensitive lipase.

Values are means ± SE of (n) separate experiments performed in duplicate.

Both radioligand binding data and enzymatic activities were expressed per cell surface area (ie, in amol/μm^2 and pU/μm^2 cells, respectively).

Significant difference before and after weight loss at $P < 0.005$, $^{*}P < 0.01$, $^{**}P < 0.05$.

Significant regional variation at $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.005$.

Gender difference for α2-AR sites at $^{\&}P < 0.05$.

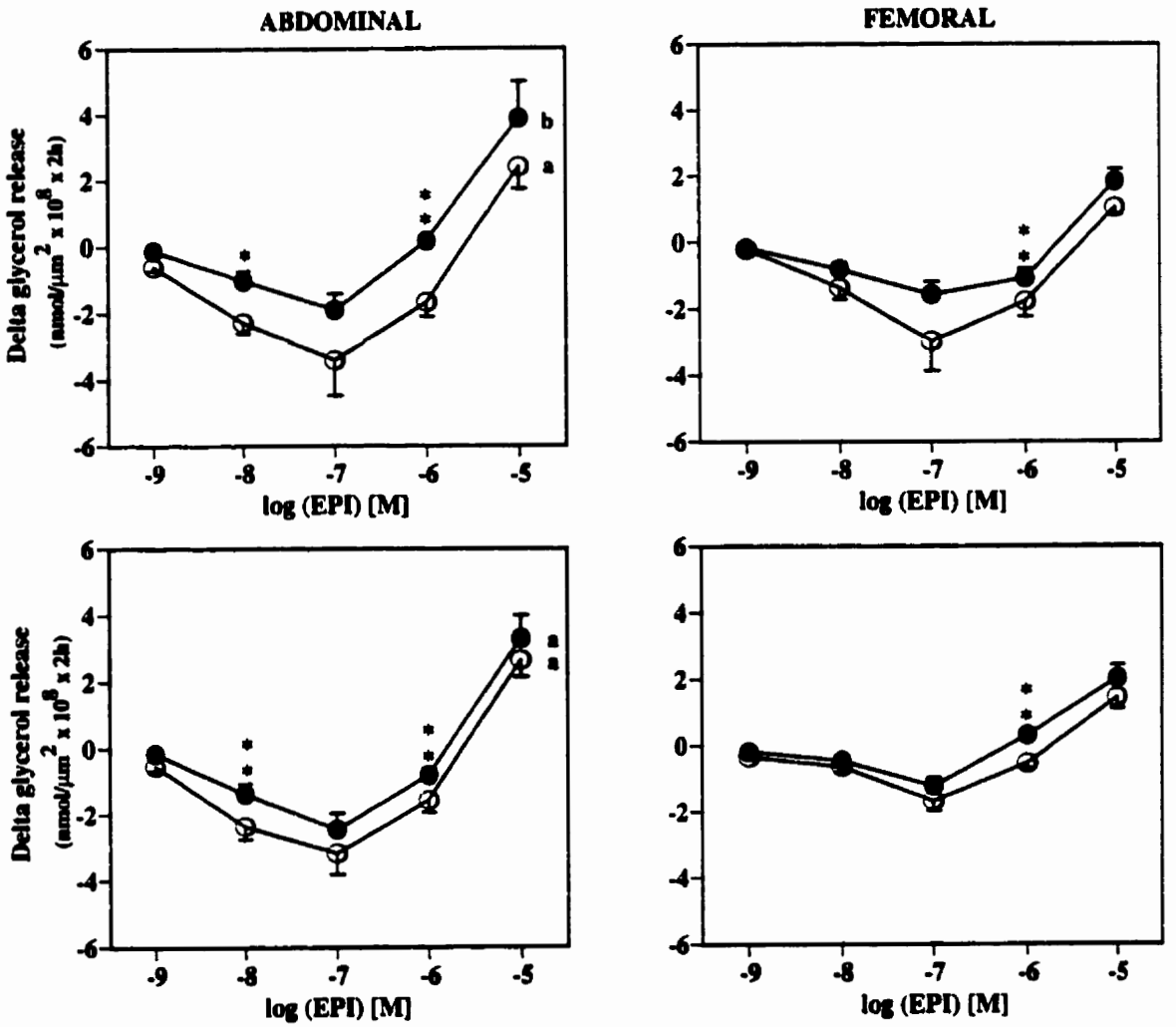

ABDOMINAL
FEMORAL
Delta glycerol release
(nmol/µm² x 10⁸ x 2h)
log (EPI) [M]
b
a
a
a
-9
-8
-7
-6
-5
6
4
2
0
-2
-4
-6

Figure 1

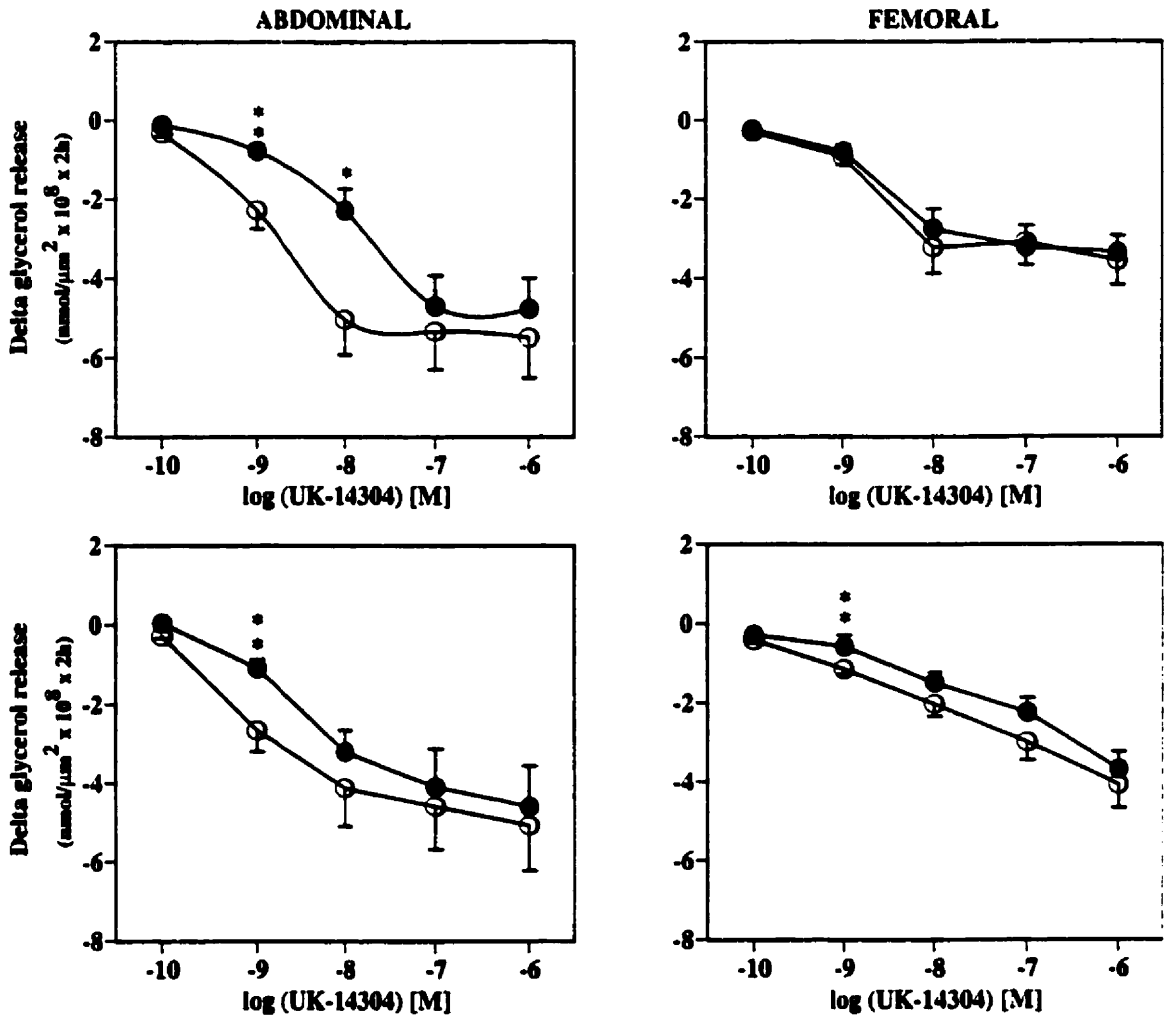

ABDOMINAL
FEMORAL
Delta glycerol release
(nmol/µm² x 10⁸ x 2h)
log (UK-14304) [M]
2
0
-2
-4
-6
-8
-10
-9
-8
-7
-6
*
**

Figure 2

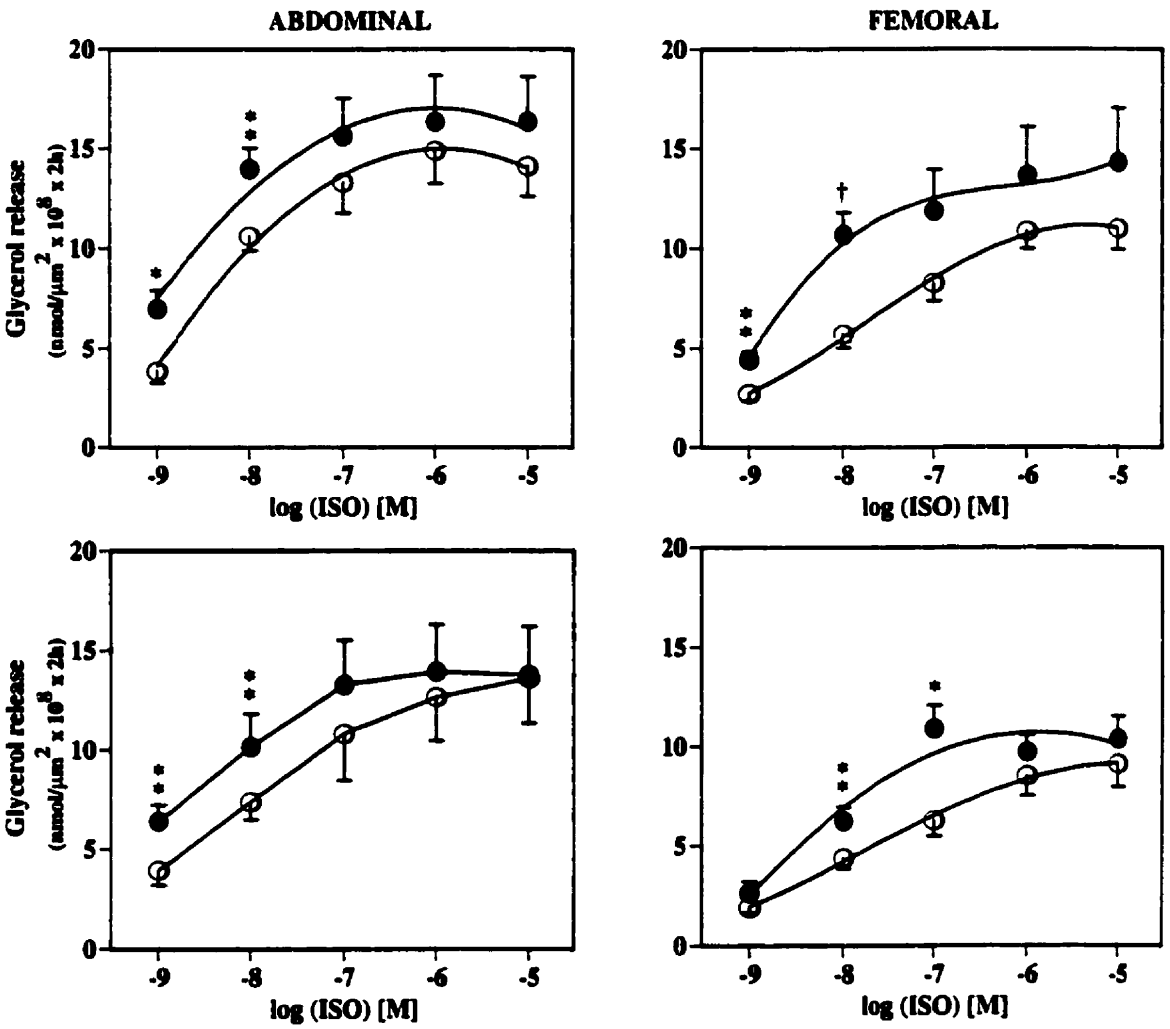

ABDOMINAL
FEMORAL
Glycerol release
(nmol/µm² x 10⁸ x 2h)
log (ISO) [M]
-9
-8
-7
-6
-5
0
5
10
15
20
*
**
†

Figure 3

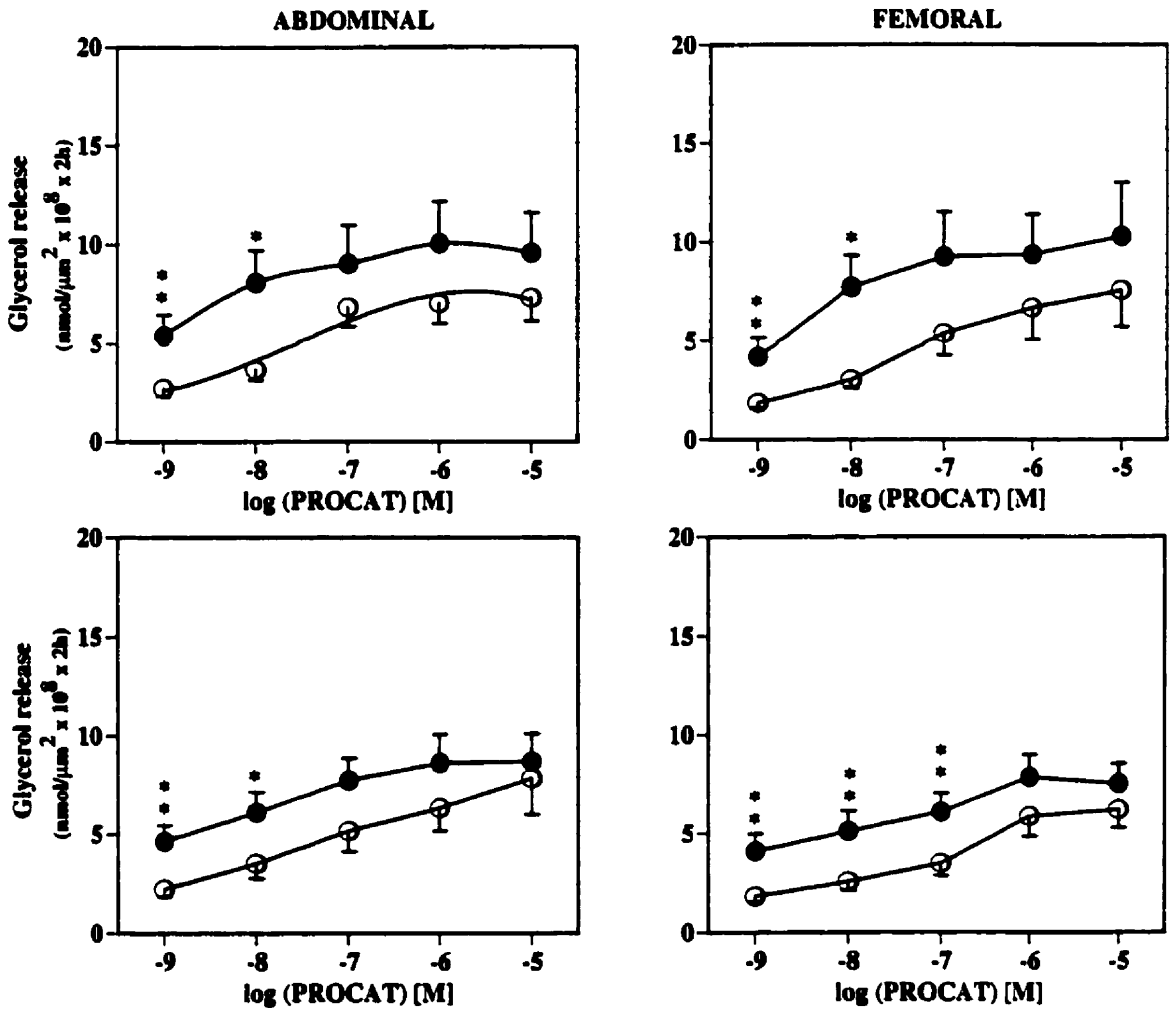

ABDOMINAL
FEMORAL
Glycerol release
(nmol/µm² x 10⁸ x 2h)
log (PROCAT) [M]
-9
-8
-7
-6
-5
0
5
10
15
20

Figure 4

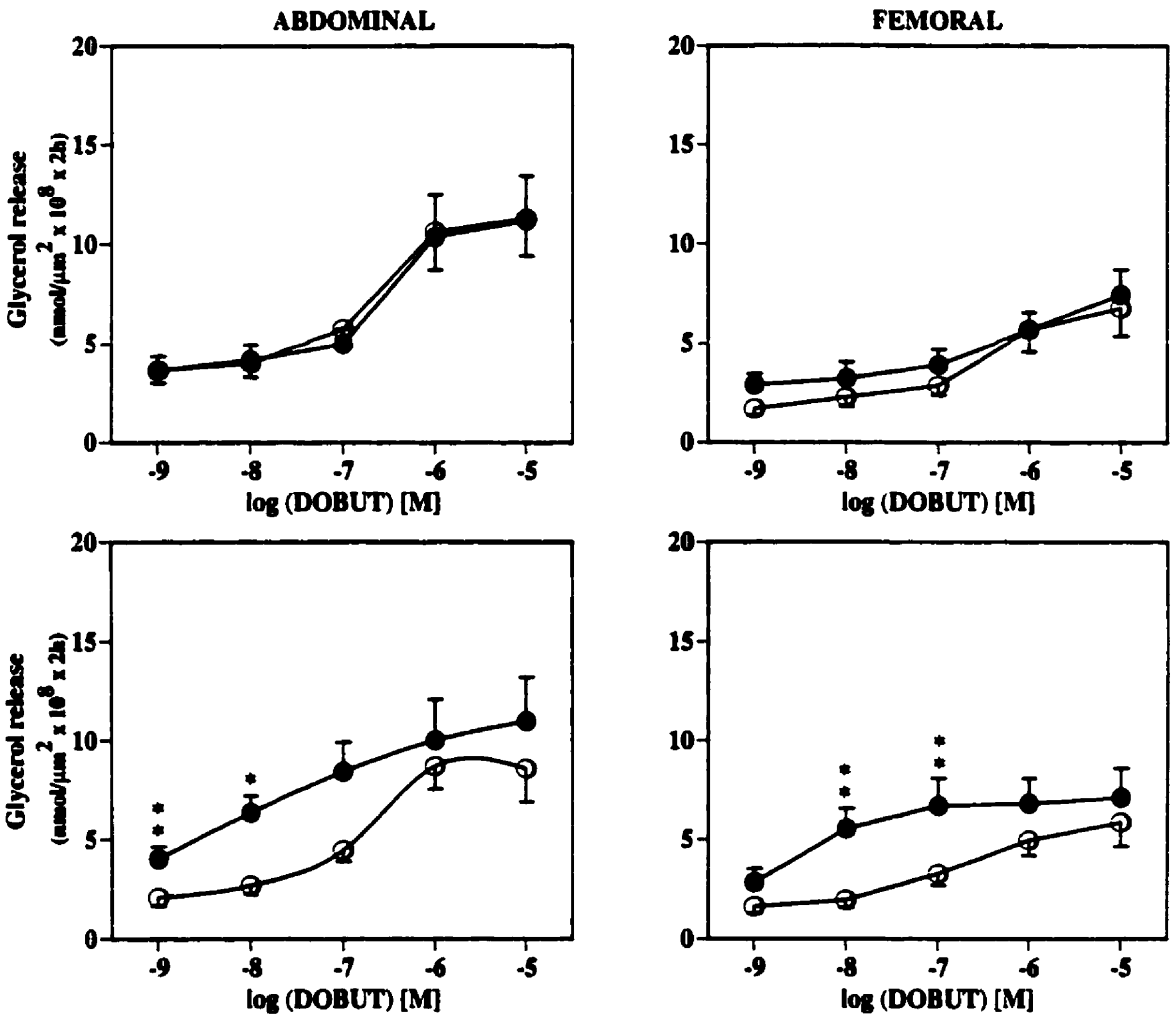

ABDOMINAL
FEMORAL
Glycerol release
(nmol/µm² x 10⁸ x 2h)
log (DOBUT) [M]
0
5
10
15
20
-9
-8
-7
-6
-5
*
**

Figure 5

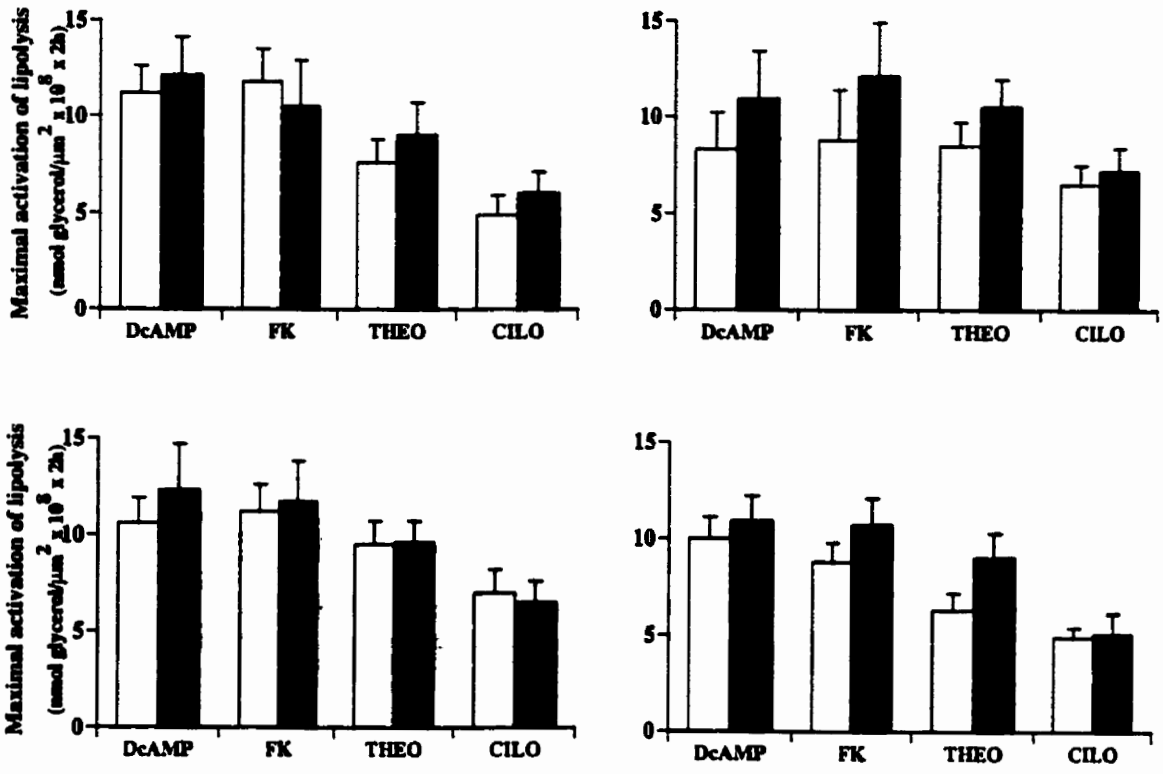

Maximal activation of lipolysis
(nmol glycerol/µm² x 10⁸ x 2h)
15
10
5
0
DcAMP
FK
THEO
CILO

Figure 6

CHAPITRE 8

MÉTABOLISME DU TISSU ADIPEUX CHEZ L'HOMME JEUNE ET MOYENNEMENT ÂGÉ PRÉSENTANT UNE ADIPOSITÉ COMPARABLE

L'article composant ce chapitre est intitulé:

"Adipose tissue metabolism in young and middle-aged men after control for total body fatness"

(sous-presse à *Journal of Clinical Endocrinology and Metabolism*)

Résumé

Cette étude consistait à comparer le métabolisme du tissu adipeux sous-cutané (sc) d'hommes jeunes (29 ± 4 ans) et d'âge moyen (57 ± 5 ans) présentant une adiposité identique. À cet égard, l'activité de la lipoprotéine lipase (LPL) des sites sc abdominal et fémoral ainsi que la réponse lipolytique d'adipocytes isolés furent mesurées chez 16 paires d'hommes d'âge différent, mais caractérisés par une masse adipeuse et une accumulation de tissu adipeux sc abdominal comparables. Aucune différence quant à l'activité de la LPL ne fut observée entre les deux groupes, et ce, indépendamment du site adipeux considéré. À de faibles concentrations (10^{-9} à 10^{-7} M), l'adrénaline a entraîné un effet antilipolytique alors qu'à fortes concentrations (10^{-6} à 10^{-5} M) un effet lipolytique de l'hormone fut observé, et ce, indépendamment du groupe et du dépôt adipeux étudié. De plus, l'action antilipolytique de l'agoniste α_2-adrénergique, UK-14304, était similaire au niveau des adipocytes sc abdominaux et fémoraux chez les deux groupes. Cependant, la lipolyse maximale induite par l'isoprénaline (agoniste β-adrénergique) ou par des agents agissant par des mécanismes postrécepteurs comme l'AMPcyclique dibutyrylé, la forskoline et la théophylline était inférieure au niveau des adipocytes des deux régions adipeuses étudiées chez les hommes d'âge moyen en comparaison à celle des sujets jeunes. À noter qu'aucune différence au niveau de la sensibilité β- ou α_2-adrénergique des adipocytes ne fut observée entre les deux groupes. Ces résultats suggèrent qu'une diminution de la capacité lipolytique des adipocytes survient avec l'âge et semble être expliquée par une altération siégeant au niveau postrécepteur.

Adipose tissue metabolism in young and middle-aged men after control for total body fatness

P. Imbeault [1], D. Prud'homme [1,2], A. Tremblay [1], J-P. Després [2,3], and P. Mauriège [1,2]

[1] Physical Activity Sciences Laboratory, Department of Social & Preventive Medicine, Laval University, [2] Lipid Research Center, Laval University Medical Research Center, [3] Quebec Heart Institute, Laval Hospital, Québec, Canada.

Running head: Adipose tissue metabolism and aging

Address for correspondence: P. Mauriège Ph.D., Lipid Research Center, Laval University Medical Research Center, 2705, boul. Laurier, Room TR-93, Ste-Foy, Québec, Canada, G1V 4G2.
Tel: (418) 654-2133; Fax (418) 654-2145; email: diabolo@internetclub.fr

Abstract

The aim of this study was to compare the subcutaneous (subc) adipose tissue metabolism of young (29 ± 4 years) vs middle-aged men (57 ± 5 years), once the concomitant variation in total adiposity was taken into account. For this purpose, subc abdominal and femoral adipose tissue lipoprotein lipase (AT-LPL) activities as well as fat cell lipolytic responses were investigated in two groups of sixteen men differing in age but displaying similar adipose tissue mass (within 2 kg) and subc abdominal adipose tissue area measured by computed tomography (within 15 cm^2). No difference was observed in AT-LPL activity of young vs middle-aged subjects, regardless of the adipose region considered. Epinephrine induced antilipolysis at low concentrations (10^{-9} to 10^{-7} M) and a net lipolytic response at higher doses (10^{-6} to 10^{-5} M), regardless of the subjects' age and the anatomic location of fat. In addition, the selective α_2-adrenergic agonist, UK-14304, promoted a similar antilipolytic response in subc abdominal and femoral adipose cells from both groups. However, maximal lipolysis induced by isoproterenol (β-adrenergic agonist) or by postadrenoceptor agents such as dibutyryl-cyclic AMP, forskolin and theophylline were lower in both adipose regions of middle-aged, as compared to young men. No difference in the β- or the α_2-adrenoceptor sensitivity of subc adipose cells was observed between groups. These results indicate that there is with age a selective decrease in the lipolytic capacity to β-adrenergic agonist which appears to be due to postadrenoceptor impairments. As subjects in the two age-groups displayed similar body fatness, these alterations are independent from the age-expected increase in total adiposity.

Keywords: adipose cells lipolysis, lipoprotein lipase activity, regional fat distribution, age.

Introduction

Advancing age is associated with a number of changes in body composition such as a reduction in lean body mass and an increase in adiposity (1). We have recently reported that a 12 year-follow-up in adult subjects was accompanied by a body fat gain, even if both a decrease in the relative fat intake and an increase in the participation in physical activity occurred (2). These results suggest that age-related effects play an important role in the regulation of fat balance since they predominate the beneficial lifestyle changes that should have promoted fat loss.

Human adipose tissue is heterogeneous in its metabolic activity and regional variation in storage and/or mobilizing potencies of adipose cells has been suggested as contributing to local differences in adiposity (3-5). Storage of fatty acids in the adipocyte is almost entirely dependent on the uptake of fatty acids released from the hydrolysis of circulating triglyceride-rich lipoproteins by lipoprotein lipase (LPL) (6). On the other hand, lipid mobilization in humans is stimulated by hormones such as catecholamines which act on cell-surface receptors and control cAMP production, and thus lipolytic activity through hormone sensitive lipase activation (HSL) (7,8). It is therefore possible that alterations in these regulatory aspects of adipose tissue metabolism could contribute to the age-related effects on body composition. In this regard, previous studies have already shown that catecholamine-induced lipolysis was reduced in adipocytes of elderly subjects (9,10). More recently, Lönnqvist et al. (11) have proposed that this impaired lipolytic response of fat cells to catecholamines with advancing age might be due to a defective activation of the hormone-sensitive lipase complex. This hypothesis was supported by the fact that all the lipolytic agents used induced about 50% lower responses in elderly as compared to young subjects, even if both the α_2- and β-adrenoceptor number and affinity remained unchanged with age.

However, to the best of our knowledge, no study has attempted to identify primary alterations in subcutaneous adipose tissue metabolism with advancing age, once the concomitant variation in total adiposity is taken into account. Indeed, previous experiments that have been conducted on young and elderly subjects (9-11) have compared the lipolytic activity of

adipocytes from individuals whose body fat distribution was different. Therefore, the aim of the present study was to examine whether differences in subcutaneous abdominal and femoral AT-LPL activities and adipose cell lipolysis exist in 16 pairs of men who display similar body fatness and subcutaneous fat accumulation but differed in age.

Material and Methods

Subjects

Thirty-two healthy Caucasian men were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. Sixteen pairs of subjects who displayed similar levels of subcutaneous AT area (within 15 cm^2) measured by computed tomography (CT) and adipose tissue mass (within 2 kg) but differed in age were compared for potential differences in subcutaneous AT-LPL activity and adipose cell lipolysis. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication which could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc) were excluded from the study. All participants were sedentary (< 2 exercise sessions of 30 min/week), non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last six months prior to the study.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique and percent body fat was derived from body density (12). Pulmonary residual volume was measured using the helium dilution method (13). Fat mass was calculated as total body weight minus fat free mass. Waist girth was measured according to the procedures recommended at the Airlie Conference (14). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology previously described by Sjöström et al. (15). Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) level, using an abdominal scout radiograph to establish the position of the scan to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing the AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) (16). Abdominal visceral AT area was determined by drawing a line within the muscle

wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Adipocyte isolation and lipolysis

After an overnight fast, participants underwent biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the anterior midthigh level (femoral site). A small cutaneous incision (1 cm) was performed in both sites following local anesthesia (1% lidocaine, without epinephrine) and about 350 mg of subcutaneous adipose tissue were surgically removed from the two fat depots.

Samples of 250 mg of adipose tissue from each site were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (17) in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus 1 mg/ml collagenase, as previously described (18). Digestion took place in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2, for 40 min at 37^{0}C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 µl.

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. 50 µl aliquots of the continuously stirred cell suspension were placed in 1.5 ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 10 µl KRB were immediately placed on ice and provided evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation or inhibition were added just before starting the incubation in 10 µl portions in order to obtain the desired final concentration. After a 2h incubation at 37^{0}C in a shaking water bath, under 95% O_2 and 5% CO_2 gas phase, 50 µl HCl (1N) were added to all tubes to stop the reaction, then 50 µl NaOH (1N) were added to neutralize the medium. All tubes were stored at –20^{0}C until glycerol determination and NADH concentration was measured by bioluminescence with luciferase solution, using an automated 2250 Dynatech luminometer (18,19). For each concentration of stimulator or inhibitor agents, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Glycerol measurement by bioluminescence is very sensitive and especially

well adapted when only small amounts of adipose tissue are available (18,19). Adipose cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ, USA). Mean fat cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described (20).

The lipolytic activity of isolated fat cells was tested with epinephrine which is a mixed agonist (α_2/β) with a higher affinity for α_2- than for β-adrenoceptor (AR) sites (21), UK-14304 (selective α_2-AR agonist) and isoproterenol (non selective β-AR agonist) (19). Ascorbic acid (0.1 mmol/l) was included in the medium in order to prevent catecholamine degradation. Some experiments were conducted with forskolin (direct activator of adenylate cyclase), dibutyryl adenosine 3', 5' cyclic monophosphate (dibutyryl c-AMP, stimulator of the protein kinase A hormone-sensitive lipase complex and phophodiesterase-resistant cyclic AMP analogue) and theophylline (mainly inhibitor of cyclic GMP-inhibited phosphodiesterase, cGI-PDE) (19). When antilipolytic effects were investigated, the incubation buffer was supplement with 5 μg/ml adenosine deaminase (ADA) to remove adenosine released into the incubation medium by the isolated fat cells, this procedure allowing better investigations of α_2-AR mediated antilipolytic effects (18,19). Lipolysis was expressed either per cell number (ie, in μmol of glycerol/10^6 cells x 2h) or per unit of cell surface area (ie, in nmol of glycerol/μm^2 x 10^8 x 2h), the latter mode of expression being used to correct for variation in fat cell size which is a well-known modulator of lipolysis (19). In case where complete dose-response curves were obtained, they were compared for both responsiveness and sensitivity. The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-5} M isoproterenol or forskolin, 10^{-3} M dibutyryl cAMP or theophylline). The β-adrenergic sensitivity was considered as the concentration of isoproterenol giving half-maximal stimulation of lipolysis (EC_{50}), whereas the α_2-adrenergic sensitivity was calculated as the dose of UK-14304 which produced half-maximal inhibition lipolysis (IC_{50}). Both were evaluated by logarithmic conversion of each dose-response curve. The higher was the EC_{50} (isoproterenol) or the IC_{50} (UK-14304) value, the lower was the β- or the α_2-adrenergic sensitivity, respectively.

Adipose tissue lipoprotein lipase (AT-LPL) activity

Samples of approximately 100 mg of adipose tissue from each region were immediately frozen in liquid nitrogen and stored at -80^0C for later measurement of heparin-releasable LPL activity, according to Savard et al. (22). AT-LPL activity was expressed as micromoles of free-fatty acids (FFA) released per hour per 10^6 cells. Since AT-LPL activity is associated with fat cell size (6,22), AT-LPL activity was also expressed per cell surface area (ie, nanomoles FFA per hour per micrometer squared times 10^8).

Drugs and chemicals

Collagenase, bovine serum albumin, ADA and enzymes for glycerol assays were obtained from Boehringer Mannheim (Canada). Ascorbic acid, theophylline, forskolin, DcAMP, (-)-isoproterenol bitartrate, (-)-epinephrine bitartrate and cold triolein were purchased from Sigma Chemical (St-Louis, MO). ^{14}C-triolein was obtained from Dupont NEN (Canada). 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK-14304) was generously provided by Dr. D.A. Faulkner (Pfizer, Sandwich, UK). All other chemicals and organic solvents were of the highest purity grade commercially available. The same batches of hormones, pharmacological agents, collagenase and albumin were used in all experiments.

Statistical analyses

Two subgroups of 16 men displaying similar levels of subcutaneous abdominal AT measured by CT and adipose tissue mass but differing in age were compared. The Student's t-test was utilized for comparisons of anthropometric variables, basal and ADA-stimulated lipolysis as well as AT-LPL activity between young and middle-aged subjects. The effects of age (young vs middle-aged) and site (abdominal vs femoral) on adipose tissue lipolytic curves were tested by a two-way analysis of variance for repeated measures. Post hoc comparisons were handled with a Student's t-test. Lipolysis measurements expressed per unit of cell surface area (ie, nmol glycerol/ μm^2 x 10^8 x 2h) were obtained by the following formula by assuming that an adipocyte has a spherical shape:

$$\frac{\text{glycerol release } (\mu\text{mol}/\ 10^6 \text{ cells x 2h}) \times 10^3 \text{ (nmol)}}{\pi[D/2]^2\ (\mu m^2) \times 1\ (\mu\text{mol}) \times 10^8}$$

; where "D" represents the mean diameter (μm) of about 500 adipose cells and "10^8" is a factor used to simplify presentation of data. All analyses were performed using the Jump version 3.2.2 program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers.

Results

Subjects' characteristics

Subjects' physical characteristics are presented in Table 1. As expected, a significant difference was observed in the subjects' age ($P < 0.0001$). Regarding the various body fatness and fat distribution variables, comparison between groups revealed that both young and middle-aged subjects displayed similar body weight, fat mass, waist girth and subcutaneous abdominal adipose tissue accumulation measured by CT. However, middle-aged subjects had a lower fat free mass and a higher visceral adipose tissue accumulation (P values ranging from 0.01 to 0.05), as compared to young individuals. As shown in Figure 1, mean fat cell weights from both depots did not differ between groups, although subcutaneous abdominal adipocytes tended to be larger in middle-aged than in young subjects ($P = 0.06$). No regional variation in adipocyte size was found within both groups. In order to control for the trend observed in the variation of fat cell weight, all lipolysis measurements have been further corrected for variation in cell surface area. However, it should be noted that similar results were obtained when expressed on a per cell basis (not shown).

Insert Table 1 and Figure 1

Adipocyte function

As shown in Figure 2, the basal lipolytic rate of both adipose regions was not affected by age. Moreover, no regional variation in basal lipolysis was observed within both groups. As expected, the addition of ADA (5 μg/ml) in the incubation medium increased the basal lipolytic rate by approximately 1.5 to 2.5 fold (Figure 2) with no further increment at higher doses of this enzyme, in all cell types. ADA-stimulated lipolysis was similar in both adipose regions between young and middle-aged subjects. No regional variation was observed in the lipolytic response to this enzyme within groups.

Insert Figure 2

The effect of epinephrine, which is known for its mixed agonist (α_2/β) properties on lipolysis was examined in the presence of ADA. As shown in Figure 3, the catecholamine initiated a similar biphasic responsiveness in subcutaneous abdominal and femoral adipocytes from both groups: antilipolysis was observed at low concentrations (10^{-9} to 10^{-7} M), this effect being completely reversed at higher doses at which the hormone exerted a lipolytic response (10^{-6} to 10^{-5} M).

Insert Figure 3

The selective α_2-agonist, UK-14304, was also tested on ADA-stimulated lipolysis to characterize the α_2-adrenoceptor component (Figure 4). A similar antilipolytic effect was observed in subcutaneous abdominal and femoral adipocytes from both groups. The α_2-adrenergic sensitivity (IC_{50}) estimated as the half-maximal antilipolysis induced by UK-14304 was also similar in both adipose depots between groups (Table 2). Moreover, middle-aged subjects were characterized by a greater α_2-adrenergic sensitivity in femoral than in subcutaneous abdominal adipose cells ($P < 0.05$) (Table 2), whereas such a regional variation was not observed in young men.

Insert Figure 4 and Table 2

To study the influence of the β-adrenoceptor component, the lipolytic effect of the β-agonist isoproterenol was examined on basal lipolysis (Figure 5). The lipolytic responses observed at both 10^{-6} and 10^{-5} M were significantly lower in middle-aged than in young subjects ($P < 0.01$). No variation between sites was observed for lipolysis initiated by isoproterenol in middle-aged subjects, whereas young subjects displayed a greater isoproterenol-induced lipolysis in the subcutaneous abdominal region ($P < 0.05$). Moreover, no difference between groups was observed in the β-adrenergic adipose cell lipolytic sensitivity of both regions (Table 2). The β-adrenergic sensitivity was also greater in subcutaneous abdominal than in

femoral adipose cells of middle-aged subjects ($P < 0.05$), whereas such regional variation was not observed in young individuals.

Furthermore, as any step of the lipolytic cascade may be responsible for the differences observed in catecholamine responsiveness, the effects of agents acting at different postreceptor levels were also investigated (Figure 6). The maximal lipolytic responses to either DcAMP (10^{-3} M), forskolin (10^{-5} M) or theophylline (10^{-3} M) were markedly decreased in subcutaneous adipocytes of middle-aged, as compared to young subjects (P values ranging to 0.01 to 0.05). Moreover, no regional variation was observed when lipolysis was stimulated by these postreceptor agents within both groups.

Insert Figures 5 and 6

Finally, no difference was observed in both abdominal and femoral AT-LPL activities between young and middle-aged subjects. No regional variation in this enzyme activity was also found in both groups of men (Figure 7).

Insert Figure 7

Discussion

This study was performed to compare adipose tissue metabolism of young and middle-aged men who displayed similar subcutaneous fat accumulation and fat cell size. This matching procedure was important due to the well-known influence of regional fat distribution and fat cell size on adipose tissue metabolism (3-5). Our results clearly showed that middle-aged subjects were characterized by a lower lipolytic capacity in both subcutaneous abdominal and femoral adipose regions, as compared to young individuals.

The α_2-antilipolytic adrenergic responses to UK-14304 and epinephrine were not altered by aging in the present study. The α_2-adrenergic sensitivity (IC_{50}) induced by UK-14304 was also similar in both adipose depots and groups, suggesting that intrinsic properties of α_2-adrenoceptors in human fat cells do not seem to be influenced by age, a notion concordant with previous observations (11). Taken together, these results are consistent with the fact that the increase in fat cell size, rather than aging itself, is one critical factor modulating the α_2-adrenergic responsiveness and the α_2-adrenoceptor number (4,23).

The fact that maximal lipolytic effect of epinephrine did not differ betweeen groups may appear discordant with the lower isoproterenol-stimulated maximal adipose cell lipolysis observed in middle-aged individuals, as compared to young subjects. In this regard, it could be hypothesized that the strong α_2-adrenergic antilipolytic component of catecholamine could not entirely be compensated by the β-adrenergic activity of the hormone (18,19,21), even after matching subjects for fat cell size and subcutaneous fat accumulation. Such a finding remains difficult to explain as the α_2-adrenoceptor responsiveness assessed either by epinephrine (mixed α_2/β-agonist) or by UK-14304 (highly selective α_2-agonist) did not appear to be more pronounced in subcutaneous adipose cells from middle-aged as compared to young men.

Our results also demonstrate that maximal adipose cell lipolytic response to the β-agonist, isoproterenol, was lower in middle-aged than in young individuals, whereas the β-adrenergic

receptor sensitivity was similar in both groups. As only a few available β-adrenoceptors need to be occupied to obtain a maximal effect, the alteration in responsiveness is usually linked to changes in hormone action at further intracellular steps in the pathway of the signal, whereas changes in sensitivity may reflect alterations in hormone action that are located at/or near the receptor level (24). Therefore, the impaired β-adrenergic response to isoproterenol observed in adipocytes from middle-aged individuals suggests a defect located at the postreceptor levels. In this regard, Lönnqvist et al. (11) have already proposed that hormone-sensitive lipase (HSL), the enzyme which hydrolyzes triacylglycerol from the lipid droplet of adipocytes in glycerol and non-esterified fatty acids (7,8), could play a key role in the age-related difference in catecholamine-stimulated lipolysis. Indeed, since HSL activity is activated by agents that promote an increase in intracellular level of cAMP, such as isoproterenol via its activation of β-adrenoceptors, DcAMP, forskolin or theophylline, a lower lipolytic effect of these agents could reflect a decreased enzyme activity. A recent study has also shown that the maximal lipolytic capacity determined by the adipose cell lipolytic response to isoproterenol was significantly correlated with HSL activity (25). Therefore, the decreased isoproterenol-induced lipolysis that we observed in adipose cells from middle-aged subjects is possibly the consequence of an altered HSL activity. A loss of the ability to translocate HSL to the lipid droplet has also recently been suggested to contribute to the diminished lipolytic response to catecholamines with age in rats (26).

However, alterations located at postreceptor levels other than HSL cannot be excluded at the present time. Indeed, that basal lipolytic rate was similar between young and middle-aged individuals suggests that the suspected HSL defect in older subjects is not major, since basal lipolytic rate has been reported to be a strong correlate of basal HSL activity (27,28). In this regard, the impaired maximal lipolytic effects of all postreceptor agents tested in adipocytes from middle-aged subjects do not exclude the possibility that these individuals are characterized by a high adipose cell phosphodiesterase activity. This finding has already been reported in old rats by some investigators (29), whereas others did not observe any change in this enzyme activity (30,31). Further studies are therefore needed to clarify this issue.

Finally, there is extensive evidence showing that adipose tissue lipoprotein lipase (AT-LPL) activity is an enzyme involved in the regulation of fat cell storage (4-6). Rebuffé-Scrive et al. (32) have already reported an absence of regional variation in AT-LPL activity of middle-aged men, a finding consistent with our results. However, no study to our knowledge has attempted to verify the impact of aging on this enzyme activity. Based on the hypothesis that AT-LPL activity may contribute to regional fat distribution (33), it was therefore expected to observe a similar subcutaneous AT-LPL activity in both groups of subjects because of their similar subcutaneous fat accumulation. Indeed, AT-LPL activity did not appear to be influenced by advancing age, once the concomitant variation in body fatness is taken into account.

Conclusion

This study demonstrated that middle-aged men display a reduced lipolytic capacity in both subcutaneous abdominal and femoral adipocytes when compared to young individuals of similar body weight and subcutaneous fat distribution. This age-related difference in lipolytic activity was mostly explained by alterations located at different postreceptor levels and is probably attributed to a decreased activation of the hormone-sensitive lipase complex. The important visceral adipose tissue accumulation observed in middle-aged subjects could also be a potential factor explaining their reduced subcutaneous adipose tissue lipolytic capacity. Taken together, these results indicate that advancing age is not associated with any major change in adipose tissue LPL activity, but rather to an altered lipid mobilizing capacity.

Acknowledgements

The authors wish to express their gratitude to Sylvie St-Pierre, Éric Doucet, Martine Marcotte, France Levasseur and Henri Bessette for their collaboration at various stages of the study and to Drs Gilles Lortie and Germain Thériault for their medical supervision. Thanks are also expressed to Suzanne Brulotte of the Department of Radiology (Laval University Hospital, Québec, Canada) for her help with the use of the computed tomograph. The subjects are also gratefully acknowledged. Supported by the Medical Research Council of Canada and the Fonds FCAR-Québec.

References

1. Schwartz RS. 1998 Obesity in the elderly. *In* Handbook of obesity. Bray GA, Bouchard C, James WPT. Marcel Dekker, Inc., New York. 103-114.
2. Tremblay A, Drapeau V, Doucet E, Alméras N, Després JP, Bouchard C. 1998 Fat balance and ageing: results from the Québec family study. Br J Nutr. 79: 413-418.
3. Arner P. 1996 Regulation of lipolysis in fat cells. Diabetes Rev. 4: 1-13.
4. Bouchard C, Després JP, Mauriège P. 1993 Genetic and nongenetic determinants of regional fat distribution. Endocr Rev. 14: 72-93.
5. Leibel RL, Edens NK, Fried SK. 1989 Physiologic basis for the control of body fat distribution in humans. Annu Rev Nutr. 9: 417-443.
6. Eckel RH. 1989 Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N Engl J Med. 320: 1060-1068.
7. Lafontan M, Berlan M. 1993 Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res. 34: 1057-1091.
8. Langin D, Holm C, Lafontan M. 1996 Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. Proc Nutr Soc. 55: 93-109.
9. Ostman J, Efendic S, Arner P. 1969 Catecholamines and metabolism of human adipose tissue. Acta Medica Scandinavica. 186: 241-246.
10. James RC, Burns TW, Chase GR. 1971 Lipolysis of human adipose cells: influence of donor factors. J Lab Clin Med. 77: 254-266.
11. Lönnqvist F, Nyberg B, Wahrenberg H, Arner P. 1990 Catecholamine-induced lipolysis in adipose tissue of the elderly. J Clin Invest. 85: 1614-1621.
12. Siri WE. 1956 The gross composition of body fat. Adv Biol Med Physiol. 4: 239-280.
13. Meneely GR, Kaltreider NL. 1949 Volume of the lung determined by helium dilution. J Clin Invest. 28: 129-139.
14. Lohman TG, Roche AF, Martorell R. 1988 The Airlie (VA) consensus conference. *In* Anthropometric Standardisation Reference Manual. Lohman TG, Roche AF, Martorell R. Human Kinetics Publishers, Champaign (Illinois). 39-80.

15. Sjöström L, Kvist H, Cederblad A, Tylen U. 1986 Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. Am J Physiol. 250: E736-E786.

16. Ferland M, Després JP, Tremblay A, et al. 1989 Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr. 61: 139-148.

17. Rodbell M. 1964 Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem. 239: 375-380.

18. Mauriège P, Després JP, Prud'homme D, et al. 1991 Regional variation in adipose tissue lipolysis in lean and obese men. J Lipid Res. 32: 1625-1633.

19. Mauriège P, Prud'homme D, Lemieux S, Tremblay A, Després JP. 1995 Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. Am J Physiol. 269: 341-350.

20. Després JP, Bouchard C, Bukowiecki L, Savard R, Lupien J. 1983 Morphology and metabolism of human fat cells: a reliability study. Int J Obesity. 7: 231-240.

21. Mauriège P, Galitzky J, Berlan M, Lafontan M. 1987 Heterogeneous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. Eur J Clin Invest. 17: 156-165.

22. Savard R, Deshaies Y, Després JP, et al. 1984 Lipogenesis and lipoprotein lipase activity in human adipose tissue: reproducibility of measurements and relationship with fat cell size. Can J Physiol Pharmacol. 62: 1448-1452.

23. Lafontan M, Berlan M. 1995 Fat cell alpha 2-adrenoceptors: the regulation of fat cell function and lipolysis. Endocr Rev. 16: 716-738.

24. Arner P. 1988 Control of lipolysis and its relevance to the development of obesity in man. Diabetes Metab Rev. 4: 507-515.

25. Large V, Arner P, Reynisdottir S, et al. 1998 Hormone-sensitive lipase expression and activity in relation to lipolysis in human fat cells. J Lipid Res. 39: 1688-1695.

26. Clifford GM, Londos C, Kraemer FB, Vernon RG, Yeaman SJ. 2000 Translocation of Hormone-sensitive Lipase and Perilipin upon Lipolytic Stimulation of Rat Adipocytes. J Biol Chem. 275: 5011-5015.

27. Reynisdottir S, Langin D, Carlstrom K, Holm C, Rossner S, Arner P. 1995 Effects of weight reduction on the regulation of lipolysis in adipocytes of women with upper-body obesity. Clin Sci. 89: 421-429.

28. Stich V, Harant I, De Glisezinski I, et al. 1997 Adipose tissue lipolysis and hormone-sensitive lipase expression during very-low-calorie diet in obese female identical twins. J Clin Endocrinol Metab. 82: 739-744.

29. Forn J, Schonhofer PS, Skidmore IF, Krishna G. 1970 Effect of aging on the adenyl cyclase and phosphodiesterase activity of isolated fat cells of rat. Biochim Biophys Acta. 208: 304-309.

30. Gettys TW, Rohlfs EM, Prpic V, Daniel KW, Taylor IL, Collins S. 1995 Age-dependent changes in beta-adrenergic receptor subtypes and adenylyl cyclase activation in adipocytes from Fischer 344 rats. Endocrinology. 136: 2022-2032.

31. Hoffman BB, Chang H, Farahbakhsh ZT, Reaven GM. 1984 Age-related decrement in hormone-stimulated lipolysis. Am J Physiol. 247: E772-777.

32. Rebuffé-Scrive M, Lönnroth P, Marin P, Wesslau C, Björntorp P, Smith U. 1987 Regional adipose tissue metabolism in men and postmenopausal women. Int J Obesity. 11: 347-355.

33. Arner P, Lithell H, Wahrenberg H, Brönnegard M. 1991 Expression of lipoprotein lipase in different subcutaneous adipose tissue regions. J Lipid Res. 32: 423-429.

Legends to figures

Figure 1

Distribution of subcutaneous abdominal and femoral fat cell weights of young and middle-aged men. Horizontal lines represent mean values.

Figure 2

Basal lipolytic rate and adenosine deaminase (ADA)-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal and femoral regions of young and middle-aged subjects. Values are means ± SE of 16 experiments performed in duplicate.

Figure 3

Effect of epinephrine (EPI) on adenosine deaminase (ADA)-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal and femoral regions of young and middle-aged subjects. Values are means ± SE of 16 experiments performed in duplicate. Glycerol release was expressed as difference between stimulated (with EPI) and basal (i.e. in presence of 5 μg/ml of ADA) values. Negative values reflect inhibition of lipolysis.

Figure 4

UK14304-induced inhibition of ADA-stimulated lipolysis in isolated adipocytes from the subcutaneous abdominal and femoral regions of young and middle-aged subjects. Values are means ± SE of 16 experiments performed in duplicate. Fat cells were incubated in the presence of ADA (5 μg/ml). Antilipolysis is given as the difference between values in the presence of UK and basal values (with ADA). Agonist concentrations required for half-maximal inhibition of lipolysis (IC_{50}) were determined from these dose-response curves.

Figure 5

Isoproterenol (ISO)-induced lipolysis in isolated adipocytes from the subcutaneous abdominal and femoral regions of young and middle-aged subjects. Fat cells were incubated without ADA (i.e., standard conditions) and values are means ± SE of 16 experiments performed in duplicate. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these dose-response curves. Significant difference between groups at † $P < 0.01$.

Figure 6

Lipolytic responsiveness to post-adrenoceptor agents in isolated adipocytes from the subcutaneous abdominal and femoral regions of young and middle-aged subjects. Fat cells were incubated without ADA, in presence of either dibutyryl-cyclicAMP (DcAMP; 10^{-3} M), forskolin (FK; 10^{-5} M) or theophylline (THEO; 10^{-3} M). Previous experiments revealed that the concentrations of different drugs used were maximally effective doses. Values are means ± SE of 16 experiments performed in duplicate and basal glycerol release has already been subtracted. Significant difference between groups at * $P < 0.05$ and † $P < 0.01$.

Figure 7

Subcutaneous abdominal and femoral adipose tissue lipoprotein lipase (AT-LPL) activities expressed per adipocyte surface area of young (n = 15) and middle-aged men (n = 14). Values are means ± SE.

Table 1. Physical characteristics of subjects.

	Young (n = 16)		Middle-aged (n = 16)	
	Mean ± SD	Range	Mean ± SD	Range
Age (years)	29 ± 4	22-35	57 ± 5 ‡	50-65
Anthropometric variables				
Body weight (kg)	89 ± 12	66-113	85 ± 11	62-103
BMI (kg/m^2)	29 ± 4	21-34	29 ± 4	23-40
% Fat	26 ± 9	10-37	29 ± 7	17-45
Fat mass (kg)	23 ± 10	7-36	25 ± 9	13-45
Fat free mass (kg)	65 ± 7	53-79	59 ± 6 *	50-72
Waist girth (cm)	96 ± 11	76-113	100 ± 10	85-121
Adipose tissue areas measured by CT (cm^2)				
Abdomen (L4-L5)				
Subcutaneous	251 ± 116	69-402	266 ± 95	120-481
Visceral	115 ± 59	38-226	175 ± 43 †	104-244

Values are means ± standard deviation (SD).

BMI = body mass index; CT = computed tomography.

Significant difference between groups at * $P< 0.05$, † $P< 0.01$ and ‡ $P< 0.0001$.

Table 2. Sensitivity for α_2- and β-adrenoceptor agonists estimated from in vitro lipolysis studies on subcutaneous abdominal and femoral adipocytes of young and middle-aged subjects.

	Subcutaneous adipose cell sensitivity			
	Young		**Middle-aged**	
	Abdominal	Femoral	Abdominal	Femoral
IC_{50} (UK-14304) (nM)	7.4 ± 3.8	4.3 ± 1.0	7.9 ± 2.2 [a]	2.4 ± 0.5
EC_{50} (isoproterenol) (nM)	38.5 ± 13.0	77.1 ± 27.8	21.7 ± 9.8 [a]	69.0 ± 25.0

Values are means ± standard error (SE) of 16 experiments performed in duplicate.

The α_2-adrenergic sensitivity estimated by the concentration of UK-1430, required for half-maximal inhibition of lipolysis (IC50) was calculated from each dose-response curve (10-10 to 10-6 M).

The β-adrenergic sensitivity estimated by the concentration of isoproterenol required for half-maximal stimulation of lipolysis (EC_{50}) was calculated from each dose-response curve (10^{-9} to 10^{-5} M).

Significant regional variation at [a] $P < 0.05$.

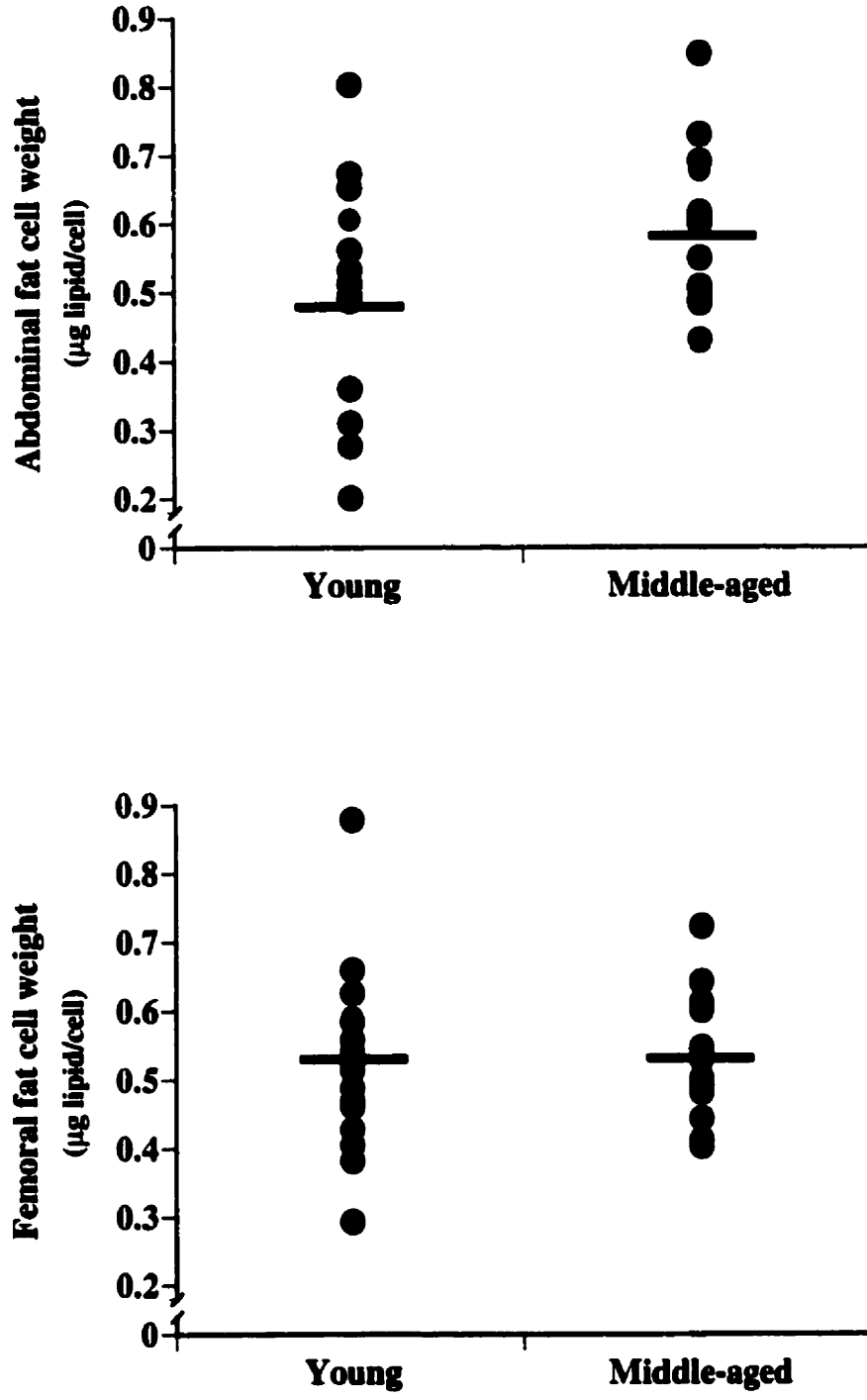

Abdominal fat cell weight
(µg lipid/cell)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
Young
Middle-aged
Femoral fat cell weight
(µg lipid/cell)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
Young
Middle-aged

Figure 1

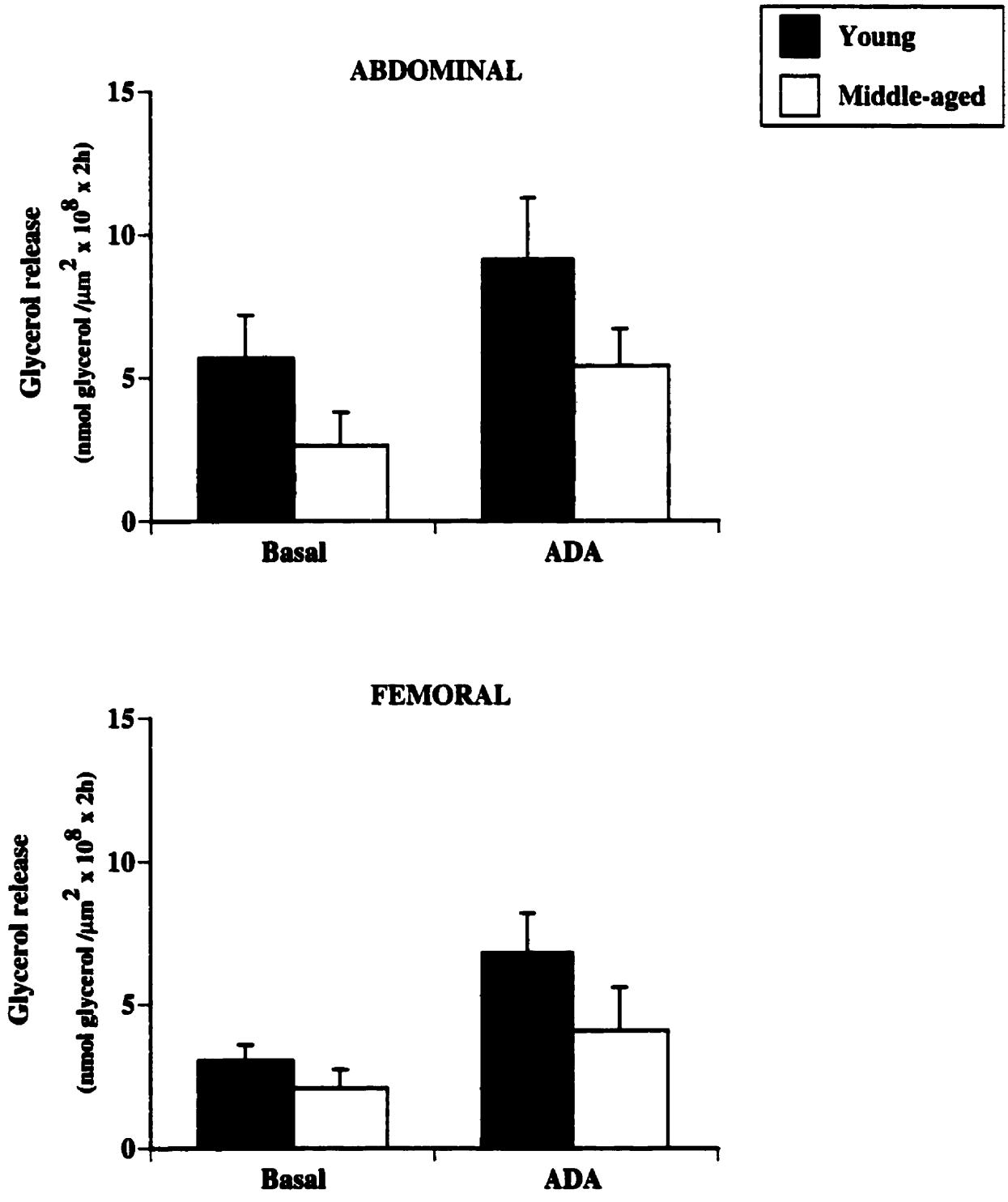

Young
Middle-aged
ABDOMINAL
Glycerol release
(nmol glycerol /µm² x 10⁸ x 2h)
15
10
5
0
Basal
ADA
FEMORAL
Glycerol release
(nmol glycerol /µm² x 10⁸ x 2h)
15
10
5
0
Basal
ADA

Figure 2

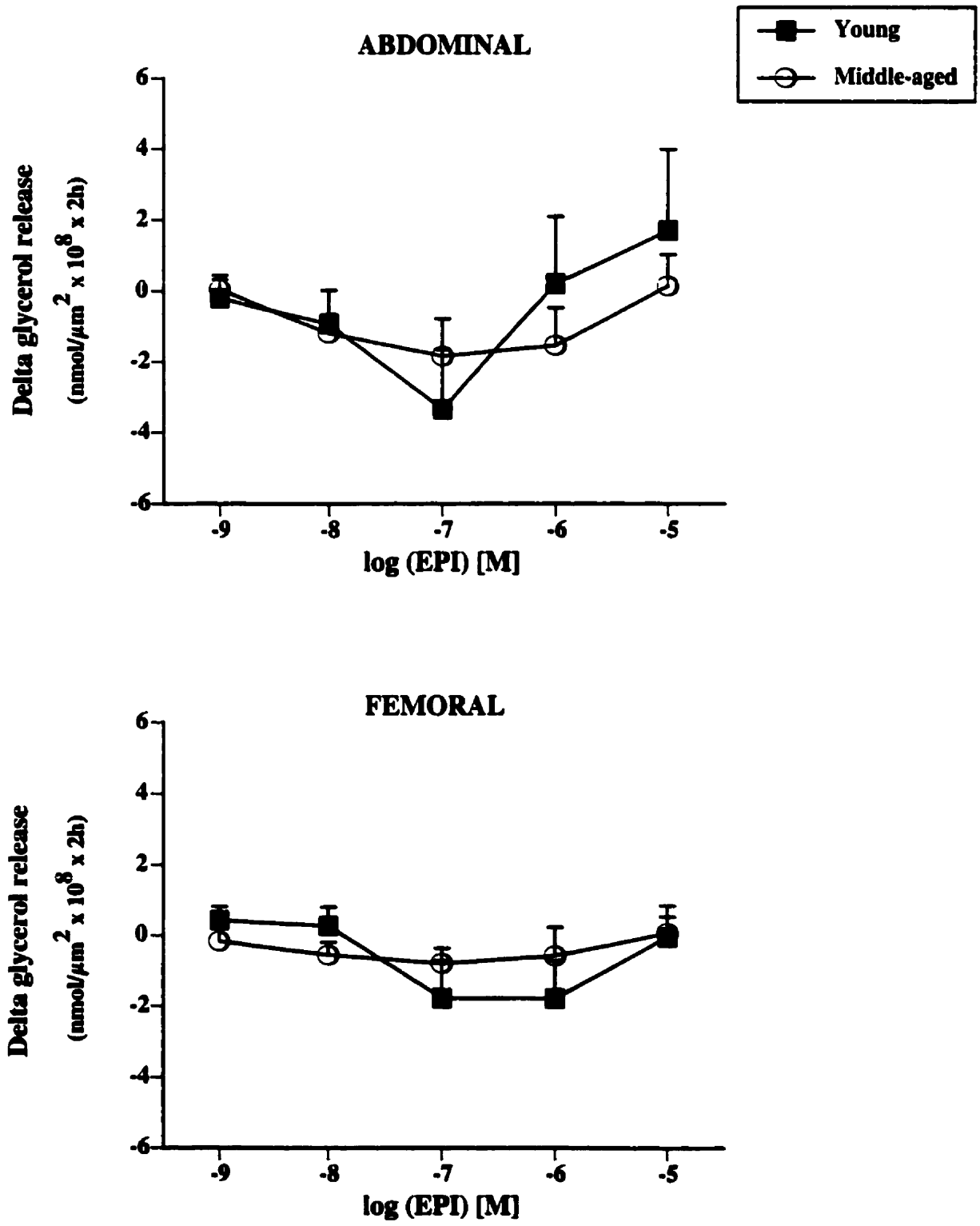

ABDOMINAL
Young
Middle-aged
Delta glycerol release
(nmol/µm 2 x 10 8 x 2h)
6
4
2
0
-2
-4
-6
-9
-8
-7
-6
-5
log (EPI) [M]
FEMORAL
Delta glycerol release
(nmol/µm 2 x 10 8 x 2h)
6
4
2
0
-2
-4
-6
-9
-8
-7
-6
-5
log (EPI) [M]

Figure 3

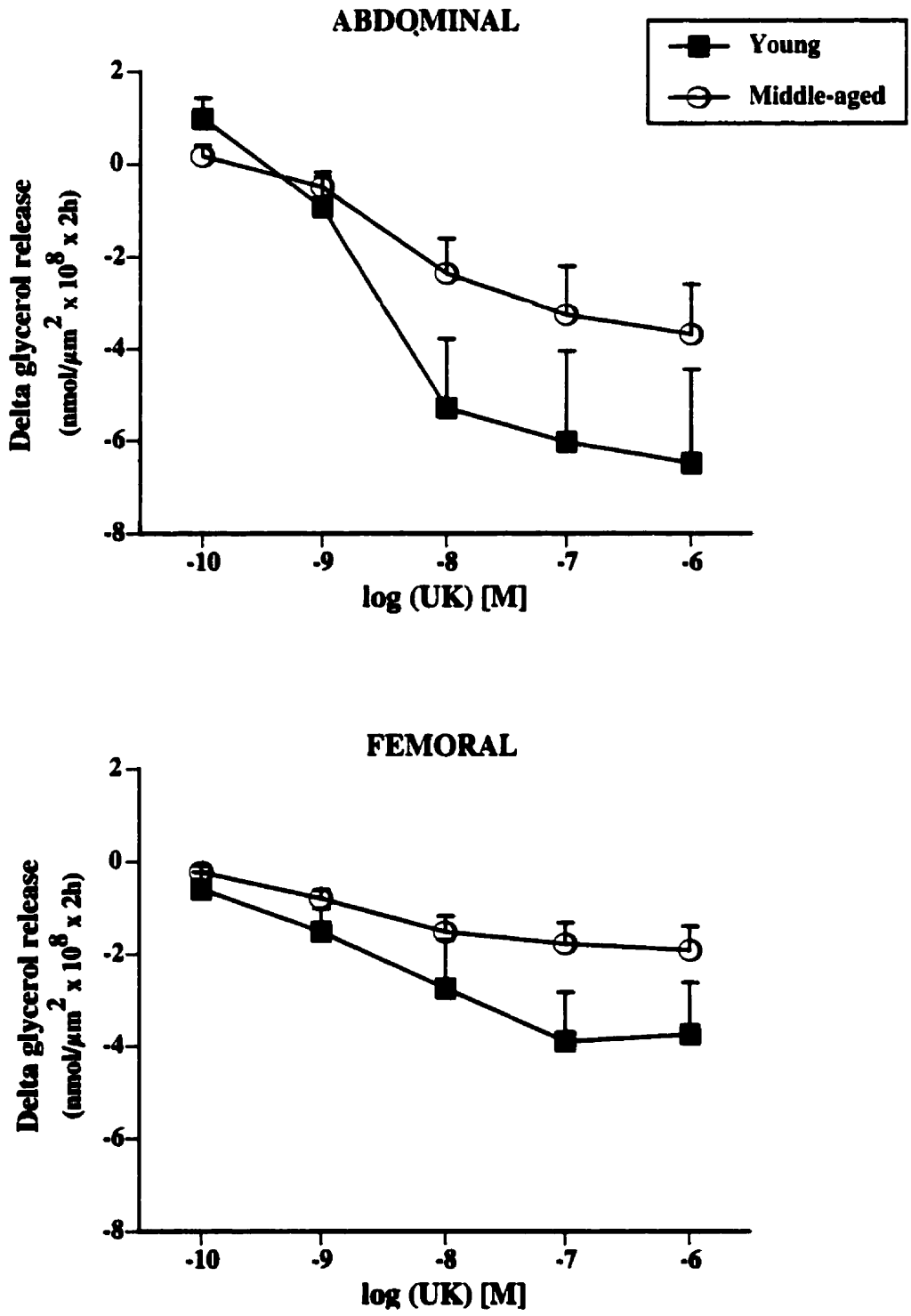

ABDOMINAL
Young
Middle-aged
Delta glycerol release
(nmol/µm^2 x 10^8 x 2h)
2
0
-2
-4
-6
-8
-10
-9
-8
-7
-6
log (UK) [M]
FEMORAL
Delta glycerol release
(nmol/µm^2 x 10^8 x 2h)
2
0
-2
-4
-6
-8
-10
-9
-8
-7
-6
log (UK) [M]

Figure 4

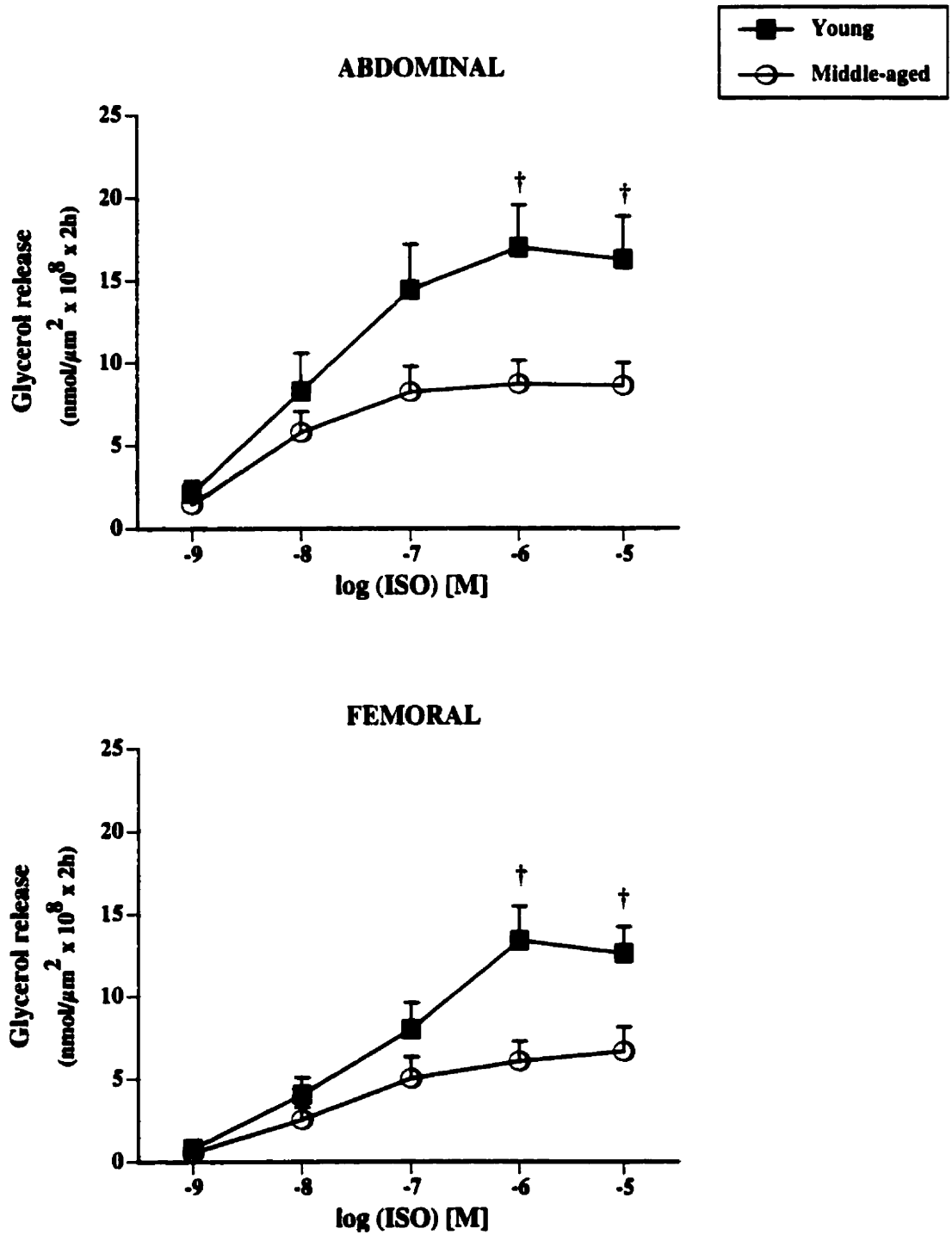

Young
Middle-aged
ABDOMINAL
Glycerol release
(nmol/μm 2 x 10 8 x 2h)
25
20
15
10
5
0
-9
-8
-7
-6
-5
log (ISO) [M]
†
†
FEMORAL
Glycerol release
(nmol/μm 2 x 10 8 x 2h)
25
20
15
10
5
0
-9
-8
-7
-6
-5
log (ISO) [M]
†
†

Figure 5

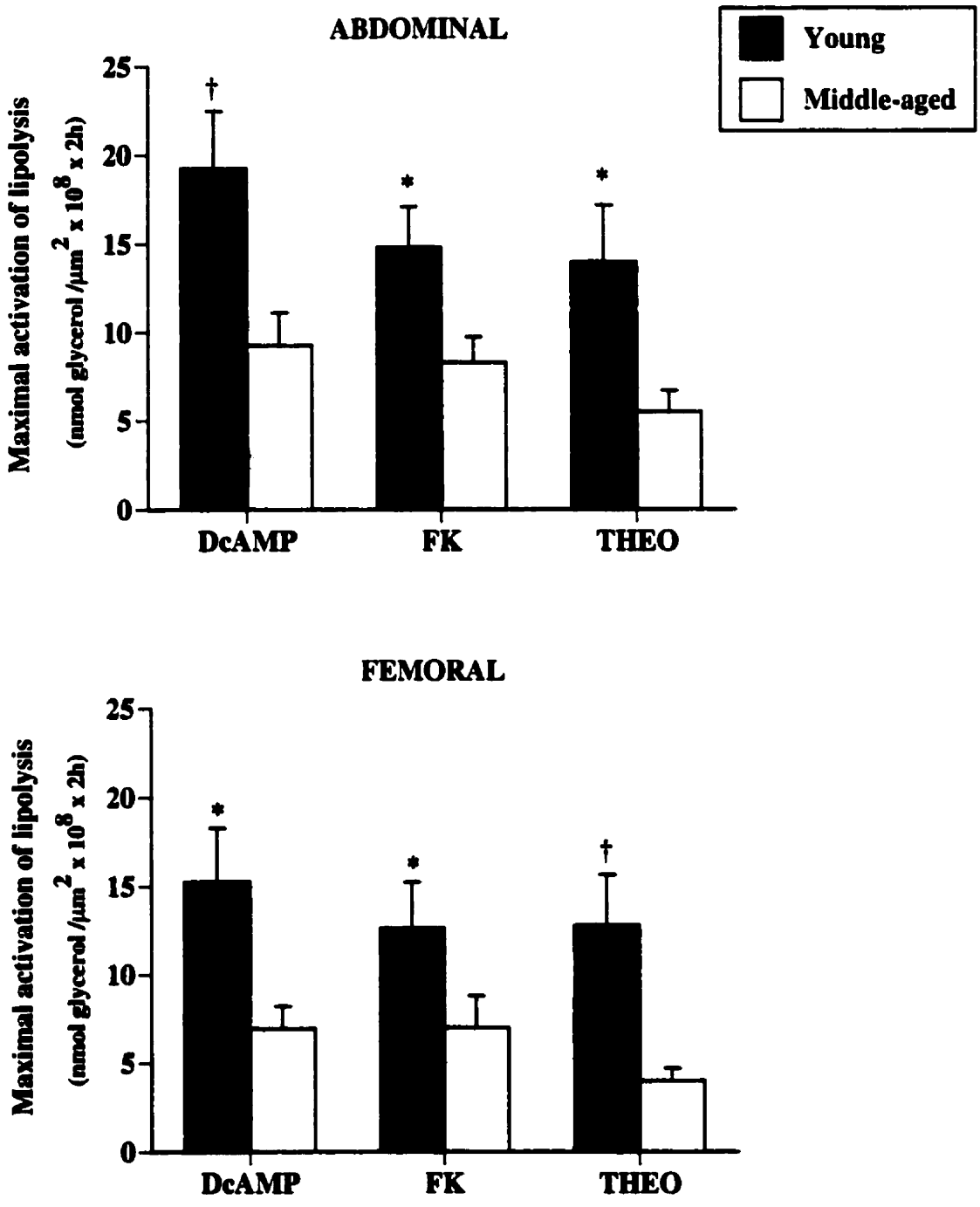

ABDOMINAL
Young
Middle-aged
Maximal activation of lipolysis
(nmol glycerol /µm² x 10⁸ x 2h)
25
20
15
10
5
0
†
*
*
DcAMP
FK
THEO
FEMORAL
Maximal activation of lipolysis
(nmol glycerol /µm² x 10⁸ x 2h)
25
20
15
10
5
0
*
*
†
DcAMP
FK
THEO

Figure 6

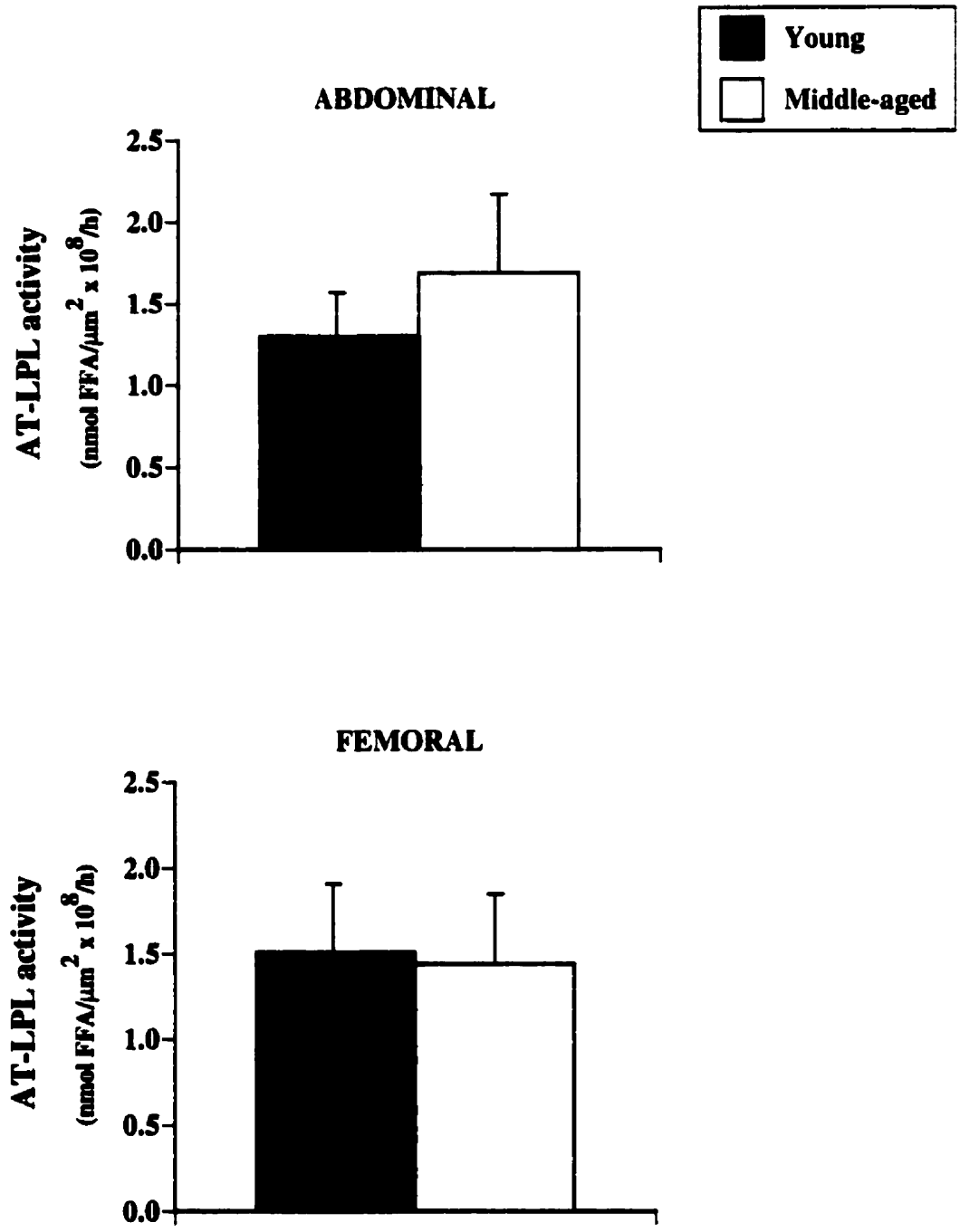

Young
Middle-aged
ABDOMINAL
AT-LPL activity
(nmol FFA/μm² x 10⁸/h)
2.5
2.0
1.5
1.0
0.5
0.0
FEMORAL
AT-LPL activity
(nmol FFA/μm² x 10⁸/h)
2.5
2.0
1.5
1.0
0.5
0.0

Figure 7

CHAPITRE 9

DIFFÉRENCES ASSOCIÉES AU VIEILLISSEMENT AU NIVEAU DE L'EXPRESSION DE PROTÉINES CLÉ IMPLIQUÉES DANS LE MÉTABOLISME DE LA CELLULE ADIPEUSE

L'article composant ce chapitre est intitulé:

"Age-related differences in mRNA expression of key proteins involved in adipose cell differentiation and metabolism"

(soumis pour publication à *American Journal of Physiology*)

Résumé

Le but de cette étude était de comparer les niveaux d'expression (ARNm) de protéines clé telles que la lipoprotéine lipase (LPL), la lipase hormono-sensible (LHS), le complément 3a (C3a) et le *peroxisome proliferator-activated receptor gamma* (PPARγ) impliquées dans le contrôle du métabolisme du tissu adipeux sous-cutané abdominal chez des hommes jeunes (n = 13) et moyennement âgés (n = 16). L'activité de la LPL du site sous-cutané abdominal et la lipolyse d'adipocytes isolés du même dépôt ont également été mesurées. Les deux groupes présentaient un poids corporel et une accumulation de tissu sous-cutané abdominal similaires. Cependant les hommes d'âge moyen étaient caractérisés par une adiposité plus élevée que les sujets jeunes (28 ± 5 vs 22 ± 7 %, $P < 0.05$). Aucune différence n'a été observée au niveau de l'activité de la LPL. À l'inverse, la lipolyse maximale des adipocytes sous-cutanés abdominaux en réponse à l'isoprénaline (agoniste β-adrénergique) ou par des agents postrécepteurs tels que l'AMPcyclique dibutyrylé, la forskoline et la théophylline était inférieure chez les hommes moyennement âgés en comparaison à celle des individus jeunes ($P < 0.05$). L'expression de la LPL était similaire au niveau des deux groupes. Cependant, les niveaux d'ARNm de la LHS, de C3a et de PPARγ étaient plus élevés chez les hommes d'âge moyen que chez les sujets jeunes. Après avoir contrôlé statistiquement les niveaux d'ARNm pour l'adiposité des sujets, seule l'expression de la LHS et de C3a est demeurée plus élevée chez les hommes moyennement âgés ($P < 0.05$). Dans le groupe entier, aucune relation n'a été observée entre l'activité et l'expression de la LPL. De plus, l'expression de la LHS n'était pas reliée à la capacité lipolytique des adipocytes. En conclusion, ces résultats suggèrent que le vieillissement entraîne un effet "uprégulateur" sur l'expression de la LHS et de C3a, alors que les niveaux d'ARNm de PPARγ semblent principalement dépendre d'une augmentation de l'adiposité.

Age-related differences in mRNA expression of key proteins involved in adipose cell differentiation and metabolism

Pascal Imbeault [1], Hubert Vidal [2], Angelo Tremblay [1], Nathalie Vega [2], André Nadeau [3] Jean-Pierre Després [4, 5] and Pascale Mauriège [1, 5]

[1] Physical Activity Sciences Laboratory, Department of Social & Preventive Medicine, Laval University, Ste-Foy, Québec, Canada; [2] INSERM U.449, Faculté de Médecine R.T.H. Laënnec, Lyon, France. [3] Diabetes Research Unit, Laval Medical Research Center, Québec, Canada [4] Quebec Heart Institute, Laval Hospital, Québec, Canada; [5] Lipid Research Center, Laval University Medical Research Center, Québec, Canada.

Running head: Adipose tissue gene expression and aging in men

Supported by the Medical Research Council of Canada and the Fonds FCAR-Québec.

Address for correspondence: P. Mauriège Ph.D.,
Lipid Research Center
Laval University Medical Research Center
2705, boul. Laurier, Room TR-93
Ste-Foy, Québec, Canada, G1V 4G2.
Tel: (418) 654-2133
Fax (418) 654-2145
email: diabolo@internetclub.fr

Abstract

This study was performed to compare the expression of key proteins (lipoprotein lipase (LPL), hormone sensitive lipase (HSL), complement 3a (C3a) and peroxisome proliferator-stimulated receptor-gamma (PPARγ)) involved in the control of subcutaneous abdominal (subc abd) adipose tissue (AT) metabolism of young (n = 13) vs middle-aged (n = 16) men. Subc abd AT-LPL activity as well as fat cell lipolysis were also measured in both groups of men. Young and middle-aged men displayed similar body weight and subc abd fat accumulation measured by computed tomography. However, middle-aged men were characterized by a higher percent body fat (28 ± 5 vs 22 ± 7 %, $P < 0.05$) than young subjects. No difference between groups was observed in subc abd adipose tissue LPL activity. On the other hand, maximal lipolytic response of subc abd adipocytes to isoproterenol (β-adrenergic agonist) or to postadrenoceptor agents such as dibutyryl-cyclic AMP, forskolin and theophylline were lower in middle-aged than in young men ($P < 0.05$). AT-LPL mRNA levels were similar regardless of the subjects' age. However, HSL, C3a and PPARγ mRNA levels were higher in middle-aged than in young individuals (P values ranging from 0.01 to 0.05). After correction for percent body fat, only HSL and C3a mRNA levels remained significantly different between groups ($P < 0.05$). Taken together, these results suggest that aging has an effect on the upregulation of HSL and C3a mRNA levels, whereas PPARγ expression seems to be mostly related to an increased adiposity.

Keywords: aging, adipocyte, lipolysis.

Introduction

Substantial changes in body fat distribution and function occur with aging (22, 42). Indeed, the peak of fat mass is attained by middle age and further declines during senescence (42). This phenomenon is paralleled by a decline in the intrinsic preadipocyte replication potential as well as by an impaired capacity of human preadipocytes to differentiate (18). Studies from animals have also revealed that the preadipocyte differentiation program is impaired with senescence since mRNA levels of β-actin and α-tubulin (early differentiation markers), lipoprotein lipase (LPL) (midway marker) and glycerol-3-phophate dehydrogenase (G3P) (late marker) were decreased in preadipocytes cultured from rats of various age (23).

As opposed to young individuals, middle-aged men commonly present a higher body fatness (42). However, little is known about changes occurring in fat cell metabolism at this specific period of human life (40-65 years). We have recently reported that middle-aged vs young men displayed a selective decrease in the subcutaneous adipose tissue lipolytic capacity which appears to be related to postreceptor alterations rather than to an increased adiposity (20).

In the last years, the discovery of proteins expressed and secreted by adipocytes has confered to adipose tissue a more active role in the control of energy balance (17). In the adipocyte, lipid storage is almost entirely dependent on the uptake of fatty acids released from the hydrolysis of circulating triglyceride-rich lipoproteins by lipoprotein lipase (LPL) (13). Conversely, adipose tissue lipid mobilization is stimulated by hormones such as catecholamines which activate cell-surface receptors increasing thereby the cellular concentration of cAMP which induces adipocyte lipolytic activity via the phosphorylation of hormone sensitive lipase (HSL) (26). Moreover, adipocyte produces acylation stimulating protein (C3a), a potent stimulator of glucose transport and triglyceride (TG) synthesis in adipocytes (5). These latter adipose cell enzymes are to some extent responsible for the control of the adipose tissue lipolysis/lipogenesis balance. More recently, characterization of transcription factor-binding sites led to the identification of a protein family that plays an important role in the induction of the fully differentiated adipocyte, the peroxisome proliferator-activated receptors (PPAR) (11, 41). Among these latter, PPARγ has been

identified to play a key role in adipocyte differentiation (45). To the best of our knowledge, few studies have examined the impact of aging on potential targets involved in the control of lipolysis/lipogenesis adipose tissue balance as well as of adipocyte differentiation in humans. In order to verify whether aging could be related to changes in the expression of genes involved in the control of adipocyte metabolism, we have quantified the mRNA levels of LPL, LHS, C3a and PPARγ in the subcutaneous abdominal fat depot of young vs middle-aged men. We have also investigated whether variation in the expression of these key proteins may contribute to explain changes in body composition and/or metabolic profile observed in middle-aged men.

Material and Methods

Subjects. Twenty-nine healthy Caucasian men were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. Thirteen young and sixteen middle-aged men were compared for potential differences in subcutaneous adipose tissue mRNA levels of genes involved in the control of adipocyte differentiation and metabolism. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication which could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc) were excluded from the study. All participants were sedentary, non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last six months prior to the study.

Total body fatness and regional fat distribution. Body density was determined by the underwater weighing technique and percent body fat was derived from body density (43). Pulmonary residual volume was measured using the helium dilution method (34). Fat mass and fat free mass was derived from the derived percentage of body fat and total body weight. Waist girth was measured according to procedures recommended at the Airlie Conference (29). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology previously described by Sjöström et al. (44). Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) level, using an abdominal scout radiograph to establish the position of the scans to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) (15). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was determined by subtracting the visceral AT area from the total abdominal AT area.

Plasma determinations. Blood samples were obtained in the morning after a 12-h fast from an antecubital vein. Plasma triglyceride levels were measured enzymatically on a RA-1000 automated analyzer (Technicon Instruments Corporation, Tarrytown, NY, USA). Fasting plasma glucose and free-fatty acid (FFA) levels were measured enzymatically (12, 36), whereas plasma insulin concentration was determined by radioimmunoassay with polyethylene glycol separation (9). A 75 g OGTT was performed in the morning after an overnight fast. Blood samples were collected in tubes containing EDTA and Trasylol (Miles Pharmaceutics, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein at -15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. The total glucose and insulin areas under the curve measured during the OGTT were calculated using the trapezoid method.

Adipocyte isolation and lipolysis. After an overnight fast, participants underwent a biopsy of subcutaneous fat in the periumbilical region. A small cutaneous incision (1 cm) was performed in the abdominal site following local anesthesia (1% lidocaine, without epinephrine) and about 400 mg of subcutaneous adipose tissue were surgically removed from the fat depot.
Samples of 250 mg of adipose tissue were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (38) in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus 1 mg/ml collagenase, as previously described (32). Digestion took place in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2, for 40 min at 37^0C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 µl.

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. 50 µl aliquots of the continuously stirred cell suspension were placed in 1.5 ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 10 µl KRB were immediately placed on ice and provided evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation were added just before starting the incubation in 10 µl portions in order to obtain the desired final concentration. After a 2h incubation at 37^0C in a shaking water bath, under 95% O_2 and 5% CO_2 gas phase, 50 µl HCl (1N) were added to

all tubes to stop the reaction, then 50 µl NaOH (1N) were added to neutralize the medium. All tubes were stored at –20°C until glycerol determination. NADH concentration was measured by bioluminescence with a luciferase solution, using an automated 2250 Dynatech luminometer (32, 33). For each concentration of stimulator agent, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Glycerol measurement by bioluminescence is very sensitive and especially well adapted when only small amounts of adipose tissue are available (32, 33). Adipose cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ, USA). Mean fat cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described (10).

The lipolytic activity of isolated fat cells was tested with isoproterenol (non selective β-AR agonist) (33). Ascorbic acid (0.1 mmol/l) was included in the medium in order to prevent catecholamine degradation. Some experiments were conducted with forskolin (direct activator of adenylate cyclase), dibutyryl adenosine 3', 5' cyclic monophosphate (dibutyryl c-AMP, stimulator of the protein kinase hormone-sensitive lipase complex and phophodiesterase-resistant cyclic AMP analogue) and theophylline (mainly inhibitor of cyclic GMP-inhibited phosphodiesterase, cGI-PDE) (33). Lipolysis was expressed either per cell number (ie, in µmol of glycerol/10^6 cells x 2h) or per unit of cell surface area (ie, in nmol of glycerol/µm^2 x 10^8 x 2h), the latter mode of expression being used to correct for variation in fat cell size which is well-known to influence the rate of lipolysis (1, 33). The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-5} M isoproterenol or forskolin, 10^{-3} M dibutyryl cAMP or theophylline).

Adipose tissue lipoprotein lipase (AT-LPL) activity. Samples of approximately 100 mg of adipose tissue from the abdominal site was immediately frozen in liquid nitrogen and stored at –80°C. Heparin-releasable LPL activity was measured within one month of freezing storage, according to Savard et al. (39). AT-LPL activity was expressed as micromoles of free-fatty

acids (FFA) released per hour per 10^6 cells. Since AT-LPL activity is associated with fat cell size (13, 39), AT-LPL activity was also expressed per cell surface area (ie, nanomoles FFA per hour per micrometer squared times 10^5).

Total RNA preparation. Total RNA from approximately 50 mg of adipose tissue was prepared using the RNeasy total RNA kit from Quiagen (Courtaboeuf, France), as previously described (4). Total RNA concentration was determined by absorbance measurement at 260 nm. The 260/280 nm absorption of all preparations ranged between 1.8 and 2.0. The average yield of total RNA was 1.6 ± 0.7 μg/100 mg of adipose tissue and no significant difference was observed between groups.

Quantification of mRNA. LPL, HSL, C3a and PPARγ adipose tissue mRNA levels were determined by reverse transcription reaction followed by competitive polymerase chain reaction (RT-competitive PCR). Briefly, this method relies on the addition of a known amount of a competitor DNA molecule in the PCR to standardize the amplification process. The construction of the competitors, the validation of assays and the conditions of the RT-PCR reactions have previously been described in detail (3, 37). For each mRNA, the specific first strand cDNA was synthesized from 0.1 μg of total RNA. During the PCR, sense primers with the 5'-end labelled with Cy-5fluorescent dye (Eurogentec, Seraing, Belgium) were used. The use of these primers allowed the synthesis of fluorescent PCR products which were analyzed with an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia, Upsala, Sweden) in 4% denaturating polyacrylamide gels. The initial concentration of target mRNA was determined at the competition equivalence point, as previously described (4).

Drugs and chemicals. Collagenase, bovine serum albumin and enzymes for glycerol assays were obtained from Boehringer Mannheim (Canada). Ascorbic acid, theophylline, forskolin, DcAMP, (-)-isoproterenol bitartrate, (-)-epinephrine bitartrate and cold triolein were purchased from Sigma Chemical (St-Louis, MO, USA). ^{14}C-triolein was obtained from Dupont NEN (Canada). All other chemicals and organic solvents were of the highest purity grade

commercially available. The same batches of hormones, pharmacological agents, collagenase and albumin were used in all experiments.

Statistical analyses. The Student's t-test was utilized for comparisons between young and middle-aged subjects. An analysis of covariance was used to determine whether there were significant differences between mRNA levels once the effect of body fat percentage was removed. Associations between two variables were quantified by using the Pearson's product-moment correlation coefficients. All analyses were performed using the Jump version 3.2.2 program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers.

Results

Subjects' physical characteristics are presented in Table 1. As expected, a significant difference was observed in the subjects' age ($P < 0.001$). Both groups of men displayed similar body weight, body mass index, fat mass, fat free mass and subcutaneous adipose tissue accumulation measured by CT. However, middle-aged men had higher percent body fat, waist girth and visceral adipose tissue accumulation than young subjects (P values ranging from 0.01 to 0.05). Moreover, subcutaneous abdominal adipocytes were larger in middle-aged vs young individuals ($P < 0.01$). As shown in Table 2, middle-aged men were characterized by higher fasting plasma glucose levels as well as glucose and insulin areas than young subjects (P values ranging from 0.01 to 0.05). On the other hand, no difference between groups was observed in fasting plasma TG and insulin levels.

Insert Tables 1 and 2

The rank order of expression of the selected key proteins studied was: LPL > HSL > C3a > PPARγ irrespective of the subjects'age. AT-LPL expression and activity were similar in both groups (Figure 1). No significant relationship was observed between the mRNA level and the activity of this enzyme in the whole group ($r = 0.34$; NS). As shown in Figure 2, mRNA levels of HSL were higher in middle-aged than in young men ($P < 0.01$). However, no difference was observed in basal lipolysis of subcutaneous adipocytes between groups whereas maximal lipolytic responses of subcutaneous adipose cells to isoproterenol (β-agonist) and to postreceptor agents such as DcAMP, forskolin and theophylline were lower in middle-aged than in young men (P values ranging from 0.01 to 0.05). Furthermore, HSL expression was not related to either basal or stimulated lipolysis ($-0.10 < r < -0.22$; NS). Finally, middle-aged men displayed higher mRNA levels of C3a and of PPARγ than young subjects ($P < 0.01$) (Figure 3).

Insert Figures 1, 2 and 3

As reported in Table 3, positive relationships were observed between subjects' body fatness as well as visceral adipose tissue accumulation and PPARγ expression ($0.48 < r < 0.66$; P values ranging from 0.001 to 0.05). Moreover, fasting plasma glucose and TG levels were positively related to HSL expression in the whole group ($0.39 < r < 0.51$; P values ranging from 0.01 to 0.05). As middle-aged men showed a greater percent body fat than young individuals, values of each mRNA target corrected for body fat percentage have been compared between groups. As shown in Figure 4, mRNA levels of PPARγ did not remain different between groups, whereas HSL and C3a mRNA levels were still significantly higher in middle-aged than in young men even after adjustment for variation in subjects' body fatness ($P < 0.05$). Similar results were found when mRNA levels were adjusted for visceral adipose tissue accumulation (not shown).

Insert Table 3 and Figure 4

Discussion

In this study, the expression of key proteins involved in the control of adipose tissue metabolism was compared between young and middle-aged men. Our results showed that subcutaneous adominal adipose tissue mRNA levels of LPL were not different regardless of the subjects' age, whereas HSL, C3a and PPARγ mRNAs levels were higher in middle-aged than in young individuals. However, only HSL and C3a mRNAs remained elevated in middle-aged men after correction for body fat percentage variation.

Advancing in age has already been reported to be accompanied by important changes in body fat distribution in men (42). More precisely, the passage of youth to middle age is associated with the peak in fat mass (42) and numerous studies have documented that fat deposition is principally concentrated in the abdominal region of middle-aged men (8, 14, 25). We have recently observed a body fat gain during a follow-up period of 12 years in men aged 44 ± 5 years at baseline even if both a decrease in their reported relative fat intake and an increase in their reported participation in physically activity have occurred (46). These results support the notion that the middle age period (40-59 years) is accompanied by some metabolic events playing an important role in the regulation of fat balance. In this regard, we have recently reported a reduced lipolytic capacity in both subcutaneous abdominal and femoral adipocytes of middle-aged vs young men of similar body weight and subcutaneous fat distribution (20). Results of the present study also reemphasize this notion since middle-aged men showed impaired maximal isoproterenol- and postreceptor-induced lipolysis as compared to young men, although the former had larger subcutaneous fat cells. A loss of the ability to translocate HSL to lipid droplet has recently been suggested to explain the diminished lipolytic response to catecholamines with age in rats (7). The fact that middle-aged individuals presented higher HSL mRNA levels suggests that a high degradation rate of this transcript may occur with aging, thus leading to a decreased HSL protein level and a reduced adipose cell lipolytic capacity. As a novel form of human HSL produced by alternative splicing has recently been discovered (27), it is also possible that the decreased adipose tissue lipolytic capacity observed in middle-aged men could result from an increase in the proportion of the short but inactive

transcript HSL as compared to the long active HSL form. Further studies will be needed to explore this possibility.

Both AT-LPL mRNA content and enzyme activity were not altered by aging in the present study. Recently, LPL mRNA levels have been reported to be inversely related to age in rhesus monkeys (19). In the latter study, the downregulation of LPL expresssion could be due to the decreased expression of PPARγ and adipocyte determination- and differentiation-dependent factor 1/sterol regulatory element binding protein 1 (ADD1/SREBP), which are known to regulate LPL expression (21, 40). One possible explanation for this controversy might be related to the wide age range of the animals studied (7 to 30 years). Indeed, adipocytes of senescent animals have been shown to have a decline in fat cell lipogenic activity which may thus explain the decrease in fat mass observed in these animals (22). In addition, Hauner et al. (18) have previously reported a reduced potential of differentiation of stromal-vascular cells obtained from old human donors. Further studies are however needed to verify whether older individuals than subjects of the present study would display a decreased AT-LPL expression. On the other hand, the fact that LPL mRNA levels were not related to LPL activity is concordant with the important posttranscriptional and posttranslational mechanisms involved in the regulation of this enzyme activity, as previously reported (2, 30, 35). On the basis of the fact that adipose tissue LPL activity may contribute to regional fat distribution (2), it was thus expected to observe a similar subcutaneous adipose tissue LPL activity in young and middle-aged men of the present study because of their comparable adipose tissue accumulation.

To the best of our knowledge, this study is the first to show that C3a mRNA levels are specifically upregulated in subcutaneous adipose tissue of middle-aged as compared to young men. This finding is based on the fact that C3a expression remained significantly higher in middle-aged than in young individuals, even after correction for body fat variation, an important factor influencing plasma ASP levels (6). Although we are aware of the fact that mRNA content of C3a does not necessarily reflect the functional activity of the ASP protein, it is tempting to speculate that the upregulation of C3a expression may favor fat gain during the middle age period in humans. Indeed, ASP has recently been shown to inhibit basal and norepinephrine-stimulated FFA release from adipocytes by its marked increase in the

fractional FFA re-esterification and to a lesser extent its inhibition of FFA produced during lipolysis (47). These effects were mediated by phosphodiesterase3 (PDE3) and by PDE4, which are known to hydrolyze cAMP in fat cells (31). To some extent, this finding may explain the impaired adipose tissue lipolytic capacity of the middle-aged men of the present study.

Even if PPARγ expression was higher in middle-aged than in young men, this difference disappeared after correction for body fatness variation. This finding suggests that PPARγ expression is not upregulated during middle age but seems to be mostly related to increased obesity, as previously reported (28, 48). It would have been expected that adipose tissue of middle-aged men presents lower PPARγ mRNA levels than that of young subjects in regard to the impaired capacity of preadipocytes to differentiate with aging (18). Once again, it is likely that subjects of the present study were not old enough to observe such an effect. One could also raise the fact that the use of whole adipose tissue complicates the interpretation of our results since PPARγ may have an entirely different role in preadipocytes (ie, promotion of differentiation) versus mature adipocytes (regulation of metabolically important genes), as the proportion of these cells changes with aging (16, 24). However, this does not exclude that PPARγ expression might be associated to the expansion of fat mass observed during middle age in humans. Further studies are therefore warranted to clarify the role of PPARγ in the middle age-related obesity phenomenon.

In conclusion, the present study demonstrates that subcutaneous abdominal adipose tissue LPL expression is similar in both middle-aged and young men. On the other hand, HSL and C3a mRNA levels are upregulated by aging whereas PPARγ expression is mostly related to increased adiposity. These results suggest that the middle age period in humans affects subcutaneous abdominal adipose tissue metabolism but it appears to be gene specific.

Acknowledgments

The authors wish to express their gratitude to the staff of the Physical Activity Sciences Laboratory and of the Lipid Research Center for data collection. We also like to thank Marie Tremblay and Rachelle Duchesne of the Diabetes Research Unit for their assistance. The subjects are also gratefully acknowledged.

References

1. Arner, P. Control of lipolysis and its relevance to the development of obesity in man. Diabetes Metab. Rev. 4: 507-515, 1988.
2. Arner, P., H. Lithell, H. Wahrenberg, and M. Brönnegard. Expression of lipoprotein lipase in different subcutaneous adipose tissue regions. J. Lipid Res. 32: 423-429, 1991.
3. Auboeuf, D., J. Rieusset, L. Fajas, P. Vallier, V. Frering, J. P. Riou, B. Staels, J. Auwerx, M. Laville, and H. Vidal. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. Diabetes 46: 1319-1327, 1997.
4. Auboeuf, D., and H. Vidal. The use of the reverse transcription-competitive polymerase chain reaction to investigate the in vivo regulation of gene expression in small tissue samples. Anal. Biochem. 245: 141-148, 1997.
5. Baldo, A., A. D. Sniderman, S. St-Luce, R. K. Avramoglu, M. Maslowska, B. Hoang, J. C. Monge, A. Bell, S. Mulay, and K. Cianflone. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. J. Clin. Invest. 92: 1543-1547, 1993.
6. Cianflone, K., D. Kalant, E. B. Marliss, R. Gougeon, and A. D. Sniderman. Response of plasma ASP to a prolonged fast. Int. J. Obesity 19: 604-609, 1995.
7. Clifford, G. M., C. Londos, F. B. Kraemer, R. G. Vernon, and S. J. Yeaman. Translocation of Hormone-sensitive Lipase and Perilipin upon Lipolytic Stimulation of Rat Adipocytes. J. Biol. Chem. 275: 5011-5015, 2000.
8. Couillard, C., S. Lemieux, S. Moorjani, P. J. Lupien, G. Thériault, D. Prud'homme, A. Tremblay, C. Bouchard, and J. P. Després. Associations between 12 year changes in body fatness and lipoprotein- lipid levels in men and women of the Québec Family Study. Int. J. Obesity 20: 1081-1088, 1996.
9. Desbuquois, B., and G. D. Aurbach. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J. Clin. Endocrinol. Metab. 37: 732-738, 1971.

10. Després, J. P., C. Bouchard, L. Bukowiecki, R. Savard, and J. Lupien. Morphology and metabolism of human fat cells: a reliability study. Int. J. Obesity 7: 231-240, 1983.

11. Desvergne, D., A. Ijpenberg, P. R. Devchand, and W. Wahli. The peroxisome proliferator-activated receptors at the cross-road of diet and hormonal signalling. J. Steroid Biochem. Molec. Biol. 65: 65-74, 1998.

12. Dole, V. P., and H. Meinertz. Microdetermination of long chain fatty acids in plasma and tissues. J. Lipid Res. 235: 2595-2599, 1960.

13. Eckel, R. H. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N. Engl. J. Med. 320: 1060-1068, 1989.

14. Enzi, G., M. Gasparo, P. R. Biondetti, D. Fiore, M. Semisa, and F. Zurlo. Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. Am. J. Clin. Nutr. 44: 739-746, 1986.

15. Ferland, M., J. P. Després, A. Tremblay, S. Pinault, A. Nadeau, S. Moorjani, P. J. Lupien, G. Thériault, and C. Bouchard. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br. J. Nutr 61: 139-148, 1989.

16. Fraselle-Jacobs, A., M. Jeanjean, N. Hauser, and C. Remacle. Effect of aging on the morphology of epididymal adipose tissue in the rat. Exp. Gerontol. 22: 389-408, 1987.

17. Fried, S. K., and C. D. Russell; Diverse roles of adipose tissue in the regulation of systematic metabolism and energy balance. Bray G. A., Bouchard C. and James W. P. T.; Handbook of obesity; New York; Marcel Dekker, Inc.; 1998, 397-413.

18. Hauner, H., G. Entenmann, M. Wabitsch, D. Gaillard, G. Ailhaud, R. Négrel, and E. F. Pfeiffer. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J. Clin. Invest. 84: 1663-1670, 1989.

19. Hotta, K., N. L. Bodkin, T. A. Gustafson, S. Yoshioka, H. K. Ortmeyer, and B. C. Hansen. Age-related adipose tissue mRNA expression of ADD1/SREBP1, PPARgamma, lipoprotein lipase, and GLUT4 glucose transporter in rhesus monkeys. J. Gerontol. A Biol. Sci. Med. Sci. 54: B183-188, 1999.

20. Imbeault, P., D. Prud'homme, A. Tremblay, J. P. Després, and P. Mauriège. Adipose tissue metabolism in young and middle-aged men after control for body fatness. J. Clin. Endocrinol. Metab. In press: 2000.

21. Kim, J. B., and B. M. Spiegelman. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 10: 1096-1107, 1996.

22. Kirkland, J. L., and D. E. Dobson. Preadipocyte function and aging: links between age-related changes in cell dynamics and altered fat tissue function. J. Am. Geriatr. Soc. 45: 959-967, 1997.

23. Kirkland, J. L., C. H. Hollenberg, and W. S. Gillon. Ageing, differentiation, and gene expression in rat epididymal preadipocytes. Biochem Cell Biol 71: 556-561, 1993.

24. Kirkland, J. L., C. H. Hollenberg, S. Kindler, and W. S. Gillon. Effects of age and anatomic site on preadipocyte number in rat fat depots. J. Gerontol. A Biol. Sci. Med. Sci. 49: B31-35, 1994.

25. Kohrt, W. M. Abdominal obesity and associated cardiovascular comorbidities in the elderly. Coronary Artery Dis. 9: 489-494, 1998.

26. Langin, D., C. Holm, and M. Lafontan. Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. Proc. Nutr. Soc. 55: 93-109, 1996.

27. Laurell, H., J. Grober, C. Vindis, T. Lacombe, M. Dauzats, C. Holm, and D. Langin. Species-specific alternative splicing generates a catalytically inactive form of human hormone-sensitive lipase. Biochem. J. 328: 137-143, 1997.

28. Lefebvre, A. M., M. Laville, N. Vega, J. P. Riou, L. van Gaal, J. Auwerx, and H. Vidal. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 47: 98-103, 1998.

29. Lohman, T. G., A. F. Roche, and R. Martorell; The Airlie (VA) consensus conference. Lohman T. G., Roche A. F. and Martorell R.; Anthropometric Standardisation Reference Manual; Champaign (Illinois); Human Kinetics Publishers; 1988, 39-80.

30. Maheux, P., S. Azhar, P. A. Kern, Y.-D. I. Chen, and G. M. Reaven. Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. Diabetologia 40: 850-858, 1997.

31. Manganiello, V. C., M. Taira, E. Degerman, and P. Belfrage. Type III cGMP-inhibited cyclic nucleotide phosphodiesterases (PDE3 gene family). Cell Signal 7: 445-455, 1995.

32. Mauriège, P., J. P. Després, D. Prud'homme, M. C. Pouliot, M. Marcotte, A. Tremblay, and C. Bouchard. Regional variation in adipose tissue lipolysis in lean and obese men. J. Lipid Res. 32: 1625-1633, 1991.

33. Mauriège, P., D. Prud'homme, S. Lemieux, A. Tremblay, and J. P. Després. Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. Am. J. Physiol. 269: 341-350, 1995.

34. Meneely, G. R., and N. L. Kaltreider. Volume of the lung determined by helium dilution. J. Clin. Invest. 28: 129-139, 1949.

35. Ong, J. M., and P. A. Kern. Effect of feeding and obesity on lipoprotein lipase activity, immunoreactive protein, and messenger RNA levels in human adipose tissue. J. Clin. Invest. 84: 305-311, 1989.

36. Richterich, R., and H. Dauwwalder. Zur bestimmung der plasmaglukose-konzentration mit der hexokinase-glucose-6-phosphat-deshydrogenase-methode. Schweiz Med Wochenschr 101: 615-618, 1971.

37. Rieusset, J., J. Auwerx, and H. Vidal. Regulation of gene expression by activation of the peroxisome proliferator-activated receptor gamma with rosiglitazone (BRL 49653) in human adipocytes. Biochem. Biophys. Res. Commun. 265: 265-271, 1999.

38. Rodbell, M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 239: 375-380, 1964.

39. Savard, R., Y. Deshaies, J. P. Després, M. Marcotte, L. J. Bukowiecki, C. Allard, and C. Bouchard. Lipogenesis and lipoprotein lipase activity in human adipose tissue: reproducibility of measurements and relationship with fat cell size. Can. J. Physiol. Pharmacol. 62: 1448-1452, 1984.

40. Schoonjans, K., J. Peinado-Onsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. E.M.B.O. J. 15: 5336-5348, 1996.

41. Schoonjans, K., B. Staels, and J. Auwerx. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. J. Lipid Res. 37: 907-925, 1996.

42. Silver, A. J., C. P. Guillen, M. J. Kahl, and J. E. Morley. Effect of aging on body fat. J. Am. Geriatr. Soc. 41: 211-213, 1993.

43. Siri, W. E. The gross composition of body fat. Adv. Biol. Med. Physiol. 4: 239-280, 1956.

44. Sjöström, L., H. Kvist, A. Cederblad, and U. Tylen. Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. Am. J. Physiol. 250: E736-E786, 1986.

45. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8: 1224-1234, 1994.

46. Tremblay, A., V. Drapeau, E. Doucet, N. Alméras, J. P. Després, and C. Bouchard. Fat balance and ageing: results from the Québec family study. Br. J. Nutr 79: 413-418, 1998.

47. van Harmelen, V., S. Reynisdottir, K. Cianflone, E. Degerman, J. Hoffstedt, K. Nilsell, A. Sniderman, and P. Arner. Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. J. Biol. Chem. 274: 18243-18251, 1999.

48. Vidal-Puig, A. J., R. V. Considine, M. Jimenez-Linan, A. Werman, W. J. Pories, J. F. Caro, and J. S. Flier. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. J. Clin. Invest. 99: 2416-2422, 1997.

Legends to figures

Figure 1

Adipose tissue mRNA levels and activity of lipoprotein lipase (LPL) from the subcutaneous abdominal depot of young (n = 13) and middle-aged (n = 16) men. AT-LPL activity is expressed per adipocyte surface area. Values are means ± SE.

Figure 2

Adipose tissue mRNA levels of HSL as well as basal and maximal lipolytic response of subcutaneous abdominal adipocytes to isoproterenol and postadrenoceptor agents in young (n = 13) and middle-aged (n = 16) men. Fat cells were incubated in the presence of either isoproterenol (ISO; 10^{-5} M), dibutyryl-cyclicAMP (DcAMP; 10^{-3} M), forskolin (FK; 10^{-5} M) or theophylline (THEO; 10^{-3} M). Basal glycerol release has been subtracted from maximal lipolytic response. Values are means ± SE. Significant difference between groups at * $P < 0.05$, † $P < 0.01$.

Figure 3

Adipose tissue mRNA levels of C3a and PPARγ from the subcutaneous abdominal depot of young (n = 13) and middle-aged (n = 16) men. Values are means ± SE. Significant difference between groups at † $P < 0.01$.

Figure 4

Adipose tissue mRNA levels of LPL, HSL, C3a and PPARγ from the subcutaneous abdominal depot of young (n = 13) and middle-aged (n = 16) men after correction for body fatness variation. Values are means ± SE. Significant difference between groups at • $P < 0.05$.

Table 1. Physical characteristics of subjects.

	Young (n = 13)	Middle-aged (n = 16)
Age (years)	31 ± 5	56 ± 6 ‡
Anthropometric variables		
Body weight (kg)	85 ± 16	86 ± 11
BMI (kg/m^2)	27 ± 4	29 ± 2
Body fat (%)	22 ± 7	28 ± 5 *
Fat mass (kg)	19 ± 9	24 ± 6
Fat free mass (kg)	64 ± 9	61 ± 7
Waist girth (cm)	91 ± 12	99 ± 7 *
Adipose tissue areas measured by CT (cm^2)		
Abdomen (L4-L5)		
Subcutaneous	200 ± 142	254 ± 70
Visceral	89 ± 52	170 ± 50 †
Fat cell weight		
Abdominal (μg lipid/cell)	0.43 ± 0.15	0.59 ± 0.10 †

Value are means ± standard deviation (SD).

BMI = body mass index; CT = computed tomography.

Significant difference between groups at * $P< 0.05$, † $P< 0.01$ and ‡ $P< 0.001$.

Table 2. Metabolic profile of subjects.

	Young (n = 13)	Middle-aged (n = 16)
Fasting variables		
Glucose (mmol/l)	5.1 ± 0.4	5.6 ± 0.5 *
Insulin (pmol/l)	75.5 ± 42.6	76.6 ± 45.8
FFA (mmol/l)	0.50 ± 0.12	0.55 ± 0.15
TG (mmol/l)	1.9 ± 1.5	2.2 ± 1.3
Metabolic responses to an oral glucose load		
Glucose area	1.08 ± 0.20	1.31 ± 0.21 †
Insulin area	57.6 ± 34.8	97.5 ± 55.2 *

Value are means ± standard deviation (SD).

Significant difference between groups at * $P< 0.05$, † $P< 0.01$, ‡ $P< 0.001$.

TG = triglycerides; FFA = free fatty acids;

Glucose and insulin areas represent integrated plasma concentrations measured for 3h after an oral glucose load (75g/OGTT). Glucose area is expressed in (mmol/l/min) x 10^{-3} whereas insulin area is expressed in (pmol/l/min) x 10^{-3}.

Table 3. Correlation coefficients between subcutaneous abdominal adipose tissue (AT) mRNA levels and selected anthropometric and metabolic variables in the whole sample of men.

	AT mRNA levels			
	LPL	**HSL**	**C3a**	**PPARγ**
Body weight	-0.16	-0.15	-0.07	0.09
Body fat %	0.23	0.34	0.27	0.66 ‡
Subcutaneous abdominal AT area	-0.01	0.06	0.18	0.38
Visceral abdominal AT area	0.28	0.28	0.17	0.48 *
Abdominal fat cell weight	0.32	0.29	0.27	0.28
Fasting glucose	0.28	0.39 *	0.28	0.33
Fasting insulin	0.23	0.01	0.15	0.08
Fasting TG	0.29	0.51 †	-0.05	0.54 †

* $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$.

For abbreviations, see footnotes to Tables 1 and 2.

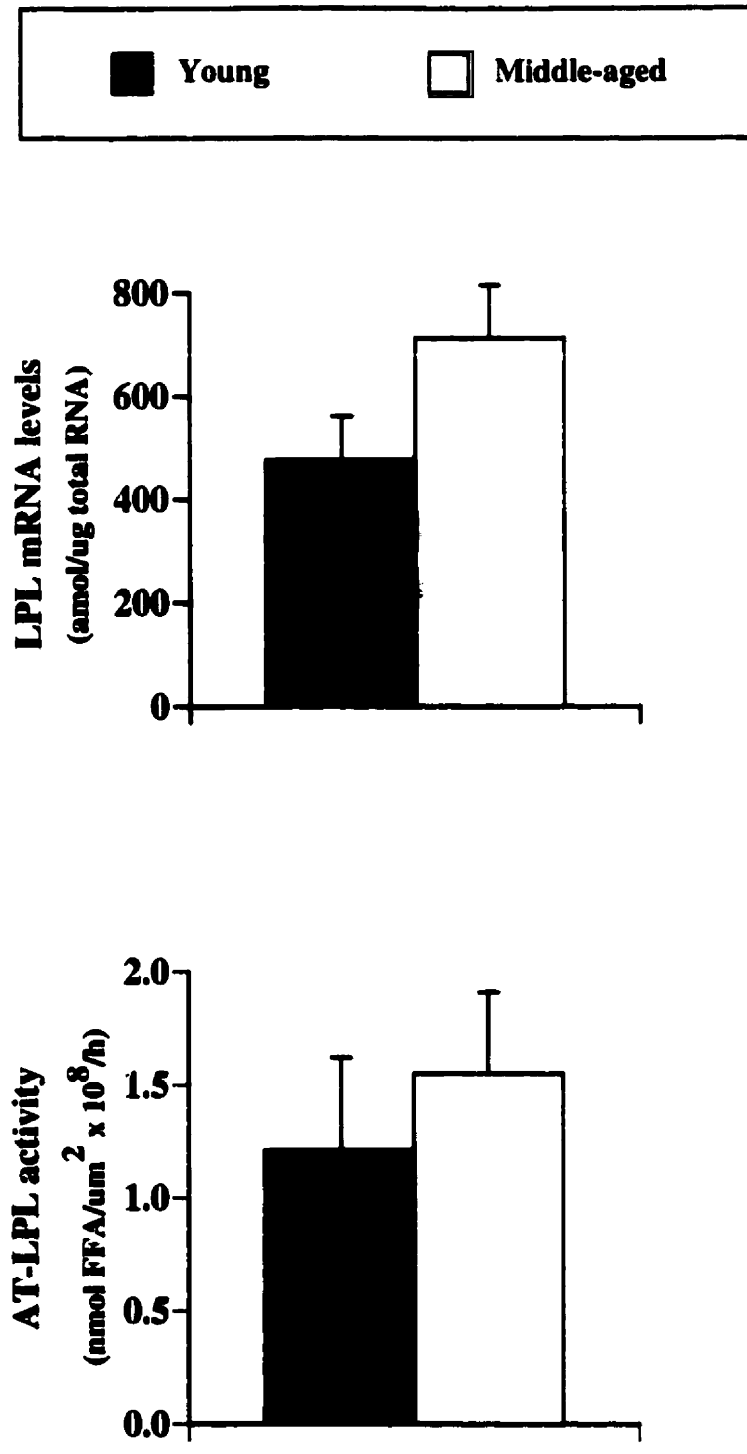

Young
Middle-aged
LPL mRNA levels
(amol/ug total RNA)
800
600
400
200
0
AT-LPL activity
(nmol FFA/um 2 x 10 8/h)
2.0
1.5
1.0
0.5
0.0

Figure 1

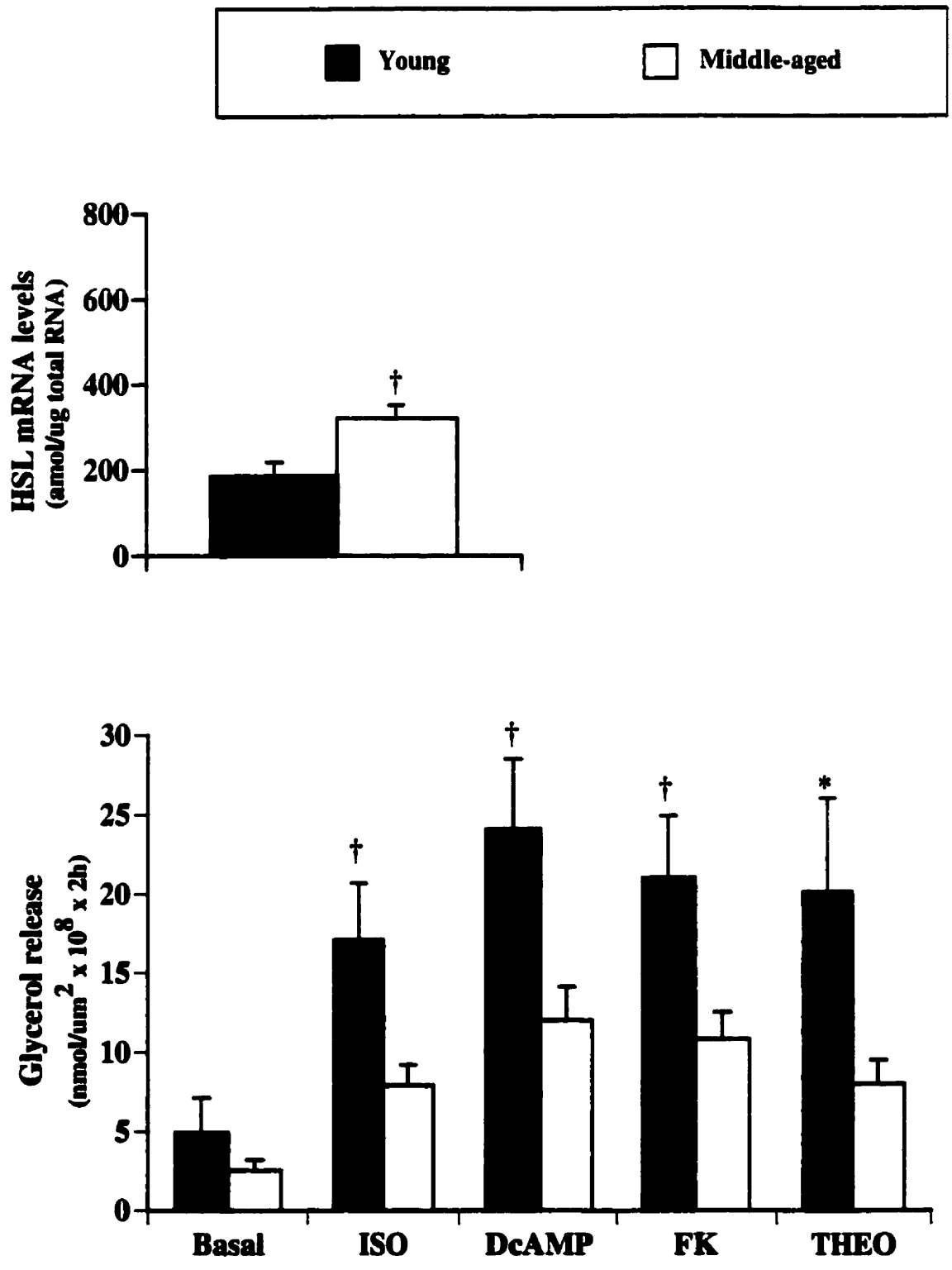

Young
Middle-aged
HSL mRNA levels
(amol/ug total RNA)
800
600
400
200
0
†
Glycerol release
(nmol/um^2 x 10^8 x 2h)
30
25
20
15
10
5
0
†
†
†
*
Basal
ISO
DcAMP
FK
THEO

Figure 2

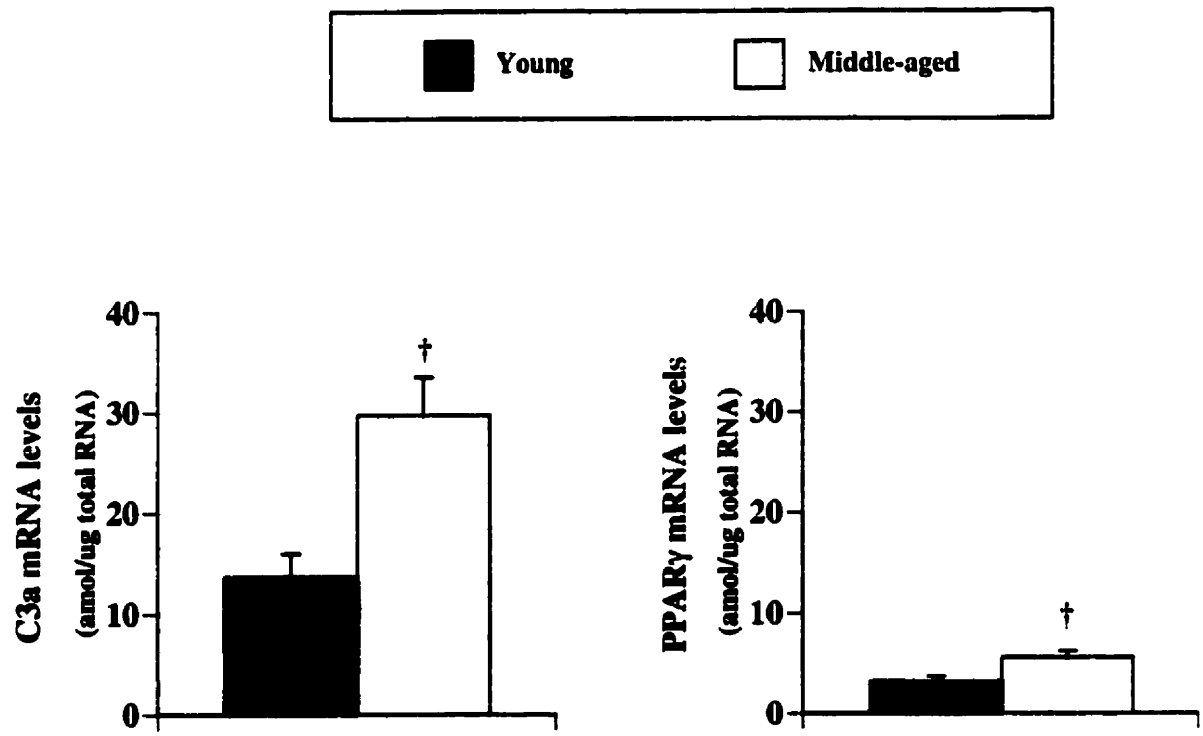

Young
Middle-aged
C3a mRNA levels
(amol/ug total RNA)
40
30
20
10
0
†
PPARγ mRNA levels
(amol/ug total RNA)
40
30
20
10
0
†

Figure 3

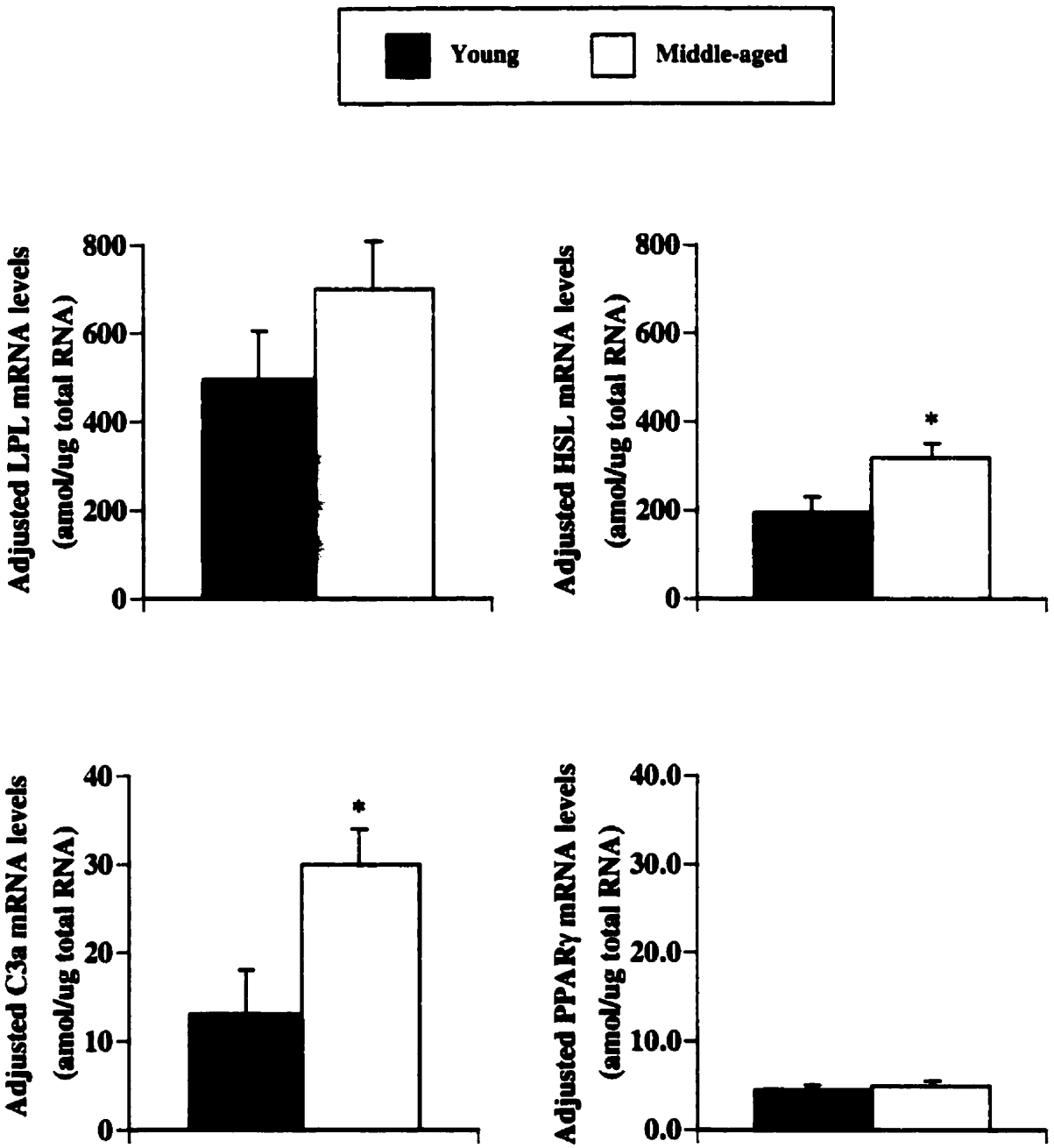

Young
Middle-aged
Adjusted LPL mRNA levels (amol/ug total RNA)
800
600
400
200
0
Adjusted HSL mRNA levels (amol/ug total RNA)
800
600
400
200
0
*
Adjusted C3a mRNA levels (amol/ug total RNA)
40
30
20
10
0
*
Adjusted PPARγ mRNA levels (amol/ug total RNA)
40.0
30.0
20.0
10.0
0.0

Figure 4

CHAPITRE 10

MÉTABOLISME DU TISSU ADIPEUX À LA MÉNOPAUSE: IMPORTANCE DE L'ADIPOSITÉ ET DE LA DISTRIBUTION RÉGIONALE DU TISSU ADIPEUX

L'article composant ce chapitre est intitulé:

"Subcutaneous adipose tissue metabolism at menopause: importance of body fatness and regional fat distribution"

(en révision à *Journal of Clinical Endocrinology and Metabolism*)

Résumé

Le but de cette étude était d'examiner la contribution de la ménopause au niveau du métabolisme du tissu adipeux sous-cutané de femmes pré (n = 8, 37 ± 5 ans) et postménopausées (n = 8, 57 ± 6 ans) présentant une accumulation et une distribution régionale de tissu adipeux similaires. À cet égard, l'activité de la lipoprotéine lipase du tissu adipeux (LPL) et la lipolyse d'adipocytes isolés des sites sous-cutanés abdominal et fémoral ont été déterminées au sein des deux groupes. L'adrénaline a induit un effet antilipolytique à de faibles concentrations et un effet lipolytique à de plus fortes concentrations chez les deux groupes et au niveau des deux sites adipeux étudiés. La réponse lipolytique maximale à l'isoprénaline (agoniste β-adrénergique) aussi bien que l'effet antilipolytique maximal de l'UK-14304 (agoniste α_2-adrénergique) des adipocytes des deux sites étaient semblables chez les femmes pré et postménopausées. De plus, aucune différence n'a été observée au niveau de la sensibilité β- et α_2-adrénergique des adipocytes entre les deux groupes étudiés. Finalement, la lipolyse maximale stimulée par les agents postrécepteurs ainsi que l'activité de la LPL n'ont varié ni selon la région ni suivant l'âge des sujets. Cette étude montre que malgré un état endocrinien différent, des femmes pré ou postménopausées caractérisées par une distribution du tissu adipeux similaire présentent une balance lipolytique/lipogénique comparable.

Subcutaneous adipose tissue metabolism at menopause: importance of body fatness and regional fat distribution

P. Mauriège [1,3], P. Imbeault [3], D. Prud'homme [1,3], A. Tremblay [3],
A. Nadeau [2], and J.P. Després [1,4].

[1] Lipid Research Center, [2] Diabetes Research Unit, CHUQ Medical Research Center, [3] Physical Activity Sciences Laboratory, Department of Social & Preventive Medicine, Laval University, and
[4] Québec Heart Institute, Laval Hospital, Québec, Canada.

Supported by the Medical Research Council of Canada.

Running head: Adipose tissue metabolism at menopause

Address correspondence to: Pascale Mauriège, Ph.D.,
Lipid Research Center,
Laval University Medical Research Center
CHUL, 2705 Blvd Laurier, Room TR-93,
Ste-Foy, Québec, CANADA G1V 4G2
Tel: 418-654-2133
Fax: 418-654-2145
e-mail: diabolo@internetclub.fr

ABSTRACT

The aim of this study was to examine the contribution of menopause *per se* on subcutaneous (subc) adipose tissue (AT) metabolism of sixteen women classified on the basis of their menopausal status: eight postmenopausal (57 ± 6 years; mean ± SD) vs eight premenopausal individuals (37 ± 5 years), these two groups being matched for subc abdominal adipose cell size (within 0.02 μg lipid/cell) and for visceral AT accumulation (within 15 cm^2) measured by computed tomography. Fasting plasma glucose and insulin levels as well as their responses to an oral glucose load were similar regardless of the women's hormonal status. Subc abdominal and femoral adipose tissue lipoprotein lipase (AT-LPL) activities as well as fat cell lipolysis were determined in both groups. Epinephrine induced antilipolysis at low concentrations and lipolysis at higher doses, in both adipose sites and groups. The maximal lipolytic response to epinephrine or to isoproterenol (β-adrenergic agonist) as well as the maximal antilipolytic effect of either the catecholamine or UK-14304 (α_2-adrenergic agonist) assessed in subc adipocytes were similar in pre- and postmenopausal women. In addition, neither the β-, nor the α_2-adrenoceptor sensitivity of subc adipose cells differed according to subjects' age. Finally, maximal lipolysis promoted by post-adrenoceptor agents and AT-lipoprotein lipase activity did not vary among adipose regions, nor between groups. Taken together, these results suggest that menopause per se does not seem to influence subc AT metabolism, once the variation related to adipose cell size and total body fatness is taken into account.

KEY WORDS: lipolysis, adipocytes, lipoprotein lipase, catecholamines, obesity, pre- and postmenopausal women.

INTRODUCTION

Abdominal obesity and visceral adipose tissue accumulation are well known correlates of metabolic complications predictive of an increased risk of coronary heart disease and type II diabetes, in both genders (1-3). In this regard, the increase in abdominal, and more particularly visceral fat accumulation, which occurs at menopause (4-6), is associated with a greater risk of developing an atherogenic lipid profile and/or an insulin resistant state (7,8). Regional variation in the lipid storage and/or mobilizing potencies of adipose cells has already been identified as potential mechanisms that could account for differences in body fat distribution in obesity (3,9-11). Indeed, adipose tissue lipoprotein lipase (AT-LPL) activity has been shown to be higher in gluteal or femoral adipose tissue than in subcutaneous abdominal fat depot from both lean and obese premenopausal women (12-15). Moreover, an enhanced adipose cell lipolytic response to catecholamines has commonly been reported in subcutaneous abdominal as compared to gluteo-femoral regions of non-obese and obese young women (12,13,16-18).

Although our understanding about the clustering of alterations in metabolic risk factors which accompanies menopause made progress over the last decade (19-23), there has been little investigation of the role of adipose tissue metabolism, an important factor to be considered when studying obesity-related metabolic complications. Indeed, discordant results have been reported concerning either the lack (13,24) or the presence (25) of site differences in AT-LPL activity in postmenopausal women. A lack of regional variation in lipolysis with age has also been observed by some investigators (13,26), whereas others have reported that subcutaneous abdominal fat cells were more responsive to catecholamine stimulation than gluteal adipocytes (24,27). One possible reason for these conflicting data could be that pre- and postmenopausal women compared in these previous studies (13,25) were not matched for total adiposity and regional fat distribution. Nicklas et al. (27) have recently reported that an increased visceral adipose tissue accumulation was associated with higher rates of lipolysis in both subcutaneous abdominal and gluteal adipocytes, thus suggesting an important role of regional fat distribution on the age-related variation in adipose cell lipolytic activity.

Therefore, the aims of the present study were *i)* to examine whether regional variation persists in subcutaneous adipose tissue metabolism at menopause, and *ii)* to verify whether differences in adipocyte metabolic activities between pre- and postmenopausal women would remain, once the concomitant variation in both adipose cell size and body fat distribution is taken into account.

MATERIAL AND METHODS

Study subjects

Sixteen healthy Caucasian, moderately overweight women were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. Eight pairs of subjects matched for both levels of subcutaneous abdominal adipose tissue (within 15 cm^2) measured by computed tomography and for subcutaneous abdominal fat cell weight (within 0.02 μg lipid/cell), but displaying marked differences in age (premenopausal vs postmenopausal), were compared for potential differences in subcutaneous adipose cell metabolism. All participants underwent a physical examination by a physician, which included medical history. Subjects with cardiovascular disease, diabetes or other endocrine disorders, or those on medication that could have potentially affected blood pressure, glucose or lipid metabolism were excluded. All women were sedentary (ie, they performed less than 30 min of exercise per week), non-smokers, moderate alcohol and caffeine consumers and their body weights were stable at the time of the study (ie, no subject had been involved in a weight loss program for the last six months). Women who had undergone surgical menopause were excluded from the study. Subjects were considered to be postmenopausal if they had not menstruated for at least two years, and if their plasma estradiol concentrations were lower than 120 pg/ml. None of them was taking or had ever taken hormonal replacement therapy. Premenopausal women had regular menstrual cycles and none was using oral contraceptives or was lactating at the time of the study. All measurements were performed while they were in the early follicular phase of the menstrual cycle.

Body fatness and regional fat distribution

Body weight was taken with a standard beam scale. Body density was determined by the underwater weighing technique and percent body fat was derived from body density (28). Pulmonary residual volume was measured using the helium dilution method (29). Fat mass was calculated from the derived percentage of body fat and total body weight. Fat-free mass was then simply calculated as the subtraction of fat mass from total body weight. Body density, fat mass and fat-free mass are highly reproducible variables which show reliability coefficients greater than 0.97 (30). Waist girth was measured according to the procedures recommended at the Airlie

Conference (31). Lemieux et al. (32) have already reported that the coefficient of variation between two consecutive measurements of waist girth was very low (0.32%). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, West Germany), according to the methodology previously described (33). Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) level, using an abdominal scout radiograph to establish the position of the scan to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating these areas with a graph pen and then computing the AT surfaces with an attenuation range of -190 to -30 HU (34). Abdominal visceral AT area was determined by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area. A high coefficient of reliability ($r = 0.99$) has already been reported by our group for the determination of subcutaneous and visceral fat accumulation by CT (35).

Adipose tissue biopsy procedure and lipoprotein lipase activity

After an overnight fast, women were subjected to biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the anterior midthigh level (femoral site). A small cutaneous incision (1 cm) was performed in both sites following local anesthesia (1 % lidocaine, without epinephrine) and about 350 mg of subcutaneous adipose tissue were surgically removed from the two fat depots. Samples of approximately 100 mg of adipose tissue from each region were immediately frozen in liquid nitrogen and stored at -80°C for later measurement of heparin-releasable LPL activity, according to Savard et al. (36). Adipose tissue lipoprotein lipase (AT-LPL) activity was expressed as micromoles of free-fatty acids (FFA) released per hour per 10^6 cells. Since AT-LPL activity is associated with fat cell size (36), AT-LPL was also expressed per cell surface area (ie, nanomoles FFA per hour per micrometer squared times 10^8).

Adipocyte lipolysis

Samples of approximately 250 mg of adipose tissue from each site were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (37) in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin

(KRBA) and 5 mM glucose, plus 1 mg/ml collagenase, as previously described (36). Digestion took place in a shaking water bath under an air gas phase of 95% O2 and 5% CO2, for 40 min at 37°C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5ml of KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 1000 cells per 100 µl.

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. 100 µl aliquots of the continuously stirred cell suspension were placed in 1.5 ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 20 µl KRB were immediately placed on ice and provided an evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation or inhibition were added just before the beginning of the assay in 20-µl portions in order to obtain the desired final concentration. After a 2h incubation at 37°C in a shaking water bath, under an air gas phase of 95 % O_2 and 5 % CO_2, 100 µl HCl (1N) were added to all tubes to stop the reaction, then 100 µl NaOH (1N) were added to neutralize the medium. All tubes were stoppered and stored at -20^0 C until glycerol determination according to Mauriège et al. (38). NADH concentration was measured by bioluminescence with a luciferase solution, using a 2250 Dynatech luminometer (17,18). For each concentration of stimulator or inhibitor agent, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Glycerol measurement by bioluminescence is very sensitive and especially well adapted when only small amounts of adipose tissue are available (17,18,38). Mean adipose cell diameter was assessed from the measurement of at least 500 cells per site and per subject, using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ, USA). Because of the spherical shape and high lipid content of the adipocytes (95 %), both the adipose cell volume and surface area can be calculated from the mean adipocyte diameter and the density of triolein (0.915 g/ml) was used to transform adipose cell volume into fat cell weight, as previously described (39).

The lipolytic activity of the isolated fat cells was tested with isoproterenol (non selective β-agonist), UK-14304 (selective α_2-agonist), and epinephrine which is a mixed agonist (α_2/β) with a higher affinity for α_2-adrenoceptor sites (16). Ascorbic acid (0.1 mmol/l) was included in the

incubation medium in order to prevent catecholamine degradation. Some experiments were conducted with forskolin (direct activator of adenylate cyclase), dibutyryl-cyclicAMP (stimulator of the protein kinase hormone-sensitive lipase complex and phosphodiesterase-resistant cyclic AMP analogue), theophylline (mainly inhibitor of cyclicGMP-inhibited phosphodiesterase, cGI-PDE) (17). When antilipolytic effects were investigated, the incubation buffer was supplemented with 5 μg/ml adenosine deaminase (ADA) to remove adenosine released into the incubation medium by the isolated fat cells, this procedure allowing better investigations of α_2-adrenoceptor mediated antilipolytic effects (16,18,38). Lipolysis was expressed either per cell number (ie, in μmol of glycerol/10^6 cells x 2 h) or per unit of cell surface area (ie, in nmol of glycerol/μm^2 x 10^8 x 2 h) in order to compensate for variations in fat cell size (18,38). In cases where complete concentration-response curves were obtained, they were compared for both responsiveness and sensitivity. The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-5} M isoproterenol or forskolin, 10^{-3} M dibutyryl-cyclicAMP or theophylline). The β-adrenergic sensitivity was considered as the concentration of isoproterenol giving half-maximal stimulation of lipolysis (EC_{50}), whereas the α_2-adrenergic sensitivity was calculated as the concentration of UK-14304 which produced half-maximal inhibition of lipolysis (IC_{50}). Both were evaluated by logarithmic conversion of each concentration-response curve. The higher was the EC_{50} (isoproterenol) or the IC_{50} (UK-14304) value, the lower was the β - or the α_2-adrenergic sensitivity, respectively.

Oral glucose tolerance test (OGTT)

A 75g OGTT was performed in the morning after an overnight fast. Blood samples were collected in tubes containing EDTA and Trasylol (Miles Pharmaceutics, Rexdale, Ontario, Canada) through a venous catheter from an antecubial vein at -15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. Plasma glucose was measured enzymatically (40), whereas the plasma insulin concentration was determined by radioimmunoassay with polyethylene glycol separation (41). The total glucose and insulin areas under the curve during OGTT were calculated with the trapezoid method.

Plasma lipids and lipoproteins

Blood samples were obtained in the morning after a 12-h fast from an antecubital vein. Cholesterol (CHOL) and triglyceride levels in plasma and lipoprotein fractions were measured enzymatically on a RA-1000 automated analyzer (Technicon Instruments Corporation, Tarrytown, NY) referenced to Centers for Disease Control (Atlanta, USA). Plasma very low density lipoproteins (VLDL, $d < 1.006$ g/ml) were isolated by ultra-centrifugation (42), and the high density lipoprotein (HDL) fraction was obtained after precipitation of low density lipoprotein (LDL) in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$ (43).

Drugs and chemicals

Collagenase, bovine serum albumin, adenosine deaminase, and enzymes for glycerol assays were obtained from Boehringer Mannheim (Canada). Ascorbic acid, theophylline, forskolin, dibutyryl-cyclicAMP, (-)isoproterenol bitartrate, (-)epinephrine bitartrate and cold triolein were purchased from Sigma Chemical Co. (St Louis, MO, USA). [^{14}C]-triolein was obtained from NEN Dupont (Canada). UK-14304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) was generously provided from Dr D.A. Faulkner (Pfizer, Sandwich, England). All other chemicals and organic solvents were of the highest purity grade commercially available. The same batches of hormones, pharmacological agents, collagenase and albumin were used in all experiments.

Statistical methods

Two subgroups of eight women displaying both similar subcutaneous abdominal AT area measured by CT and fat cell size, but differing in age, were compared. The Student's t-test was used for comparison of anthropometric and metabolic variables between pre- and postmenopausal women. The effects of age (young vs middle-aged) and of adipose site (abdominal vs femoral) on adipose tissue lipolytic curves were tested by a two-way analysis of variance for repeated measures. Post-hoc comparisons were handled with a Student's t-test. All statistical analyses were performed using the Jump Software 3.2.2. from the SAS Institute Inc. (Cary, NC, USA) adapted for Macintosh computers.

RESULTS

Subjects' characteristics

Physical characteristics of our sample of moderately overweight women are presented in Table 1. As expected from the design, a significant difference was observed in the subjects' age ($P < 0.0001$). However, neither the body mass index, nor fat mass differed among pre and postmenopausal women. Both groups also displayed similar visceral and subcutaneous abdominal AT areas measured by computed tomography, as well as comparable subcutaneous fat cell weights. Also, no regional variation was found in adipose cell size within both groups. Metabolic characteristics of subjects are shown in Table 2. With the exception of plasma cholesterol and LDL-CHOL concentrations being significantly lower in pre- than in postmenopausal women ($P < 0.05$), the lipid-lipoprotein profile did not differ between groups. In addition, no difference in fasting plasma glucose and insulin levels as well as in the corresponding responses to an oral glucose load was found between pre- and postmenopausal women (Figure 1).

Adipose tissue lipoprotein lipase (AT-LPL) activity

As illustrated in Figure 2, neither regional variation nor difference between groups was observed in AT-LPL activity, although it tended to be higher in pre- than in postmenopausal women (P values ranging from 0.06 to 0.09). Similar results were obtained when this enzyme activity was expressed per cell number (not shown).

Insert Tables 1 and 2, Figures 1 and 2.

Adipose cell lipolytic function

Basal lipolytic rate and ADA-stimulated lipolysis

As shown in Figure 3, basal lipolysis was not significantly different between groups and among adipose regions. Addition of adenosine deaminase (ADA) at 5 μg/ml increased the basal lipolytic rate by about 1.5 to 2 times in adipocytes of premenopausal women. However, the level of glycerol release achieved with this enzyme did not seem to vary with the adipose region in postmenopausal subjects. Indeed, although ADA-stimulated adipose cell lipolysis tended to be

higher in pre- than in postmenopausal women, this difference did not reach statistical significance (P values ranging from 0.06 to 0.09).

Epinephrine responsiveness

In the presence of ADA, epinephrine (EPI) which is known for its mixed agonist (α_2/β) adrenergic properties on lipolysis, initiated a similar biphasic responsiveness in all cell types (main effects for age $F_{1,21} = 1.09$, $P = 0.31$; main effects for site $F_{1,21} = 0.98$, $P = 0.33$) (Figure 4). Inhibition of lipolysis was observed at the lowest concentrations (from 10^{-9} to 10^{-7} M), this effect being completely reversed at higher doses (from 10^{-6} to 10^{-5} M) where the hormone exerted a lipolytic action, thus suggesting a differential recruitment of α_2-, then of β-adrenoceptors.

Insert Figures 3 and 4.

Selective α_2- and β-adrenergic effects

As epinephrine responsiveness results from both α_2- and β-adrenoceptor stimulation, selective adrenergic agonists were used to discriminate between these two antagonistic effects.

To study the influence of the α_2-adrenoceptor component, the effect of the selective α_2-agonist, UK-14304, was tested on ADA-stimulated lipolysis (Figure 5). UK-14304 exerted a similar antilipolytic response in both groups ($F_{1,13} = 1.09$, $P = 0.31$) and adipose sites ($F_{1,13} = 0.47$, $P = 0.50$). Moreover, the α_2-adrenergic sensitivity estimated as the half-maximal antilipolysis induced by UK-14304 (which clustered at 1 to 3 nM) was not significantly different among adipose depots and between the two matched groups.

The effect of the β-agonist isoproterenol was also examined on basal lipolysis to characterize the β-adrenoceptor component (Figure 6). Stimulation of lipolysis induced by isoproterenol did not differ among adipose regions ($F_{1,21} = 2.91$, $P = 0.10$) and between the two matched groups ($F_{1,21} = 0.47$, $P = 0.78$). In addition, the β-adrenergic sensitivity defined as the concentration of isoproterenol required for half-maximal lipolysis (values ranging from 20 to 60 nM), was not

significantly different, regardless of the anatomic location of fat and the subjects' age. Similar results were observed for epinephrine-, isoproterenol-, and UK 14304-induced lipolysis expressed per cell number (not shown).

Insert Figures 5 and 6.

Lipolytic responses to post-adrenoceptor agents

Despite the lack of group-difference in catecholamine responsiveness, the effects of agents that stimulate lipolysis at well-defined post-receptor levels were also investigated (Figure 7). For this purpose, adipose cells were incubated without ADA, in the presence of either DcAMP (10^{-3} M), forskolin (10^{-5} M) or theophylline (10^{-3} M). As expected, there was no regional variation, nor a difference between pre- and postmenopausal women, when lipolysis was stimulated with maximal concentrations of these post-adrenoceptor agents.

Insert Figure 7.

DISCUSSION

The objective of the present study was to examine whether subcutaneous adipose tissue metabolism differed between pre- and postmenopausal women, after control for the concomitant variation in body fatness and in adipose tissue distribution, an issue that has not been addressed in previous studies (13,25). To the best of our knowledge, our study documents for the first time that the early phase of menopause does not appear to be associated with major changes in the adipose cell mobilizing and/or storage capacities, when compared to the premenopausal status.

Postmenopausal women of the present study were characterized by lower subcutaneous abdominal and visceral adipose tissue areas measured by computed tomography, as compared to other studies (27,44). Such a discrepancy could be easily explained by the age and menopausal status of the participants, as women from the two latter studies were older than our subjects. The similar adipose tissue lipoprotein lipase activity found in subcutaneous abdominal and femoral depots of postmenopausal women (Fig 2) is largely the consequence of the absence of regional variation in fat cell size that we observed in our group (Table 1). This finding is consistent with most (13,24,26,44), but not all (25) previous findings.

The similar biphasic epinephrine response profile in all cell types (Fig 4) probably reflects the interaction of the hormone with both types of adrenoceptors and supports the notion of the differential recruitment of α_2- then of β-sites (16-18,38). These data suggest that hypertrophy of adipose cells which is commonly associated with an expanded adipose tissue mass rather than age *per se* is a critical correlate of the increased α_2-adrenoceptor component in obesity (10,45).

The absence of difference in basal lipolytic rate, regardless of either the adipose region or the group considered, is consistent with the unaltered adenosine deaminase-stimulated lipolysis (Fig 3), and agrees with previous studies conducted on middle-aged men (46) and women (13,27). The tendency for a lower ADA-simulated lipolysis in adipose cells of post- vs premenopausal women may reflect a greater inhibition of lipolysis by adenosine with aging, as already suggested in old rats (47,48). Further studies are therefore needed to address this issue. Once again, the finding that basal lipolysis did not vary among adipose regions and groups could be explained by

the similar adipose cell size, as this variable was found to be highly correlated with basal glycerol release (9). On the other hand, comparison of epinephrine- or isoproterenol-induced maximal lipolysis (at 10^{-5} M) (Figs 4 and 6, respectively) revealed no regional variation, nor any group difference. This observation was further strengthened by the similar lipolytic effect of agents acting selectively at the adenylate cyclase, the lipase-protein kinase A complex or the phosphodiesterase level (Fig 7), findings which are concordant with an unaltered post-adrenoceptor lipolytic pathway. The observation that adipose cell lipolytic capacity was not impaired in postmenopausal women seems to be in contrast with previous observations (46,49). In these studies, however, the decreased lipid mobilization, which may be linked to a defect at the hormone-sensitive lipase level observed with aging, was probably due to the fact that middle-aged men had higher amounts of visceral adipose tissue than young individuals. In the present study, pre- vs postmenopausal women had similar levels of total and abdominal fat. These differences among studies emphasize the importance of visceral fat accumulation as a critical correlate of subcutaneous adipose cell lipolysis (27).

The fact that postmenopausal women displayed higher plasma cholesterol levels (Table 2) has already been observed by others, and the higher levels of LDL-CHOL accounted for most of the increased total cholesterol concentration commonly observed at menopause (19-23). The lack of alterations in plasma insulin and glucose levels with aging (Figure 2) may be partly explained by the design of our study as both groups of women had similar levels of visceral adipose tissue. Indeed, postmenopausal women characterized by a large visceral AT deposition had higher plasma insulin levels than middle-aged women displaying a low intraabdominal fat accumulation (27). Thus, the increased visceral adiposity which is generally observed at menopause appears to be an important factor for the metabolic deteriorations which occur in women during this period. However, the increase in LDL-cholesterol levels that we observed with age, appears to be largely independent from the concomitant variation in adiposity, a finding that we have previously reported in both genders (50). Although visceral AT accumulation and its related metabolic dysfunctions are associated with changes in circulating sex steroid hormones, it is clear that high androgen levels are characteristic features of intra-abdominal obesity in pre- and postmenopausal women, while the reverse situation is generally found in men (7,8,51). In this regard, whether *i)* the similar visceral AT deposition observed in pre- and postmenopausal women may account for

the lack of difference in the lipolytic capacity of subcutaneous abdominal adipocytes, or *ii)* a third factor related to visceral adipose tissue such as an altered sex steroid hormone profile which could lead to alterations in subcutaneous adipose cell metabolism is controlled by our matching procedure, is presently unknown. Based upon the fact that abdominal obese women are hyperandrogenic irrespective of their menopausal status and that testosterone exerts a lipolytic action on subcutaneous adipose tissue (52), it seems reasonable to assume that our matching of pre- and postmenopausal women for regional fat distribution may have also allowed us to "control" the effect of sex steroids on adipose tissue lipolysis. Further studies are, however, needed to test this hypothesis.

Taken together, our data show that regional variation in subcutaneous adipose tissue metabolism is not observed in the early phase of menopause. In addition, once the concomitant variation in body fatness, body fat distribution and adipose cell size is taken into account, our sample of pre- and postmenopausal women displays similar subcutaneous adipose tissue lipolysis and lipoprotein lipase activity. These results suggest that regional fat distribution is an important determinant which should be considered when investigating age-related effects on adipose tissue metabolism in women.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to France Levasseur, Henri Bessette, Germain Thériault and Gilles Lortie for their excellent collaboration at various stages of the study. The subjects and the Physical Activity Sciences Laboratory staff are also gratefully acknowledged. We also like to thank Marie Tremblay and Rachelle Duchesne of the Diabetes Research Unit for their assistance in data collection, as well as Suzanne Brulotte of the Department of Radiology (Laval University Hospital, Québec, Canada) for her help with the use of the computed tomograph. The contribution of the staff of the Lipid Research Center is also gratefully acknowledged.

REFERENCES

1. Björntorp P. 1993 Visceral obesity: a "civilization syndrome". Obesity Res. 1: 206-222.
2. Després JP. 1994 Dyslipidaemia and obesity. Baillière's Clin Endocrinol Metabol. 8: 629-660.
3. Kissebah AH, Krakower GR. 1994 Regional adiposity and morbidity. Physiol Rev. 74: 761-811.
4. Ley CJ, Lees B, Stevenson JC. 1992 Sex- and menopause-associated changes in body-fat distribution. Am J Clin Nutr. 55: 950-954.
5. Heymsfield SB, Gallagher D, Poehlman ET, et al. 1994 Menopausal changes in body composition and energy expenditure. Exp Gerontol. 29: 377-389.
6. Tchernof A, Poehlman ET. 1998 Effects of the menopause transition on body fatness and body fat distribution. Obesity Res. 6: 246-254.
7. Björntorp P. 1996 The regulation of adipose tissue distribution in humans. Int J Obesity. 20: 291-302.
8. Björntorp P. 1996 The android woman-a risky condition. J Intern Med. 239: 105-110.
9. Arner P. 1988 Control of lipolysis and its relevance to the development of obesity in man. Diabetes Metab Rev. 4: 507-515.
10. Bouchard C, Després JP, Mauriège P. 1993 Genetic and nongenetic determinants of regional fat distribution. Endocr Rev. 14: 72-93.
11. Leibel RL, Edens NK, Fried SK. 1989 Physiologic basis for the control of body fat distribution in humans. Annu Rev Nutr. 9: 417-443.
12. Rebuffé-Scrive M, Enk L, Crona N, et al. 1985 Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy and lactation. J Clin Invest. 75: 1973-1976.
13. Rebuffé-Scrive M, Eldh J, Hafström LO, Bjorntörp P. 1986 Metabolism of mammary, abdominal, and femoral adipocytes in women before and after menopause. Metabolism. 35: 792-797.
14. Arner P, Lithell H, Wahrenberg H, Brönnegard M. 1991 Expression of lipoprotein lipase in different subcutaneous adipose tissue regions. J Lipid Res. 32: 423-429.

15. Imbeault P, Alméras N, Richard D, Després JP, Tremblay A, Mauriège P. 1999 Effect of a moderate weight loss on adipose tissue lipoprotein lipase activity and expression: existence of sexual variation and regional differences. Int J Obesity. 23: 957-965.

16. Mauriège P, Galitzky J, Berlan M, Lafontan M. 1987 Heterogeneous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. Eur J Clin Invest. 17: 156-165.

17. Mauriège P, Prud'homme D, Lemieux S, Tremblay A, Després JP. 1995 Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. Am J Physiol. 269: 341-350.

18. Mauriège P, Imbeault P, Langin D, et al. 1999 Regional and gender variations in adipose tissue lipolysis in response to weight loss. J Lipid Res. 40: 1559-1571.

19. Kuller LH, Meilahn EN, Cauley JA, Gutai JP, Matthews KA. 1994 Epidemiologic studies of menopause: changes in risk factors and disease. Exp Gerontol. 29: 495-509.

20. Stevenson JC. 1996 Metabolic effects of the menopause and oestrogen replacement. Baillière's Clin Endocrinol Metabol. 10: 449-467.

21. Barrett-Connor E. 1997 Sex differences in coronary heart disease. Why are women so superior? The 1995 Ancel Keys Lecture. Circulation. 95: 252-264.

22. Poehlman ET, Toth MJ, Ades PA, Rosen CJ. 1997 Menopause-associated changes in plasma lipids, insulin-like growth factor I and blood pressure: a longitudinal study. Eur J Clin Invest. 27: 322-326.

23. Gower BA, Nagy TR, Goran MI, Toth MJ, Poehlman ET. 1998 Fat distribution and plasma lipid-lipoprotein concentrations in pre- and postmenopausal women. Int J Obesity. 22: 605-611.

24. Rebuffé-Scrive M, Lönnroth P, Marin P, Wesslau C, Björntorp P, Smith U. 1987 Regional adipose tissue metabolism in men and postmenopausal women. Int J Obesity. 11: 347-355.

25. Raison J, Basdevant A, Sitt Y, Guy-Grand B. 1988 Regional differences in adipose tissue lipoprotein lipase activity in relation to body fat distribution and menopausal status in obese women. Int J Obesity. 12: 465-472.

26. Lindberg UB, Crona N, Silfverstolpe G, Björntorp P, Rebuffé-Scrive M. 1990 Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. Horm Metabol Res. 22: 345-351.

27. Nicklas BJ, Rogus EM, Colman EG, Goldberg AP. 1996 Visceral adiposity, increased adipocyte lipolysis, and metabolic dysfunction in obese postmenopausal women. Am J Physiol. 270: E72-78.

28. Siri WE. 1956 The gross composition of body fat. Adv Biol Med Physiol. 4: 239-280.

29. Meneely GR, Kaltreider NL. 1949 Volume of the lung determined by helium dilution. J Clin Invest. 28: 129-139.

30. Bouchard C. 1985 Reproducibility of body composition and adipose tissue measurements in humans. *In* Body composition assessments in youth and adults, Report of the sixth Ross conference on medical research. Roche AF. Ross Laboratories, Columbus, Ohio. 9-14.

31. Lohman TG, Roche AF, Martorell R. 1988 The Airlie (VA) consensus conference. *In* Anthropometric Standardisation Reference Manual. Lohman TG, Roche AF, Martorell R. Human Kinetics Publishers, Champaign (Illinois). 39-80.

32. Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Despres JP. 1996 A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. Am J Clin Nutr. 64: 685-693.

33. Sjöström L, Kvist H, Cederblad A, Tylen U. 1986 Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. Am J Physiol. 250: E736-E786.

34. Ferland M, Després JP, Tremblay A, et al. 1989 Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr. 61: 139-148.

35. Després JP, Prud'homme D, Pouliot MC, Tremblay A, Bouchard C. 1991 Estimation of abdominal fat accumulation from simple anthropometric measurements in men. Am J Clin Nutr. 54: 471-477.

36. Savard R, Deshaies Y, Després JP, et al. 1984 Lipogenesis and lipoprotein lipase activity in human adipose tissue: reproducibility of measurements and relationship with fat cell size. Can J Physiol Pharmacol. 62: 1448-1452.

37. Rodbell M. 1964 Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem. 239: 375-380.

38. Mauriège P, Després JP, Prud'homme D, et al. 1991 Regional variation in adipose tissue lipolysis in lean and obese men. J Lipid Res. 32: 1625-1633.

39. Després JP, Bouchard C, Bukowiecki L, Savard R, Lupien J. 1983 Morphology and metabolism of human fat cells: a reliability study. Int J Obesity. 7: 231-240.

40. Richterich R, Dauwwalder H. 1971 Zur bestimmung der plasmaglukose-konzentration mit der hexokinase-glucose-6-phosphat-deshydrogenase-methode. Schweiz Med Wochenschr. 101: 615-618.

41. Desbuquois B, Aurbach GD. 1971 Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab. 37: 732-738.

42. Havel RJ, Eder H, Bragdon HF. 1955 The distribution and chemical composition of utracentrifugally separated lipoproteins in human serum. J Clin Invest. 34: 1345-1353.

43. Moorjani S, Gagné C, Lupien PJ, Brun D. 1986 Plasma triglycerides related decrease in high density lipoprotein cholesterol and its association with myocardial infarction in heterozygous familial hypercholesterolemia. Metabolism. 35: 311-316.

44. Berman DM, Nicklas BJ, Rogus EM, Dennis KE, Goldberg AP. 1998 Regional differences in adrenoceptor binding and fat cell lipolysis in obese, postmenopausal women. Metabolism. 47: 467-473.

45. Lafontan M, Berlan M. 1995 Fat cell alpha 2-adrenoceptors: the regulation of fat cell function and lipolysis. Endocr Rev. 16: 716-738.

46. Imbeault P, Prud'homme D, Tremblay A, Després JP, Mauriège P. 2000 Adipose tissue metabolism in young and middle-aged men after control for body fatness. J Clin Endocrinol Metab. In press:

47. Hoffman BB, Chang H, Farahbakhsh ZT, Reaven GM. 1984 Age-related decrement in hormone-stimulated lipolysis. Am J Physiol. 247: E772-777.

48. Hoffman BB, Chang H, Farahbakhsh Z, Reaven G. 1984 Inhibition of lipolysis by adenosine is potentiated with age. J Clin Invest. 74: 1750-1755.

49. Lönnqvist F, Nyberg B, Wahrenberg H, Arner P. 1990 Catecholamine-induced lipolysis in adipose tissue of the elderly. J Clin Invest. 85: 1614-1621.

50. Després JP, Tremblay A, Leblanc C, Bouchard C. 1988 Effect of the amount of body fat on the age-associated increase in serum cholesterol. Prev Med. 17: 423-431.

51. Tchernof A, Labrie F, Bélanger A, Després JP. 1996 Obesity and metabolic complications: contribution of dehydroepiandrostérone and other steroid hormones. J Endocrinol. 150: S155-S164.

52. Rebuffé-Scrive M, Marin P, Björntorp P. 1991 Effect of testosterone on abdominal adipose tissue in men. Int J Obesity. 15: 791-795.

Table 1. Physical characteristics of the women.

	Premenopausal (n = 8)	**Postmenopausal (n = 8)**
Age (years)	37 ± 5	57 ± 6 *
Body weight (kg)	70 ± 12	69 ± 13
BMI (kg/m^2)	29 ± 6	27 ± 3
Body fat (%)	39 ± 10	40 ± 8
Fat mass (kg)	28 ± 10	28 ± 10
Fat-free mass (kg)	42 ± 5	40 ± 4
Waist girth (cm)	83 ± 11	83 ± 11
Adipose tissue areas (cm^2)		
Abdomen (L4-L5)		
Subcutaneous	352 ± 117	339 ± 117
Visceral	94 ± 55	113 ± 57
Regional fat cell weight (μg lipid/cell)		
Abdominal	0.62 ± 0.18	0.65 ± 0.19
Femoral	0.70 ± 0.17	0.72 ± 0.20

Values are means ± standard deviation (SD). BMI = body mass index.

Statistical significance at *P < 0.0001.

Table 2. Metabolic profile of the women.

	Premenopausal (n = 8)	Postmenopausal (n = 8)
Fasting variables		
Triglycerides (mmol/l)	1.3 ± 0.5	1.7 ± 0.9
Cholesterol (mmol/l)	4.62 ± 0.65	5.51 ± 0.92 *
LDL-CHOL (mmol/l)	2.87 ± 0.57	3.78 ± 0.92 *
HDL-CHOL (mmol/l)	1.29 ± 0.30	1.20 ± 0.24

Values are means ± standard deviation (SD).

CHOL: cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein.

Statistical significance at *P < 0.05.

LEGENDS TO FIGURES

Figure 1. Plasma glucose and insulin responses to a 75 g oral glucose tolerance test (OGTT) in pre- and postmenopausal women. Bars represent the area under the curve. Glucose areas are expressed in 10^{-3} mmol/l/min whereas insulin areas are expressed in 10^{-3} pmol/l/min. Values are means ± standard error (SE).

Figure 2. Lipoprotein lipase (LPL) activity in subcutaneous abdominal and femoral adipose tissues from pre- vs postmenopausal women. Values are means ± standard error (SE) of 8 experiments run in duplicate.

Figure 3. Basal lipolytic rate and adenosine deaminase (ADA)-stimulated lipolysis in subcutaneous abdominal and femoral adipocytes from pre- vs postmenopausal women. Values are means ± SE of 8 experiments run in duplicate.

Figure 4. Effect of epinephrine (EPI) on ADA-stimulated lipolysis in subcutaneous abdominal and femoral adipocytes from pre- vs postmenopausal women. Values are means ± SE of 8 experiments run in duplicate. Glycerol release was expressed as the difference between stimulated (with epinephrine) and basal (ie, in presence of 5 μg/ml of ADA) values. Negative values reflect inhibition of lipolysis.

Figure 5. UK14304-induced inhibition of ADA-stimulated lipolysis in subcutaneous abdominal and femoral adipocytes from pre- vs postmenopausal women. Values are means ± SE of 8 experiments run in duplicate. Antilipolysis is given as the difference between values in the presence of UK14304 and ADA values. Agonist concentrations required for half-maximal inhibition of lipolysis (IC_{50}) were determined from these dose-response curves.

Figure 6. Isoproterenol (ISO)-induced lipolysis in subcutaneous abdominal and femoral adipocytes from pre- vs postmenopausal women. Fat cells were incubated without ADA (ie, in standard conditions) and values are means ± SE of 8 experiments run in duplicate. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these dose-response curves.

Figure 7. Lipolytic responsiveness to post-adrenoceptor agents of subcutaneous abdominal and femoral adipocytes from pre- vs postmenopausal women. Fat cells were incubated without ADA, in the presence of either dibutyryl-cyclicAMP (DcAMP) (10^{-3} M), forskolin (FK) (10^{-5} M) or theophylline (THEO) (10^{-3} M). Previous experiments revealed that the concentrations of the different drugs used were maximally effective doses. Values are means ± SE of 8 experiments run in duplicate and basal glycerol release has already been subtracted. Lipolytic responsiveness was calculated as the difference between lipolysis at maximum concentration of each agent minus basal lipolytic rate.

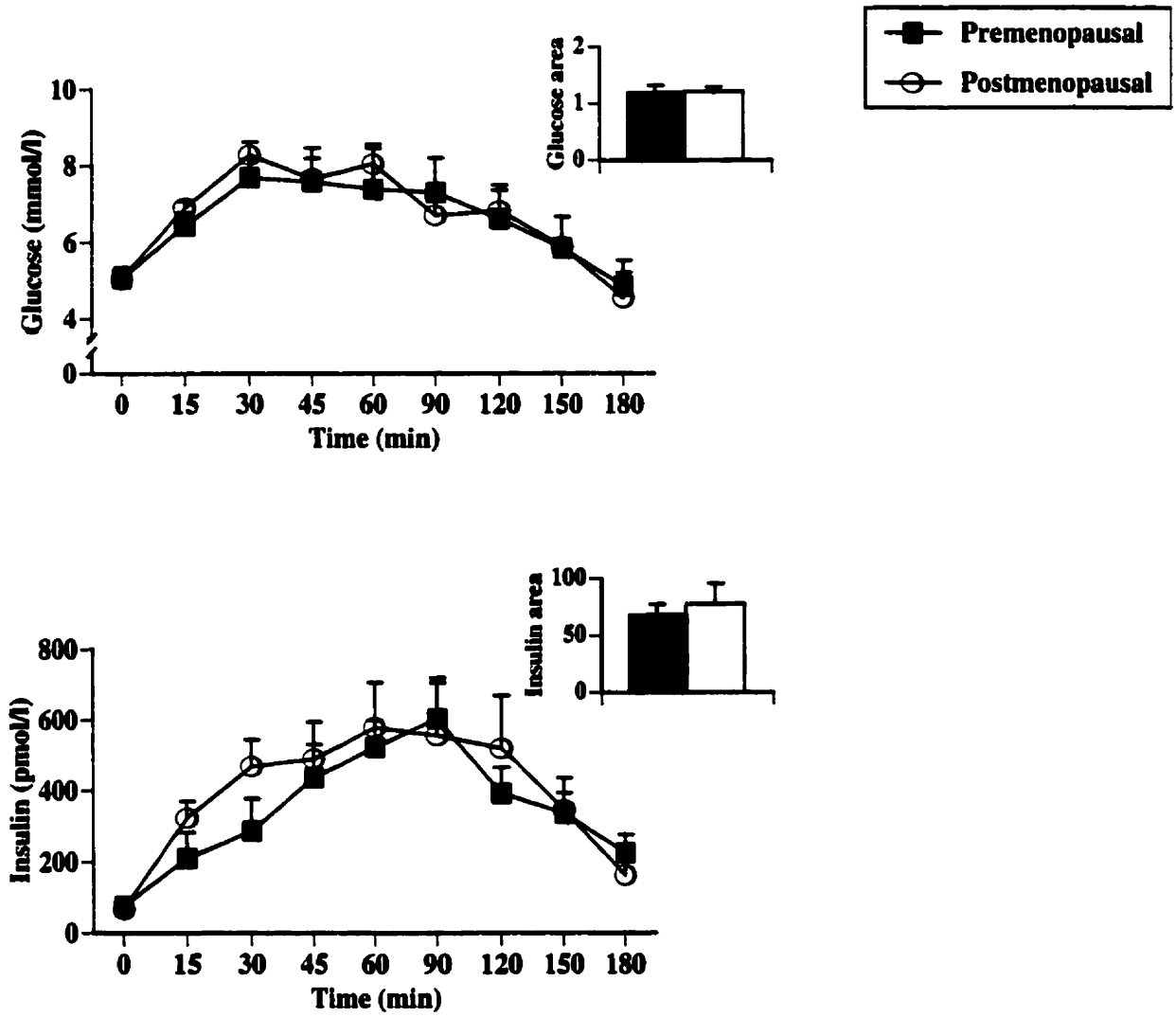

Premenopausal
Postmenopausal
Glucose (mmol/l)
10
8
6
4
0
0 15 30 45 60 90 120 150 180
Time (min)
Glucose area
2
1
0
Insulin (pmol/l)
800
600
400
200
0
0 15 30 45 60 90 120 150 180
Time (min)
Insulin area
100
50
0

Figure 1

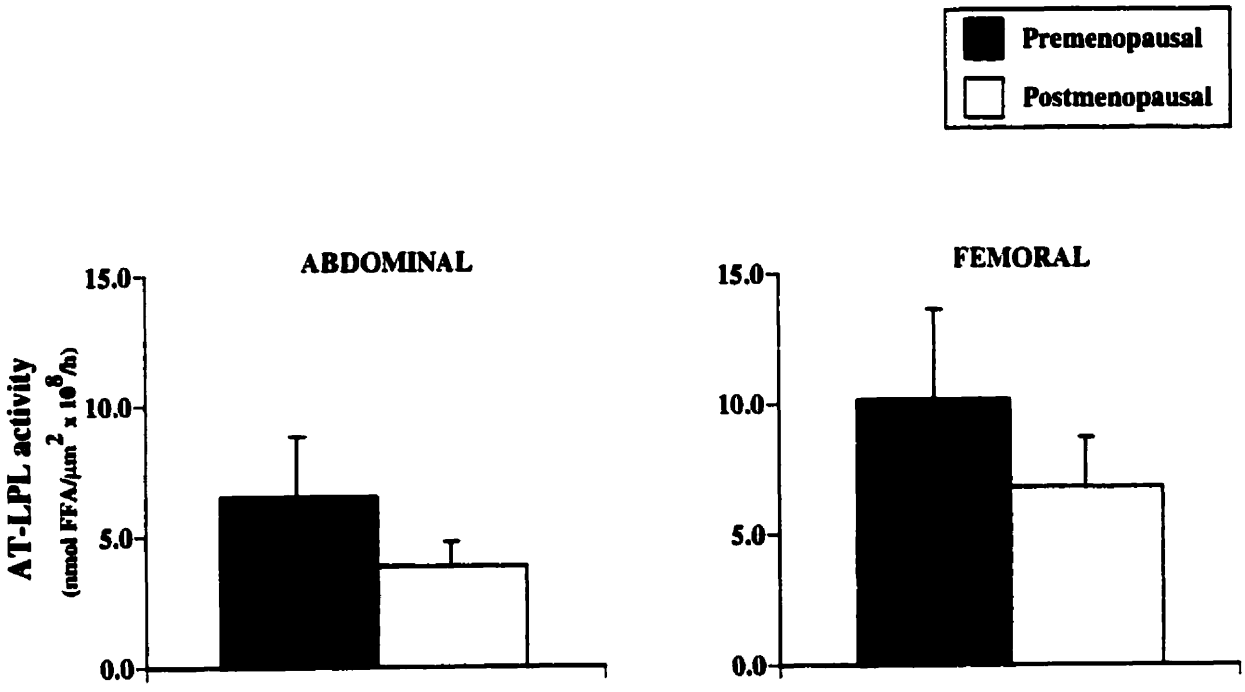

Premenopausal
Postmenopausal
ABDOMINAL
FEMORAL
AT-LPL activity
(nmol FFA/μm² x 10⁸/h)
15.0
10.0
5.0
0.0
15.0
10.0
5.0
0.0

Figure 2

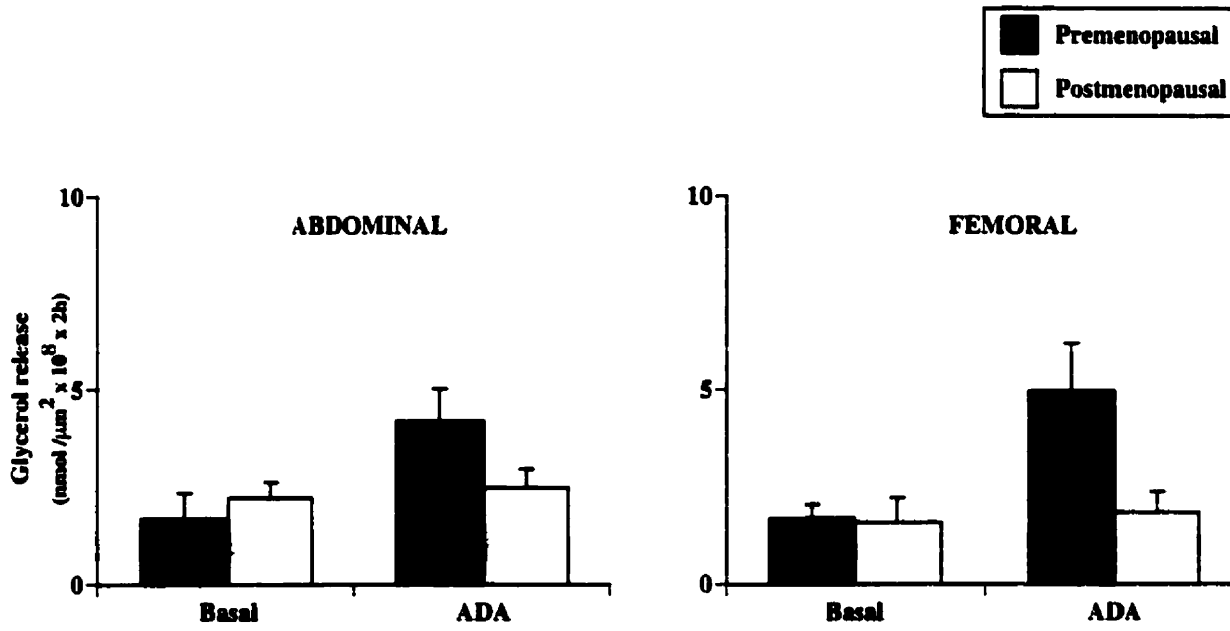

Premenopausal
Postmenopausal
ABDOMINAL
FEMORAL
Glycerol release
(nmol /µm² x 10⁸ x 2h)
10
5
0
Basal
ADA

Figure 3

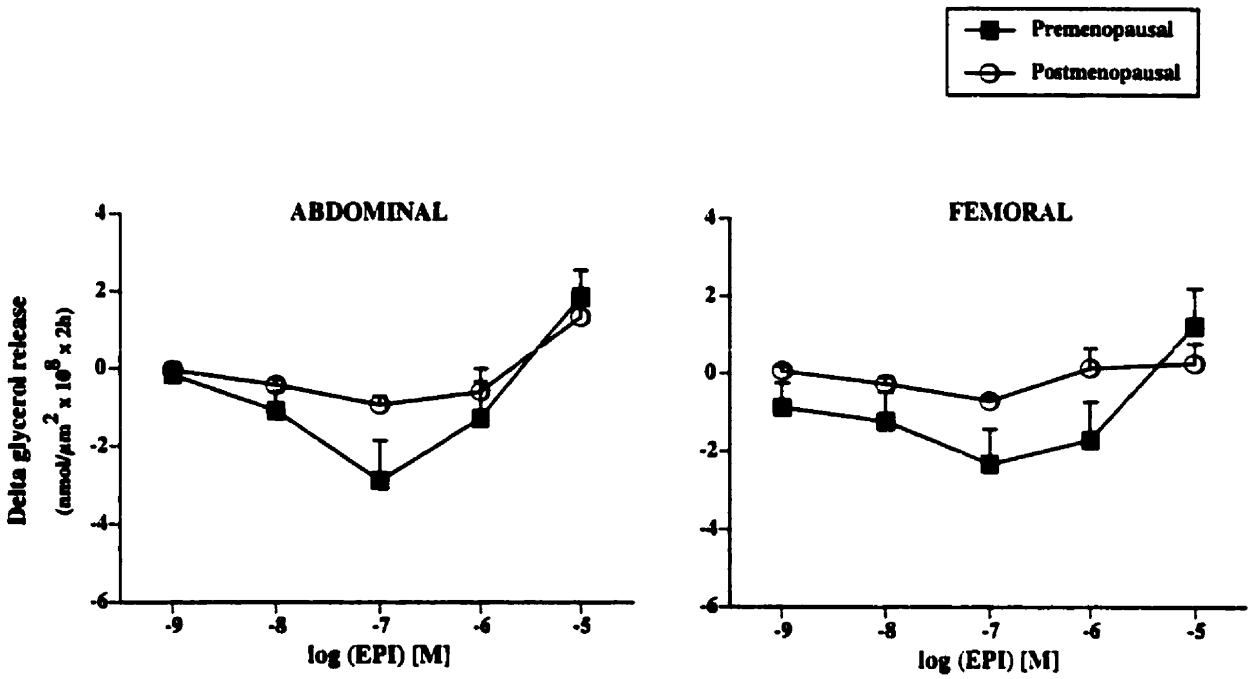

Premenopausal
Postmenopausal
ABDOMINAL
FEMORAL
Delta glycerol release
(nmol/μm² x 10⁸ x 2h)
4
2
0
-2
-4
-6
-9
-8
-7
-6
-5
log (EPI) [M]

Figure 4

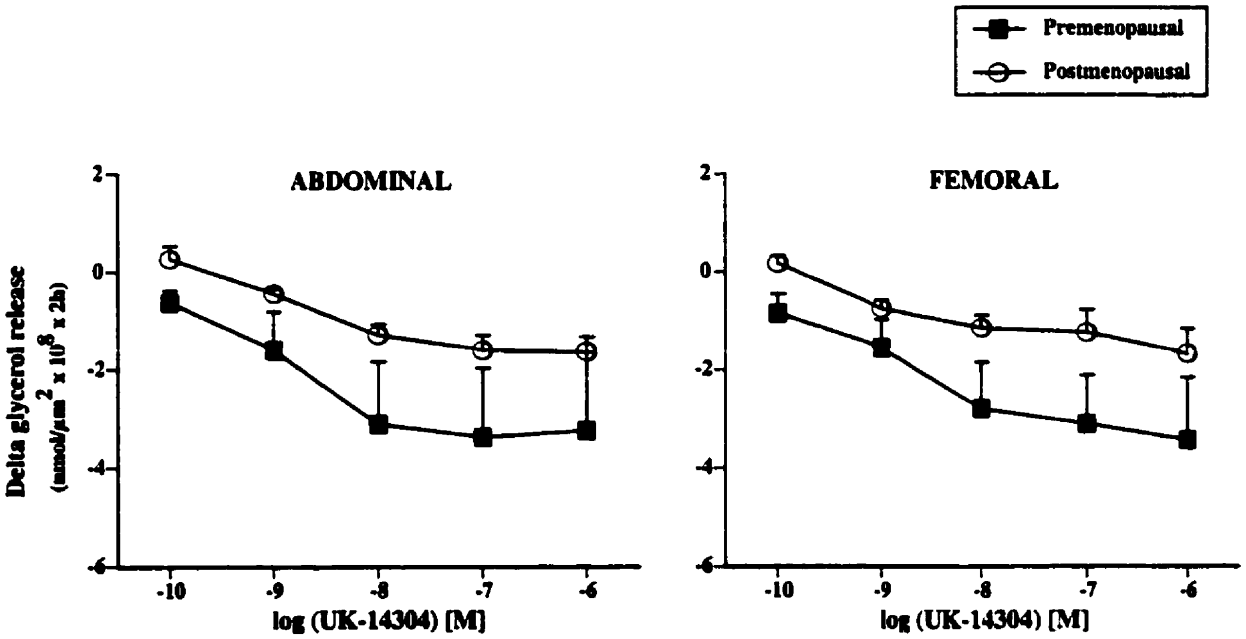

Premenopausal
Postmenopausal
ABDOMINAL
FEMORAL
Delta glycerol release
(nmol/μm² x 10⁸ x 2h)
2
0
-2
-4
-6
-10
-9
-8
-7
-6
log (UK-14304) [M]

Figure 5

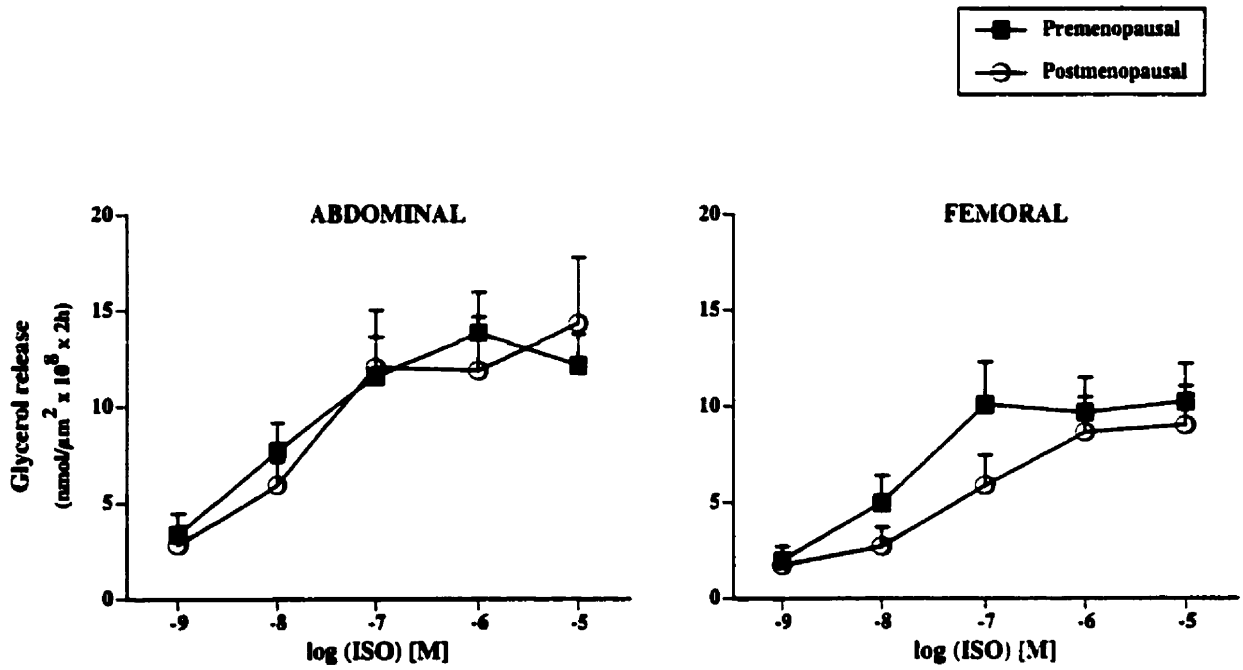

Premenopausal
Postmenopausal
ABDOMINAL
FEMORAL
Glycerol release
(nmol/μm2 x 10-8 x 2h)
20
15
10
5
0
-9
-8
-7
-6
-5
log (ISO) [M]

Figure 6

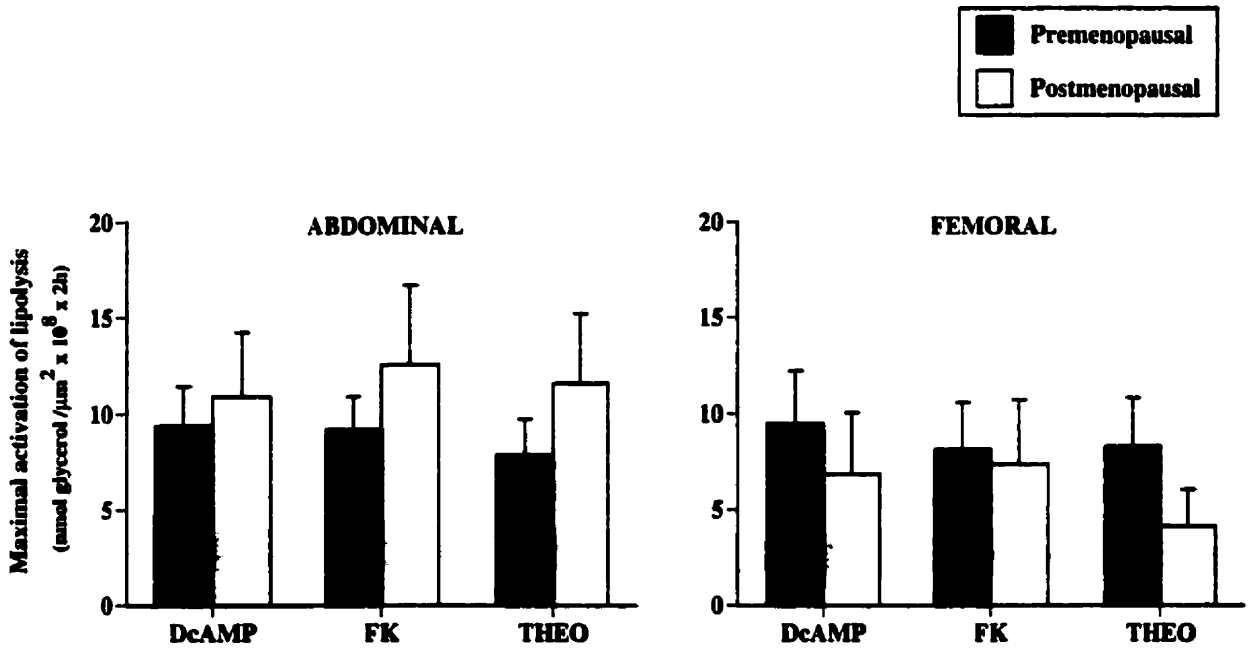

Premenopausal
Postmenopausal
Maximal activation of lipolysis
(nmol glycerol /µm² x 10⁸ x 2h)
ABDOMINAL
FEMORAL
20
15
10
5
0
DcAMP
FK
THEO

Figure 7

CHAPITRE 11

LA DIMINUTION DE LA SENSIBILITÉ α_2-ADRÉNERGIQUE DES ADIPOCYTES DU SITE SOUS-CUTANÉ ABDOMINAL AGIT COMME MODULATEUR DE LA LIPÉMIE POSTPRANDIALE CHEZ L'HOMME

L'article composant ce chapitre est intitulé:

"Reduced α_2-adrenergic sensitivity of subcutaneous abdominal adipocytes as a modulator of postprandial lipemia in men"

(en révision à *Journal of Lipid Research*)

Résumé

Cette étude examinait la lipémie postprandiale de deux groupes d'hommes présentant le même âge ainsi qu'un poids corporel et un profil de distribution de la masse adipeuse comparables, mais caractérisés par une faible (n =11) vs une forte (n = 15) sensibilité α_2-adrénergique (α_2-AR) des adipocytes sous-cutanés abdominaux. L'activité de la lipoprotéine lipase du tissu adipeux (TA-LPL) ainsi que celle de la LPL plasmatique posthéparinée (PH-LPL) ont également été mesurées après une période de jeûne de 12 heures. Ces activités enzymatiques étaient similaires quel que soit le groupe. La réponse lipolytique maximale à l'isoprénaline (agoniste β-AR) et la sensibilité β-AR des adipocytes sous-cutanés abdominaux ne différaient pas entre les deux groupes. L'agoniste α_2-AR, UK-14304, a entraîné une réponse antilipolytique similaire au sein des deux groupes. Cependant, la sensibilité α_2-AR, définie comme étant la concentration d'UK-14304 requise pour inhiber de moitié la lipolyse (IC_{50}), était significativement différente entre les groupes ($P < 0.001$). Les hommes caractérisés par une faible vs une forte sensibilité α_2-AR montraient des concentrations plasmatiques à jeun de triglycérides (TG) plus importantes. Dans le groupe entier, une relation positive fut observée entre l'IC_{50} de l'UK-14304 des adipocytes sous-cutanés abdominaux et des concentrations plasmatiques de TG ($r = 0.39$, $P < 0.05$), suggérant qu'une faible sensibilité α_2-AR antilipolytique est associée à des concentrations plasmatiques élevées de TG. Suite à la consommation d'un repas riche en lipides, les individus caractérisés par une faible sensibilité α_2-AR au niveau des adipocytes sous-cutanés abdominaux présentaient des taux de TG au niveau des fractions de lipoprotéines riches en triglycérides (TRL) totales-, de taille moyenne et petite supérieurs à ceux des hommes caractérisés par une forte sensibilité α_2-AR adipocytaire (P variant de 0.05 à 0.01). Parmi les variables indépendantes qu'étaient le poids corporel, le pourcentage de graisse, l'accumulation de tissu adipeux au niveau sous-cutané abdominal et viscéral ainsi que la sensibilité α_2-AR des adipocytes sous-cutanés abdominaux, seules les concentrations plasmatiques de TG furent retenues comme étant le meilleur prédicteur de la variance (73%) de l'aire sous la courbe des niveaux de TG de la fraction des

TRL totales. En résumé, ces résultats indiquent qu'une réduction de la sensibilité α_2-AR des adipocytes sous-cutanés abdominaux est associée à des concentrations plasmatiques de TG élevées pouvant ainsi entraîner des altérations au niveau de la mobilisation des TG postprandiaux.

Reduced α_2-adrenergic sensitivity of subcutaneous abdominal adipocytes as a modulator of fasting and postprandial triglyceride levels in men

P. Imbeault [1], C. Couillard [2], A. Tremblay [1], J-P. Després [2,3], and P. Mauriège [1,2]

[1]Physical Activity Sciences Laboratory, Department of Social & Preventive Medicine, Laval University, [2]Lipid Research Center, CHUQ Medical Research Center and [3]Québec Heart Institute, Laval Hospital Research Center, Québec, Canada.

Supported by the Medical Research Council of Canada and the Fonds FCAR-Québec.

Address for correspondence: P. Mauriège, Ph.D.
Lipid Research Center
CHUQ Medical Research Center
2705, boul. Laurier, Room TR-93
Ste-Foy, Québec, Canada, G1V 4G2
Tel: (418) 654-2133
Fax: (418) 654-2145
email: diabolo@internetclub.fr

Running head: Postprandial lipemia and abdominal adipocyte α_2-adrenergic sensitivity

Abbreviations list: ADA: adenosine deaminase; Apo-B: apolipoprotein-B; ASP: acylation stimulating protein; AT-LPL: adipose tissue lipoprotein lipase; AUC: area under the curve; AUIC: area under incremental curve; CT: computed tomography; FFA: free fatty acid; HSL: hormone-sensitive lipase; LDL: low-density-lipoprotein; PH-LPL: postheparin plasma lipoprotein lipase; RP: retinyl palmitate; TG: triglyceride; TRL: triglyceride-rich-lipoprotein; VLDL: very-low-density-lipoprotein.

Abstract

This study examined the postprandial lipemia of two groups of men displaying similar age, body weight and regional fat distribution, but characterized by either low (n = 11) vs high (n = 15) α_2-adrenergic sensitivity of subcutaneous abdominal adipocytes. In addition to fat cell lipolysis, adipose tissue lipoprotein lipase (AT-LPL) as well as postheparin plasma LPL activities were measured in the fasting state. Fasting AT-LPL and PH-LPL activities were similar in both groups. Maximal adipose cell lipolysis induced by isoproterenol (β-adrenergic agonist) as well as the β-adrenergic sensitivity did not differ between both groups of men. The selective α_2-adrenergic agonist, UK-14304, promoted a similar antilipolytic response in subcutaneous abdominal adipocytes from both groups. However, the α_2-adrenergic sensitivity, defined as the dose of UK-14304 which produced half-maximal inhibition of lipolysis (IC_{50}), was significantly different between groups ($P < 0.0001$). Men with low vs high subcutaneous abdominal fat cell α_2-adrenergic sensitivity showed higher fasting TG levels. In the whole group, a positive relationship was observed between log transformed IC_{50} UK-14304 values of subcutaneous adipocytes and fasting TG levels ($r = 0.39$, $p < 0.05$), suggesting that a low abdominal adipose cell α_2-adrenergic sensitivity is associated with high TG levels. Following the consumption of a high fat meal, subjects with low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity showed higher TG levels in total-, medium- and small-triglyceride-rich lipoproteins (TRL) fractions at 0 to 6h time points than men with high adipocyte α_2-adrenergic sensitivity (P values ranging from 0.01 to 0.05). Stepwise regression analysis showed that fasting TG concentration was the only variable retained as a significant predictor of the area under the curve of TG levels in total TRL fractions (73% of variance) among independent variables such as body weight, % body fat, visceral and subcutaneous abdominal adipose tissue accumulation measured by CT as well as subcutaneous abdominal fat cell α_2-adrenoceptor sensitivity. Taken together, these results indicate that a reduced antilipolytic sensitivity of subcutaneous abdominal adipocytes to catecholamines may increase fasting TG levels which in turn play a role in the etiology of an impaired postprandial TRL clearance in men.

Keywords: adipose cell lipolysis, lipase activities, regional fat distribution, lipoproteins.

Introduction

Alterations in plasma lipid and lipoprotein levels are prominent features of obesity, especially abdominal obesity (1-3). Indeed, individuals displaying a substantial accumulation of abdominal adipose tissue show greater plasma triglycerides (TG), very-low-density-lipoprotein (VLDL) and apolipoprotein (Apo)-B concentrations than nonobese persons. These metabolic alterations probably result from an increased free fatty acid (FFA) flux to the liver and a major culprit seems to be visceral adipose tissue, since it is characterized by a high lipolytic activity and a low antilipolytic response to insulin (4,5).

As α_2-adrenoceptors inhibit and β-adrenoceptors stimulate adipose tissue lipolysis (6,7), the fact that subcutaneous adipocytes possess greater α_2- and lower β-adrenoceptors than visceral fat cells explains in part their lower lipolytic capacity (8,9). Moreover, subcutaneous adipocytes do not display the same potential as visceral adipose cells to deliver FFA into the portal circulation due to their anatomical location. However, some *in vitro* studies have already underlined that subcutaneous fat cell lipolysis may also contribute to the development of metabolic perturbations in abdominally obese patients. In this regard, Arner et al. (10) have previously reported that low β_2-adrenoceptor sensitivity in subcutaneous abdominal fat cells was related to high plasma VLDL-TG and Apo-B levels. Furthermore, we have recently demonstrated that men with high femoral fat cell lipolysis (i.e. a low α_2-adrenergic component) were characterized by increased fasting plasma insulin, LDL-cholesterol (C) and LDL-ApoB levels (11), suggesting that a high femoral adipose tissue lipolysis may be associated with an enhanced cardiovascular disease (CVD) risk profile in men.

Evidences from *in vivo* studies have demonstrated that in the postprandial state, the enzyme responsible for adipocyte intracellular TG hydrolysis, hormone-sensitive lipase (HSL), is normally suppressed by insulin, whereas lipoprotein lipase (LPL) is activated by this hormone, thus leading to FFA release which either enter the tissue for reesterification and storage or are delivered into the systemic circulation (12). These coordinated changes appear important in the regulation of FFA movement and postprandial lipoprotein metabolism. However, to the

best of our knowledge, no study has yet verified whether the antilipolytic efficiency (ie, the α_2-adrenoceptor sensitivity) of subcutaneous abdominal fat cells measured *in vitro* could be associated with the fate of triglyceride-rich lipoproteins (TRL) in the postprandial state. Therefore, the aim of this study was to verify whether postprandial levels of various TRL fractions differ between two groups of men characterized by similar body fatness and regional fat distribution but displaying low vs high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity.

Research design and Methods

Subjects. Twenty-six Caucasian men were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. As two distinct clusters of men regarding their antilipolytic sensitivity of isolated subcutaneous abdominal adipocytes to UK-14304 (α_2-adrenoceptor agonist) were observed, a low (n = 11) and a high (n = 15) α_2-adrenoceptor sensitive groups have been compared in order to isolate the independent contribution of subcutaneous abdominal adipocyte antilipolytic sensitivity to postprandial lipemia. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication which could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc) were excluded from the study. All participants were sedentary, non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last six months prior to the study.

Total body fatness and regional fat distribution. Body density was determined by the underwater weighing technique and percent body fat was derived from body density (13). Pulmonary residual volume was measured using the helium dilution method (14). Fat mass was calculated as total body weight minus fat free mass. Waist girth was measured according to procedures recommended at the Airlie Conference (15). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology previously described by Sjöström et al. (16). Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) level, using an abdominal scout radiograph to establish the position of the scans to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) (17). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Oral lipid tolerance test. After a 12-h overnight fast, an intraveneous catheter was inserted into a forearm vein for blood sampling. Each participant was given a test meal containing 60 g fat/m^2 body surface area and 60000 IU of vitamin A (Aquasol A; Astra, Westborough, MA) (18). The meal consisted of eggs, cheese, toast, peanut butter, peaches, whipped cream and milk. Composition of the meal was 64% fat, 18% carbohydrate and 18% protein. The test meal was well tolerated by all subjects. After the meal, subjects were not allowed to eat for the next 8 h but were given free access to water. Blood samples were drawn before the meal and every 2 h after the meal over an 8-h period; samples were handled in a dimmed light to avoid deterioration of vitamin A.

Adipocyte isolation and lipolysis. After an overnight fast, participants underwent a biopsy of subcutaneous fat in the periumbilical region. A small cutaneous incision (1 cm) was performed in the abdominal site following local anesthesia (1% lidocaine, without epinephrine) and about 350 mg of subcutaneous adipose tissue were surgically removed from the fat depot.
Samples of 250 mg of adipose tissue were used for the measurement of fat cell lipolysis. Adipocytes were isolated according to the method of Rodbell (19) in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus 1 mg/ml collagenase, as previously described (20). Digestion took place in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2, for 40 min at 37^0C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 µl.

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. 50 µl aliquots of the continuously stirred cell suspension were placed in 1.5 ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 10 µl KRB were immediately placed on ice and provided evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation or inhibition were added just before starting the incubation in 10 µl portions in order to obtain the desired final concentration. After a 2h incubation at 37^0C in a shaking water bath, under 95% O_2 and 5% CO_2 gas phase, 50 µl HCl

(1N) were added to all tubes to stop the reaction, then 50 µl NaOH (1N) were added to neutralize the medium. All tubes were stored at -20^0C until glycerol determination and NADH concentration was measured by bioluminescence with a luciferase solution, using an automated 2250 Dynatech luminometer (20,21). For each concentration of stimulator or inhibitor, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Glycerol measurement by bioluminescence is very sensitive and especially well adapted when only small amounts of adipose tissue are available (20,21). Adipose cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ, USA). Mean fat cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described (22).

The lipolytic activity of the isolated fat cells was tested with epinephrine which is a mixed agonist (α_2/β) with a higher affinity for α_2- than for β-adrenoceptor (AR) sites (8), UK-14304 (selective α_2-AR agonist), isoproterenol (non selective β-AR agonist) (21). Ascorbic acid (0.1 mmol/l) was included in the medium in order to prevent catecholamine degradation. When antilipolytic effects were investigated, the incubation buffer was supplement with 5 µg/ml adenosine deaminase (ADA) to remove adenosine released into the incubation medium by the isolated fat cells, this procedure allowing better investigations of α_2-AR mediated antilipolytic effects (20,21). Lipolysis was expressed either per cell number (ie, in µmol of glycerol/10^6 cells x 2h) or per unit of cell surface area (ie, in nmol of glycerol/μm^2 x 10^8 x 2h), the latter mode of expression being used to correct for variation in fat cell size which is well-known to influence the rate of lipolysis (21). In case where complete dose-response curves were obtained, they were compared for both responsiveness and sensitivity. The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-5} M isoproterenol or forskolin, 10^{-3} M dibutyryl cAMP or theophylline). The β-adrenergic sensitivity was considered as the concentration of isoproterenol giving half-maximal stimulation of lipolysis (EC_{50}), whereas the α_2-adrenergic sensitivity was calculated as the dose of UK-14304 which produced half-maximal inhibition of lipolysis (IC_{50}). Both were evaluated by logarithmic conversion of each dose-response curve.

The higher was the EC_{50} (isoproterenol) or the IC_{50} (UK-14304) value, the lower was the β- or the α_2-adrenergic sensitivity, respectively.

Adipose tissue lipoprotein lipase (AT-LPL) activity. Samples of approximately 100 mg of adipose tissue from the abdominal site were immediately frozen in liquid nitrogen and stored at -80^0C. Heparin-releasable LPL activity was measured within one month of freezing storage, according to Savard et al. (23). AT-LPL activity was expressed as micromoles of free-fatty acids (FFA) released per hour per 10^6 cells. Since AT-LPL activity is associated with fat cell size (23,24), AT-LPL activity was also expressed per cell surface area (ie, nanomoles FFA per hour per micrometer squared times 10^4).

Postheparin plasma LPL (PH-LPL) activity. Plasma LPL activity was measured on one occasion after a 12-h overnight fast, 10 min after an intravenous injection of heparin (60 IU/kg body weight). The lipase activity was measured using a modification of the method of Nilsson-Ehle and Ekman (25), as previously described (26), and expressed as nanomoles of oleic acid released per milliliter of plasma per minute.

Fasting and postprandial plasma lipoprotein concentrations. Plasma was separated immediately after blood collection by centrifugation at 3000 rpm for 10 minutes at 4°C and placed in aluminium foil wrapped tubes. Triglyceride and cholesterol concentrations in total plasma were determined enzymatically on a RA-1000 Auto-Analyzer (Technicon Instruments Corporation, Tarrytown, NY), as previously described (27). Each plasma sample (4 ml) was then subjected to a 12-hour ultracentrifugation (50 000 rpm) in a Beckman 50.3Ti rotor (Palo Alto, CA) at 4°C, in 6 ml Beckman Quickseal tubes, which yielded two fractions: the top fraction containing TRL ($d < 1.006$ g/ml; TOTAL) and the bottom fraction consisting of triglyceride-poor lipoproteins ($d > 1.006$ g/ml). Using the distilled water layering technique and modified method of Ruotolo et al. (28), the TOTAL-TRL fraction was further separated, by a 5-minute spin (40 000 rpm) at 4°C using the same tubes and rotor, into three subclasses of TRL namely: LARGE, MEDIUM, and SMALL. A small volume (100 µl) of a $d = 1.019$ g/ml saline solution was added to the TOTAL-TRL fraction to facilitate water layering. The

LARGE-TRL fraction was collected by tube slicing and made up to a final volume of 1 ml with 0.15M NaCl. The next 3 ml of the middle layer were collected by aspiration as MEDIUM-TRL and the final 2 ml were considered as the SMALL-TRL fraction. HDL particles were isolated from the bottom fraction (d > 1.006 g/ml) after precipitation of apo B-containing lipoproteins with heparin and $MnCl_2$ (29). The triglyceride and cholesterol contents of each fraction, e.g. LARGE, MEDIUM and SMALL-TRL as well as HDL, were quantified on the Auto-Analyzer. All lipoprotein isolation procedures were completed within 2-3 days of the fat load, and samples protected from light at all times for later assays. Plasma FFA levels were measured at 0, 2, 4, 6 and 8 hours using a colorimetric method (30).

Glucose and insulin concentrations. Fasting and postprandial plasma glucose levels were determined using the glucose oxidase assay (31) (Sigma, St-Louis, MO). Plasma insulin concentrations were measured by a commercial double antibody radioimmunoassay (Linco Research, St-Louis, MO) that shows little cross-reactivity (< 0.02%) with pro-insulin (32).

Retinyl palmitate (RP) measurements. As retinyl esters, predominantly RP, serve as useful estimates of the intestinally derived component of postprandial lipemia, the RP content of TOTAL as well as of LARGE, MEDIUM and SMALL-TRL fractions was analyzed using high-performance liquid chromatography (HPLC) as previously described (28). Briefly, aliquots of 100 µl of TOTAL and LARGE-TRL as well as 500 µl of MEDIUM and SMALL-TRL were used for the analysis. The volume of total and LARGE-TRL fractions was adjusted to 500 µl with 0.15M NaCl. A volume of 200 µl of retinyl acetate (RA; 200 ng/ml; SIGMA, St-Louis, MO) was added to each sample as internal standard. The extraction of RP from the samples was obtained by addition of 500 ml of methanol followed by 500 µl of mobile phase buffer prepared from 90 ml of hexane, 15 ml of n-butyl chloride, 5 ml of acetonitrile and 0.01 ml of acetic acid (82:13:5 by volume with 0.01 ml of acetic acid). Tubes were mixed thoroughly after each addition step. All solvents used were HPLC graded (Caledon Laboratories Ltd., Georgetown, Ontario, Canada). Samples were then centrifuged for 15 minutes at 1500 rpm (room temperature). This procedure yielded two distinct phases. The upper phase, containing the RP and RA, was carefully removed and placed in separate autosampler vials. Vials were then placed in an autosampler from Shimadzu Corporation

(Kyoto, Japan) and samples analyzed with a HPLC system from Waters (Waters Associates, Milford, MA). The RP and RA peaks were detected at 325 nm. The RP concentration (in RA equivalent) of every fraction was calculated according to the equation of Ruotolo et al. (28).

RP (ng RA/ml) = (RP peak area/RA peak area) x (1/volume of sample used) x 40 ng RA

Drugs and chemicals. Collagenase, bovine serum albumin, ADA and enzymes for glycerol assays were obtained from Boehringer Mannheim (Canada). Ascorbic acid, theophylline, forskolin, DcAMP, (-)-isoproterenol bitartrate and (-)-epinephrine bitartrate and cold triolein were purchased from Sigma Chemical (St-Louis, MO, USA). ^{14}C-triolein was obtained from Dupont NEN (Canada). 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK-14304) was generously provided by Dr. D.A. Faulkner (Pfizer, Sandwich, UK). All other chemicals and organic solvents were of the highest purity grade commercially available. The same batches of hormones, pharmacological agents, collagenase and albumin were used in all experiments.

Statistical analyses. The Student's t-test was utilized for comparisons between men with low and high subcutaneous abdominal adipocyte α_2-adrenergic sensitivity. Associations between two variables were quantified by Pearson's product-moment correlation coefficients. Stepwise multiple regression was also performed to enhance predictability of fasting TG levels and areas under the curve (AUC) of TG. Analysis of variance for repeated measures was used to verify the overall differences in total-, large-, medium- and small-TRL fractions over time. The same procedure was performed with plasma insulin, glucose and FFA concentrations. The AUC of TG, glucose, insulin, FFA and RP were determined by the trapezoid method. All analyses were performed using the Jump version 3.2.2 program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers.

Results

Physical characteristics and fasting metabolic variables of both groups are presented in Table 1. Men with high or low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity displayed similar age, body weight, fat mass and regional adipose tissue distribution measured by CT. Moreover, no difference in subcutaneous abdominal fat cell size was observed between groups. Men with high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity showed lower fasting plasma TG levels than men with low adipose cell α_2-adrenoceptor sensitivity ($P < 0.01$). However, fasting plasma insulin, FFA and glucose levels were similar in both groups.

Insert Table 1

AT- and PH-LPL activities did not differ between men with low vs high α_2-adrenoceptor sensitivity (Figure 1). Moreover, neither the basal lipolytic rate nor the ADA-stimulated lipolysis was significantly different between groups (Figure 2). As shown in Figure 3A, the effect of epinephrine, which is known for its mixed agonist (α_2/β) properties on lipolysis was examined in the presence of ADA. The catecholamine initiated a similar biphasic response profile in subcutaneous abdominal adipocytes from both groups: antilipolysis was observed at low concentrations (10^{-9} to 10^{-7} M), this effect being completely reversed at higher doses at which the hormone exerted a lipolytic response (10^{-6} to 10^{-5} M), thus suggesting a differential recruitment of α_2-, then of β–adrenoceptors.

Insert Figures 1 and 2

In order to characterize the α_2-adrenoceptor component, the selective α_2-agonist, UK-14304, was also tested on ADA-stimulated lipolysis (Figure 3B). A similar antilipolytic effect was observed in subcutaneous abdominal adipocytes from both groups. However, the α_2-adrenergic sensitivity (IC_{50}) estimated as the half-maximal antilipolysis induced by UK-14304 was significantly different between groups ($P < 0.001$) (Figure 4). Indeed, men with high

subcutaneous abdominal fat cell α_2-adrenoceptor sensitivity showed a 16-fold lower IC_{50} value than men with low α_2-adrenoceptor sensitivity. In the whole group, a positive relationship was observed between log transformed IC_{50} (UK-14304) values and fasting TG levels, suggesting that a low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity was associated with high TG levels ($r = 0.39$, $P < 0.05$) (not shown).

To study the influence of the β-adrenoceptor component, the lipolytic effect of the β-agonist isoproterenol was also examined on basal lipolysis (Figure 3C). No difference in the maximal lipolytic response to isoproterenol (defined at 10^{-5} M) was observed between groups. Moreover, the β-adrenergic adipose cell lipolytic sensitivity was similar in both groups (22 ± 9 vs 51 ± 21 nM in men with high vs low α_2-adrenoceptor adipocyte sensitivity).

Insert Figures 3A, 3B, 3C and 4

Figure 5 shows TG concentrations in total- as well as in large-, medium- and small-TRL fractions before and after the consumption of the high fat meal. Men with high subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity displayed lower values of total-, medium- and small-TRL fractions at 0 to 6h time points than men with low abdominal adipocyte antilipolytic sensitivity (P values ranging from 0.01 to 0.05). However, no difference between groups was observed in the respective response above baseline values (AUIC) of the TRL fractions.

Changes in RP over the meal in total- as well as in large-, medium- and small-TRL fractions are presented in Figure 6. Although some group differences were observed in RP of large and medium TRL fractions, no difference in RP contained in total and small TRL fractions was observed between men with high vs low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Both groups of men also showed similar RP AUIC for all TRL fractions. Moreover, postprandial insulin, glucose and FFA levels were similar in both groups (not shown).

Insert Figures 5 and 6

To estimate the respective contribution of regional adiposity and subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity in accounting for variance in the AUC of TG levels in total TRL fractions, stepwise multiple regression analysis was performed. Our model included body weight, % body fat, visceral and subcutaneous abdominal adipose tissue accumulation measured by CT, fasting TG concentration as well as subcutaneous abdominal fat cell α_2-adrenoceptor sensitivity. Fasting TG concentration was the only variable retained in the model and accounted for 73% of the variance in the AUC of TG levels in total TRL fractions ($P < 0.0001$) (not shown).

Discussion

Results of the present study suggest a potential role of subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity in the etiology of altered postprandial lipemia. Indeed, men with low antilipolytic efficiency showed higher fasting TG levels which therefore impaired postprandial metabolism following the ingestion of a rich fat meal compared to men with a high adipose cell sensitivity to an α_2-adrenoceptor agonist. This finding is reinforced by the perfect control of factors which could have contributed to adipose tissue lipolysis variation or postprandial lipemia between our groups such as age, body fat, visceral adipose tissue accumulation and subcutaneous fat cell size.

We have recently demonstrated that an excess of visceral adipose tissue accumulation was associated with an impaired postprandial TRL clearance in men (33). The important flux of FFA to the liver from visceral adipocytes is a well-known correlate of this postprandial metabolic alteration. Although subcutaneous abdominal adipocytes do not present the same capacity than visceral fat cells to deliver FFA into the portal circulation (5,8,9), large subcutaneous abdominal adipocytes have also been reported to alter the metabolic risk profile in men (34). The rationale behind this finding was that large adipocytes are generally characterized by a high lipolytic rate (20,35,36) which could lead to an increased adipocyte-hepatocyte FFA flux and therefore to metabolic disturbances. The most striking feature of the present study was the significant contribution of subcutaneous abdominal adipocytes to fasting and therefore to postprandial metabolism of TRL fractions, even after controlling for both fat cell size and visceral adipose tissue accumulation. From a clinical standpoint, all these data suggest that an individual with a high visceral fat deposition and a low α_2-adrenoceptor subcutaneous abdominal adipocyte sensitivity may be at greater risk for alteration of postprandial lipemia.

We found that subcutaneous abdominal adipose cell lipolytic response was similar after stimulation of either α_2- or β-adrenoceptors in both groups of men. The β-adrenergic sensitivity was also comparable between men with low vs high subcutaneous abdominal

adipocyte α_2-adrenoceptor sensitivity. Taken together, these results suggest that the difference between groups observed in the subcutaneous abdominal adipocyte α_2-adrenergic sensitivity reflects principally alterations in hormone action that are located at/or near the α_2-adrenoceptor level (35). Since a selective α_2-adrenergic stimulation promotes antilipolysis, it is possible that at given low catecholamine concentrations, subjects with low subcutaneous abdominal adipocyte α_2-adrenergic sensitivity display a greater fasting FFA release than men with high adipose cell α_2.antilipolytic sensitivity. Consequently, an inappropriated elevated FFA availability might result in an increased esterification of FFA and a reduced hepatic degradation of apolipoprotein B, leading to an increased synthesis and secretion of VLDL particles. This model is supported by the high fasting TG levels observed in men with low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. These results are in good accordance with a previous study showing that an acute elevation of plasma FFA stimulates VLDL production in humans (37).

In the postprandial phase, men with low abdominal adipose cell α_2-adrenergic sensitivity could also be characterized by an impaired suppression of HSL activity, resulting in a continued FFA release at a time when FFA mobilization might be substantially reduced in the high α_2-adrenergic sensitivity group. This increased FFA availability could, once again, contribute to the reduced clearance of TRL fractions observed in subjects with low α_2-adrenergic sensitivity. To some extent, these results are concordant with a previous *in vivo* study reporting that adipose tissue HSL activity of obese subjects fails to respond to insulin in the postprandial state, a potential maladaptation in terms of lipoprotein metabolism and risk for coronary heart disease (38). On the basis of our results, we cannot exclude that the *in vitro* antilipolytic response of subcutaneous abdominal adipocytes to insulin was different between our groups. Further studies are needed to investigate this issue.

Another important factor explaining the release of FFA in the postprandial state is the chylomicron hydrolysis by the action of LPL in adipose tissue capillaries. As previously proposed, a good proportion of FFA release in the postprandial period originates from this

pathway rather than from the intracellular action of HSL (12). Based on studies conducted by his team, Frayn (39) has suggested that FFA generated by LPL will flow into adipocytes only if the concentration gradient is in the appropriate direction. In other words, after an overnight fasted state, when HSL is most active, LPL-derived FFA are principally released into plasma. On the other hand, in the fed state, HSL activity is suppressed by insulin and this may favor entrapment by adipose tissue of LPL-derived FFA, leading then to fat store replenishment. Based on these evidences, the regulation of HSL activity appears an important determinant of the fate of LPL-derived FFA. Our results showed that men with low adipose cell α_2-adrenergic sensitivity display similar fasting AT-LPL as well as PH-LPL activities than men with high adipocyte α_2-adrenergic sensitivity. Moreover, both groups of men did not show any difference in basal lipolysis, a well-know determinant of HSL activity (40,41). Postprandially, our results are concordant with the above Frayn's group hypothesis (12). Indeed, men with low abdominal fat cell α_2-adrenergic sensitivity might be characterized by an inappropriate release of FFA into the circulation due to their expected lower HSL activity suppression induced by the meal. That no difference between groups was observed in postprandial plasma FFA levels may be explained by the fact that FFA are rapidly taken up by hepatocytes and induce hepatic VLDL production which, in turn, alters postprandial lipoprotein clearance, as previously reported (42). Another recently discovered adipose tissue regulator of postprandial FFA metabolism which might have an impact on our observations is the acylation stimulating protein (ASP) (43). Indeed, ASP production in adipose tissue has recently been related to postprandial TG clearance and uptake of FFA into adipocyte (44).

Regarding TRL RP concentrations, it is tempting to speculate that high fasting TG levels found in the low fat cell α_2-adrenergic sensitivity group may be the result of the stimulation of hepatic VLDL-TG secretion several hours after meal ingestion. Indeed, the increased TG levels in small TRL observed in men with low adipose cell α_2-adrenergic sensitivity was not accompanied by a higher RP concentrations in this TRL fraction, as compared to men with high adipocyte α_2-adrenergic sensitivity. This observation supports the fact that TG from endogenous TRL, presumably VLDL particles, account for most of the increase in small-TRL TG levels observed late through the postprandial period (33,42).

In conclusion, the present study shows that men with low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity show elevated postprandial TG concentrations as compared to men with high adipose cell α_2-adrenergic sensitivity. This difference is mainly due to the fact that a reduced subcutaneous abdominal adipose cell α_2-adrenergic sensitivity may increase fasting TG level which in turn is an important predictor of postprandial TG concentration. These results confer to subcutaneous abdominal adipose cell α_2-adrenergic sensitivity an indirect role in the regulation of postprandial lipemia via its impact on fasting TG levels.

Acknowledgements

The authors wish to express their gratitude to Sylvie St-Pierre, Éric Doucet and Henri Bessette for their collaboration at various stages of the study and to Dr Gilles Lortie for his medical supervision. The subjects and the staff of the Lipid Research Center are also gratefully acknowledged.

References

1. Björntorp, P. 1991. Metabolic implications of body fat distribution. *Diabetes Care.* **14:** 1132-1143.
2. Després, J. P., S. Moorjani, P. J. Lupien, A. Tremblay, A. Nadeau, and C. Bouchard. 1990. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis.* **10:** 497-511.
3. Kissebah, A. H., and G. R. Krakower. 1994. Regional adiposity and morbidity. *Physiol. Rev.* **74:** 761-811.
4. Bolinder, J., L. Kager, J. Ostman, and P. Arner. 1983. Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes.* **32:** 117-123.
5. Mauriège, P., A. Marette, C. Atgié, C. Bouchard, G. Thériault, L. J. Bukowiecki, P. Marceau, S. Biron, A. Nadeau, and J. P. Després. 1995. Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J. Lipid Res.* **36:** 672-684.
6. Arner, P. 1996. Regulation of lipolysis in fat cells. *Diabetes Rev.* **4:** 1-13.
7. Lafontan, M., and M. Berlan. 1995. Fat cell alpha 2-adrenoceptors: the regulation of fat cell function and lipolysis. *Endocr. Rev.* **16:** 716-738.
8. Mauriège, P., J. Galitzky, M. Berlan, and M. Lafontan. 1987. Heterogeneous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. *Eur. J. Clin. Invest.* **17:** 156-165.
9. Hellmér, J., C. Marcus, T. Sonnefeld, and P. Arner. 1992. Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. *J. Clin. Endocrinol. Metab.* **75:** 15-20.
10. Arner, P., H. Wahrenberg, F. Lönnqvist, and B. Angelin. 1993. Adipocyte β-adrenoceptor sensitivity influences plasma lipid levels. *Arterioscler. Thromb.* **13:** 967-972.
11. Mauriège, P., J. P. Després, S. Moorjani, D. Prud'homme, B. Lamarche, C. Bouchard, A. Nadeau, A. Tremblay, and P. J. Lupien. 1993. Abdominal and femoral adipose tissue lipolysis and cardiovascular disease risk factors in men. *Eur. J. Clin. Invest.* **23:** 729-740.

12. Frayn, K. N., S. Shadid, R. Hamlani, S. M. Humphreys, M. L. Clark, B. A. Fielding, O. Boland, and S. W. Coppack. 1994. Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am. J. Physiol.* **266:** E308-317.

13. Siri, W. E. 1956. The gross composition of body fat. *Adv. Biol. Med. Physiol.* **4:** 239-280.

14. Meneely, G. R., and N. L. Kaltreider. 1949. Volume of the lung determined by helium dilution. *J. Clin. Invest.* **28:** 129-139.

15. Lohman, T. G., A. F. Roche, and R. Martorell. 1988. The Airlie (VA) consensus conference. *In* Anthropometric Standardisation Reference Manual. Lohman, T. G., Roche, A. F. and Martorell, R. Human Kinetics Publishers, Champaign (Illinois). 39-80.

16. Sjöström, L., H. Kvist, A. Cederblad, and U. Tylen. 1986. Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. *Am. J. Physiol.* **250:** E736-E786.

17. Ferland, M., J. P. Després, A. Tremblay, S. Pinault, A. Nadeau, S. Moorjani, P. J. Lupien, G. Thériault, and C. Bouchard. 1989. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br. J. Nutr.* **61:** 139-148.

18. Krasinski, S., J. Cohn, R. Russell, and E. Schaefer. 1990. Postprandial plasma vitamin A in humans: a reassessment of the use of plasma retinyl esters as a markers for intestinally derived chylomicrons and their remnants. *Metabolism.* **39:** 357-365.

19. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375-380.

20. Mauriège, P., J. P. Després, D. Prud'homme, M. C. Pouliot, M. Marcotte, A. Tremblay, and C. Bouchard. 1991. Regional variation in adipose tissue lipolysis in lean and obese men. *J. Lipid Res.* **32:** 1625-1633.

21. Mauriège, P., D. Prud'homme, S. Lemieux, A. Tremblay, and J. P. Després. 1995. Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. *Am. J. Physiol.* **269:** 341-350.

22. Després, J. P., C. Bouchard, L. Bukowiecki, R. Savard, and J. Lupien. 1983. Morphology and metabolism of human fat cells: a reliability study. *Int. J. Obesity.* **7:** 231-240.

23. Savard, R., Y. Deshaies, J. P. Després, M. Marcotte, L. J. Bukowiecki, C. Allard, and C. Bouchard. 1984. Lipogenesis and lipoprotein lipase activity in human adipose tissue:

reproducibility of measurements and relationship with fat cell size. *Can. J. Physiol. Pharmacol.* **62:** 1448-1452.

24. Eckel, R. H. 1989. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* **320:** 1060-1068.
25. Nilsson-Ehle, P., and R. Ekman. 1978. Specific assays for lipoprotein lipase and hepatic lipase activities of post-heparin plasma. *In* Protides of Biological Fluids. Peeters, H. Pergamon, Oxford, U.K. 243-246.
26. St-Amand, J., J. P. Després, S. Lemieux, B. Lamarche, S. Moorjani, D. Prud'homme, C. Bouchard, and P. J. Lupien. 1995. Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women? *Metabolism.* **44:** 491-498.
27. Moorjani, S., A. Dupont, F. Labrie, P. J. Lupien, D. Brun, C. Gagné, M. Giguère, and A. Bélanger. 1987. Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. *Metabolism.* **36:** 244-250.
28. Ruotolo, G., H. Zhang, V. Bentsianov, and N. A. Le. 1992. Protocol for the study of the metabolism of retinyl esters in plasma lipoproteins during postprandial lipemia. *J. Lipid Res.* **33:** 1541-1549.
29. Burstein, M., and J. Samaille. 1960. Sur un dosage rapide du cholestérol lié aux b-lipoprotéines du sérum. *Clin. Chim. Acta.* **5:** 609-610.
30. Noma, A., H. Okabe, and M. Kita. 1973. A new colorimetric microdetermination of free fatty acids in serum. *Clin Chim Acta.* **43:** 317-320.
31. Raabo, E., and T. C. Terkildsen. 1960. On the enzymatic determination of blood glucose. *Scand J Clin Lab Invest.* **12:** 402-407.
32. Morgan, C. R., and A. Lazarow. 1963. Immunoassay of insulin-Two antibody system. Plasma insulin levels in normal, subdiabetics rats. *Diabetes.* **12:** 115-126.
33. Couillard, C., N. Bergeron, D. Prud'homme, J. Bergeron, A. Tremblay, C. Bouchard, P. Mauriège, and J. P. Després. 1998. Postprandial triglyceride response in visceral obesity in men. *Diabetes.* **47:** 953-960.
34. Imbeault, P., S. Lemieux, D. Prud'homme, A. Tremblay, A. Nadeau, J. P. Després, and P. Mauriège. 1999. Relationship of visceral adipose tissue to metabolic risk factors for coronary heart disease: is there a contribution of subcutaneous fat cell hypertrophy? *Metabolism.* **48:** 355-362.

35. Arner, P. 1988. Control of lipolysis and its relevance to the development of obesity in man. *Diabetes Metab. Rev.* **4:** 507-515.

36. Kissebah, A. H., N. Vydelingum, R. Murray, D. J. Evans, A. J. Hartz, R. K. Kalkhoff, and P. Adams. 1982. Relation of body fat distribution to metabolic complications of obesity. *J. Clin. Endocrinol. Metab.* **54:** 254-260.

37. Lewis, G. F., K. D. Uffelman, L. W. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest.* **95:** 158-166.

38. Coppack, S. W., R. D. Evans, R. M. Fisher, K. N. Frayn, G. F. Gibbons, S. M. Humphreys, M. L. Kirk, J. L. Potts, and T. D. Hockaday. 1992. Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. *Metabolism.* **41:** 264-272.

39. Frayn, K. N. 1998. Non-esterified fatty acid metabolism and postprandial lipaemia. *Atherosclerosis.* **141:** S41-S48.

40. Stich, V., I. Harant, I. De Glisezinski, F. Crampes, M. Berlan, M. Kunesova, V. Hainer, M. Dauzats, D. Rivière, M. Garrigues, C. Holm, M. Lafontan, and D. Langin. 1997. Adipose tissue lipolysis and hormone-sensitive lipase expression during very-low-calorie diet in obese female identical twins. *J. Clin. Endocrinol. Metab.* **82:** 739-744.

41. Reynisdottir, S., D. Langin, K. Carlstrom, C. Holm, S. Rossner, and P. Arner. 1995. Effects of weight reduction on the regulation of lipolysis in adipocytes of women with upper-body obesity. *Clin. Sci.* **89:** 421-429.

42. Lewis, G. F., N. M. O'Meara, P. A. Soltys, J. D. Blackman, P. H. Iverius, W. L. Pugh, G. S. Getz, and K. S. Polonsky. 1991. Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *J. Clin. Endocrinol. Metab.* **72:** 934-944.

43. Baldo, A., A. D. Sniderman, S. St-Luce, R. K. Avramoglu, M. Maslowska, B. Hoang, J. C. Monge, A. Bell, S. Mulay, and K. Cianflone. 1993. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J. Clin. Invest.* **92:** 1543-1547.

44. Saleh, J., L. K. Summers, K. Cianflone, B. A. Fielding, A. D. Sniderman, and K. N. Frayn. 1998. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol

clearance by human adipose tissue in vivo in the postprandial period. *J. Lipid Res.* **39:** 884-891.

Legends to figures

Figure 1

Adipose tissue lipoprotein lipase (AT-LPL) activity of subcutaneous abdominal region as well as postheparin plasma LPL activity in men with high vs low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE.

Figure 2

Basal lipolytic rate and adenosine deaminase (ADA)-stimulated lipolysis in isolated adipocytes from the abdominal region of men with high vs low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE.

Figure 3A

Effect of epinephrine (EPI) on adenosine deaminase (ADA)-stimulated lipolysis in subcutaneous abdominal adipocytes of men with high vs low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE. Glycerol release was expressed as the difference between stimulated (with EPI) and ADA values. Negative values reflect inhibition of lipolysis.

Figure 3B

UK14304-induced inhibition of ADA-stimulated lipolysis in subcutaneous abdominal adipocytes of men with high vs low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Values are means ± SE. Fat cells were incubated in the presence of ADA (5 µg/ml). Antilipolysis is given as the difference between values in the presence of UK and ADA values. Agonist concentrations required for half-maximal inhibition of lipolysis (IC_{50}) were determined from these dose-response curves.

Figure 3C

Isoproterenol (ISO)-induced lipolysis in subcutaneous abdominal adipocytes of men with high vs low subcutaneous abdominal adipose cell α_2-adrenergic sensitivity. Fat cells were incubated without ADA and values are means ± SE. Agonist concentrations required for half-maximal stimulation of lipolysis (EC_{50}) were determined from these dose-response curves.

Figure 4

Individual values of subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity of high or low sensitive men. Significant difference between groups at ‡ $P < 0.0001$. Horizontal lines represent mean values.

Figure 5

Postprandial triglyceride concentrations of total, large, medium and small TRL in men with high (open symbols; n = 15) vs low (filled symbols; n = 11) subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Bars represent the areas under incremental curves (responses) of each subgroup. Data are means ± SE. Significant difference between groups at * $P < 0.05$, † $P < 0.01$.

Figure 6

Postprandial RP concentrations of total, large, medium and small TRL in men with high (open symbols; n = 15) vs low (filled symbols; n = 11) subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity. Bars represent the areas under incremental curves (responses) of each subgroup. Data are means ± SE. Significant difference between groups at * $P < 0.05$.

Table 1. Physical characteristics and fasting metabolic profile of men with high or low subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity.

	Subcutaneous abdominal adipocyte α_2-adrenoceptor sensitivity	
	High (n = 15)	**Low (n = 11)**
Age (years)	45 ± 10	48 ± 11
Body weight (kg)	90 ± 16	86 ± 11
BMI (kg/m^2)	30 ± 5	29 ± 3
Fat mass (kg)	24 ± 10	24 ± 7
% body fat	26 ± 7	27 ± 5
Abdominal adipose tissue areas (cm^2)		
Subcutaneous	291 ± 109	260 ± 57
Visceral	169 ± 50	159 ± 39
Abdominal fat cell weight (μg lipid/cell)	0.56 ± 0.12	0.56 ± 0.11
Triglycerides (mmol/l)	2.0 ± 0.7	3.4 ± 1.8 †
FFA (mmol/l)	0.7 ± 0.1	0.8 ± 0.1
Insulin (pmol/l)	106 ± 45	104 ± 43
Glucose (mmol/l)	5.2 ± 0.5	5.2 ± 0.7

Data are means ± SD

BMI = body mass index; FFA = free fatty acids

Significant difference between groups at † $P < 0.01$.

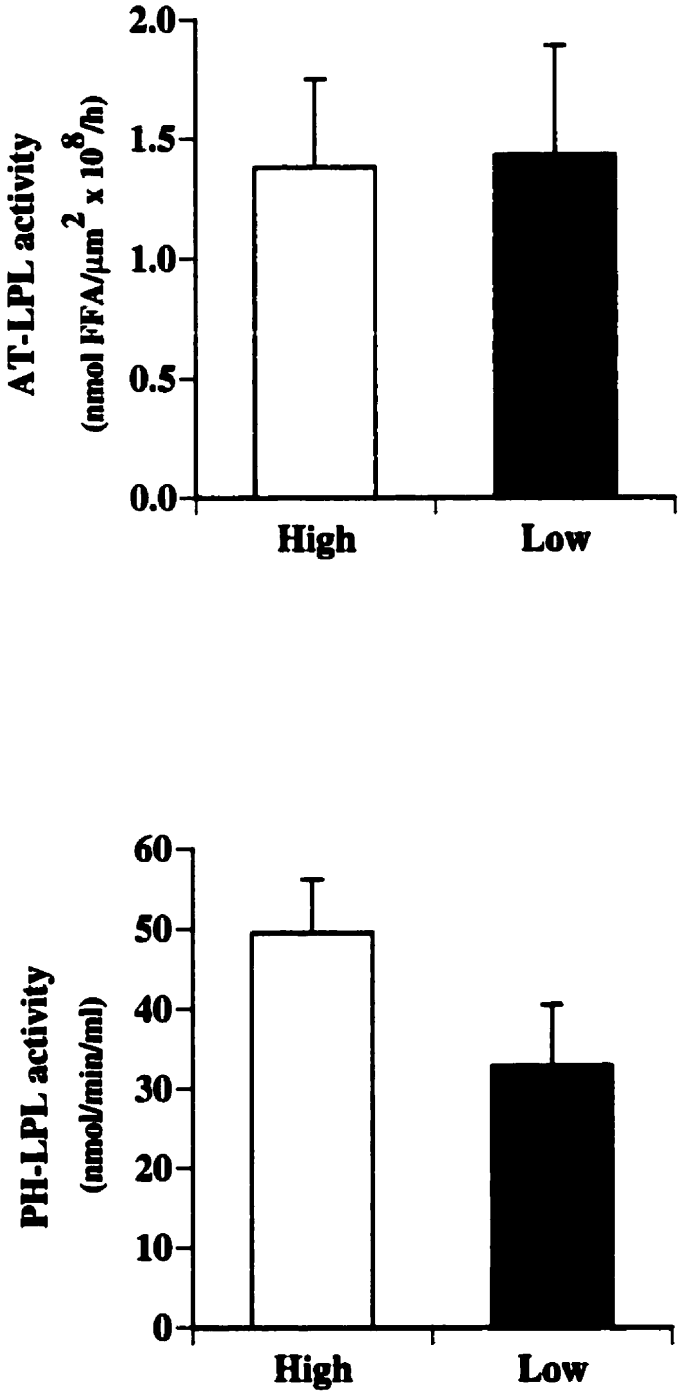

AT-LPL activity
(nmol FFA/μm^2 x 10^8/h)
2.0
1.5
1.0
0.5
0.0
High
Low
PH-LPL activity
(nmol/min/ml)
60
50
40
30
20
10
0
High
Low

Figure 1

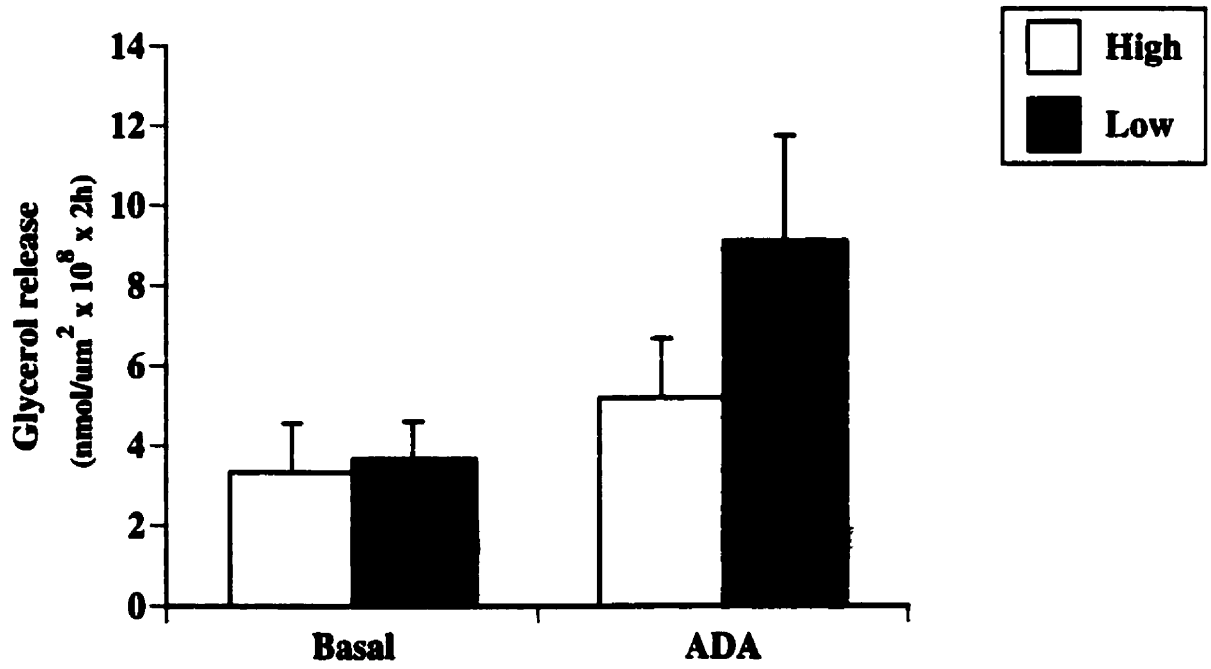

High
Low
Glycerol release
(nmol/um^2 x 10^8 x 2h)
14
12
10
8
6
4
2
0
Basal
ADA

Figure 2

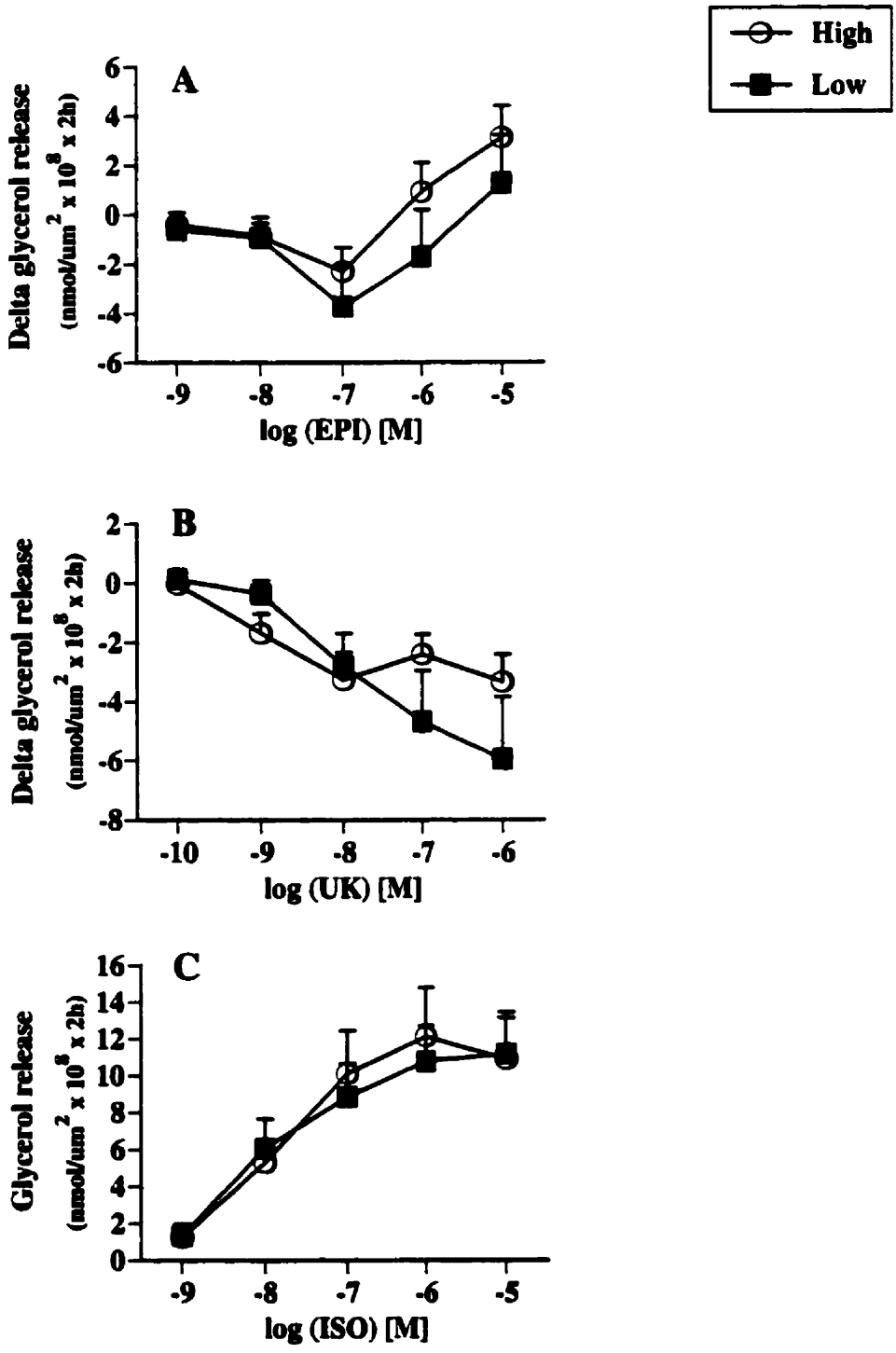

High
Low
A
Delta glycerol release
(nmol/um^2 x 10^8 x 2h)
6
4
2
0
-2
-4
-6
-9
-8
-7
-6
-5
log (EPI) [M]
B
Delta glycerol release
(nmol/um^2 x 10^8 x 2h)
2
0
-2
-4
-6
-8
-10
-9
-8
-7
-6
log (UK) [M]
C
Glycerol release
(nmol/um^2 x 10^8 x 2h)
16
14
12
10
8
6
4
2
0
-9
-8
-7
-6
-5
log (ISO) [M]

Figure 3

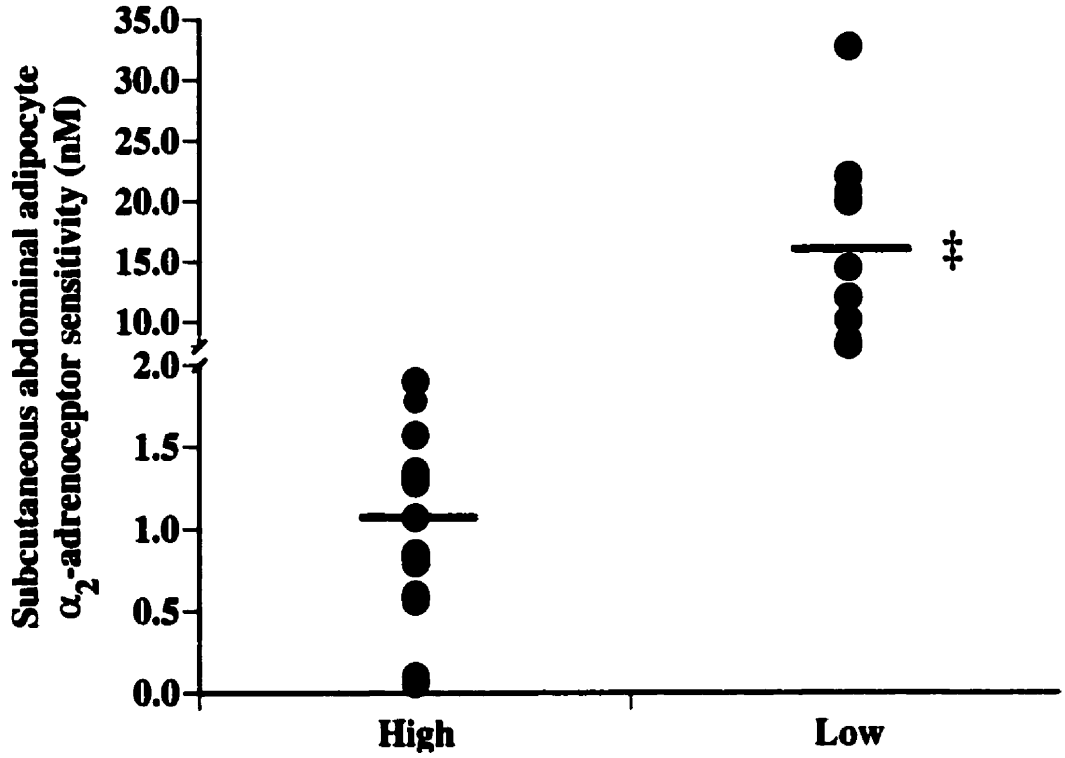

Subcutaneous abdominal adipocyte
α_2-adrenoceptor sensitivity (nM)
35.0
30.0
25.0
20.0
15.0
10.0
2.0
1.5
1.0
0.5
0.0
‡
High
Low

Figure 4

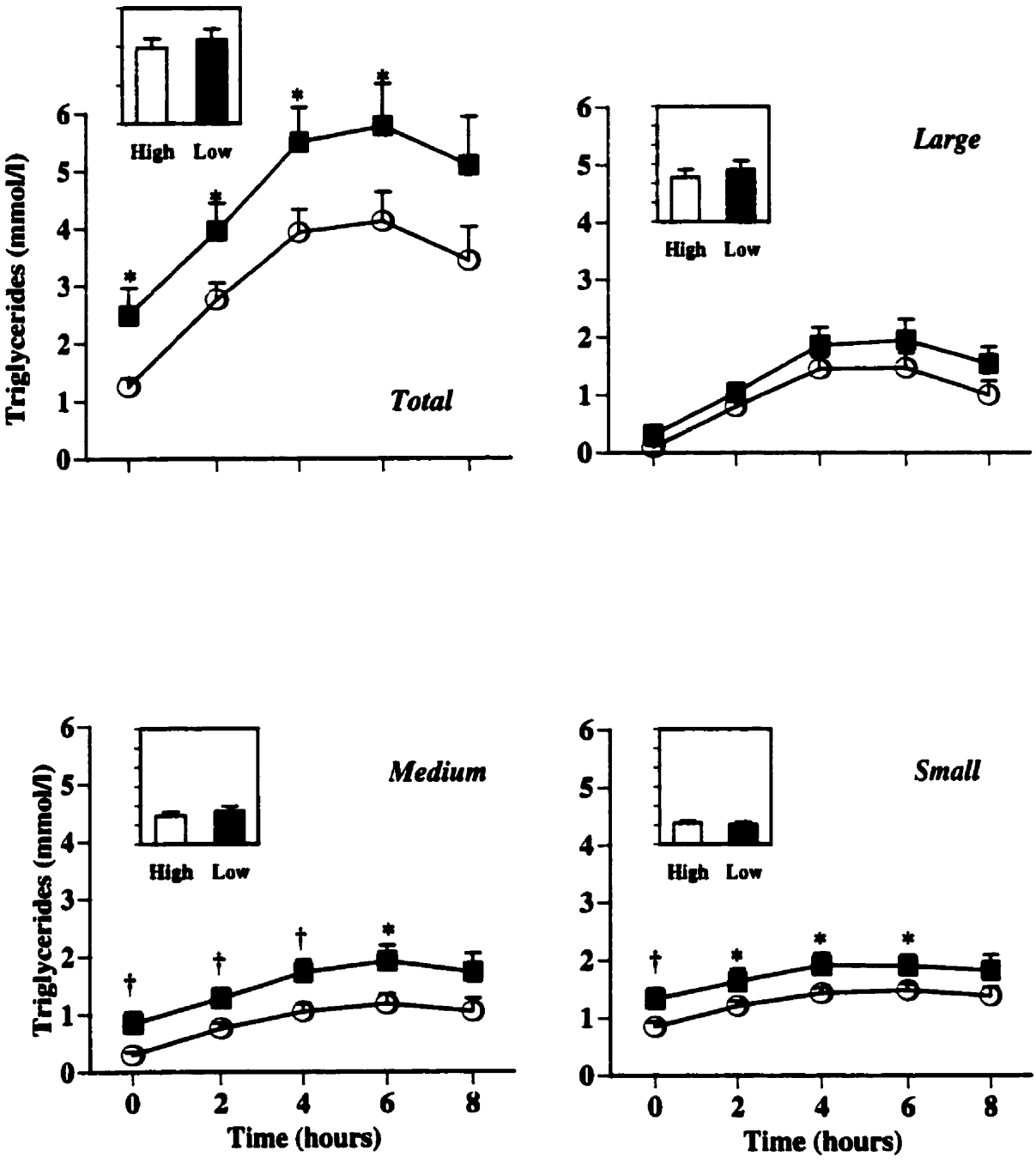

Triglycerides (mmol/l)
High Low
Total
Large
Medium
Small
Time (hours)
0
2
4
6
8

Figure 5

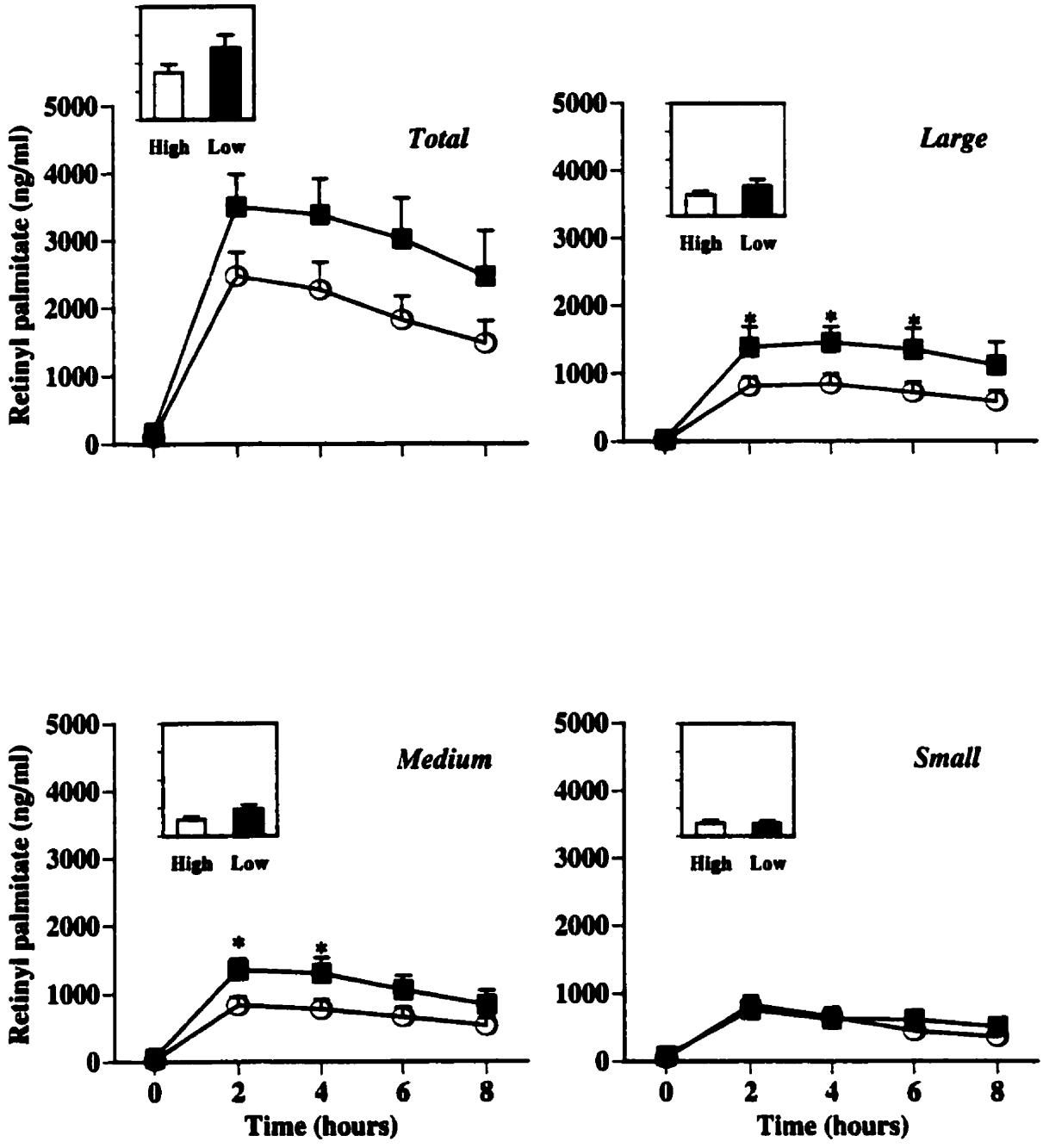

Total
Large
Medium
Small
High
Low
Retinyl palmitate (ng/ml)
5000
4000
3000
2000
1000
0
0
2
4
6
8
Time (hours)
*

Figure 6

CHAPITRE 12

LA STIMULATION LIPOLYTIQUE β-ADRÉNERGIQUE DES ADIPOCYTES DU SITE SOUS-CUTANÉ ABDOMINAL AGIT COMME DÉTERMINANT DE L'OXYDATION LIPIDIQUE CHEZ L'HOMME OBÈSE

L'article composant ce chapitre est intitulé:

"β-adrenoceptor-stimulated lipolysis of subcutaneous abdominal adipocytes as a determinant of fat oxidation in obese men"

(*European Journal of Clinical Investigation* 30; 290-296, 2000)

Résumé

Afin de vérifier si une altération de la capacité lipolytique des adipocytes sous-cutanés pouvait contribuer à une faible oxydation lipidique, les relations entre la lipolyse d'adipocytes isolés à partir des sites sous-cutanés (sc) abdominal et fémoral et le quotient respiratoire (QR) de 20 sujets obèses ont été étudiées. La réponse lipolytique maximale à l'isoprénaline était plus élevée au niveau des adipocytes sc abdominaux que fémoraux ($P < 0.01$). Par ailleurs, aucune différence régionale ne fut observée au niveau de la lipolyse stimulée par les agents postrécepteurs. La réponse lipolytique maximale à l'isoprénaline des adipocytes du site abdominal, mais non fémoral, était inversement reliée au QR ($r = -0.61$, $P < 0.01$) et positivement corrélée à l'oxydation lipidique ($r = 0.57$, $P < 0.01$). L'effet lipolytique maximal de l'isoprénaline au niveau des adipocytes sous-cutanés abdominaux fut la seule variable retenue comme prédicteur significatif de la variance du RQ (38%) et de l'oxydation lipidique (30 %) parmi les autres variables étudiées telles que la masse grasse, la masse maigre, la circonférence de taille et l'accumulation de tissu adipeux sous-cutané. En résumé, ces résultats suggèrent que l'activité lipolytique des adipocytes de la région sous-cutanée abdominale agit à titre de déterminant au niveau de l'oxydation lipidique chez des hommes obèses.

β-adrenoceptor-stimulated lipolysis of subcutaneous abdominal adipocytes as a determinant of fat oxidation in obese men.

Pascal Imbeault [1], Angelo Tremblay[1], Jean-Pierre Després [2,3], and Pascale Mauriège [1,3]

[1] Physical Activity Sciences Laboratory, Department of Social & Preventive Medicine, Laval University, [2] Quebec Heart Institute, Laval Hospital and [3] Lipid Research Center, CHUQ Medical Research Center, Québec, Canada.

Running head: Adipose tissue lipolysis and fat oxidation

Supported by Servier Canada, by the Fonds FCAR-Québec and by the Medical Research Council of Canada

Address for correspondence: Pascale Mauriège Ph.D., Physical Activity Sciences Laboratory, PEPS, Laval University, Ste-Foy, Québec, Canada, G1K 7P4
Tel: (418) 656-3851; Fax (418) 656-2441.

Abstract

Background: To verify whether an impaired lipolytic capacity of subcutaneous adipocytes may contribute to low rate of fat oxidation.

Design: Relationships between adipose tissue lipolysis of subcutaneous (subc) abdominal and femoral isolated adipocytes and respiratory quotient (RQ) were investigated in 20 obese men (age: 44 ± 5 yr; means ± SD) studied in the fasting state.

Results: Maximal isoproterenol-induced lipolysis was greater in subcutaneous abdominal than in femoral fat cells even if glycerol release was corrected for variation in cell surface area ($P < 0.01$). On the other hand, no regional variation was observed in the adipose cell lipolytic responses to postadrenoceptor agents such as dibutyryl-cyclic AMP, forskolin and theophylline. Maximal isoproterenol-induced lipolysis of subc abdominal, but not of femoral adipocytes, was inversely related to RQ ($r = -0.61$; $P < 0.01$) and positively associated to fat oxidation ($r = 0.57$; $P < 0.01$). These relationships were independent of possible confounding factors such as fat mass, fat-free mass, waist girth and subc abdominal adipose tissue accumulation assessed by computed tomography since maximal isoproterenol-induced lipolysis of subcutaneous abdominal adipocytes was the only variable retained as a significant predictor of RQ levels (38% of variance) and of fat oxidation (30% of variance).

Conclusion: These results suggest that adipose tissue lipolytic activity of subc abdominal adipocytes acts as a determinant of fat oxidation in obese men.

Key words: lipolysis, human adipocytes, respiratory quotient, lipid oxidation

Introduction

Fat balance appears to be the component of macronutrient balance which is exposed to greatest deviations, since dietary fat does not seem to present the ability to acutely promote its oxidation [1] and has a weak potential to inhibit subsequent energy intake [2-4]. Previous studies have also shown that fat is the substrate whose oxidation mostly depends on sympathetic stimulation, especially via β-adrenoceptors [5, 6]. This determinant role of the sympathetic nervous system activity in the regulation of fat metabolism has recently received further support from results showing that a low muscle sympathetic nerve activity is indepedently related to a reduced lipid oxidation [7]. Moreover, a low lipid oxidation capacity has, in turn, been shown to be a factor predisposing to weight gain over time [8, 9].
Evidence also supports the idea that obese and reduced-obese individuals display an impaired fat utilization in response to β-adrenergic stimulation and that this is mainly reflected by a decreased skeletal muscle nonesterified fatty acid (NEFA) uptake as well as by a reduced mobilization of NEFA and glycerol from adipose tissue [10-12]. Based on the hypothesis that a low adipose cell lipolytic response might be involved in the development of obesity, Snitker et al. [13] have recently reported that an impaired lipolytic response to isoproterenol measured by microdialysis in abdominal subcutaneous adipose tissue was associated with a low rate of sleeping fat oxidation in men.

In an attempt to verify whether *in vivo* lipid oxidation could also be related to *in vitro* adipose tissue metabolism, we examined whether maximal lipolysis in subcutaneous abdominal and femoral isolated adipocytes was related to respiratory quotient measured in the resting state in 20 obese men.

Material and Methods

Subjects

Twenty healthy Caucasian obese men were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication which could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc) were excluded from the study. All participants were sedentary (participate at no more than two continuous exercise sessions of 30 minutes per week), non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight reducing program, and their body weight had been stable during at least six months prior to the study.

Total body fatness and regional fat distribution

Body density was determined by the underwater weighing technique and percent body fat was derived from body density [14]. Pulmonary residual volume was measured using the helium dilution method [15]. Fat mass was calculated as total body weight minus fat-free mass. Waist girth was measured according to procedures recommended at the Airlie Conference [16].

Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology previously described by Sjöström et al. [17]. Subjects were examined in the supine position with both arms stretched above their head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) level, using an abdominal scout radiograph to establish the position of the scans to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing the AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) [18]. Abdominal visceral AT area was determined by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Adipocyte isolation and lipolysis

After an overnight fast, participants underwent biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the anterior midthigh level (femoral site). A small cutaneous incision (1 cm) was performed at both sites following local anesthesia (1% lidocaine, without epinephrine) and about 200 mg of subcutaneous adipose tissue were surgically removed from the two fat depots for the measurement of fat cell lipolysis.

Adipocytes were isolated according to the method of Rodbell [19] in a Krebs-Ringer bicarbonate buffer (pH 7.4) (KRB) containing 4% bovine serum albumin and 5 mM glucose (KRBA), plus 1 mg/ml collagenase, as previously described [20]. Digestion took place in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2, for 40 min at 37^0C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml KRBA. Isolated adipocytes were finally re-suspended in KRBA, in order to obtain a final concentration of approximately 500 cells per 50 µl.

Extracellular glycerol release was used as the indicator of adipocyte lipolysis. 50 µl aliquots of the continuously stirred cell suspension were placed in 1.5 ml conical tubes. Two of these tubes were used for cell counting and sizing; two others containing 10 µl KRB were immediately placed on ice and provided evaluation of the initial concentration of glycerol in the medium. Agents for lipolysis stimulation were added just before starting the incubation in 10 µl portions in order to obtain the desired final concentration. After a 2h incubation at 37^0C in a shaking water bath, under 95% O_2 and 5% CO_2 gas phase, 50 µl HCl (1N) were added to all tubes to stop the reaction, then 50 µl NaOH (1N) were added to neutralize the medium. All tubes were stored at -20^0C until glycerol determination and NADH concentration was measured by bioluminescence with luciferase solution, using an automated 2250 Dynatech luminometer [20, 21]. For each concentration of stimulator agent, the amount of glycerol was taken as the average of the quantities obtained from the two incubated tubes. Glycerol measurement by bioluminescence is very sensitive and especially well adapted when only small amounts of adipose tissue are available [20, 21]. Adipose cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ, USA).

Mean fat cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, as previously described [22].

The lipolytic activity of the isolated fat cells was tested with isoproterenol [21]. Complete dose-response curves (from 10^{-9} to 10^{-5} M) were performed with this non selective β-adrenoceptor agonist. The β-adrenergic sensitivity was considered as the concentration of isoproterenol giving half-maximal stimulation of lipolysis (EC_{50}) and was evaluated by logarithmic conversion of each dose-response curve. The higher was the EC_{50} (isoproterenol) value, the lower was the β-adrenergic sensitivity. Some experiments were also conducted with forskolin (direct activator of adenylate cyclase), dibutyryl adenosine 3', 5' cyclic monophosphate (dibutyryl cAMP) (stimulator of the protein kinase hormone-sensitive lipase complex and phophodiesterase-resistant cyclic AMP analogue) and theophylline (mainly inhibitor of cyclic GMP-inhibited phosphodiesterase, cGI-PDE) [21]. Lipolysis was expressed either per cell number (ie, in μmol of glycerol/10^6 cells x 2h) or per unit of cell surface area (ie, in nmol of glycerol/μm^2 x 10^8 x 2h). The latter mode of expression was used to correct lipolytic values for variation in fat cell size which is a well-known correlate of lipolysis [21]. The responsiveness was expressed as the difference between basal glycerol release and the lipolytic rate at maximum effective concentration of the agents tested (10^{-6} M isoproterenol, 10^{-5} M forskolin, 10^{-3} M dibutyryl cAMP or theophylline). It shoud be noted that maximal isoproterenol-induced lipolysis was obtained at 10^{-6} M instead of 10^{-5} M, as previously shown by others [23], and then explained why we further used the former lipolytic response in our analyses.

Respiratory quotient and lipid oxidation

The relative amounts of macronutrients oxidized by the subjects was reflected by the respiratory quotient (RQ), the molar ratio between CO_2 produced and O_2 consumed. RQ was measured by indirect calorimetry after an overnight fast of at least 10 hours. Subjects were asked to be sedentary 48h before the resting metabolic measurements. After a 15-min resting period, expired gas collection was achieved through a mouthpiece, while the nose was clipped

for a 15-min period. Oxygen and carbon dioxide concentrations were determined by non-dispersive infrared analysis (Uras 10 E, Hartmann & Braun, Germany) whereas pulmonary ventilation determination was assessed with a S-430A measurement system (Ventura, CA). The calculation of rates of fat oxidation was derived according to the following equation by assuming that protein oxidation was negligible [24]:

$$\text{Fat oxidation (g/min)} = 1.67 \times VO_2 \text{ (l/min)} - 1.67 \times VCO_2 \text{ (l/min)}$$

Statistical analyses

Regional variation for adipocyte size as well as maximal lipolytic capacity were tested with the Student's t-test. Associations between two variables were quantified by the Pearson's product-moment correlation coefficients. Stepwise multiple regression analyses were also performed to evaluate predictability of RQ and fat oxidation. All analyses were performed using the Jump program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers (version 3.2.2).

Results

Characteristics of the subjects are presented in Table 1. Subcutaneous (subc) adipocytes were larger in the abdominal than in the femoral depot ($P < 0.05$). The wide range of adiposity of our sample was reflected by the large range of adipocyte size observed in both depots (0.40 - 0.92 and 0.35 - 0.72 μg lipid/cell for the abdominal and the femoral sites, respectively).

Insert Table 1

Adipose tissue lipolysis

As illustrated in Figure 1, the basal lipolytic rate tended to be higher in subc abdominal than in femoral adipose cells, but this difference did not reach significant level ($P = 0.07$). The maximal lipolytic responses to isoproterenol (β-agonist) and to different postadrenoceptor agents were also investigated in both subc abdominal and femoral adipocytes (Figure 1). With the exception of isoproterenol-induced lipolysis which was greater in abdominal than in femoral adipocytes ($P < 0.01$), no further regional variation was observed when lipolysis was maximally stimulated with DcAMP, forskolin or theophylline. However, the β-adrenergic sensitivity (EC_{50}) was greater in subc abdominal than in femoral adipocytes (21 ± 4 vs 35 ± 6 nM; $P < 0.05$).

Insert Figure 1

Correlational analyses

As shown in Figure 2, the maximum lipolytic response of subc abdominal adipocytes to isoproterenol (10^{-6} M) was negatively related to basal RQ ($r = -0.61$; $p < 0.01$) but positively associated to subjects' lipid oxidation ($r = 0.57$; $P < 0.01$). Moreover, subc abdominal fat cell β-adrenergic sensitivity assessed by the EC_{50} (isoproterenol) was neither related to basal RQ, nor to lipid oxidation ($-0.15 < r < 0.12$; NS). On the other hand, maximal femoral adipose cell lipolytic response to the β-agonist and the β-adrenergic sensitivity were not significantly

related to subjects' RQ and lipid oxidation. No relationship was observed between basal lipolytic rate of either abdominal or femoral adipocytes and subjects' RQ ($-0.21 < r < 0.21$; NS) (not shown).

Insert Figure 2

Stepwise multiple regression analysis

To estimate the respective contribution of regional adiposity and of maximal isoproterenol-induced lipolysis of subc abdominal adipocytes in accounting for variance in basal RQ and fat oxidation, stepwise multiple regression analysis was performed. Our model included age, fat mass, fat-free mass, subc abdominal adipose tissue accumulation measured by CT as well as maximal isoproterenol-induced lipolysis of subc abdominal adipocytes. Maximal subc abdominal adipocyte isoproterenol-induced lipolysis was the only variable retained in the model and accounted for 38% and 30% of the variance in basal RQ and fat oxidation, respectively ($P < 0.01$).

Discussion

The present study was performed to verify whether *in vitro* maximal isoproterenol induced-lipolysis in subcutaneous abdominal and femoral adipocytes was related to basal RQ in 20 obese men. Our results showed that a low maximal isoproterenol-induced lipolytic response of subcutaneous abdominal adipocytes is related to a low rate of fat oxidation, a finding consistent with the recent observation that *in situ* abdominal adipose tissue lipolytic response to isoproterenol was inversely related to basal RQ [13].

The impaired ability to use fat has previously been identified as a factor related to the development of obesity [8, 9]. Evidence also indicates that fat oxidation is mainly dependent on sympathetic activity [5, 6], a notion which has recently been reemphasized by the inverse relationship found between 24-h RQ and muscle sympathetic nerve activity [7]. Moreover, the sympathetic nervous system plays an important role in the regulation of lipolysis since norepinephrine and epinephrine act as stimulators of lipolysis via their action on β-adrenoceptors in human adipocytes [25, 26]. Blaak et al. have well demonstrated that during infusion of isoproterenol, obese [10] and reduced-obese subjects [11] had an impaired lipolytic response as well as a low fat utilization capacity. Furthermore, a low epinephrine-induced lipolysis was observed in ex-obese runners even if they were performing a large amount of weekly aerobic training [12]. This is also consistent with the finding that resting RQ is increased in reduced-obese subjects showing resistance to lose fat while being physically active [27]. These results suggest that a low adipose tissue lipolytic activity may be partly responsible for a low fat oxidation. As demonstrated in a recent study [13] and in the present one, an impaired β-adrenergic-stimulated maximal lipolysis, which highly reflects adipose tissue lipolytic activity, is associated with a low rate of fat utilization.

The lower maximal-induced lipolysis observed in femoral as compared to subcutaneous abdominal adipocytes of our sample is in accordance with previous studies [28] and probably attests for a decreased β-adrenergic component in femoral fat cells [29]. Moreover, the fact

that β-adrenergic sensitivity was greater in subcutaneous abdominal than femoral adipocytes indicates that abdominal adipocytes of obese men display a more efficient β-adrenergic component than femoral fat cells. Based on the notion that lipolytic rate is a determinant of body fat oxidation [30], the low β-adrenergic-mediated lipolysis of femoral adipocytes might explain their negligible contribution to variations in basal RQ.

As recently reported [13], a high *in situ* lipolytic response to isoproterenol in subcutaneous abdominal adipose tissue is related to a high rate of fat oxidation, regardless of the subjects' body fatness. This notion is also confirmed by the fact that isoproterenol-induced maximal lipolysis in subcutaneous abdominal adipocytes was the only predictor of RQ. However, one should be aware that only 38% of the variance in basal RQ was predicted by isoproterenol-induced maximal lipolysis in subcutaneous abdominal adipocytes. This finding implies that other important factors contributing to lipid oxidation exist and remain to be identified. In this regard, an impaired ability of muscle to take up NEFA has already been pointed out as an important culprit to a low rate of fat oxidation [10, 31]. The effect of age should also be considered since aging has been related to an impaired adipose tissue lipolytic capacity [32, 33] as well as a reduced capacity of skeletal muscle to oxidize fatty acids [34]. In the present study, we did not observe any significant effect of age on the maximal isoproterenol-induced lipolysis as well as on the lipid oxidation of subjects, but this might be explained by the narrow age range of the subjects.

In summary, the present study shows that a low maximal lipolytic response of subcutaneous abdominal adipocytes to isoproterenol is associated with a reduced fat oxidation rate in obese men. This finding supports a recent *in vivo* study involving abdominal adipose tissue lipolysis in the determination of whole-body fat/carbohydrate oxydation rate. On the other hand, the maximal lipolytic activity of femoral adipocytes does not appear to contribute to the variation of fat oxidation, probably because of their lower β-adrenoceptor component, as compared to subcutaneous abdominal fat cells.

Acknowledgements

The authors wish to express their gratitude to Sylvie St-Pierre, Éric Doucet and Henri Bessette for their collaboration at various stages of the study as well as to Dr Gilles Lortie for his medical supervision. Thanks are also expressed to Suzanne Brulotte of the Department of Radiology (Laval University Hospital, Québec, Canada) for her help with the use of the computed tomograph. The subjects and the staff of the Lipid Research Center are also gratefully acknowledged.

References

1. Flatt JP, Ravussin E, Acheson KJ and Jequier E. Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 1985; 76: 1019-24.
2. Lawton CL, Burley VJ, Wales JK and Blundell JE. Dietary fat and appetite control in obese subjects: weak effects on satiation and satiety. Int J Obesity 1993; 17: 409-16.
3. Blundell JE, Burley VJ, Cotton JR and Lawton CL. Dietary fat and the control of energy intake: evaluating the effects of fat on meal size and postmeal satiety. Am J Clin Nutr 1993; 57: S772-S7.
4. Stubbs RJ, Murgatroyd PR, Goldberg GR and Prentice AM. Carbohydrate balance and the regulation of day-to-day food intake in humans. Am J Clin Nutr 1993; 57: 897-903.
5. Tremblay A, Coveney JP, Després JP, Nadeau A and Prud'homme D. Increased resting metabolic rate and lipid oxidation in exercise-trained individuals: evidence for a role of beta adrenergic stimulation. Can J Physiol Pharmacol 1992; 70: 1342-7.
6. Acheson KJ, Ravussin E, Schoeller DA, Christin L, Bourquin L, Baertschi P et al. Two-week stimulation or blockade of the sympathetic nervous system in man: influence on body weight, body composition, and twenty four-hour energy expenditure. Metabolism 1988; 37: 91-8.
7. Snitker S, Tataranni PA and Ravussin E. Respiratory quotient is inversely associated with muscle sympathetic nerve activity. J Clin Endocrinol Metab 1998; 83: 3977-9.
8. Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF et al. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. Am J Physiol 1990; 259: E650-7.
9. Seidell JC, Muller DC, Sorkin JD and Andres R. Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore longitudinal study on aging. Int J Obesity 1992; 16: 667-74.
10. Blaak EE, Van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA and Saris WH. Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. Am J Physiol 1994; 267: E306-15.

11. Blaak EE, Van Baak MA, Kemerink GJ, Pakbiers MT, Heidendal GA and Saris WH. Beta-adrenergic stimulation of skeletal muscle metabolism in relation to weight reduction in obese men. Am J Physiol 1994; 267: E316-22.

12. Tremblay A, Després JP and Bouchard C. Adipose tissue characteristics of ex-obese long-distance runners. Int J Obesity 1984; 49: 799 - 805.

13. Snitker S, Hellmér J, Boschmann M, Monroe MB and Ravussin E. Whole body fat oxidation is related to in situ adipose tissue lipolytic response to isoproterenol in males. Am J Physiol 1998; 275: E400-4.

14. Siri WE. The gross composition of body fat. Adv Biol Med Physiol 1956; 4: 239-80.

15. Meneely GR and Kaltreider NL. Volume of the lung determined by helium dilution. J Clin Invest 1949; 28: 129-39.

16. Lohman TG, Roche AF and Martorell R. The Airlie (VA) consensus conference. In: Lohman TG, Roche AF and Martorell R, eds. Anthropometric Standardisation Reference Manual. Champaign (Illinois): Human Kinetics Publishers, 1988: 39-80.

17. Sjöström L, Kvist H, Cederblad A and Tylen U. Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. Am J Physiol 1986; 250: E736-E86.

18. Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, Moorjani S et al. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr 1989; 61: 139-48.

19. Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 1964; 239: 375-80.

20. Mauriège P, Després JP, Prud'homme D, Pouliot MC, Marcotte M, Tremblay A et al. Regional variation in adipose tissue lipolysis in lean and obese men. J Lipid Res 1991; 32: 1625-33.

21. Mauriège P, Prud'homme D, Lemieux S, Tremblay A and Després JP. Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. Am J Physiol 1995; 269: 341-50.

22. Després JP, Bouchard C, Bukowiecki L, Savard R and Lupien J. Morphology and metabolism of human fat cells: a reliability study. Int J Obesity 1983; 7: 231-40.

23. Wahrenberg H, Lönnqvist F and Arner P. Mechanisms underlying regional differences in lipolysis in human adipose tissue. J Clin Invest 1989; 84: 458-67.

24. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 1983; 55: 628-34.

25. Lafontan M and Berlan M. Fat cell adrenergic receptors and the control of white and brown fat cell function. J Lipid Res 1993; 34: 1057-91.

26. Arner P. Regulation of lipolysis in fat cells. Diabetes Rev 1996; 4: 1-13.

27. Doucet E, Imbeault P, Alméras N and Tremblay A. Physical activity and low-fat diet: Is it enough to maintain weight stability in the reduced-obese individual following weight loss by drug therapy and energy restriction? Obesity Res 1999; 7: 323-33.

28. Leibel RL and Hirsch J. Site- and sex-related differences in adrenoceptor status of human adipose tissue. J Clin Endocrinol Metab 1987; 64: 1205-10.

29. Arner P, Hellström L, Wahrenberg H and Brönnegard M. Beta-adrenoceptor expression in human fat cells from different regions. J Clin Invest 1990; 86: 1595-600.

30. Groop LC, Bonadonna RC, Simonson DC, Petrides AS, Shank M and DeFronzo RA. Effect of insulin on oxidative and nonoxidative pathways of free fatty acid metabolism in human obesity. Am J Physiol 1992; 263: E79-E84.

31. Colberg SR, Simoneau JA, Leland Theate F and Kelley DE. Skeletal muscle utilization of free fatty acids in women with visceral obesity. J Clin Invest 1995; 95: 1846-53.

32. Lönnqvist F, Nyberg B, Wahrenberg H and Arner P. Catecholamine-induced lipolysis in adipose tissue of the elderly. J Clin Invest 1990; 85: 1614-21.

33. Imbeault P, Prud'homme D, Tremblay A, Després JP and Mauriège P. Adipose tissue metabolism in men: a clue regarding age-related obesity. Int J Obesity 1999; 23: S69.

34. Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM et al. Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women. J Gerontol 1992; 47: B71-6.

Legends to figures

Figure 1

Maximal lipolytic responses of subcutaneous abdominal and femoral adipocytes to isoproterenol and postadrenoceptor agents. Fat cells were incubated in the presence of either isoproterenol (ISO; 10^{-6} M), dibutyryl-cyclicAMP (DcAMP; 10^{-3} M), forskolin (FK; 10^{-5} M) or theophylline (THEO; 10^{-3} M). Previous experiments revealed that the concentrations of different drugs used were maximally effective doses. Values are means ± SE of 19-20 experiments performed in duplicate and basal glycerol release has already been subtracted. Significant regional variation at * $P < 0.01$.

Figure 2

Relationships between isoproterenol-induced maximal lipolysis (left panels) or β-adrenoceptor sensitivity reflected by EC_{50} (isoproterenol) (right panels) of subcutaneous abdominal adipocytes and basal respiratory quotient (RQ) or lipid oxidation. Maximal lipolytic response to isoproterenol was calculated as the difference between glycerol release at 10^{-6} M of the β-agonist and the basal lipolytic rate.

Table 1. Characteristics of subjects

	Means ± SD	Range
Age (years)	44 ± 4	35-50
Anthropometric variables		
Body weight (kg)	100 ± 12	82-122
BMI (kg/m^2)	33 ± 3	27-40
Fat mass (kg)	34 ± 9	14-44
Body fat (%)	34 ± 6	17-44
Fat-free mass (kg)	66 ± 8	52-79
Waist girth (cm)	108 ± 9	92-124
Adipose tissue areas measured by CT (cm^2)		
Abdomen (L4-L5)		
Subcutaneous	364 ± 103	185-594
Visceral	180 ± 55	93-282
Regional subcutaneous fat cell weight (µg lipid/cell)		
Abdominal	0.61 ± 0.11 [a]	0.40-0.92
Femoral	0.55 ± 0.10	0.35-0.72
Indirect calorimetry		
Basal RQ	0.81 ± 0.05	0.72-0.89

Values are means ± standard deviation (SD) of 20 men.

BMI = body mass index; CT = computed tomography; RQ = respiratory quotient.

Significant regional variation at [a] $P < 0.05$.

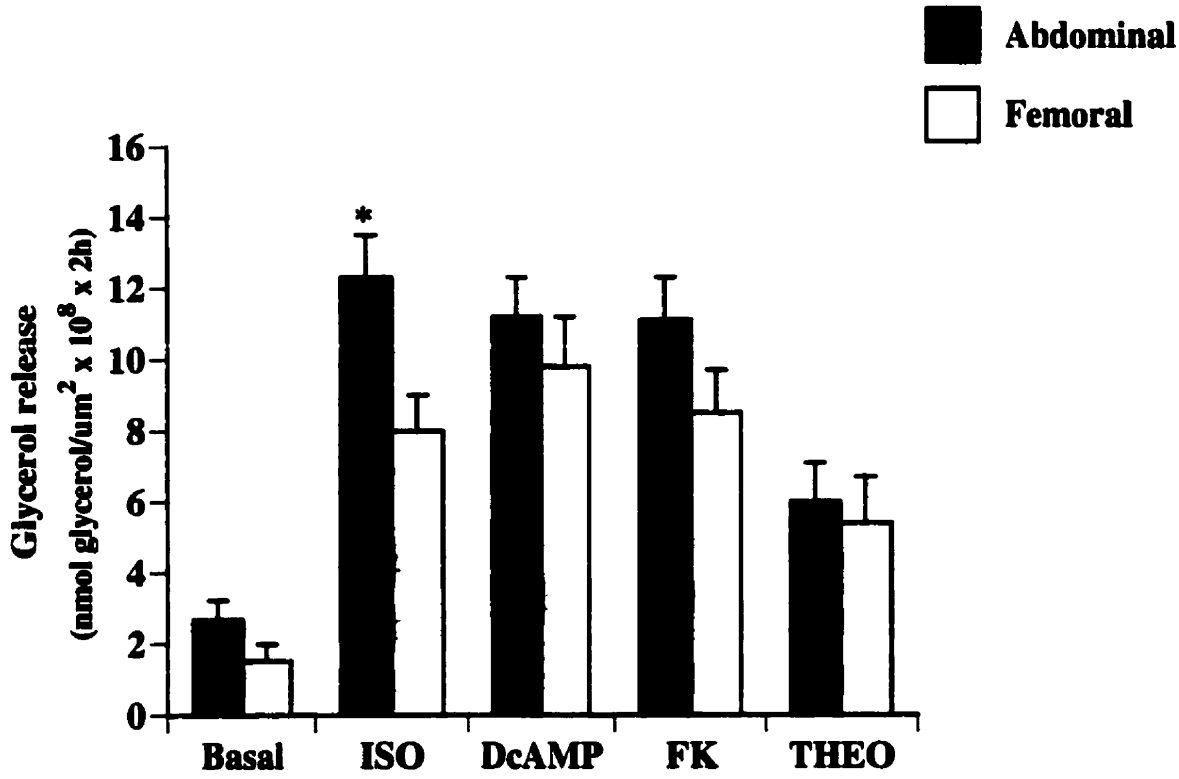

Abdominal
Femoral
Glycerol release
(nmol glycerol/um^2 x 10^8 x 2h)
16
14
12
10
8
6
4
2
0
*
Basal
ISO
DcAMP
FK
THEO

Figure 1

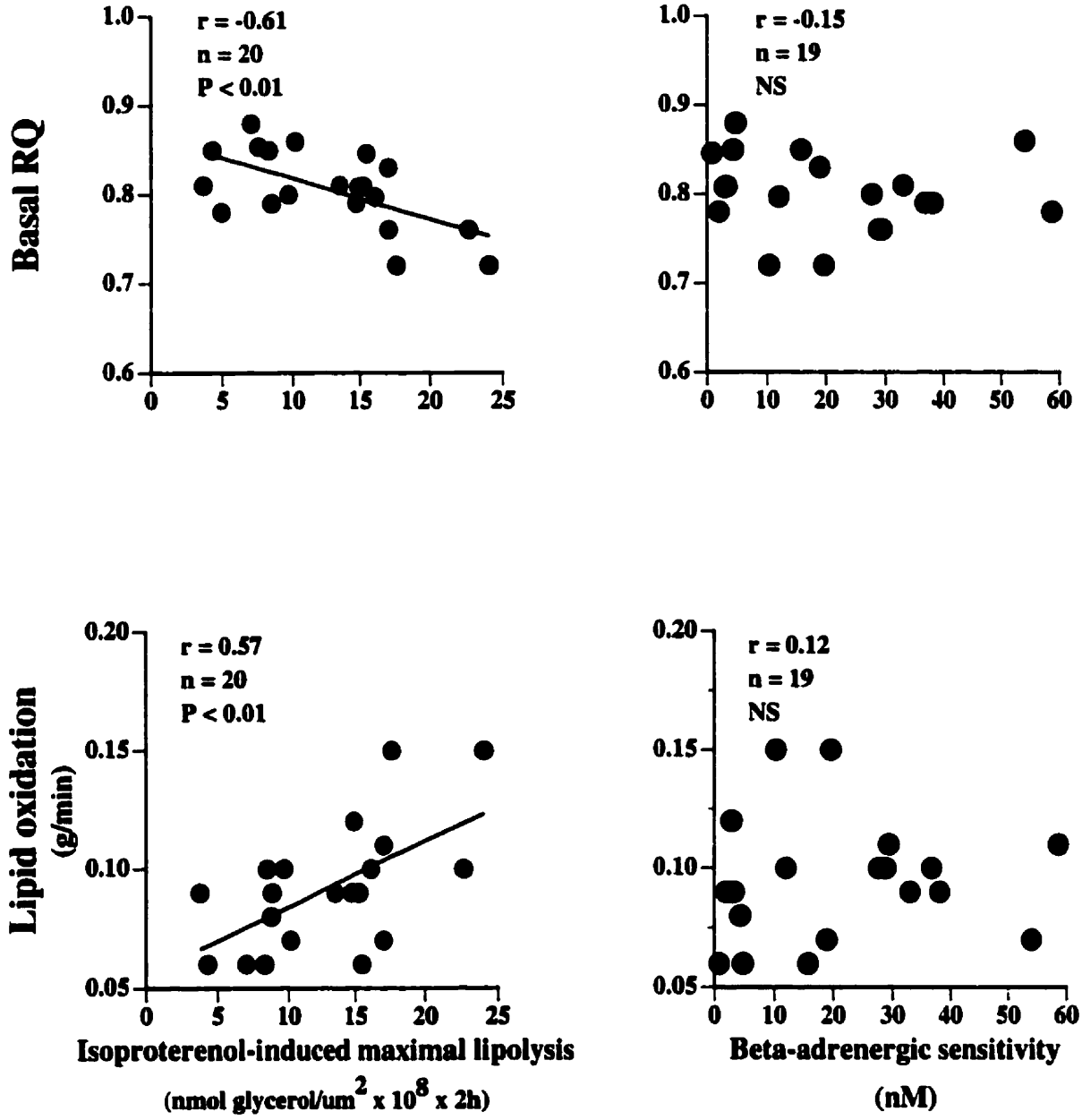

Basal RQ
r = -0.61
n = 20
P < 0.01
r = -0.15
n = 19
NS
Lipid oxidation (g/min)
r = 0.57
n = 20
P < 0.01
r = 0.12
n = 19
NS
Isoproterenol-induced maximal lipolysis
(nmol glycerol/um2 x 10^8 x 2h)
Beta-adrenergic sensitivity
(nM)

Figure 2

CHAPITRE 13

RÉGULATION DU TRANSCRIT ADIPOCYTAIRE PPARγ EN RÉPONSE À UNE PERTE DE POIDS CHEZ L'HUMAIN: ABSENCE DE RELATIONS AVEC LES CONCENTRATIONS PLASMATIQUES ET DU TISSU ADIPEUX DE POLLUANTS

L'article composant ce chapitre est intitulé:

"Regulation of adipose tissue PPARγ gene expression in response to weight loss in humans: absence of interaction with plasma and adipose tissue pollutant levels"

(en préparation)

Résumé

La présente étude consistait à examiner les effets d'une perte de poids sur l'expression de PPARγ (*peroxisome proliferator-activated receptor gamma)*, un facteur transcriptionnel important impliqué dans la différenciation adipocytaire, au niveau des tissus adipeux sous-cutanés abdominal et fémoral. De plus, nous avons vérifié s'il existait des relations entre les changements induits par la perte de poids au niveau de l'expression de PPARγ du tissu adipeux et les variations de concentrations en organochlorés (OC) plasmatiques et du tissu adipeux chez 7 sujets obèses. La mesure de l'expression de PPARγ par RT-PCR compétitive à partir d'échantillons de tissu adipeux ainsi que les concentrations plasmatiques et adipeuses de polluants plasmatiques furent mesurées avant et après perte de poids (durée de 15 semaines; déficit énergétique de 500-800 kcal/jour). Tel que prévu, la restriction énergétique a entraîné une diminution significative de la masse corporelle ($P < 0.001$) et de la masse grasse ($P < 0.01$). En réponse à la perte de poids, l'expression de PPARγ ne diminua de façon significative qu'au niveau du site sous-cutané abdominal ($P < 0.05$). La concentration plasmatique de 10 des 17 OC étudiés et de 4 des 14 composés détectés au niveau des sites adipeux fut augmentée. Aucune relation significative ne fut observée entre les changements de PPARγ induits par la perte de poids et ceux des concentrations d'OC plasmatiques ($-0.63 < rho < 0.70$) et du tissu adipeux ($-0.80 < rho < 0.52$). Ces résultats montrent qu'une perte de poids entraîne une réduction significative du transcrit PPARγ au niveau du dépôt adipeux sous-cutané abdominal. L'absence de relations entre l'augmentation des concentrations d'OC plasmatiques et du tissu adipeux et les changements d'expression de PPARγ en réponse à une perte de poids suggère que les OC étudiés ne semblent pas influencer directement l'expression de ce facteur transcriptionnel de la différenciation adipocytaire.

Regulation of adipose tissue PPARγ gene expression in response to weight loss in humans: absence of interaction with plasma and adipose tissue pollutant levels

Pascal Imbeault [1], Hubert Vidal [2], Jonathan Chevrier [1], Éric Dewailly [1], Pierre Ayotte [1], Angelo Tremblay [1] and Pascale Mauriège [1,3]

[1] Department of Social & Preventive Medicine, Laval University, Ste-Foy, Québec, Canada; [2] INSERM U.449, Faculté de Médecine R.T.H. Laënnec, Lyon, France; [3] Lipid Research Center, Laval University Medical Research Center, Québec, Canada.

Running head: Adipose tissue PPARγ mRNA levels, weight loss and pollutants.

Supported by the Fonds FCAR-Québec.

Address for correspondence: P. Mauriège Ph.D.,
Department of Social & Preventive Medecine
Division of Kinesiology
Laval University, Ste-Foy, Québec
Canada, G1K 7P4
Tel: (418) 656-3851
Fax (418) 654-2441
email: diabolo@internetclub.fr

Abstract

Objective: To examine the effect of a body weight loss on PPARγ mRNA levels of subcutaneous abdominal and femoral adipose depots and to verify the existence of a relationship between weight loss-induced changes in PPARγ mRNA levels of subcutaneous abdominal and femoral adipose depots and those in plasma and adipose tissue concentrations of organochlorine pollutants in 7 obese subjects.

Research Methods and Procedures: Subjects underwent a 15-week weight-reducing program (energy deficit: 500-800 kcal/day below subjects' estimated sedentary energy expenditure). PPARγ mRNA levels of adipose tissue from the abdominal and femoral depots, various fat distribution parameters (assessed by computed tomography and anthropometry) and plasma or adipose tissue concentrations of organochlorine compounds were measured at baseline and after the 15-week weight reducing program.

Results: Dietary restriction induced a reduction in body weight ($P < 0.001$), fat mass ($P < 0.01$), subcutaneous abdominal and midthigh adipose tissue areas ($P < 0.001$). In response to weight loss, PPARγ mRNA levels were significantly reduced in the subcutaneous abdominal adipose tissue site ($P < 0.05$), whereas this decrease did not reach statistical significance in the femoral region. In plasma, 10 of the 17 organochlorine pollutants were significantly increased in response to weight loss. On the other hand, statistically significant increases were observed only in 6 of the 14 detected compounds of both subcutaneous abdominal and femoral fat depots. In general, no significant relationship was found between weight loss-induced changes in PPARγ mRNA levels and those in plasma or adipose tissue organochlorine pollutants.

Discussion: Weight reduction induced a significant decrease of PPARγ mRNA levels in subcutaneous abdominal adipose tissue but not in the femoral depot. The weight loss-induced increases in plasma and subcutaneous abdominal adipose tissue concentrations of organochlorine pollutants were generally not related to changes in PPARγ mRNA levels, which may suggest that pollutants are not directly associated with adipose cell differentiation through PPARγ gene expression interaction.

Introduction

Quality of life and comfort have considerably increased during the industrialization era for most of Western countries. However, this period of time has also been characterized by the production of cheap petrochemical compounds displaying high resistance to degradation. These compounds were used as highly effective pesticides, dielectrics, fire retardants and were included in ink, plastic and rubber mixtures. Moreover, due to their lipophilicity, these compounds preferentially bioaccumulate and biomagnify in higher trophic levels of the food chain (24,29). Consequently, although the use of these substances has been banned or restricted in Western countries, they are still found in virtually every person on the planet and might play adverse effects in humans (29).

Based on the fact that pollutants are stored in fat, it has been postulated that adipose tissue loss could result in increased organ and blood concentrations of these compounds (16). A previous study has confirmed this hypothesis by reporting increased dichlorodiphenyl trichloroethane (DDT)-related compounds concentrations in blood, adipose tissue, heart, lung, spleen and brain in starved mice which were a priori treated with a load of ^{14}C-DDT (27). In a recent study, we have examined the impact of body weight loss on plasma and adipose tissue organochlorine compounds (OCs) in obese individuals. As expected, body weight loss induced a 10 to 29% increase in plasma concentrations of detected OCs (7). This study also raises concerns about undesired and potentially harmful side effects of weight loss in some obese subjects since reduced-obese individuals had greater concentrations of these contaminants as compared to lean controls.

In the last years, many studies have focused on nuclear receptors due to their critical roles in several aspects of vertebrate development by converting the effects of small lipophilic hormones, the peroxisome proliferators, into transcriptional responses (4,22). Peroxisome proliferators are a diverse group of chemicals that include hypolipidemic drugs, herbicides and industrial plasticizers (9,13). Recent studies have shown that many genes affected by peroxisome proliferators are regulated by a novel member of the steroid hormone receptor superfamily, the peroxisome proliferator-activated receptor (PPAR) (31). The PPAR family

comprises three closely related gene products: PPARα, PPARγ and PPARβ/δ. The three PPARs have different expression patterns: PPARα is most abundantly expressed in liver, kidney, heart and muscle; PPARγ is mainly expressed in fat cells, large intestine and cells of the monocyte lineage; and the distribution of PPARβ/δ is ubiquitous (1,31). It has previously been demonstrated that the ligand-binding of Xenopus laevis PPARγ was activated by trichloroacetic acid, one of the secondary metabolites of trichloroethylene which is a widely used pollutant in dry cleaning and paint stripping (9). More recently, investigation of human PPARγ revealed that the transcriptional activity of this receptor was stimulated by mono(2-ethylhexyl)phthalate (MEHP), a metabolite of the industrial plasticizer di(2-ethylhexyl)phthalate (DEHP) (21). The fact that OCs are lipophilic may implicate a role of these latter agents in the regulation of adipose tissue metabolism. To the best of our knowledge, no study has yet considered the potential interaction of contaminants on PPARγ expression in human adipose tissue, a key transcriptional factor of adipocyte differentiation (35). The major reason for this is that ethically, it is unacceptable to study the impact of contaminants by purposely exposing individuals to suspected pollutants. Nevertheless, the increase in OCs that we have recently observed following weight loss in obese individuals (7) represents a valuable model to verify whether PPARγ expression in adipose tissue might be influenced by pollutants. Therefore, the aim of this study was a) to examine the effect of a body weight loss on PPARγ mRNA levels of subcutaneous abdominal and femoral adipose depots and b) to verify the existence of a relationship between weight loss-induced changes in PPARγ mRNA levels of subcutaneous abdominal and femoral adipose depots and those in plasma and adipose tissue concentrations of organochlorine pollutants in 7 obese subjects.

Material and Methods

Subjects. From the thirthy-nine healthy obese inviduals who participated in the weight loss program intervention before and after which organochlorine compounds were measured in adipose tissue and blood samples (7), sufficient amounts of adipose tissue for the measurement of PPARγ expression were obtained from seven subjects (i.e. 3 women and 4 men). Subjects were recruited through the media and gave their written informed consent to participate in this study, which was approved by the Laval University Medical Ethics Committee. All individuals underwent a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, endocrine disorders, or those on medication which could have influenced triglyceride metabolism (β-blockers, antihypertensive drugs, etc) were excluded from the study. Women had regular menstrual cycles and none was using oral contraceptives or lactating at the time of the study. All measurements were performed while women were in the early follicular phase of their menstrual cycle. All participants were sedentary, non-smokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight-reducing program, and their body weight had been stable during the last six months prior to the study.

All subjects participated in a 15-week weight loss program induced by a moderate caloric restriction which took into account the individual macronutrient composition of subjects, as reported in their three-day dietary record before the study (18-19% protein, 38-39% fat, 41-46% carbohydrate and 1-2% alcohol of total energy intake). This non-macronutrient specific energy restricted diet was fixed in part with a resting metabolic rate (RMR) measurement to which an activity factor of 1.4 (37) was multiplied to estimate daily energy expenditure (DEE) of subjects who where sedentary at the onset of the program. Thus, DEE was calculated as follows:

$$DEE = RMR\ (kcal/minute) \times 1440\ minute/day \times 1.4$$

To fix energy intake of the weight-reducing program, 700 kcal were subtracted from DEE.

Total body fatness and regional fat distribution. Body density was determined by the underwater weighing technique and percent body fat was derived from body density (32). Pulmonary residual volume was measured using the helium dilution method (25). Fat mass was calculated as total body weight minus fat free mass. Waist girth was measured according to procedures recommended at the Airlie Conference (20). Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlangen, Germany), according to the methodology previously described by Sjöström et al. (33). Briefly, subjects were examined in the supine position with both arms stretched above the head. CT scans were performed at the abdominal (between L4 and L5 vertebrae) and at the femoral (midthigh) levels, using an abdominal scout radiograph to establish the position of the scans to the nearest millimeter. Total adipose tissue (AT) areas were calculated by delineating the abdomen with a graph pen and then computing AT surfaces with an attenuation range of -190 to -30 Hounsfield units (HU) (11). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was determined by subtracting the visceral AT area from the total abdominal AT area.

Adipose tissue biopsy procedure. After an overnight fast, participants were subjected to biopsies of subcutaneous fat, one performed in the periumbilical region (abdominal site) and the other at the anterior midthigh level (femoral site). Local anesthesia (1% xylocaine, without epinephrine) was performed in such a way that it did not influence the metabolic activity of excised AT (23). Biopsies were performed before and 4-6 weeks after the end of the treatment when all subjects were weight stable. A small cutaneous incision (1 cm) was performed in both sites and approximately 50 mg of adipose tissue from each region were removed and immediately frozen in liquid nitrogen for later RNA preparation.

Total RNA preparation. Total RNA was isolated using guanidinium thiocyanate phenol chloroform extraction and alcohol precipitation (8). The absorption ratio (260:280 nm) ranged between 1.6 and 1.9 for all preparations. The average yield of total RNA was similar before and after weight loss (17 ± 5 µg/g vs 15 ± 6 µg/g adipose tissue, respectively). Total RNA was stored at -80^{0}C until quantification of the target mRNA.

Construction of the competitor. The construction of the competitor has previously been described in detail (1). Briefly, the PPARγ competitor was directly constructed in the expression vector pSG5-hPPARγ by deleting a 74 bp-long fragment (nucleotides +433 to +507 of the PPARγ2 coding sequence) by digestion (HindIII) and ligation. The PPARγ competitor was then subcloned into pBS (fragment -34 to +843) to give pBSCompPPARg.

Quantification of mRNA. PPARγ adipose tissue mRNA levels were determined by reverse transcription reaction followed by competitive polymerase chain reaction (RT-competitive PCR). Briefly, this method relies on the addition of a known amount of a competitor DNA molecule in the PCR to standardize the amplification process. The specific first strand cDNA was synthesized from 0.1 μg of total RNA in the presence of 15 pmol of the designed antisense primer, with 2.5 U of a thermostable reverse transcriptase (*Tth* DNA polymerase; Promega, Charbonnière, France) in conditions described previously (2). After the reaction, the RT medium (10 μl) was added to a PCR mix (10 mmol/l Tris-HCl, pH 8.3, 100 mmol/l KCl, 0.75 mol/l EGTA, 5 % glycerol, 0.2 mmol/l deoxynucleoside triphosphates, 45 pmol specific sense primers with the 5'-end labelled with Cy-5fluorescent dye (Eurogentec, Seraing, Belgium), 37.5 pmol antisense primer and 5 U *Taq* polymerase) of 100 μl of final volume. Then, 4 aliquots (20 μl) of the mixture were transferred to microtubes containing a different, but known, amount of competitor. After 120 s at 95^{0}C, the tubes were subjected to 40 cycles of amplification (Omnigene, Hybaid, Taddington, U.K.), including denaturation for 40 s at 95^{0}C, hybridization for 50 s at 55^{0}C and elongation for 50 s at 72^{0}C.

Analysis of the PCR products. The fluorescent-labeled PCR products were analyzed with an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia, Upsala, Sweden) in a 4% denaturating polyacrylamide gel. The amount of PCR products was calculated by integrating the peak area using the Fragment Manager software from Pharmacia. To determine the concentration of the target mRNA, the logarithm of the peak surface ratio of competitor to target cDNA was plotted versus the logarithm of the amount of competitor added into the PCR medium. The initial concentration of the target mRNA was determined at the competition equivalence point, as previously described (2).

Chemical analysis. Nine PCB congeners (International Union of Pure and Applied Chemistry nos.: 28, 99, 118, 138, 153, 156, 170, 180 and 187), one commercial PCB mixture formerly used in electrical transformers, Aroclor 1260 (A1260) and seven chlorinated pesticides (beta-hexachlorocyclohexane (β-HCH), dichlorodiphenyl dichloroethene (p,p'-DDE), dichlorodiphenyl trichloroethene (p,p'-DDT), hexachlorobenzene (HCB), mirex, oxychlordane and transnonachlor were determined in plasma and adipose tissue samples. Blood and adipose tissue samples were taken before and following weight loss. Samples were first spiked with the internal standard (PCB congener no. 198), homogenized in hexane:acetone (2:1, v/v) and the resulting organic phase washed with water to remove the bulk of the acetone. An aliquot of the hexane extract was used for lipid determination by gravimetry and the rest of the extract was defatted with concentrated sulfuric acid. The defatted hexane was successively washed with water and aqueous potassium hydroxide prior to filtration through anhydrous sodium sulfate. The filtrate was then concentrated and cleaned up by chromatography on an acidic silica gel column and a deacrivated (0.5%) Florisil column. Organochlorines were eluted from the columns using methylene chloride:hexane (25:75, v/v) and analyzed on a HP-5890 gas chromatograph equipped with dual capillary columns (Ultra-1 and Ultra-2) and dual ^{63}Ni electron detectors. Adipose tissue analyses were made using similar methods. Depending on the lipid content and the available quantity of tissues, detection limits varied from 6.4 to 43 μg/kg and from 0.02 to 0.3 μg/l in adipose tissue and plasma, respectively.

Statistical analyses. The Wilcoxon signed rank test was used to compare means before and after weight loss. The Spearman rho test was utilized to assess the degree of relationship between variables. All analyses were performed using the Jump version 3.2.2 program (SAS Institute Inc., Cary, NC) adapted for Macintosh computers.

Results

Subjects' physical characteristics before and after the weight-reducing program are presented in Table 1. As expected, body weight, BMI, fat mass, fat free mass and waist girth were reduced after weight loss (P values ranging from 0.001 to 0.05). The 10% reduction in body weight was also accompanied by a significant decrease of all the adipose tissue areas measured by CT (P values ranging from 0.001 to 0.01). In contrast to femoral adipocytes, subcutaneous abdominal fat cells were significantly reduced following weight loss ($P < 0.001$). No regional variation in fat cell size was observed before and after treatment.

In response to weight loss, PPARγ mRNA levels were significantly reduced in the subcutaneous abdominal region (4.7 ± 1.9 vs 2.6 ± 1.9 amol/ug total RNA before and after weight reduction, respectively; $P < 0.05$) (Figure 1). A decrease in PPARγ mRNA levels was also observed in the femoral depot (3.6 ± 1.9 vs 2.5 ± 2.2 amol/ug total RNA before and after weight loss, respectively; NS), but this effect did not reach statistical significance. Moreover, weight loss induced changes in adipose tissue PPARγ mRNA levels and those in anthropometric variables such as body weight, fat mass, subcutaneous abdominal and midthigh adipose tissue areas were not significantly related ($-0.07 < r < 0.54$; NS) (not shown).

Insert Table 1 and Figure 1

As shown in Table 2, 10 of the 17 plasma organochlorine pollutants were significantly increased in response to weight loss. Similar results were observed when organochlorine concentrations were expressed on a plasma lipid-adjusted basis (not shown). Adipose tissue organochlorine concentrations were also increased following weight loss (Table 3). However, statistically significant increases were observed only in 6 of the 14 detected compounds in both subcutaneous abdominal and femoral fat depots.

Insert Tables 2 and 3

As pollutants might act as peroxisome proliferators, the association between changes in subcutaneous abdominal adipose tissue PPARγ mRNA levels and those in plasma or adipose tissue organochlorine levels was also investigated. A positive relationship would suggest that the higher the increase in organochlorine concentrations, the greater the increase in PPARγ mRNA levels. With the exception of the significant positive relationship found between variations in PCB 99 and those of PPARγ mRNA levels of the femoral depot (rho = 0.89, $P < 0.01$), no further significant association was observed between variations in PPARγ mRNA levels and changes in plasma organochlorine concentrations following weight loss ($-0.63 <$ rho < 0.52; NS) (Table 4). As shown in Table 5, no significant relationship was found between changes in subcutaneous abdominal adipose tissue organochlorine concentrations and changes in PPARγ mRNA levels of this adipose region in response to weight loss. The only significant relationship observed between changes in femoral PPARγ mRNA levels was that with PCB 156 (rho = 0.87, $P < 0.05$) (Table 6).

Insert Tables 4, 5 and 6

Dicussion

The present study demonstrates that in response to weight loss, PPARγ expression is significantly decreased in the subcutaneous abdominal adipose tissue region. This effect was also seen in the femoral adipose tissue site but not to a statistically significant extent. Our results also show that plasma and adipose tissue organochlorine concentrations were not associated with variations in subcutaneous abdominal and femoral adipose tissue PPARγ mRNA levels in response to weight loss. Based on these data, it is tempting to suggest that pollutants do not seem to directly influence the regulation of subcutaneous adipose tissue PPARγ expression.

Several studies have previously shown that herbicides and industrial plasticizers act as peroxisome proliferators (9,13). However, most of the latter experiments have more particularly focused on liver since administration of organochlorine compounds such as A1260 to rodents results in a dramatic proliferation of hepatic peroxisomes as well as liver hyperplasia (28,34). To the best of our knowledge, the present study was the first to investigate the potential role of the increased pollutant levels on the regulation of adipose tissue mRNA levels of PPARγ in response to weight loss. The fact that no relationship was observed between changes in plasma and adipose tissue pollutant concentrations and those in subcutaneous adipose tissue PPARγ mRNA levels does not exclude the possibility that pollutants may act directly or indirectly on other target genes modulating adipose tissue metabolism. In this regard, a recent study has demonstrated that endrin, a chlorinated cyclodiene pesticide, inhibits adipocyte differentiation not by its direct action on PPARγ but through the specific suppression of C/EBPα (26), an indispensable nuclear transcriptional factor triggering the entire process of adipocyte differentiation (17,38).

From a clinical standpoint, experimental evidence tends to support the idea that exposure to pollutants may trigger adipose tissue mechanisms which could lead to weight gain over time. Indeed, as exposure to PCB has been observed to affect thyroid function in humans (14) and as low thyroid levels have previously been reported to increase adipose tissue LPL activity

(12,30) and reduce epinephrine-stimulated lipolysis (30), one could suggest that some pollutants may induce adaptations leading to long-term fat storage. Conversely, other reports suggest that some pollutants such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) induced impairments of synthesis and storage of metabolic reserves by inhibiting glucose transport (10,18), LPL activity (5) and fatty acid synthesis (15). Further studies are therefore required to evaluate whether body fat gain may be a protective adaptation of the body to attenuate pollutants exposure or whether body fat loss in response to pollutant exposure may represent a protective reaction to avoid further lipophilic pollutant accumulation.

These apparently conflicting evidences may be in agreement with the wide range of correlations that we observed between changes in PPARγ mRNA levels and those in organochlorine compounds in plasma and in abdominal and femoral adipose tissue samples. Indeed, the organochlorines measured in this study represent an heterogenous group of compounds whose variations do not likely have the same impact on PPARγ mRNA levels. We are aware that our correlational analyses were not performed in the optimal statistical context due to our small sample of subjects. However, the range of correlations that we document may also be the reflect of a real biological phenomenon by which some pollutants exert a stimulating effetc on PPARγ mRNA levels whereas others might rather promote an inhibitory effect. This hypothesis is obviously quite speculative, but it can serve as a template for future experimentation into the specificity of the effect by which organochlorines modulate PPARγ mRNA levels.

That body weight loss induced a decrease in PPARγ mRNA levels of the subcutaneous abdominal depot is consistent with previous studies (3,36). However, the present study is the first to report a regional difference in the changes of PPARγ expression following weight loss. The mechanism (s) by which adipose tissue PPARγ mRNA levels decreased in response to weight loss is (are) still unclear. Some authors have suggested that a down-regulation of PPARγ gene expression might be involved in the reduction of fat mass during dieting (3). On the basis of the fact that adipogenic factors (i.e. C/EBPs and PPARγ) probably interact with

each other in a complex fashion (6), it is also logical to speculate that the inhibition of C/EBPα by some pollutants (19,26) exerts a negative feedback action on adipogenic factors such as PPARγ in response to the weight loss-induced increase in plasma and adipose tissue pollutant concentrations. However , this hypothesis deserves further investigation.

Conclusion

In summary, the present study showed a significantly reduced expression of subcutaneous abdominal adipose tissue PPARγ mRNA levels after a 10% reduction in body weight, whereas such a decrease did not reach statistical significance in the femoral depot. The weight loss-induced increase in plasma and subcutaneous abdominal adipose tissue pollutant concentrations was not related to changes in PPARγ mRNA levels, suggesting that pollutants investigated are not directly associated with the regulation of PPARγ mRNA levels.

Acknowledgments

The authors wish to express their gratitude to the staff of the Physical Activity Sciences Laboratory and of the Lipid Research Center for data collection. We are grateful to Nathalie Vega and Paulette Vallier from the INSERM Unit 449 of Lyon (France) for technical help on this work. The contribution of subjects is also gratefully acknowledged.

References

1. **Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H.** Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes.* 1997;46: 1319-1327.
2. **Auboeuf D, Vidal H.** The use of the reverse transcription-competitive polymerase chain reaction to investigate the in vivo regulation of gene expression in small tissue samples. *Anal Biochem.* 1997;245: 141-148.
3. **Bastard JP, Hainque B, Dusserre E, Bruckert E, Robin D, Vallier P, Perche S, Robin P, Turpin G, Jardel C, Laville M, Forest C, Vidal H.** Peroxisome proliferator activated receptor-gamma, leptin and tumor necrosis factor-alpha mRNA expression during very low calorie diet in subcutaneous adipose tissue in obese women. *Diabetes Metab Res Rev.* 1999;15: 92-98.
4. **Blumberg B, Evans RM.** Orphan nuclear receptors--new ligands and new possibilities. *Genes Dev.* 1998;12: 3149-3155.
5. **Brewster DW, Matsumura F.** Reduction of adipose tissue lipoprotein lipase activity as a result of in vivo administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin to the guinea pig. *Biochem Pharmacol.* 1988;37: 2247-2253.
6. **Brun RP, Kim JB, Hu E, Spiegelman BM.** Peroxisome proliferator-activated receptor gamma and the control of adipogenesis. *Curr Opin Lipidol.* 1997;8: 212-218.
7. **Chevrier J, Dewailly E, Ayotte P, Mauriège P, Després JP, Tremblay A.** Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *Int J Obesity.* 2000;In press:
8. **Chomezynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thyocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162: 156-159.
9. **Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W.** Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell.* 1992;68: 879-887.

10. **Enan E, Liu PC, Matsumura F.** 2,3,7,8-Tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig. *J Biochem Chem.* 1992;267: 19785-19791.

11. **Ferland M, Després JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, Lupien PJ, Thériault G, Bouchard C.** Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. *Br J Nutr.* 1989;61: 139-148.

12. **Gavin LA, McMahon F, Moeller M.** Modulation of adipose lipoprotein lipase by thyroid hormone and diabetes. The significance of the low T3 state. *Diabetes.* 1985;34: 1266-1271.

13. **Issemann I, Green S.** Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature.* 1990;347: 645-650.

14. **Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ.** Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatr Res.* 1994;36: 468-473.

15. **Lakshman MR, Chirtel SJ, Chambers LL, Coutlakis PJ.** Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on lipid synthesis and lipogenic enzymes in the rat. *J Pharmacol Exp Ther.* 1989;248: 62-66.

16. **Laug EP, Fitzburgh OG.** 2, 2-bis (p-chlorophenyl)-1,1,1-trichloroethane (DDT) in the tissues of the rat following oral ingestion for periods of six months to two years. *J Pharmacol Exp Ther.* 1946;98: 268-273.

17. **Lin FT, Lane MD.** CCAAT/enhancer binding protein alpha is sufficient to initiate the 3T3- L1 adipocyte differentiation program. *Proc Natl Acad Sci.* 1994;91: 8757-8761.

18. **Liu PC, Matsumura F.** Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the "adipose- type" and "brain-type" glucose transporters in mice. *Mol Pharmacol.* 1995;47: 65-73.

19. **Liu PC, Phillips MA, Matsumura F.** Alteration by 2,3,7,8-Tetrachlorodibenzo-p-dioxin of CCAAT/enhancer binding protein correlates with suppression of adipocyte differentiation in 3T3-L1 cells. *Mol Pharmacol.* 1996;49: 989-997.

20. **Lohman TG, Roche AF, Martorell R.** *The Airlie (VA) consensus conference.* In: Anthropometric Standardisation Reference Manual. Human Kinetics Publishers, Champaign (Illinois), 1988: 39-80.

21. **Maloney EK, Waxman DJ.** trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol.* 1999;161: 209-218.

22. **Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, et al.** The nuclear receptor superfamily: the second decade. *Cell.* 1995;83: 835-839.

23. **Mauriège P, Després JP, Prud'homme D, Pouliot MC, Marcotte M, Tremblay A, Bouchard C.** Regional variation in adipose tissue lipolysis in lean and obese men. *J Lipid Res.* 1991;32: 1625-1633.

24. **McFarland VA, Clarke JU.** Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener- specific analysis. *Environ Health Perspect.* 1989;81: 225-239.

25. **Meneely GR, Kaltreider NL.** Volume of the lung determined by helium dilution. *J Clin Invest.* 1949;28: 129-139.

26. **Moreno-Aliaga MJ, Matsumura F.** Endrin inhibits adipocyte differentiation by selectively altering expression pattern of CCAAT/enhancer binding protein-alpha in 3T3-L1 cells. *Mol Pharmacol.* 1999;56: 91-101.

27. **Ohmiya Y, Nakai K.** Effect of starvation on excretion, distribution and metabolism of DDT in mice. *Tohoku J Exp Med.* 1977;122: 143-153.

28. **Reddy JK, Rao MS, Azarnoff DL, Sell S.** Mitogenic and carcinogenic effects of a hypolipidemic peroxisome proliferator, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14, 643), in rat and mouse liver. *Cancer Res.* 1979;39: 152-161.

29. **Safe SH.** Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol.* 1994;24: 87-149.

30. **Saffari B, Ong JM, Kern PA.** Regulation of adipose tissue lipoprotein lipase gene expression by thyroid hormone in rats. *J Lipid Res.* 1992;33: 241-249.

31. **Schoonjans K, Staels B, Auwerx J.** Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res.* 1996;37: 907-925.

32. **Siri WE.** The gross composition of body fat. *Adv Biol Med Physiol.* 1956;4: 239-280.

33. **Sjöström L, Kvist H, Cederblad A, Tylen U.** Determination of total adipose tissue and body fat in women by computed tomography, ^{40} K and tritium. *Am J Physiol.* 1986;250: E736-E786.

34. **Styles JA, Kelly M, Pritchard NR, Elcombe CR.** A species comparison of acute hyperplasia induced by the peroxisome proliferator methylclofenapate: involvement of the binucleated hepatocyte. *Carcinogenesis.* 1988;9: 1647-1655.

35. **Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM.** mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* 1994;8: 1224-1234.

36. **Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Flier JS.** Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest.* 1997;99: 2416-2422.

37. **White MD, Bouchard G, Buemann B, Alméras N, Després JP, Bouchard C, Tremblay A.** Energy and macronutrient balances for humans in a whole body metabolic chamber without control of preceding diet and activity level. *Int J Obesity.* 1997;21: 135-140.

38. **Yeh WC, Cao Z, Classon M, McKnight SL.** Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev.* 1995;9: 168-181.

Legend to figure

Figure 1

PPARγ mRNA levels of subcutaneous abdominal and femoral adipose tissues before and after weight loss in 7 obese subjects. Significant difference before and after weight loss at * $P < 0.05$. Horizontal lines represent mean values.

Table 1. Physical characteristics of subjects before and after weight loss.

	Before	After
Anthropometric variables		
Body weight (kg)	97 ± 19	87 ± 16 ‡
BMI (kg/m^2)	35 ± 2	31 ± 2 ‡
Body fat (%)	42 ± 5	38 ± 7 *
Fat mass (kg)	41 ± 6	33 ± 5 †
Fat free mass (kg)	56 ± 14	54 ± 15 *
Waist girth (cm)	127 ± 63	105 ± 57 ‡
Adipose tissue areas measured by CT (cm^2)		
Abdomen (L4-L5)		
Subcutaneous	459 ± 76	359 ± 72 ‡
Visceral	191 ± 82	116 ± 44 †
Midthigh subcutaneous	127 ± 63	105 ± 57 ‡
Regional fat cell weight (μg lipid/cell)		
Abdominal	0.66 ± 0.13	0.52 ± 0.08 †
Femoral	0.63 ± 0.17	0.57 ± 0.09

Value are means ± standard deviation (SD).

BMI = body mass index; CT = computed tomography.

Significant difference before and after weight loss at * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Table 2. Plasma organochlorine levels of subjects before and after a moderate weight loss.

	Before (μg/l)	After (μg/l)
β-HCH	0.109 ± 0.055	0.151 ± 0.067
p,p'-DDE	2.386 ± 2.246	2.919 ± 2.740 *
p,p'-DDT	0.037 ± 0.020	0.037 ± 0.017
HCB	0.107 ± 0.020	0.117 ± 0.016
Mirex	0.020 ± 0.008	0.023 ± 0.011
Oxychlordane	0.053 ± 0.026	0.059 ±0.020
Transnonachlor	0.063 ± 0.034	0.074 ± 0.028 *
Aroclor 1260	2.34 ± 0.98	2.67 ± 0.86 *
PCB 28	0.029 ± 0.022	0.031 ± 0.022
PCB 99	0.057 ± 0.041	0.066 ± 0.036 *
PCB 118	0.061 ± 0.016	0.074 ± 0.011 †
PCB 138	0.191 ± 0.031	0.219 ± 0.76 *
PCB 153	0.260 ± 0.107	0.297 ± 0.093 *
PCB 156	0.039 ± 0.011	0.037 ± 0.008
PCB 170	0.057 ± 0.010	0.067 ± 0.023 *
PCB 180	0.139 ± 0.063	0.164 ± 0.060 †
PCB 187	0.036 ± 0.013	0.041 ± 0.011 *

Values are means ± standard deviation (SD).

Significant difference before and after weight loss at * $P < 0.05$, † $P < 0.01$.

Table 3. Adipose tissue organochlorine levels in subcutaneous abdominal and femoral adipose tissues of subjects before and after a moderate weight loss.

	Abdominal		Femoral	
	Before (μg/kg)	**After (μg/kg)**	**Before (μg/kg)**	**After (μg/kg)**
β-HCH	22.0 ± 8.5	27.8 ± 12.0	25.7 ± 11.7	32.7 ± 20.8
p,p'-DDE	439.0 ± 283.1	577.0 ± 502.8	406.0 ± 249.9	604.8 ± 434.5
p,p'-DDT	19.4 ± 12.2	19.0 ± 6.5	24.4 ± 14.4	30.8 ± 9.4
HCB	22.2 ± 2.6	26.8 ± 8.4	25.0 ± 9.7	32.4 ± 12.2
Oxychlordane	20.8 ± 4.8	26.0 ± 12.5	20.9 ± 11.7	28.0 ± 12.5 *
Transnonachlor	28.8 ± 5.9	35.8 ± 15.7	28.3 ± 13.7	34.8 ± 13.8
Aroclor 1260	0.62 ± 0.12	0.81 ± 0.27 *	0.69 ± 0.20	0.88 ± 0.27 &
PCB 118	15.0 ± 3.8	18.1 ± 4.9 &	16.8 ± 5.0	21.6 ± 3.5
PCB 138	52.7 ± 8.4	67.0 ± 18.0 *	57.0 ± 14.2	71.8 ± 19.6 *
PCB 153	67.0 ± 16.0	87.7 ± 31.4 *	75.5 ± 23.7	97.6 ± 32.0
PCB 156	11.4 ± 3.1	14.0 ± 5.6 &	13.4 ± 8.6	16.8 ± 8.6
PCB 170	15.7 ± 6.6	21.4 ± 11.2 &	16.3 ± 7.9	23.2 ± 10.6 †
PCB 180	38.7 ± 11.2	51.3 ± 21.6 *	42.5 ± 15.1	54.8 ± 24.3 &
PCB 187	14.3 ± 2.8	17.1 ± 7.8	14.6 ± 6.0	19.8 ± 7.5 †

Values are means ± standard deviation (SD).

Significant difference before and after weight loss at * $P < 0.05$, † $P < 0.01$; & $P = 0.06$

Table 4. Relationships between changes (Δ) in plasma organochlorine concentrations and changes in PPARγ mRNA levels of subcutaneous abdominal and femoral depots in response to weight loss.

	Δ PPARγ mRNA	
	Abdominal	Femoral
Δ β-HCH	-0.25	0.01
Δ p,p'-DDE	-0.02	-0.63
Δ p,p'-DDT	0.02	-0.23
Δ HCB	0.02	-0.13
Δ Mirex	0.09	-0.07
Δ Oxychlordane	0.18	0.43
Δ Transnonachlor	0.01	0.52
Δ Aroclor 1260	-0.11	0.09
Δ PCB 28	0.47	-0.04
Δ PCB 99	0.33	0.89 †
Δ PCB 118	0.45	0.30
Δ PCB 138	0.14	-0.14
Δ PCB 153	-0.14	0.14
Δ PCB 156	0.20	-0.31
Δ PCB 170	0.16	0.13
Δ PCB 180	-0.29	0.31
Δ PCB 187	-0.07	0.48

† $P < 0.01$.

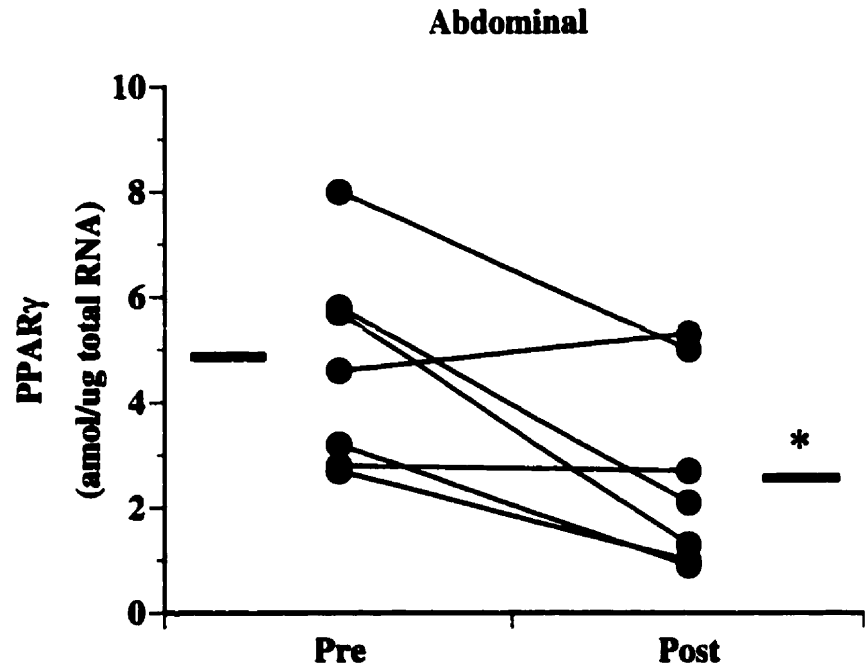
Table 5. Relationships between changes (Δ) in subcutaneous abdominal adipose tissue organochlorine concentrations and changes in PPARγ mRNA levels of this depot in response to weight loss.

	Δ PPARγ mRNA levels of abdominal adipose tissue
Δ β-HCH	0.20
Δ p,p'-DDE	-0.60
Δ p,p'-DDT	-0.80
Δ HCB	-0.71
Δ Oxychlordane	-0.60
Δ Transnonachlor	-0.60
Δ Aroclor 1260	-0.20
Δ PCB 99	-0.09
Δ PCB 118	-0.46
Δ PCB 138	-0.35
Δ PCB 153	-0.20
Δ PCB 156	-0.09
Δ PCB 170	0.20
Δ PCB 180	-0.18
Δ PCB 187	-0.06

Table 6. Relationships between changes (Δ) in femoral adipose tissue organochlorine concentrations and changes in PPARγ mRNA levels of this depot in response to weight loss.

	Δ PPARγ mRNA levels of femoral adipose tissue
Δ β-HCH	0.70
Δ p,p'-DDE	-0.80
Δ p,p'-DDT	0.70
Δ HCB	0.21
Δ Oxychlordane	0.30
Δ Transnonachlor	0.10
Δ Aroclor 1260	0.10
Δ PCB 99	-0.20
Δ PCB 118	0.70
Δ PCB 138	-0.30
Δ PCB 153	-0.30
Δ PCB 156	0.87 *
Δ PCB 170	0.10
Δ PCB 180	-0.50
Δ PCB 187	0.21

* $P < 0.05$.


Abdominal
PPARγ
(amol/ug total RNA)
10
8
6
4
2
0
*
Pre
Post

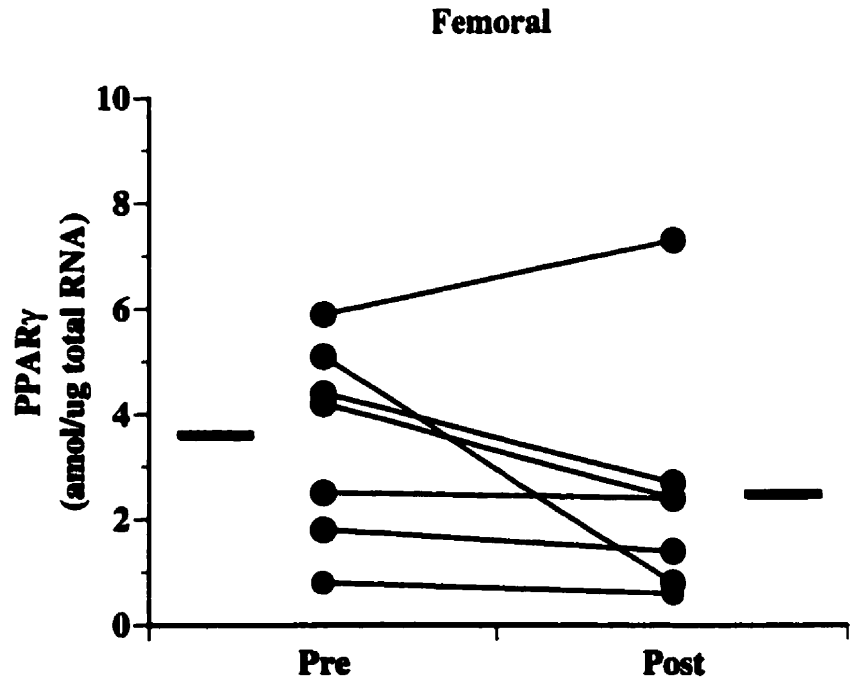

Femoral
PPARγ
(amol/ug total RNA)
10
8
6
4
2
0
Pre
Post

Figure 1

CHAPITRE 14

CONCLUSIONS ET PERSPECTIVES

Ce travail a permis de caractériser les systèmes de contrôle du métabolisme adipocytaire (lipolyse/lipogenèse) au cours de diverses situations physiologiques comme la perte de poids et le vieillissement chez l'humain. Par ailleurs, nos résultats ont mis en évidence i) la contribution de l'hypertrophie adipocytaire dans la détérioration du profil métabolique ainsi que ii) l'implication du système lipolytique et/ou antilipolytique de l'adipocyte au niveau de la capacité oxydative lipidique in vivo et du métabolisme de la lipémie postprandiale.

Il est maintenant bien connu que le tissu adipeux sécrète un bon nombre de signaux dont certains présentent une activité endocrine alors que d'autres agissent plutôt de façon autocrine/paracrine. Ce réseau complexe permet au tissu adipeux d'effectuer des ajustements particuliers au niveau de l'utilisation des substrats métaboliques par l'organisme. À ce titre, plusieurs conséquences métaboliques néfastes associées à l'obésité peuvent provenir d'un mauvais fonctionnement du "répertoire" endocrinien du tissu adipeux. Les résultats du Chapitre 5 montrent que, pour une accumulation donnée de tissu adipeux viscéral, les hommes caractérisés par une hypertrophie des adipocytes sous-cutanés abdominaux présentent une détérioration accrue de leur profil métabolique comparativement à des individus présentant de plus petits adipocytes du même site. À la lumière de ces résultats, il n'est pas impossible que l'hypertrophie adipocytaire soit associée à une synthèse accrue d'un signal ou de signaux altérant le profil métabolique de l'homme. À titre d'exemple, il convient de souligner la surexpression du facteur de nécrose tumorale-alpha (TNF-α) et de ses deux récepteurs chez l'individu obèse (Kern et al., 1995; Mohamed-Ali et al., 1999) en se rappelant du rôle

important que joue cette cytokine dans le développement de la résistance à l'insuline. De plus, mentionnons l'inhibiteur de l'activateur du plasminogène (PAI-1) qui voit son expression augmenter au niveau de l'adipocyte sous-cutané abdominal hypertrophié (Eriksson et al., 1998) et dont la sécrétion accrue pourrait contribuer à l'augmentation de problèmes vasculaires (Shimomura et al., 1996). De futures études pourraient être entreprises pour vérifier si, pour une distribution régionale de tissu adipeux donnée, des individus caractérisés par une hypertrophie des adipocytes sous-cutanés abdominaux exprimeraient des niveaux supérieurs de PAI-1 et/ou de TNF-α comparativement à des individus présentant de petits adipocytes.

En raison d'une diminution drastique des travaux physiques auxquels l'homme était autrefois contraint et de l'augmentation du phénomène de suralimentation auquel il est maintenant confronté, il est bien connu que l'obésité est de plus en plus prépondérante au sein des pays industrialisés (Flegal et al., 1998). De plus, plusieurs études rapportent qu'une accumulation excesssive de tissu adipeux, notamment au niveau abdominal, est fortement associée à des complications métaboliques préjudiciables pour la santé (Björntorp, 1991b; Després, 1991; Kissebah et al., 1994). Ces observations incitent ainsi le clinicien à adopter des stratégies favorisant la prévention et/ou le traitement de l'obésité et des altérations métaboliques qui en découlent. La restriction calorique est l'une des stratégies communément utilisées qui entraîne des changements métaboliques particuliers au niveau de l'adipocyte. L'étude du Chapitre 6 relate qu'en réponse à une perte de poids modérée induite par une restriction calorique de 15 semaines, les changements de l'activité de la lipoprotéine lipase (LPL) diffèrent selon le sexe de l'individu. Jusqu'à maintenant, les mécanismes explicatifs de cette divergence sexuelle au niveau de la variation de l'activité de la LPL demeurent inconnus. Il est toutefois possible que celle-ci soit en partie expliquée par les variations de concentrations d'hormones sexuelles chez les hommes et les femmes en réponse à la perte de poids (Zumoff & Strain, 1994). Il convient de noter que l'ampleur de la perte de poids induite par une restriction calorique semble être à l'origine des divergences de la littérature en ce qui a trait aux effets de la perte de poids sur l'activité de la LPL (Rebuffé-Scrive et al., 1983). À ce titre, rappelons que certaines études ont rapporté une augmentation de l'activité de la LPL du tissu adipeux après une perte substantielle de masse corporelle (Kern et al., 1990; Schwartz et al., 1981), pouvant ainsi expliquer la prédisposition au regain de poids à laquelle les individus "post-obèses" sont

souvent confrontés. Sur la base de ces résultats, il serait pertinent d'étudier l'activité de la LPL périodiquement (par exemple, toutes les 2 semaines) et sur une longue échéance lors d'un programme de perte de poids sévère pour déterminer le moment à partir duquel la machinerie enzymatique de l'adipocyte semble être prédisposée à favoriser la resynthèse des graisses. Une autre cible intéressante que l'on pourrait envisager d'étudier lors de cette stratégie de perte de poids progressive serait le TNF-α. Tout comme l'activité de la LPL, une certaine controverse existe quant à l'effet d'une perte pondérale sur l'expression du TNF-α. En effet, des travaux ont rapporté une diminution significative de l'expression du TNF-α suite à une perte massive du poids corporel (i.e. perte moyenne de 35 kg) (Kern et al., 1995), alors que d'autres ont montré une augmentation du transcrit TNF-α après une perte de poids modérée de 6 kg (Bastard et al., 1999). Ces résultats concordent bien avec les divergences qui existent au niveau de l'activité de la LPL suite à une perte de poids en se rappelant que l'expression du TNF-α exerce un effet inhibiteur sur l'activité de la LPL (Kern et al., 1995). Enfin, une meilleure connaissance de la quantité perdue de poids corporel à laquelle le métabolisme de l'adipocyte se réorganiserait pour favoriser la resynthèse des graisses permettrait de mieux comprendre le phénomène de résistance à l'amaigrissement qui peut survenir avant l'atteinte d'une normalisation pondérale.

Nous avons également montré dans le Chapitre 7 qu'en réponse à une perte pondérale induite par une restriction calorique de 15 semaines, la sensibilité β-adrénergique lipolytique (plus particulièrement β2-adrénergique) des adipocytes sous-cutanés abdominaux et fémoraux d'hommes et de femmes obèses augmente de façon significative. Cette amélioration de la sensibilité est notamment expliquée par une élévation du nombre de récepteurs β-adrénergiques au niveau des deux dépôts adipeux étudiés. À l'inverse, nous avons observé une diminution de la sensibilité α_2-adrénergique des adipocytes sous-cutanés du site abdominal chez l'homme et des adipocytes des deux dépôts chez la femme en réponse à la perte pondérale, ceci n'étant toutefois pas associée à une baisse des récepteurs α_2-adrénergiques. Ces travaux nous auront permis de bien caractériser les effets d'une perte de poids modérée sur la lipolyse induite par les catécholamines au niveau d'adipocytes isolés. Des études additionnelles seraient toutefois nécessaires pour approfondir les effets d'une perte pondérale

sur les voies d'inhibition de la lipolyse. En effet, peu d'études jusqu'à maintenant ont documenté l'impact d'une restriction calorique sur les voies antilipolytiques stimulées par l'insuline, les prostaglandines de type E2 ou le peptide YY. Sur la base du fait que le tissu adipeux humain est caractérisé par la présence de récepteurs aux androgènes (Dieudonné et al., 1998; Miller et al., 1990) et aux oestrogènes (Mizutani et al., 1994; Price & O'Brien, 1993), il serait également intéressant d'examiner les effets directs des stéroïdes sexuels sur la lipolyse d'adipocytes isolés avant et après une perte pondérale.

Dans un autre volet de cette thèse, nous avons étudié l'effet de l'âge sur le métabolisme du tissu adipeux. À ce titre, nous avons rapporté une diminution significative de la capacité lipolytique des adipocytes sous-cutanés abdominaux et fémoraux d'individus moyennement âgés comparativement à des sujets jeunes, bien que les deux groupes présentaient une masse grasse identique (Chapitre 8). De plus, nous avons observé dans le Chapitre 9 que l'âge semble jouer un rôle activateur au niveau des transcrits de la lipase hormono-sensible (LHS) et du complément 3a (C3a). À l'inverse, nous avons rapporté des niveaux d'ARNm de la LPL et de PPARγ similaires au niveau du tissu adipeux sous-cutané abdominal. On peut donc penser que la capacité fonctionnelle de l'adipocyte à mobiliser des graisses semble être altérée chez l'homme moyennement âgé, bien que ceci ne soit pas expliqué par un défaut au niveau transcriptionnel de la LHS. Il n'est pas impossible que cette altération de la capacité lipolytique puisse intervenir dans le phénomène de gain de poids corporel associé au vieillissement chez l'homme. D'un point de vue mécanistique, il est probable que l'altération de la capacité lipolytique des hommes d'âge moyen soit expliquée par un défaut du transporteur intracellulaire des acides gras nommé aP2 (*adipocyte lipid-binding protein*). Cette hypothèse est à la base du fait que l'invalidation du gène aP2 chez des souris entraîne une diminution significative de la lipolyse basale et stimulée des adipocytes isolés en comparaison à des souris contrôles (Coe et al., 1999; Scheja et al., 1999). L'accumulation prononcée des acides gras à l'intérieur des adipocytes de souris mutées dont la protéine aP2 est inactive entraînerait un effet inhibiteur bien connu sur la LHS (Fredrikson et al., 1981; Jepson & Yeaman, 1992). L'observation récente de la formation d'un complexe entre la LHS et l'aP2 procure une base solide selon laquelle la lipolyse est contrôlée à l'intérieur même de l'adipocyte par l'aP2 (Shen et al., 1999). À la lumière de ces résultats, il serait donc pertinent de mesurer les niveaux

protéiques d'aP2 chez des sujets jeunes et d'âge moyen pour vérifier si ce facteur adipocytaire peut être impliqué dans l'altération de la capacité lipolytique associée au vieillissement chez l'homme.

Parallèlement, nous avons étudié le métabolisme du tissu adipeux sous-cutané de femmes pré et postménopausées. En effet, l'étude du Chapitre 10 nous a permis de montrer que malgré un état endocrinien différent, des femmes pré et postménopausées caractérisées par une distribution régionale du tissu adipeux similaire présentent une balance lipolytique/lipogénique comparable. Cette expérience nous a ainsi permis de mettre en évidence l'importance de considérer la distribution régionale du tissu adipeux lorsque l'on désire caractériser l'équilibre lipolytique/lipogénique de l'adipocyte. Des études futures pourraient évaluer si les mécanismes lipolytiques/lipogéniques de la cellule adipeuse varient en fonction des changements possibles de la distribution régionale du tissu adipeux conséquents à une réversibilité de l'état ménopausé induite par substitution hormonale. En effet, nous pourrions étudier le métabolisme du tissu adipeux sous-cutané de femmes ménopausées caractérisées par une accumulation de tissu adipeux au niveau abdominal avant et après une thérapie oestro-progestative d'une durée nécessaire pour induire des changements morphologiques de la distribution du tissu adipeux à celui d'un groupe témoin.

Le Chapitre 11 qui compose cette thèse repose principalement sur le fait que les capacités lipolytiques et lipogéniques de l'adipocyte agissent de façon coordonnée et qu'elles influencent de façon importante la régulation du métabolisme des acides gras et des lipoprotéines plasmatiques. Nous avons rapporté que des individus caractérisés par une faible sensibilité α_2-adrénergique (i.e., antilipolytique) au niveau des adipocytes sous-cutanés abdominaux en situation de jeûne présentent des niveaux plasmatiques élevés de TG à jeun qui, par conséquent, entraînent des altérations importantes au niveau de la mobilisation postprandiale de TG. Nos travaux ont ainsi permis de mettre en évidence l'impact de la sensibilité antilipolytique α_2-adrénergique des adipocytes de la région sous-cutanée abdominale sur le devenir postprandial des lipoprotéines riches en TG chez l'homme. Il serait de mise dans le futur de vérifier si la capacité antilipolytique de l'insuline au niveau d'adipocytes isolés après une période de jeûne pourrait aussi être prédictrice des modulations postprandiales des

concentrations de lipoprotéines riches en TG. De plus, des études additionnelles pourraient être entreprises pour vérifier si nos observations s'appliquent chez la femme préménopausée. Jusqu'à présent, quelques travaux ont permis d'identifier l'âge comme étant un facteur qui altère le métabolisme des lipoprotéines plasmatiques en situation postprandiale (Cohn et al., 1988; Krasinski et al., 1990; van Beek et al., 1999). Toutefois, les mécanismes expliquant cette observation sont encore inconnus. En considérant les effets du vieillissement sur le métabolisme du tissu adipeux rapportés précédemment, il serait donc intéressant d'étudier le métabolisme des lipoprotéines plasmatiques en situation postprandiale de sujets jeunes et d'âge moyen pour vérifier si les capacités lipolytiques et lipogéniques adipocytaires de ces derniers peuvent contribuer à l'altération de la lipémie postprandiale.

En plus de sa capacité antilipolytique qui est notamment médiée par les récepteurs α_2-adrénergiques, l'adipocyte dispose également de récepteurs β-adrénergiques à caractère lipolytique qui sont recrutés par l'isoprénaline. Nous avons montré dans le Chapitre 12 que la réponse lipolytique maximale à ce β-agoniste s'avère un déterminant non négligeable de l'oxydation lipidique (30% de la variance) mesurée par calorimétrie indirecte chez des sujets obèses. Ces observations confirment en quelque sorte les résultats d'une étude antérieure rapportant que les individus obèses présentent une altération dans leur capacité oxydative lipidique en réponse à une stimulation β-adrénergique systémique (Blaak et al., 1994a). Outre l'exercice qui présente le potentiel d'augmenter la faculté lipomobilisatrice de l'adipocyte en réponse à une stimulation β-adrénergique, il pourrait être intéressant de développer des composés chimiques (ou même des neutraceutiques) pouvant stimuler les récepteurs β-adrénergiques des tissus musculaires et adipeux afin de favoriser la mobilisation des graisses au niveau de l'adipocyte et d'induire la thermogénèse via les récepteurs β-adrénergiques du muscle squelettique de l'individu présentant une surcharge pondérale. Des stratégies futures pour le traitement de l'obésité pourraient également reposer leur approche sur la combinaison d'une pratique régulière d'exercices et la prise de composés aux propriétés lipomobilisatrices. À ce titre, nous pourrions comparer l'efficacité d'un programme de perte de poids en comparant 2 groupes d'individus obèses caractérisés initialement par la même distribution régionale de tissu adipeux; l'un soumis à un programme d'entraînement d'une durée de 15

semaines (séances aérobiques 3-4 fois/sem, 30 min/séance à 65% de la VO_2max), l'autre soumis au même programme d'entraînement combiné à la prise quotidienne d'un composé aux propriétés lipomobilisatrices.

Finalement, les travaux qui composent le Chapitre 13 ont montré qu'une perte de poids entraîne une réduction significative de l'expression du facteur transcriptionnel impliqué dans la différenciation adipocytaire, PPARγ au niveau du dépôt sous-cutané abdominal. Par ailleurs, cette étude a été réalisée sur la base du fait que la perte pondérale chez des sujets obèses entraîne une augmentation des concentrations plasmatiques et tissulaires d'organochlorés (Backman & Kolmodin-Hedman, 1978; Chevrier et al., 2000; Walford et al., 1999) et que certains polluants exercent un pouvoir prolifératéur sur les peroxysomes (Dreyer et al., 1992). Sachant que l'expression de plusieurs gènes est affectée par les prolifératéurs de peroxysomes sous la régulation du récepteur nucléaire hormonal stéroïdien, PPARγ, nous avons vérifié si les changements de concentrations d'organochlorés du plasma et du tissu adipeux en réponse à une perte de poids avaient le potentiel d'affecter le transcrit PPARγ. L'absence de relations entre l'augmentation des concentrations plasmatiques et adipeuses d'organochlorés et les changements d'expression de PPARγ en réponse à une perte de poids suggère que les organochlorés étudiés ne semblent pas influencer directement l'expression de ce facteur transcriptionnel de la différenciation adipocytaire. D'autres facteurs transcriptionnels tels que les C/EBP et l'ADD1/SREBP1 peuvent être cependant influencés par l'augmentation des concentrations d'organochlorés subséquente à une perte de poids. De plus, il est fort probable que l'augmentation des polluants plasmatiques et tissulaires en réponse à la perte de poids ne soit pas suffisante pour entraîner des changements d'expresssion de ces facteurs transcriptionnels de la différenciation adipocytaire. Des études impliquant des pertes de poids drastiques seraient ainsi de mise pour vérifier l'hypothèse selon laquelle l'augmentation des niveaux de polluants suite à une diminution des réserves de graisses corporelles pourrait entraîner des changements au niveau du métabolisme de l'adipocyte humain. Il serait d'autant plus intéressant d'étudier directement les effets potentiels des organochlorés sur le programme de la différenciation adipocytaire du métabolisme de la cellule adipeuse par la technique de culture préadipocytaire. En d'autres mots, nous pourrions vérifier si certains organochlorés

agissent à titre d'activateurs de la différenciation adipocytaire ou encore à titre de facteurs apoptotiques.

Étant donné l'accumulation potentielle d'organochlorés au niveau du tissu adipeux, il est possible que ce tissu joue un rôle protecteur contre les éventuels désordres endocriniens et les facteurs de risque de développement de cancers causés par les organochlorés. Une stratégie de recherche qui pourrait être mise de l'avant pour vérifier cette hypothèse consisterait à soumettre des animaux maigres et obèses à une diète contaminée par de tels agents. Ainsi, nous pourrions vérifier s'il existe des divergences temporelles dans le développement des désordres endocriniens entre les deux souches d'animaux. Par ailleurs, le développement récent de souris lipoatrophiques de type A-ZIP/F-1, qui se caractérisent par leur absence de tissu adipeux (Moitra et al., 1998), pourrait également être utile pour vérifier si une greffe de tissu adipeux prémunirait ces souris des éventuels risques endocriniens ou de développement de cancers reliés à la consommation d'une diète enrichie de contaminants.

En conclusion, nos travaux ont permis de caractériser les effets de la perte pondérale et du vieillissement sur la balance lipolytique/lipogénique des adipocytes isolés des régions sous-cutanées. De plus, nos études ont montré le rôle que joue la cellule adipeuse dans la régulation du profil métabolique et de l'équilibre énergétique chez l'être humain. L'ensemble des données obtenues a donc conduit à une meilleure compréhension des facteurs (i.e. sexe, âge, composition corporelle) qui influencent les mécanismes régulateurs des processus de stockage et de mobilisation des lipides et pourrait ainsi favoriser le développement de nouvelles stratégies cliniques dans le traitement de l'obésité.

CHAPITRE 15

RÉFÉRENCES DES CHAPITRES 1, 2, 3, 4 et 14

1. Abate N., Garg A., Peshock R. M., Stray-Gundersen J. & Grundy S. M. (1995). Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest* 96: 88-98.
2. Ailhaud G. (1990). Cellular and secreted lipoprotein lipase revisited. *Clin Biochem* 23: 343-347.
3. Alessi M. C., Peiretti F., Morange P., Henry M., Nalbone G. & Juhan-Vague I. (1997). Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes* 46: 860-867.
4. Amri E. Z., Bonino F., Ailhaud G., Abumrad N. A. & Grimaldi P. A. (1995). Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J Biochem Chem* 270: 2367-2371.
5. Arner P. (1993). Adenosine, prostaglandins and phosphodiesterase as targets for obesity pharmacotherapy. *Int J Obesity* 17 Suppl 1: S57-59.
6. Arner P., Lithell H., Wahrenberg H. & Brönnegard M. (1991). Expression of lipoprotein lipase in different subcutaneous adipose tissue regions. *J Lipid Res* 32: 423-429.
7. Astrup A., Buemann B., Christensen N. J. & Toubro S. (1994). Failure to increase lipid oxidation in response to increasing dietary fat content in formerly obese women. *Am J Physiol* 266: E592-599.
8. Auboeuf D., Rieusset J., Fajas L., Vallier P., Frering V., Riou J. P., Staels B., Auwerx J., Laville M. & Vidal H. (1997). Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in

humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* 46: 1319-1327.

9. Auwerx J. (1999). PPARgamma, the ultimate thrifty gene. *Diabetologia* 42: 1033-1049.
10. Backman L. & Kolmodin-Hedman B. (1978). Concentration of DDT and DDE in plasma and subcutaneous adipose tissue before and after intestinal bypass operation for treatment of obesity. *Toxicol Appl Pharmacol* 46: 663-669.
11. Baldo A., Sniderman A. D., St-Luce S., Avramoglu R. K., Maslowska M., Hoang B., Monge J. C., Bell A., Mulay S. & Cianflone K. (1993). The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J Clin Invest* 92: 1543-1547.
12. Bastard J. P., Hainque B., Dusserre E., Bruckert E., Robin D., Vallier P., Perche S., Robin P., Turpin G., Jardel C., Laville M., Forest C. & Vidal H. (1999). Peroxisome proliferator activated receptor-gamma, leptin and tumor necrosis factor-alpha mRNA expression during very low calorie diet in subcutaneous adipose tissue in obese women. *Diabetes Metab Res Rev* 15: 92-98.
13. Bengtsson G. & Olivecrona T. (1982). On the pH dependency of the lipoprotein lipase activity. *Biochem Biophys Acta* 712: 196-199.
14. Benoist F., Lau P., McDonnell M., Doelle H., Milne R. & McPherson R. (1997). Cholesteryl ester transfer protein mediates selective uptake of high density lipoprotein cholesteryl esters by human adipose tissue. *J Biochem Chem* 272: 23572-23577.
15. Berg M., Fraker D. L. & Alexander H. R. (1994). Characterization of differentiation factor/leukaemia inhibitory factor effect on lipoprotein lipase activity and mRNA in 3T3-L1 adipocytes. *Cytokine* 6: 425-432.
16. Berlan M., Dang-Tran L., Lafontan M. & Denard Y. (1981). Influence of hypocaloric diet on alpha-adrenergic responsiveness of obese human subcutaneous adipocytes. *Int J Obesity* 5: 145-153.
17. Björntorp P. (1991a). Adipose tissue distribution and function. *Int J Obesity* 15: 67-81.
18. Björntorp P. (1991b). Metabolic implications of body fat distribution. *Diabetes Care* 14: 1132-1143.
19. Björntorp P. (1994). Fatty acids, hyperinsulinemia, and insulin resistance: which comes first? *Curr Opin Lipidol* 5: 166-174.

20. Björntorp P. (1995). Endocrine abnormalities of obesity. *Metabolism* 44: 21-23.

21. Blaak E. E., Van Baak M. A., Kemerink G. J., Pakbiers M. T., Heidendal G. A. & Saris W. H. (1994a). Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. *Am J Physiol* 267: E306-315.

22. Blaak E. E., Van Baak M. A., Kemerink G. J., Pakbiers M. T., Heidendal G. A. & Saris W. H. (1994b). Beta-adrenergic stimulation of skeletal muscle metabolism in relation to weight reduction in obese men. *Am J Physiol* 267: E316-322.

23. Blundell J. E., Burley V. J., Cotton J. R. & Lawton C. L. (1993). Dietary fat and the control of energy intake: evaluating the effects of fat on meal size and postmeal satiety. *Am J Clin Nutr* 57: S772-S777.

24. Boden G. (1997). Role of fatty acids in the pathogenesis of insulin resistance and NIDDM [published erratum appears in Diabetes 1997 Mar;46(3):536]. *Diabetes* 46: 3-10.

25. Bolinder J., Kager L., Ostman J. & Arner P. (1983). Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes* 32: 117-123.

26. Brewster D. W. & Matsumura F. (1988). Reduction of adipose tissue lipoprotein lipase activity as a result of in vivo administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin to the guinea pig. *Biochem Pharmacol* 37: 2247-2253.

27. Brouwer A., Morse D. C., Lans M. C., Schuur A. G., Murk A. J., Klasson-Wehler E., Bergman A. & Visser T. J. (1998). Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol Ind Health* 14: 59-84.

28. Brun R. P., Tontonoz P., Forman B. M., Ellis R., Chen J., Evans R. M. & Spiegelman B. M. (1996). Differential activation of adipogenesis by multiple PPAR isoforms. *Genes Dev* 10: 974-984.

29. Burton A. & Fried S. K. (1992). Effects of insulin and dexamethasone on lipoprotein lipase in human adipose tissue. *Am J Physiol* 262: E695-E699.

30. Byrne C. D., Brindle N. P. J., Wang T. W. M. & Hales C. N. (1991). Interaction of non-esterified fatty acid and insulin in control of triacylglycerol secretion by Hep G2 cells. *Biochem J* 280: 99-104.

31. Campbell P. J., Carlson M. G. & Nurjhan N. (1994). Fat metabolism in human obesity. *Am J Physiol* 266: E600-605.

32. Carlsen E., Giwercman A., Keiding N. & Skakkebaek N. E. (1992). Evidence for decreasing quality of semen during past 50 years. *Br Med J* 305: 609-613.

33. Castan I., Valet P., Larrouy D., Voisin T., Remaury A., Daviaud D., Laburthe M. & Lafontan M. (1993). Distribution of PYY receptors in human fat cells: an antilipolytic system alongside the alpha 2-adrenergic system. *Am J Physiol* 265: E74-80.

34. Chevrier J., Dewailly E., Ayotte P., Mauriège P., Després J. P. & Tremblay A. (2000). Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *Int J Obesity* In press:

35. Choy L. N., Rosen B. S. & Spiegelman B. M. (1992). Adipsin and an endogenous pathway of complement from adipose cells. *J Biochem Chem* 267: 12736-12741.

36. Chung J. & Scanu A. M. (1977). Isolation, molecular properties, and kinetic characterization of lipoprotein lipase from rat heart. *J Biol Chem* 252: 4202-4209.

37. Coe N. R., Simpson M. A. & Bernlohr D. A. (1999). Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res* 40: 967-972.

38. Cohn J. S., McNamara J. R., Cohn S. D., Ordovas J. M. & Schaefer E. J. (1988). Postprandial plasma lipoprotein changes in human subjects of different ages. *J Lipid Res* 29: 469-479.

39. Colberg S. R., Simoneau J. A., Leland Theate F. & Kelley D. E. (1995). Skeletal muscle utilization of free fatty acids in women with visceral obesity. *J Clin Invest* 95: 1846-1853.

40. Coleman D. L. (1978). Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* 14: 141-148.

41. Coppack S. W., Evans R. D., Fisher R. M., Frayn K. N., Gibbons G. F., Humphreys S. M., Kirk M. L., Potts J. L. & Hockaday T. D. (1992). Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. *Metabolism* 41: 264-272.

42. Coppack S. W., Jensen M. D. & Miles J. M. (1994). In vivo regulation of lipolysis in humans. *J Lipid Res* 35: 177-193.

43. Couillard C., Bergeron N., Prud'homme D., Bergeron J., Tremblay A., Bouchard C., Mauriège P. & Després J. P. (1998). Postprandial triglyceride response in visceral obesity in men. *Diabetes* 47: 953-960.

44. Crampes F., Marceron M., Beauville M., Rivière D., Garrigues M., Berlan M. & Lafontan M. (1989). Platelet alpha 2-adrenoceptors and adrenergic adipose tissue responsiveness after moderate hypocaloric diet in obese subjects. *Int J Obesity* 13: 99-110.

45. Crandall D. L., Quinet E. M., Morgan G. A., Busler D. E., McHendry-Rinde B. & Kral J. G. (1999). Synthesis and secretion of plasminogen activator inhibitor-1 by human preadipocytes. *J Clin Endocrinol Metab* 84: 3222-3227.

46. Darlington G. J., Ross S. E. & MacDougald O. A. (1998). The role of C/EBP genes in adipocyte differentiation. *J Biol Chem* 273: 30057-30060.

47. de Glisezinski I., Crampes F., Harant I., Berlan M., Hejnova J., Langin D., Rivière D. & Stich V. (1998). Endurance training changes in lipolytic responsiveness of obese adipose tissue. *Am J Physiol* 275: E951-956.

48. Després J. P. (1991). Obesity and lipid metabolism: relevance of body fat distribution. *Curr Opin Lipidol* 2: 5-15.

49. Després J. P., Bouchard C., Savard R., Tremblay A., Marcotte M. & Thériault G. (1984a). The effect of a 20-week endurance training program on adipose-tissue morphology and lipolysis in men and women. *Metabolism* 33: 235-239.

50. Després J. P., Bouchard C., Savard R., Tremblay A., Marcotte M. & Thériault G. (1984b). Level of physical fitness and adipocyte lipolysis in humans. *J Appl Physiol* 56: 1157-1161.

51. Després J. P., Moorjani S., Ferland M., Tremblay A., Lupien P. J., Nadeau A., Pinault S., Thériault G. & Bouchard C. (1989a). Adipose tissue distribution and lipoprotein levels in obese women: importance of intra-abdominal fat. *Arteriosclerosis* 9: 203-210.

52. Després J. P., Moorjani S., Lupien P. J., Tremblay A., Nadeau A. & Bouchard C. (1990). Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. *Arteriosclerosis* 10: 497-511.

53. Després J. P., Nadeau A., Trembaly A., M. F., Moorjani S., Lupien P. J., Thériault G., Pinault S. & Bouchard C. (1989b). Role of deep abdomianl fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. *Diabetes* 38: 304-309.

54. Desvergne B. & Wahli W. (1999). Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 20: 649-688.

55. Dewailly E., Mulvad G., Pedersen H. S., Ayotte P., Demers A., Weber J. P. & Hansen J. C. (1999). Concentration of organochlorines in human brain, liver, and adipose tissue autopsy samples from Greenland. *Environ Health Perspect* 107: 823-828.

56. Dieudonné M. N., Pecquery R., Boumediene A., Leneveu M. C. & Giudicelli Y. (1998). Androgen receptors in human preadipocytes and adipocytes: regional specificities and regulation by sex steroids. *Am J Physiol* 274: C1645-1652.

57. Dreyer C., Krey G., Keller H., Givel F., Helftenbein G. & Wahli W. (1992). Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 68: 879-887.

58. Eckel R. H. (1989). Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 320: 1060-1068.

59. Eckel R. H. & Yost T. J. (1987). Weight reduction increases adipose tissue lipoprotein lipase responsiveness in obese women. *J Clin Invest* 80: 992-997.

60. Ek I., Arner P., Bergqvist A., Carlstrom K. & Wahrenberg H. (1997). Impaired adipocyte lipolysis in nonobese women with the polycystic ovary syndrome: a possible link to insulin resistance? *J Clin Endocrinol Metab* 82: 1147-1153.

61. Enan E., Liu P. C. & Matsumura F. (1992). 2,3,7,8-Tetrachlorodibenzo-p-dioxin causes reduction of glucose transporting activities in the plasma membranes of adipose tissue and pancreas from the guinea pig. *J Biochem Chem* 267: 19785-19791.

62. Eriksson P., Reynisdottir S., Lönnqvist F., Stemme V., Hamsten A. & Arner P. (1998). Adipose tissue secretion of plasminogen activator inhibitor-1 in non- obese and obese individuals. *Diabetologia* 41: 65-71.

63. Evans D. J., Hoffmann R. G., Kalkhoff R. K. & Kissebah A. H. (1983). Relationship of androgenic activity to body fat topography, fat cell morphology, and metabolic aberrations in premenopausal women. *J Clin Endocrinol Metab* 57: 304-310.

64. Farooqi I. S., Jebb S. A., Langmack G., Lawrence E., Cheetham C. H., Prentice A. M., Hughes I. A., McCamish M. A. & O'Rahilly S. (1999). Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 341: 879-884.

65. Felts J. M., Itakura H. & Crane T. R. (1975). The mechanism of assimilation of constituents of chylomicrons, very low density lipoproteins and remnants. A new theory. *Biochem Biophys Res Commun* 66: 1467-1475.

66. Ferraro R. T., Eckel R. H., Larson D. E., Fontvieille A. M., Rising R., Jensen D. R. & Ravussin E. (1993). Relationship between skeletal muscle lipoprotein lipase activity and 24- hour macronutrient oxidation. *J Clin Invest* 92: 441-445.

67. Flatt J. P. (1987). Dietary fat, carbohydrate balance, and weight maintenance: effects of exercise. *Am J Clin Nutr* 45: 296-306.

68. Flegal K. M., Carroll M. D., Kuczmarski R. J. & Johnson C. L. (1998). Overweight and obesity in the United States: prevalence and trends, 1960-1994. *Int J Obesity* 22: 39-47.

69. Forman B. M., Chen J. & Evans R. M. (1997). Hypolipidemic drugs, polyunsaturated fatty acids and eicosanoids are ligands for PPARα and PPARδ. *Proc Natl Acad Sci* 94: 4312-4317.

70. Forman B. M., Tontonoz P., Chen J., Brun R. P., Spiegelman B. M. & Evans R. M. (1995). 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 83: 803-812.

71. Frayn K. N., Shadid S., Hamlani R., Humphreys S. M., Clark M. L., Fielding B. A., Boland O. & Coppack S. W. (1994). Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 266: E308-317.

72. Frayn K. N., Williams C. M. & Arner P. (1996). Are increased plasma non-esterified fatty acid concentrations a risk marker for coronary heart disease and other chronic diseases? *Clin Sci* 90: 243-253.

73. Frederich R. C., Kahn B. B., Peach M. J. & Hlier J. S. (1992). Tissue-specific nutritional regulation of angiotensinogen in adipose tissue. *Hypertension* 19: 339-344.

74. Fredrikson G., Stralfors P., Nilsson N. O. & Belfrage P. (1981). Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J Biol Chem* 256: 6311-6320.

75. Fried S. K., Bunkin D. A. & Greenberg A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab* 83: 847-850.

76. Fried S. K. & Kral J. G. (1987). Sex differences in regional distribution of fat cell size and lipoprotein lipase activity in morbidly obese patients. *Int J Obesity* 11: 129-140.

77. Fried S. K., Russell C. D., Grauso N. L. & Brolin R. E. (1993). Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J Clin Invest* 92: 2191-2198.

78. Garfinkel A. S., Nilsson-Ehle P. & Schotz C. (1976). Regulation of lipoprotein lipase. Induction by insulin. *Biochem Biophys Acta* 424: 264-273.

79. Gasic S., Tian B. & Green A. (1999). Tumor necrosis factor alpha stimulates lipolysis in adipocytes by decreasing Gi protein concentrations. *J Biochem Chem* 274: 6770-6775.

80. Glickman R. M. & Green P. H. R. (1977). The intestine as a source of apolipoprotein A1. *Proc Natl Acad Sci USA* 74: 2569-2573.

81. Goodpaster B. H., Thaete F. L., Simoneau J. A. & Kelley D. E. (1997). Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* 46: 1579-1585.

82. Green A., Swenson S., Johnson J. L. & Partin M. (1989). Characterization of human adipocyte adenosine receptors. *Biochem Biophys Res Commun* 163: 137-142.

83. Grégoire F. M., Smas C. M. & Sul H. S. (1998). Understanding adipocyte differentiation. *Physiol Rev* 78: 783-809.

84. Grundy S. M. (1990) Cholesterol and atherosclerosis: diagnosis and treatment. J.B. Lippincott CO, Philadelphia.

85. Harp J. B. & DiGirolamo M. (1995). Components of the renin-angiotensin system in adipose tissue: changes with maturation and adipose mass enlargement. *J Gerontol* 50A: B270-B276.

86. Hauner H., Petrusche T., Russ M., Rohrig K. & Eckel J. (1995). Effects of tumor necrosis factor alpha (TNFα) on glucose transport and lipid metabolism of newly-differentiated human fat cell culture. *Diabetologia* 38: 764-771.

87. Heiling V. J. & Jensen M. D. (1992). Free fatty acid metabolism in the follicular and luteal phases of the menstrual cycle. *J Clin Endocrinol Metab* 74: 806-810.

88. Hellmér J., Marcus C., Sonnefeld T. & Arner P. (1992). Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. *J Clin Endocrinol Metab* 75: 15-20.

89. Hellström L., Langin D., Reynisdottir S., Dauzats M. & Arner P. (1996a). Adipocyte lipolysis in normal weight subjects with obesity among first- degree relatives. *Diabetologia* 39: 921-928.

90. Hellström L., Reynisdottir S., Langin D., Rossner S. & Arner P. (1996b). Regulation of lipolysis in fat cells of obese women during long-term hypocaloric diet. *Int J Obesity* 20: 745-752.

91. Hellström L., Wahrenberg H., Reynisdottir S. & Arner P. (1997). Catecholamine-induced adipocyte lipolysis in human hyperthyroidism. *J Clin Endocrinol Metab* 82: 159-166.

92. Hennes M. M. I., Shrago E. & Kissebah A. H. (1990). Receptor and postreceptor effects of free fatty acids (FFA) on hepatocyte insulin dynamics. *Int J Obesity* 14: 831-841.

93. Heymsfield S. B., Greenberg A. S., Fujioka K., Dixon R. M., Kushner R., Hunt T., Lubina J. A., Patane J., Self B., Hunt P. & McCamish M. (1999). Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 282: 1568-1575.

94. Higgins J. M. & Fielding C. J. (1975). Lipoprotein lipase mechanism of formation of triglycerides-rich remnant particles from very low density lipoproteins and chylomicrons. *Biochemistry* 14: 2288-2293.

95. Hodgetts V., Coppack S. W., Frayn K. N. & Hockaday T. D. (1991). Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise. *J Appl Physiol* 71: 445-451.

96. Hoffstedt J., Arner P., Hellers G. & Lonnqvist F. (1997). Variation in adrenergic regulation of lipolysis between omental and subcutaneous adipocytes from obese and non-obese men. *J Lipid Res* 38: 795-804.

97. Holm C., Belfrage P. & Fredrikson G. (1989). Human adipose tissue hormone-sensitive lipase: identification and comparison with other species. *Biochem Biophys Acta* 1006: 193-197.

98. Hotamisligil G. S., Arner P., Caro J. F., Atkinson R. L. & Spiegelman B. M. (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95: 2409-2415.

99. Hotamisligil G. S., Shargill N. S. & Spiegelman B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259: 87-91.

100. James R. C., Burns T. W. & Chase G. R. (1971). Lipolysis of human adipose cells: influence of donor factors. *J Lab Clin Med* 77: 254-266.

101. Jepson C. A. & Yeaman S. J. (1992). Inhibition of hormone-sensitive lipase by intermediary lipid metabolites. *FEBS* 310: 197-200.

102. Kadesch T. (1993). Consequences of heteromeric interactions among helix-loop-helix proteins. *Cell Growth Differ* 4: 49-55.

103. Kather H., Wieland E., Fischer B. & Schlierf G. (1985a). Antilipolytic effects of N6-phenylisopropyladenosine and prostaglandin E2 in fat-cells of obese volunteers before and during energy restriction. *Biochem J* 231: 531-535.

104. Kather H., Wieland E., Fischer B., Wirth A. & Schlierf G. (1985b). Adrenergic regulation of lipolysis in abdominal adipocytes of obese subjects during caloric restriction: reversal of catecholamine action caused by relief of endogenous inhibition. *Eur J Clin Invest* 15: 30-37.

105. Keifer M. C. & Mahurin R. K. (1997). Chronic neurologic effects of pesticide overexposure. *Occup Med* 12: 291-304.

106. Kelling C. K., Christian B. J., Inhorn S. L. & Peterson R. E. (1985). Hypophagia-induced weight loss in mice, rats, and guinea pigs treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam Appl Toxicol* 5: 700-712.

107. Kern P. A., Ong J. M., Saffari B. & Carty J. (1990). The effects of weight loss on the activity and expression of adipose-tissue lipoprotein lipase in very obese humans. *N Engl J Med* 322: 1053-1059.

108. Kern P. A., Saghizadeh M., Ong J. M., Bosch R. J., Deem R. & Simsolo R. B. (1995). The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 95: 2111-2119.

109. Kim J. B. & Spiegelman B. M. (1996). ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 10: 1096-1107.

110. Kissebah A. H. & Krakower G. R. (1994). Regional adiposity and morbidity. *Physiol Rev* 74: 761-811.

111. Kliewer S. A., Lenhard J. M., Willson T. M., Patel I., Morris D. C. & Lehmann J. M. (1995). A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell* 83: 813-819.

112. Krasinski S. D., Cohn J. S., Schaefer E. J. & Russell R. M. (1990). Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. *J Clin Invest* 85: 883-892.

113. Krey G., Braissant O., L'Horset F., Kalkhoven E., Perroud M., Parker M. G. & Wahli W. (1997). Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator- dependent receptor ligand assay. *Mol Endocrinol* 11: 779-791.

114. Labrie F. (1991). Intracrinology. *Mol Cell Endocrinol* 78: C113-118.

115. Lafontan M. & Berlan M. (1982). Characterization of physiological agonist selectivity of human fat cell alpha 2-adrenoceptors: adrenaline is the major stimulant of the alpha 2-adrenoceptors. *Eur J Pharmacol* 82: 107-111.

116. Lafontan M. & Berlan M. (1993). Fat cell adrenergic receptors and the control of white and brown fat cell function. *J Lipid Res* 34: 1057-1091.

117. Lafontan M. & Berlan M. (1995). Fat cell alpha 2-adrenoceptors: the regulation of fat cell function and lipolysis. *Endocr Rev* 16: 716-738.

118. Lakshman M. R., Chirtel S. J., Chambers L. L. & Coutlakis P. J. (1989). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on lipid synthesis and lipogenic enzymes in the rat. *J Pharmacol Exp Ther* 248: 62-66.

119. Lamarche B., Despres J. P., Moorjani S., Nadeau A., Lupien P. J., Tremblay A., Theriault G. & Bouchard C. (1993). Evidence for a role of insulin in the regulation of abdominal adipose tissue lipoprotein lipase response to exercise training in obese women. *Int J Obesity* 17: 255-261.

120. Langin D., Holm C. & Lafontan M. (1996). Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. *Proc Nutr Soc* 55: 93-109.

121. Larrouy D., Galitzky J. & Lafontan M. (1991). A1 adenosine receptors in the human fat cell: tissue distribution and regulation of radioligand binding. *Eur J Pharmacol* 206: 139-147.

122. Lawton C. L., Burley V. J., Wales J. K. & Blundell J. E. (1993). Dietary fat and appetite control in obese subjects: weak effects on satiation and satiety. *Int J Obesity* 17: 409-416.

123. Lee S. S., Pineau T., Drago J., Lee E. J., Owens J. W., Kroetz D. L., Fernandez-Salguero P. M., Westphal H. & Gonzalez F. J. (1995). Targeted disruption of the alpha isoform of the peroxisome proliferator- activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 15: 3012-3022.

124. Lewis G. F. (1997). Fatty acid regulation of very low density lipoprotein production. *Curr Opin Lipidol* 8: 146-153.

125. Lin F. T. & Lane M. D. (1994). CCAAT/enhancer binding protein alpha is sufficient to initiate the 3T3- L1 adipocyte differentiation program. *Proc Natl Acad Sci* 91: 8757-8761.

126. Lindberg U. B., Crona N., Silfverstolpe G., Björntorp P. & Rebuffé-Scrive M. (1990). Regional adipose tissue metabolism in postmenopausal women after treatment with exogenous sex steroids. *Horm Metabol Res* 22: 345-351.

127. Lindberg U. B., Leibel R. L., Silfverstolpe G., Hirsch J., Björntorp P. & Rebuffe-Scrive M. (1991). Effects of early pregnancy on regional adipose tissue metabolism. *Horm Metabol Res* 23: 25-29.

128. Lissner L., Levitsky D. A., Strupp B. J., Kalkwarf H. J. & Roe D. A. (1987). Dietary fat and regulation of energy intake in human subjects. *Am J Clin Nutr* 46: 886-892.

129. Lithell H., Boberg J., Hellsing K., Lundqvist G. & Vessby B. (1978). Lipoprotein lipase activity in human skeletal and adipose tissue in the fasting and the fed states. *Atherosclerosis* 30: 89-94.

130. Liu L. S., Spelleken M., Rohrig K., Hauner H. & Eckel J. (1998). Tumor necrosis factor-alpha acutely inhibits insulin signaling in human adipocytes: implication of the p80 tumor necrosis factor receptor. *Diabetes* 47: 515-522.

131. Liu P. C. & Matsumura F. (1995). Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the "adipose- type" and "brain-type" glucose transporters in mice. *Mol Pharmacol* 47: 65-73.

132. Liu P. C., Phillips M. A. & Matsumura F. (1996). Alteration by 2,3,7,8-Tetrachlorodibenzo-p-dioxin of CCAAT/enhancer binding protein correlates with suppression of adipocyte differentiation in 3T3-L1 cells. *Mol Pharmacol* 49: 989-997.

133. Loftus T. M. & Lane M. D. (1997). Modulating the transcriptional control of adipogenesis. *Curr Opin Genet Dev* 7: 603-608.

134. Lönnqvist F., Krief S., Strosberg A. D., Nyberg S., Emorine L. J. & Arner P. (1993). Evidence for a functional beta 3-adrenoceptor in man. *Br J Pharmacol* 110: 929-936.

135. Lönnqvist F., Nyberg B., Wahrenberg H. & Arner P. (1990). Catecholamine-induced lipolysis in adipose tissue of the elderly. *J Clin Invest* 85: 1614-1621.

136. Lönnqvist F., Thorne A., Large V. & Arner P. (1997). Sex differences in visceral fat lipolysis and metabolic complications of obesity. *Arterioscler Thromb* 17: 1472-1480.

137. Lönnroth P. & Smith U. (1986). The antilipolytic effect of insulin in human adipocytes requires activation of the phosphodiesterase. *Biochem Biophys Res Commun* 141: 1157-1161.

138. Marcus C., Bolme P., Micha-Johansson G., Margery V. & Bronnegard M. (1994). Growth hormone increases the lipolytic sensitivity for catecholamines in adipocytes from healthy adults. *Life Sci* 54: 1335-1341.

139. Marcus C., Ehren H., Bolme P. & Arner P. (1988). Regulation of lipolysis during the neonatal period. Importance of thyrotropin. *J Clin Invest* 82: 1793-1797.

140. Marcus C., Karpe B., Bolme P., Sonnenfeld T. & Arner P. (1987). Changes in catecholamine-induced lipolysis in isolated human fat cells during the first year of life. *J Clin Invest* 79: 1812-1818.

141. Marcus C., Sonnenfeld T., Karpe B., Bolme P. & Arner P. (1989). Inhibition of lipolysis by agents acting via adenylate cyclase in fat cells from infants and adults. *Pediatric Res* 26: 255-259.

142. Martin L. J., Connelly P. W., Nancoo D., Wood N., Zhang Z. J., Maguire G., Quinet E., Tall A. R., Marcel Y. L. & McPherson R. (1993). Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype. *J Lipid Res* 34: 437-446.

143. Mauriège P. (1996). Variations régionales du métabolisme du tissu adipeux: influence de l'obésité et rôle des stéroïdes sexuels (No 545). Université Paul Sabatier Toulouse III, Toulouse, pp 81.

144. Mauriège P., Brochu M., Prud'homme D., Tremblay A., Nadeau A., Lemieux S. & Després J. P. (1999a). Is visceral adiposity a significant correlate of subcutaneous adipose cell lipolysis in men? *J Clin Endocrinol Metab* 84: 736-742.

145. Mauriège P., de Pergola G., Berlan M. & Lafontan M. (1988). Human fat cell beta-adrenergic receptors: beta-agonist-dependent lipolytic responses and characterization of beta-adrenergic binding sites on human fat cell membranes with highly selective beta 1-antagonists. *J Lipid Res* 29: 587-601.

146. Mauriège P., Després J. P., Prud'homme D., Pouliot M. C., Marcotte M., Tremblay A. & Bouchard C. (1991). Regional variation in adipose tissue lipolysis in lean and obese men. *J Lipid Res* 32: 1625-1633.

147. Mauriège P., Galitzky J., Berlan M. & Lafontan M. (1987). Heterogeneous distribution of beta- and alpha$_2$-adrenoceptor binding sites in human fat cells from various deposits: functional consequences. *Eur J Clin Invest* 17: 156-165.

148. Mauriège P., Imbeault P., Langin D., Lacaille M., Alméras N., Tremblay A. & Després J. P. (1999b). Regional and gender variations in adipose tissue lipolysis in response to weight loss. *J Lipid Res* 40: 1559-1571.

149. Mauriège P., Marette A., Atgié C., Bouchard C., Thériault G., Bukowiecki L. J., Marceau P., Biron S., Nadeau A. & Després J. P. (1995a). Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J Lipid Res* 36: 672-684.

150. Mauriège P., Prud'homme D., Lemieux S., Tremblay A. & Després J. P. (1995b). Regional differences in adipose tissue lipolysis from lean and obese women: existence of postreceptor alterations. *Am J Physiol* 269: 341-350.

151. McFarland V. A. & Clarke J. U. (1989). Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ Health Perspect* 81: 225-239.

152. Miller L. K., Kral J. G., Strain G. W. & Zumoff B. (1990). Androgen binding to ammonium sulfate precipitates of human adipose tissue cytosols. *Steroids* 55: 410-415.

153. Misra A., Gard A., Abate N., Peshock R. M., Stray-Gundersen J. & Grundy S. (1997). Relationship of anterior and posterior subcutaneous abdominal fat to insulin sensitivity in nondiabetic men. *Obesity Res* 5: 93-99.

154. Mizutani T., Nishikawa Y., Adachi H., Enomoto T., Ikegami H., Kurachi H., Nomura T. & Miyake A. (1994). Identification of estrogen receptor in human adipose tissue and adipocytes. *J Clin Endocrinol Metab* 78: 950-954.

155. Mohamed-Ali V., Goodrick S., Bulmer K., Holly J. M., Yudkin J. S. & Coppack S. W. (1999). Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo. *Am J Physiol* 277: E971-975.

156. Mohamed-Ali V., Pinkney J. H. & Coppack S. W. (1998). Adipose tissue as an endocrine and paracrine organ. *Int J Obesity* 22: 1145-1158.

157. Moitra J., Mason M. M., Olive M., Krylov D., Gavrilova O., Marcus-Samuels B., Feigenbaum L., Lee E., Aoyama T., Eckhaus M., Reitman M. L. & Vinson C. (1998). Life without white fat: a transgenic mouse. *Genes Dev* 12: 3168-3181.

158. Moldes M., Lasnier F., Feve B., Pairault J. & Djian P. (1997). Id3 prevents differentiation of preadipose cells. *Mol Cell Biol* 17: 1796-1804.

159. Mukherjee R., Jow L., Croston G. E. & Paterniti J. R., Jr. (1997). Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARgamma2 versus PPARgamma1 and activation with retinoid X receptor agonists and antagonists. *J Biol Chem* 272: 8071-8076.

160. Neal R. A., Beatty P. W. & Gasiewicz T. A. (1979). Studies of the mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p- dioxin (TCDD). *Ann N Y Acad Sci* 320: 204-213.

161. Nicklas B. J., Rogus E. M., Colman E. G. & Goldberg A. P. (1996). Visceral adiposity, increased adipocyte lipolysis, and metabolic dysfunction in obese postmenopausal women. *Am J Physiol* 270: E72-78.

162. Ohisalo J. J., Ranta S. & Huhtaniemi I. T. (1984). Inhibition of adenosine 3',5'-monophosphate accumulation and lipolysis by adenosine analogs in human subcutaneous adipocytes. *J Clin Endocrinol Metab* 58: 32-35.

163. Ong J. M., Kirchgessner T. G., Schotz M. C. & Kern P. A. (1988). Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes. *J Biol Chem* 263: 12933-12938.

164. Ostman J., Arner P., Engfeldt P. & Kager L. (1979). Regional differences in the control of lipolysis in human adipose tissue. *Metabolism* 28: 1198-1205.

165. Ostman J., Arner P., Kimura H., Wahrenberg H. & Engfeldt P. (1984). Influence of fasting on lipolytic response to adrenergic agonists and on adrenergic receptors in subcutaneous adipocytes. *Eur J Clin Invest* 14: 383-391.

166. Ostman J., Efendic S. & Arner P. (1969). Catecholamines and metabolism of human adipose tissue. *Acta Medica Scandinavica* 186: 241-246.

167. Ottosson M., Vikman-Adolfsson K., Enerbäck S., Elander A., Björntorp P. & Edén S. (1995). Growth hormone inhibits lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 80: 936-941.

168. Ottosson M., Vikman-Adolfsson K., Enerbäck S., Olivecrona G. & Björntorp P. (1994). The effects of cortisol on the regulation of lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 79: 820-825.

169. Pedersen S. B., Jonler M. & Richelsen B. (1994). Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 78: 1354-1359.

170. Peiris A. N., Mueller R. A., Struve M. F., Smith G. A. & Kissebah A. H. (1987). Relationship of androgenic activity to splanchnic insulin metabolism and peripheral glucose utilization in premenopausal women. *J Clin Endocrinol Metab* 64: 162-169.

171. Pelleymounter M., Cullen M., Baker M., Hecht R., Winters D., Bone T. & Collins F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269: 540-543.

172. Peltonen P., Marniemi J., Hietanen E., Vuori I. & Ehnholm C. (1981). Changes in serum lipids, lipoproteins, and heparin releasable lipolytic enzymes during moderate physical training in man: a longitudinal study. *Metabolism* 30: 518-526.

173. Poland A. & Knutson J. C. (1982). 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 22: 517-554.

174. Pouliot M.-C., Després J. P., Moorjani S., Lupien P. J., Tremblay A., Nadeau A. & Bouchard C. (1991). Regional variation in adipose tissue lipoprotein lipase activity: association with plasma high density lipoprotein levels. *Eur J Clin Invest* 21: 398-405.

175. Pouliot M. C., Després J. P., Nadeau A., Moorjani S., Prud'homme D., Lupien P. J., Tremblay A. & Bouchard C. (1992). Visceral obesity in men: associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes* 41: 826-834.

176. Presta E., Leibel R. L. & Hirsch J. (1990). Regional changes in adrenergic receptor status during hypocaloric intake do not predict changes in adipocyte size or body shape. *Metabolism* 39: 307-315.

177. Price T. M. & O'Brien S. N. (1993). Determination of estrogen receptor messenger ribonucleic acid (mRNA) and cytochrome P450 aromatase mRNA levels in adipocytes and adipose stromal cells by competitive polymerase chain reaction amplification. *J Clin Endocrinol Metab* 77: 1041-1045.

178. Radeau T., Lau P., Robb M., McDonnell M., Ailhaud G. & McPherson R. (1995). Cholesteryl ester transfer protein (CETP) mRNA abundance in human adipose tissue: relationship to cell size and membrane cholesterol content. *J Lipid Res* 36: 2552-2561.

179. Radeau T., Robb M., Lau P., Borthwick J. & McPherson R. (1998). Relationship of adipose tissue cholesteryl ester transfer protein (CETP) mRNA to plasma concentrations of CETP in man. *Atherosclerosis* 139: 369-376.

180. Randle P. J., Garland P. B., Hales C. N. & Newsholme E. A. (1963). The glucose-fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1: 785-789.

181. Rebuffé-Scrive M., Anderson B., Olbe L. & Bjorntorp P. (1989). Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. *Metabolism* 38: 453-458.

182. Rebuffé-Scrive M., Anderson B., Olbe L. & Björntorp P. (1990). Metabolism of adipose tissue in intraabdominal depots in severely obese men and women. *Metabolism* 39: 1021-1025.

183. Rebuffé-Scrive M., Basdevant A. & Guy-Grand B. (1983). Nutritional induction of adipose tissue lipoprotein lipase in obese subjects. *Am J Clin Nutr* 37: 974-980.

184. Rebuffé-Scrive M., Eldh J., Hafström L. O. & Bjorntörp P. (1986). Metabolism of mammary, abdominal, and femoral adipocytes in women before and after menopause. *Metabolism* 35: 792-797.

185. Rebuffé-Scrive M., Enk L., Crona N., Lönnroth P., Abrahamsson L., Smith U. & Björntorp P. (1985). Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy and lactation. *J Clin Invest* 75: 1973-1976.

186. Rebuffé-Scrive M., Krotkiewski M., Elfverson J. & Björntorp P. (1988). Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J Clin Endocrinol Metab* 67: 1122-1128.

187. Rebuffé-Scrive M., Lönnroth P., Marin P., Wesslau C., Björntorp P. & Smith U. (1987). Regional adipose tissue metabolism in men and postmenopausal women. *Int J Obesity* 11: 347-355.

188. Rebuffé-Scrive M., Marin P. & Björntorp P. (1991). Effect of testosterone on abdominal adipose tissue in men. *Int J Obesity* 15: 791-795.

189. Reilly C. F., Fujita T., Hutzelmann J. E., Mayer E. J. & Shebuski R. J. (1991). Plasminogen activator inhibitor-1 suppresses endogenous fibrinolysis in a canine model of pulmonary embolism. *Circulation* 84: 287-292.

190. Reitman J. S., Kosmacos F. C. & Howard B. V. (1982). Characterization of lipase activities in obese Pima indians decreases with weight reduction. *J Clin Invest* 70: 791-797.

191. Reynisdottir S., Langin D., Carlstrom K., Holm C., Rossner S. & Arner P. (1995). Effects of weight reduction on the regulation of lipolysis in adipocytes of women with upper-body obesity. *Clin Sci* 89: 421-429.

192. Reynisdottir S., Wahrenberg H., Carlström K., Rössner S. & Arner P. (1994). Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta2-adrenoceptors. *Diabetologia* 37: 428-435.

193. Richelsen B. (1986). Increased $alpha_2$- but similar beta-adrenergic receptor activities in subcutaneous gluteal adipocytes from females compared with males. *Eur J Clin Invest* 16: 302-309.

194. Richelsen B., Eriksen E. F., Beck-Nielsen H. & Pedersen O. (1984). Prostaglandin E2 receptor binding and action in human fat cells. *J Clin Endocrinol Metab* 59: 7-12.

195. Richelsen B., Pedersen S. B., Borglum J. D., Moller-Pedersen T., Jorgensen J. & Jorgensen J. O. (1994). Growth hormone treatment of obese women for 5 wk: effect on body composition and adipose tissue LPL activity. *Am J Physiol* 266: E211-E216.

196. Rogan W. J. & Gladen B. C. (1992). Neurotoxicology of PCBs and related compounds. *Neurotoxicol* 13: 27-35.

197. Ron D. & Habener J. F. (1992). CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant- negative inhibitor of gene transcription. *Genes Dev* 6: 439-453.

198. Rosenbaum M., Presta E., Hirsch J. & Leibel R. L. (1991). Regional differences in adrenoreceptor status of adipose tissue in adults and prepubertal children. *J Clin Endocrinol Metab* 73: 341-347.

199. Roust L. R. & Jensen M. D. (1993). Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes* 42: 1567-1573.

200. Rozen R., Banegas E., Davilla M. & Apfelbaum M. (1984). Effects of a very-low-calorie diet on adrenergic responsiveness in human adipose tissue. *Int J Obesity* 8: 141-149.

201. Saleh J., Summers L. K., Cianflone K., Fielding B. A., Sniderman A. D. & Frayn K. N. (1998). Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue in vivo in the postprandial period. *J Lipid Res* 39: 884-891.

202. Samra J. S., Clark M. L., Humphreys S. M., Macdonald I. A., Matthews D. R. & Frayn K. N. (1996). Effects of morning rise in cortisol concentration on regulation of lipolysis in subcutaneous adipose tissue. *Am J Physiol* 271: E996-1002.

203. Samuelsson L., Strömberg K., Vikman K., Bjursell B. & Enerbäck S. (1991). The CCAAT/enhancer binding protein and its role in adipocyte differentiation: evidence for direct involvement in terminal adipocyte development. *Embo J* 10: 3787-3793.

204. Savard R., Després J. P., Marcotte M., Thériault G., Tremblay A. & Bouchard C. (1987). Acute effects of endurance training exercise on human adipose tissue metabolism. *Metabolism* 36: 480-485.

205. Scheja L., Makowski L., Uysal K. T., Wiesbrock S. M., Shimshek D. R., Meyers D. S., Morgan M., Parker R. A. & Hotamisligil G. S. (1999). Altered insulin secretion associated with reduced lipolytic efficiency in aP2-/- mice. *Diabetes* 48: 1987-1994.

206. Schwartz R. & Brunzell J. (1978). Increased adipose tissue lipoprotein lipase activity in moderately obese men after weight reduction. *Lancet* 1: 1230-1231.

207. Schwartz R. & Brunzell J. (1981). Increase of adipose tissue LPL activity with weight loss. *J Clin Invest* 67: 1425-1430.

208. Seidell J. C. (1997). What is the cost of obesity? *Int J Obesity* 21: S1.

209. Seip R. L., Angelopoulos T. J. & Semenkovich C. F. (1995). Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue. *Am J Physiol* 268: E229-E236.

210. Shen W. J., Sridhar K., Bernlohr D. A. & Kraemer F. B. (1999). Interaction of rat hormone-sensitive lipase with adipocyte lipid- binding protein. *Proc Natl Acad Sci* 96: 5528-5532.

211. Shimomura I., Funahashi T., Takahashi M., Maeda K., Kotani K., Nakamura T., Yamashita S., Miura M., Fukuda Y., Takemura K., Tokunaga K. & Matsuzawa Y. (1996). Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nature Med* 2: 800-803.

212. Silver A. J., Guillen C. P., Kahl M. J. & Morley J. E. (1993). Effect of aging on body fat. *J Am Geriatr Soc* 41: 211-213.

213. Simoneau J. A. & Bouchard C. (1995a). Skeletal muscle metabolism and body fat content in men and women. *Obesity Res* 3: 23-29.

214. Simoneau J. A., Colberg S. R., Thaete F. L. & Kelley D. E. (1995b). Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *Faseb J* 9: 273-278.

215. Simsolo R. B., Ong J. M. & Kern P. A. (1993). The regulation of adipose tissue and muscle lipoprotein lipase in runners by detraining. *J Clin Invest* 92: 2124-2130.

216. Sniderman A. D. & Cianflone K. (1995). Metabolic disruptions in the adipocyte-hepatocyte fatty acid axis as the causes of hyperapoB. *Int J Obesity* 19: S27-S33.

217. Snitker S., Hellmér J., Boschmann M., Monroe M. B. & Ravussin E. (1998). Whole body fat oxidation is related to in situ adipose tissue lipolytic response to isoproterenol in males. *Am J Physiol* 275: E400-404.

218. Sörbris R., Petersson B.-G. & Nilsson-Ehle P. (1981). Effects of weight reduction on plasma lipoproteins and adipose tissue metabolism in obese subjects. *Eur J Clin Invest* 11: 491-498.

219. Spiegelman B. M. (1998). PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47: 507-514.

220. Spiegelman B. M. & Flier J. S. (1996). Adipogenesis and obesity: rounding out the big picture. *Cell* 87: 377-389.

221. St-Amand J., Després J. P., Lemieux S., Lamarche B., Moorjani S., Prud'homme D., Bouchard C. & Lupien P. J. (1995). Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women? *Metabolism* 44: 491-498.

222. Starnes H. F., Jr., Warren R. S., Jeevanandam M., Gabrilove J. L., Larchian W., Oettgen H. F. & Brennan M. F. (1988). Tumor necrosis factor and the acute metabolic response to tissue injury in man. *J Clin Invest* 82: 1321-1325.

223. Stephens T. W., Basinski M., Bristow P. K., Bue-Valleskey J. M., Burgett S. G., Craft L., Hale J., Hoffmann J., Hsiung H. M., Kriauciunas A. & et al. (1995). The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377: 530-532.

224. Stich V., de Glisezinski I., Galitzky J., Hejnova J., Crampes F., Rivière D. & Berlan M. (1999). Endurance training increases the beta-adrenergic lipolytic response in subcutaneous adipose tissue in obese subjects. *Int J Obesity* 23: 374-381.

225. Stich V., Harant I., De Glisezinski I., Crampes F., Berlan M., Kunesova M., Hainer V., Dauzats M., Rivière D., Garrigues M., Holm C., Lafontan M. & Langin D. (1997). Adipose tissue lipolysis and hormone-sensitive lipase expression during very-low-calorie diet in obese female identical twins. *J Clin Endocrinol Metab* 82: 739-744.

226. Stubbe I., Hansson P., Gustafson A. & Nilsson-Ehle P. (1983). Plasma lipoproteins and lipolytic enzyme activities during endurance training in sedentary men: changes in high-density lipoprotein subfractions and composition. *Metabolism* 32: 1120-1128.

227. Stubbs J. R., Harbon G. H., Murgatroyd P. R. & Prenctice A. M. (1995). Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake in men eating ad libitum. *Am J Clin Nutr* 62: 316-329.

228. Svedberg J., Björntorp P., Smith U. & Lönnroth P. (1990). Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. *Diabetes* 39: 570-574.

229. Tall A. (1995). Plasma lipid transfer proteins. *Annu Rev Biochem* 64: 235-257.

230. Tanaka T., Yoshida N., Kishimoto T. & Akira S. (1997). Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. *Embo J* 16: 7432-7443.

231. Taniguchi A., Kataoka K., Kono T., Oseko F., Okuda H., Nagata I. & Imura H. (1987). Parathyroid hormone-induced lipolysis in human adipose tissue. *J Lipid Res* 28: 490-494.

232. Taskinen M. R. & Nikkila E. A. (1980). Effect of acute vigorous exercise on lipoprotein lipase activity of adipose tissue and skeletal muscle in physically active men. *Artery* 6: 471-483.

233. Taskinen M. R. & Nikkilä E. A. (1981). Lipoprotein lipase of adipose tissue and skeletal muscle in human obesity: Response to glucose and to semistarvation. *Metabolism* 30: 810-817.

234. Taskinen M. R. & Nikkilä E. A. (1987). Basal and postprandial lipoprotein lipase activity in adipose tissue during caloric restriction and refeeding. *Metabolism* 36: 625-630.

235. Tavernier G., Barbe P., Galitzky J., Berlan M., Caput D., Lafontan M. & Langin D. (1996). Expression of beta3-adrenoceptors with low lipolytic action in human subcutaneous white adipocytes. *J Lipid Res* 37: 87-97.

236. Tchernof A., Labrie F., Bélanger A. & Després J. P. (1996). Obesity and metabolic complications: contribution of dehydroepiandrostérone and other steroid hormones. *J Endocrinol* 150: S155-S164.

237. Tchernof A., Labrie F., Bélanger A., Prud'homme D., Bouchard C., Tremblay A., Nadeau A. & Després J. P. (1997). Relationships between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. *Atherosclerosis* 133: 235-244.

238. Tchernof A., Toth M. J. & Poehlman E. T. (1999). Sex hormone-binding globulin levels in middle-aged premenopausal women. Associations with visceral obesity and metabolic profile. *Diabetes Care* 22: 1875-1881.

239. Thomas C. D., Peters J. C., Reed G. W., Abumrad N. N., Sun M. & Hill J. O. (1992). Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. *Am J Clin Nutr* 55: 934-942.

240. Tontonoz P., Hu E., Graves R. A., Budavari A. I. & Spiegelman B. M. (1994). mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8: 1224-1234.

241. Tontonoz P., Kim J. B., Graves R. A. & Spiegelman B. M. (1993). ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. *Mol Cell Biol* 13: 4753-4759.

242. Torti F. M., Dieckmann B., Beutler B., Cerami A. & Ringold G. M. (1985). A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. *Science* 229: 867-869.

243. Tremblay A., Després J. P. & Bouchard C. (1984). Adipose tissue characteristics of ex-obese long-distance runners. *Int J Obesity* 49: 799 - 805.

244. Tremblay A., Drapeau V., Doucet E., Alméras N., Després J. P. & Bouchard C. (1998). Fat balance and ageing: results from the Québec family study. *Br J Nutr* 79: 413-418.

245. Tremblay A., Lavallée N., Alméras N., Allard L., Després J. P. & Bouchard C. (1991). Nutritional determinants of the increase in energy intake associated with a high-fat diet. *Am J Clin Nutr* 53: 1134-1137.

246. Tremblay A., Plourde G., Després J. P. & Bouchard C. (1989). Impact of dietary fat content and fat oxidation on energy intake in humans. *Am J Clin Nutr* 49: 799-805.

247. Tsutsumi C., Okuno M., Tannous L., Piantedosi R., Allan M., Goodman D. S. & Blaner W. S. (1992). Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biochem Chem* 267: 1805-1810.

248. Umek R. M., Friedman A. D. & McKnight S. L. (1991). CCAAT-enhancer binding protein: a component of a differentiation switch. *Science* 251: 288-292.

249. Vague J. (1956). The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am J Clin Nutr* 4: 20-34.

250. Valet P., Berlan M., Beauville M., Crampes F., Montastruc J. L. & Lafontan M. (1990). Neuropeptide Y and peptide YY inhibit lipolysis in human and dog fat cells through a pertussis toxin-sensitive G protein. *J Clin Invest* 85: 291-295.

251. van Beek A. P., de Ruijter-Heijstek F. C., Erkelens D. W. & de Bruin T. W. (1999). Menopause is associated with reduced protection from postprandial lipemia. *Arterioscler Thromb* 19: 2737-2741.

252. Vaughan D. E., Declerck P. J., Van Houtte E., De Mol M. & Collen D. (1992). Reactivated recombinant plasminogen activator inhibitor-1 (rPAI-1) effectively prevents thrombolysis in vivo. *Thromb Haemost* 68: 60-63.

253. Wahrenberg H., Engfeldt P., Bolinder J. & Arner P. (1987). Acute adaptation in adrenergic control of lipolysis during physical exercise in humans. *Am J Physiol* 253: E383-E390.

254. Wahrenberg H., Lönnqvist F. & Arner P. (1989). Mechanisms underlying regional differences in lipolysis in human adipose tissue. *J Clin Invest* 84: 458-467.

255. Walford R. L., Mock D., MacCallum T. & Laseter J. L. (1999). Physiologic changes in humans subjected to severe, selective calorie restriction for two years in biosphere 2: health, aging, and toxicological perspectives. *Toxicol Sci* 52: S61-65.

256. Wang N. D., Finegold M. J., Bradley A., Ou C. N., Abdelsayed S. V., Wilde M. D., Taylor L. R., Wilson D. R. & Darlington G. J. (1995). Impaired energy homeostasis in C/EBP alpha knockout mice. *Science* 269: 1108-1112.

257. Wang Q., Bing C., Al-Barazanji K., Mossakowaska D. E., Wang X. M., McBay D. L., Neville W. A., Taddayon M., Pickavance L., Dryden S., Thomas M. E., McHale M. T., Gloyer I. S., Wilson S., Buckingham R., Arch J. R., Trayhurn P. & Williams G. (1997). Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* 46: 335-341.

258. Wu Z., Bucher N. L. & Farmer S. R. (1996). Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. *Mol Cell Biol* 16: 4128-4136.

259. Xu X. & Björntorp P. (1990). The effects of steroid hormones on adipocyte development. *Int J Obesity* 14: 159-163.

260. Yeh W. C., Cao Z., Classon M. & McKnight S. L. (1995). Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev* 9: 168-181.

261. Yeo G. S., Farooqi I. S., Aminian S., Halsall D. J., Stanhope R. G. & O'Rahilly S. (1998). A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat Genet* 20: 111-112.

262. Yost J. Y. & Eckel R. H. (1992). Regional similarities in the metabolic regulation of adipose tissue lipoprotein lipase. *Metabolism* 41: 33-36.

263. Zhang Y., Proenca R., Maffei M., Barone M., Leopold L. & Friedman J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432.

264. Zierath J. R., Livingston J. N., Thorne A., Bolinder J., Reynisdottir S., Lönnqvist F. & Arner P. (1998). Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway. *Diabetologia* 41: 1343-1354.

265. Zilversmit D. B. (1979). Atherogenesis: a postprandial phenomenon. *Circulation* 60: 473-485.

266. Zumoff B. & Strain G. W. (1994). A perspective on the hormonal abnormalities of obesity: are they cause or effect? *Obesity Res* 2: 56-67.

267. Zurlo F., Larson K., Bogardus C. & Ravussin E. (1990a). Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* 86: 1423-1427.

268. Zurlo F., Lillioja S., Esposito-Del Puente A., Nyomba B. L., Raz I., Saad M. F., Swinburn B. A., Knowler W. C., Bogardus C. & Ravussin E. (1990b). Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol* 259: E650-657.