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OBÉSITÉ, DISTRIBUTION DU TISSU ADIPEUX ET COMPLICATIONS MÉTABOLIQUES :

ASSOCIATIONS AVEC LE PRODUIT DU GÈNE OB ET LE MÉTABOLISME DES LIPOPROTÉINES EN PÉRIODE POST-PRANDIALE

Thèse
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À Chantal
et
à notre nouvelle vie qui commence

RÉSUMÉ BREF

La présente thèse porte sur les associations entre l'obésité ainsi que la distribution régionale du tissu adipeux (TA), et ses complications métaboliques tant en période de jeûne qu'en période post-prandiale de même qu'avec les concentrations plasmatiques de leptine. Les résultats indiquent que de faibles concentrations de HDL-cholestérol sont plus étroitement liées au syndrome plurimétabolique des niveaux réduits d'apolipoprotéine A-I. L'accumulation importante de TA viscéral est associée à la détérioration de la tolérance aux lipides, et la différence sexuelle dans l'accumulation de TA viscéral semble être impliquée dans la lipémie post-prandiale de plus grande amplitude chez l'homme que chez la femme. Par ailleurs, les femmes affichent des concentrations plasmatiques de leptine plus élevées que les hommes, et ce, indépendamment du degré d'adiposité généralement plus important chez la femme. Les niveaux plasmatiques d'androgènes chez l'homme ne semblent pas expliquer la concentration faible de leptine. Cependant, la taille adipocytaire moyenne plus élevée chez la femme semble favoriser une production plus importante de leptine par le TA. Bien que fortement associée à l'adiposité, la leptinémie n'est pas un facteur de risque indépendant pour la cardiopathie ischémique.

RÉSUMÉ LONG

La distribution du tissu adipeux (TA) est une variable critique dans la détérioration métabolique associée à l'obésité. En effet, une accumulation excessive de TA viscéral est associée à un état dyslipidémique et à des altérations du métabolisme du glucose et de l'insuline. La présente thèse de doctorat a donc porté sur la détérioration métabolique de l'obésité viscérale en période de jeûne de même qu'en période post-prandiale. Les résultats de la présente thèse démontrent que les niveaux réduits de HDL-cholestérol, contrairement aux concentrations faibles d'apolipoprotéine A-I, sont plus précis dans l'identification des sujets affichant les caractéristiques du syndrome plurimétabolique. Les résultats de cette thèse démontrent également que la détérioration métabolique retrouvée dans l'obésité viscérale s'étend au métabolisme des triglycérides en période post-prandiale, De plus, l'accumulation plus importante de TA viscéral chez l'homme pourrait être à l'origine de la différence sexuelle notée dans l'amplitude de la lipémie post-prandiale chez l'humain. Par ailleurs, bien que la leptine soit fortement associée à l'adiposité chez l'humain, elle ne semble pas avoir de lien avec la distribution du TA ni même avec la détérioration métabolique fréquemment observée chez les individus obèses. Les femmes sont caractérisées par des concentrations plasmatiques plus élevées de leptine que les hommes et ce, indépendamment du degré d'adiposité. Les niveaux d'androgènes ne semblent pas associés à la leptinémie chez l'homme, mais les résultats obtenus dans cette thèse indiquent que la taille adipocytaire moyenne plus grande chez la femme contribuerait à augmenter la production de leptine par le tissu adipeux et à expliquer, du moins en partie, le dimorphisme sexuel noté dans la leptinémie chez l'humain. L'obésité est associée à un risque accru de maladies cardio-vasculaires et à l'élévation des concentrations plasmatiques de leptine. Cependant, les résultats émanant de la présente thèse indiquent que la concentration de leptine n'est pas un facteur de risque indépendant pour le développement de la cardiopathie ischémique chez l'homme.

AVANT-PROPOS

La réalisation des travaux présentés dans cette thèse n'aurait pu être possible sans l'appui, l'encadrement, la confiance, l'encouragement et l'aide d'un grand nombre de personnes. Tout d'abord, je tiens à souligner l'importance du soutien financier qui me fut accordé par le fonds pour la formation de chercheurs et l'aide à la recherche (FCAR). La poursuite de mes études doctorales n'aurait pas été possible sans l'apport financier essentiel de cet organisme. Je tiens également à remercier l'Université Laval de m'avoir donner la possibilité de faire ces études en offrant le programme de Ph.D. dans le domaine choisi. Je souhaite que, malgré toutes les coupures budgétaires des années passées et futures, ce genre de programme tant académique que boursier demeurent afin que d'autres étudiants puissent en profiter.

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Leptinemia is not a risk factor for ischemic heart disease in men: Prospective results from the Québec Cardiovascular Study

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PREMIÈRE PARTIE

CHAPITRE 1

INTRODUCTION GÉNÉRALE

Le maintien du poids corporel est intimement lié à l'équilibre de la balance énergétique, i.e. à la différence entre l'apport en nutriments et la façon de dépenser l'énergie consommée. Un changement dans cet équilibre mène à une perte ou à un gain de poids corporel. Afin d'assurer l'équilibre énergétique, des mécanismes régulateurs, au niveau desquels interagissent de nombreuses hormones (ex. insuline, catécholamines, glucocorticoïdes, etc.), sont indispensables. La complexité de cette régulation assure l'efficacité du contrôle de la balance énergétique tout en lui conférant, cependant, une certaine fragilité. Par exemple, des altérations au niveau des gènes codant pour certaines de ces hormones ou de leurs récepteurs, pourraient entraîner un déséquilibre des mécanismes impliqués dans la régulation de la prise alimentaire et de la dépense énergétique, provoquant ainsi des variations plus ou moins importantes de poids. Les progrès effectués jusqu'à maintenant dans le domaine de la biologie moléculaire, entre autres, ont permis un examen plus approfondi des bases génétiques du contrôle du poids corporel et de l'accumulation de tissu adipeux (TA).

Cependant, même si les fondements génétiques et/ou physiologiques de l'obésité ne sont pas encore connus, ses effets sur le métabolisme n'en sont pas moins évidents et néfastes. En effet, de nombreuses dyslipidémies de même que le développement du diabète non-insulino-

dépendant (diabète de type II), sont autant de conditions associées à l'accumulation excessive de tissu adipeux, et qui sont reconnues pour augmenter le risque de développement prématuré de maladies cardio-vasculaires (MCV). De plus, il est maintenant connu que des différences au niveau de la distribution régionale du tissu adipeux, i.e. une accumulation de graisse au niveau de l'abdomen ou en périphérie, font en sorte que certains individus développent ou non les nombreuses détériorations métaboliques associées à l'obésité.

Les travaux effectués dans le cadre de ce programme de doctorat ont conduit à la rédaction de sept manuscrits qui sont tous constitués de résultats originaux. Ces travaux ont porté, entre autres, sur l'impact de l'accumulation de graisse viscérale sur le profil lipidique en période de jeûne ainsi que sur la tolérance aux lipides alimentaires en période post-prandiale. De plus, la récente découverte de la leptine, hormone, qui lorsqu'administrée à des souris obèses, entraîne une perte de poids, a ouvert la voie à de nombreuses avenues de recherche. À cet effet, une partie des travaux effectués dans cette thèse a permis d'approfondir les connaissances sur les associations entre la concentration plasmatique de leptine et l'adiposité chez l'humain de même que sur les mécanismes de régulation de la leptinémie. Parmi les manuscrits ayant été rédigés, 4 sont présentement publiés ou sous presse dans les journaux scientifiques *Metabolism, Diabetologia, Diabetes* et *Diabetes Care*, et 1 autre a été soumis pour publication dans la revue *Journal of Endocrinology*. Finalement, les deux derniers manuscrits seront soumis sous peu pour publication.

CHAPITRE 2

OBÉSITÉ, DISTRIBUTION RÉGIONALE DU TISSU ADIPEUX ET COMPLICATIONS MÉTABOLIQUES

Une des caractéristiques majeures de la société nord-américaine est sans aucun doute la fréquence élevée d'individus affichant un surplus de poids [1,2]. En effet, il a été récemment démontré que 35% des Canadiens et 27% des Canadiennes présentent une accumulation excessive de poids tel qu'indiqué par un indice de masse corporelle (IMC) supérieur à 27 kg/m^2 [1]. Cette prévalence de l'obésité est également observable chez les Américains où 25% de la population sont caractérisés par un surplus de poids [2]. Les effets dommageables de l'obésité sont bien connus et varient entre l'hypertension, le diabète non-insulino-dépendant et les dyslipidémies. Il semble également plus que probable que l'augmentation du risque de développer des MCV notée chez les sujets obèses [3-7], soit médiée par les différentes altérations métaboliques qui lui sont associées [3,6,8-14]. Cependant, il est également connu que le risque de MCV varie grandement d'un individu obèse à un autre. Certains auteurs ont suggéré que la distribution régionale du TA serait le facteur déterminant dans l'expression des complications métaboliques liées à l'obésité. En effet, l'accumulation préférentielle de TA au niveau abdominal est plus étroitement liée aux différentes détériorations métaboliques énumérées que l'obésité en-soi [3,6,8-14]. Ainsi, il a été démontré que les individus ayant une mesure élevée du rapport de la circonférence de la taille sur celle des hanches (rapport taille-hanche, RTH) sont plus susceptibles de développer les complications métaboliques traditionnellement associée à l'obésité [3,6,8-14].

Le perfectionnement des techniques d'imagerie, telle que la tomographie axiale, a permis d'augmenter l'efficacité et la précision des mesures de distribution du régionale du TA. À l'aide de cette technique utilisant les différences de densité entre les tissus, il est maintenant possible de déterminer la contribution du tissu adipeux viscéral dans la détérioration métabolique associée à l'obésité. En effet, une accumulation élevée de graisse viscérale est étroitement associée à l'hyperinsulinémie et à la dégradation de la tolérance au glucose [15,16]. De plus, les individus caractérisés par une accumulation excessive de tissu adipeux viscéral, présentent un état dyslipidémique incluant des concentrations plasmatiques élevées de triglycérides et d'apolipoprotéine (apo) B de même que des niveaux faibles de HDL-cholestérol, particulièrement de HDL_2-cholestérol, et ce, même en présence de niveaux normaux de cholestérol total et de LDL-cholestérol [16,17]. De plus, en comparant des sujets obèses mais caractérisés par une accumulation faible vs élevée de graisse viscérale, il a été démontré que les individus présentant une adiposité viscérale importante, affichaient une hyperinsulinémie, une intolérance au glucose ainsi que des concentrations plasmatiques élevées de triglycérides et faibles de HDL-cholestérol à jeun [17]. Ces résultats suggèrent donc que l'obésité viscérale est associée à de nombreuses altérations métaboliques, et ce, de façon indépendante de l'augmentation de l'adiposité en-soi.

Il y a maintenant dix ans, Reaven décrivait les composantes du syndrome X (ou syndrome plurimétabolique) parmi lesquelles on retrouve la résistance à l'insuline, l'hyperinsulinémie, l'intolérance au glucose, l'hypertriglycéridémie et l'hypoalphalipoprotéinémie [18]. De plus, il fut récemment suggéré que l'obésité viscérale devait être reconnue comme une composante distincte du syndrome X et considérée dans l'augmentation du risque de MCV [19].

Par ailleurs, l'accumulation préférentielle et excessive de graisse viscérale est liée à la détérioration du profil métabolique et particulièrement des concentrations de lipoprotéines et de lipides plasmatiques. La plupart des études ayant examiné cette association ont principalement étudié les conséquences de l'obésité viscérale sur les concentrations plasmatiques de lipides à jeun. Cependant, la fréquence élevée et régulière des repas dans la société nord-américaine, nous amène à croire que les individus ne sont que rarement en période de jeûne. L'étude du métabolisme des lipoprotéines et lipides en période post-prandiale, i.e. à la suite d'un repas, peut donc fournir des informations supplémentaires relatives aux détériorations métaboliques associées à l'obésité.

CHAPITRE 3

MÉTABOLISME DES LIPOPROTÉINES EN PÉRIODE POST-PRANDIALE

L'analyse des lipides et lipoprotéines a traditionnellement été réservée à l'état de jeûne, i.e. lorsque le métabolisme des triglycérides est ralenti et que le transport des lipides a atteint un état d'équilibre. Cependant, un intérêt sans cesse croissant est accordé à l'étude du métabolisme des lipoprotéines en période post-prandiale depuis l'hypothèse émise par Zilversmit [20], selon laquelle les chylomicrons et leurs résidus d'hydrolyse, après enrichissement en esters de cholestérol, pourraient être impliqués dans le développement de l'athérosclérose [20]. Par ailleurs, peu de données sont disponibles quant aux conditions métaboliques pouvant favoriser un tel phénomène. Ainsi, il semble important de mieux comprendre comment, entre autres, des variations de composition corporelle, de même que des changements dans la sensibilité à l'insuline ou le profil lipidique, peuvent affecter la tolérance aux lipides d'un individu.

3.1 *Classification des lipoprotéines*

Le transport des lipides dans le sang est assuré par la contribution de nombreuses sous-classes de lipoprotéines. Celles-ci varient grandement en taille et en composition afin de permettre un transport et un métabolisme plus efficaces des lipides. Cependant, bien qu'il existe un équilibre dans la distribution de ces lipoprotéines en phase de jeûne, la prise alimentaire entraîne des changements majeurs dans la répartition des lipoprotéines entre les différentes sous-classes. En

effet, l'introduction des chylomicrons en période post-prandiale, lipoprotéines synthétisées et sécrétées par l'intestin, détruit cet équilibre. Le chylomicron est une lipoprotéine régulière, i.e. constituée d'une enveloppe de phospholipides, de cholestérol non-estérifié et d'apolipoprotéines, mais dans laquelle est contenu un noyau lipidique extrêmement riche en triglycérides [21]. Le rôle principal du chylomicron est d'acheminer les triglycérides alimentaires jusqu'au tissu adipeux pour le stockage, et jusqu'au muscle où ils seront utilisés en tant que source énergétique. L'arrivée massive de chylomicrons à la suite d'un repas perturbe donc l'équilibre existant dans le métabolisme des lipoprotéines en état de jeûne, et entraîne de nouvelles interactions entre les sous-classes de lipoprotéines.

3.2 *Métabolisme des lipoprotéines*

Les lipoprotéines riches en triglycérides exogènes (chylomicrons), produites par l'intestin, et endogènes (VLDL), synthétisées au niveau du foie, suivent les mêmes voies métaboliques (Figure 1). Le catabolisme des chylomicrons et des VLDL débute par l'hydrolyse du contenu en triglycérides sous l'action de la lipoprotéine lipase (LPL) qui est située à la surface des cellules endothéliales des capillaires sanguins du tissu adipeux, des muscles et de nombreux autres tissus [22,23]. Ce processus est dépendant de la présence de l'apo C-II contenue dans les chylomicrons et les VLDL, et reconnue comme un activateur de l'activité de la LPL [24]. Les résidus résultant de cet appauvrissement en triglycérides, et en d'autres composantes (phospholipides, apolipoprotéines, cholestérol non-estérifié), sont par la suite récupérés par le foie. Les

phospholipides et apolipoprotéines provenant de la dégradation des chylomicrons, entrent dans l'élaboration de la sous-classe de lipoprotéines nommée HDL.

Tableau 1: Caractéristiques principales des différentes sous-classes de lipoprotéines

Lipoprotéines	***Caractéristiques***
Chylomicrons	. Principaux transporteurs de triglycérides en période post-prandiale . Production intestinale
VLDL	. Lipoprotéines de très faible densité *(Very-Low Density Lipoproteins)* . Principaux transporteurs de triglycérides en période de jeûne . Production hépatique
LDL	. Lipoprotéine de faible densité *(Low Density Lipoproteins)* . Principaux transporteurs d'esters de cholestérol . Produites par l'hydrolyse des triglycérides des VLDL
HDL	. Lipoprotéine de densité élevée *(High Density Lipoproteins)* . Particules impliquées dans le transport inverse du cholestérol . Existent sous deux formes : HDL_2 et HDL_3 . Produites par l'hydrolyse des triglycérides des chylomicrons

De plus, l'action de nombreux enzymes, telles que la lécithine:acyl transférase (LCAT), la protéine de transfert d'esters de cholestérol (CETP) et la lipase hépatique (HL), et agissant sur les résidus de VLDL et les HDL, permet la formation d'une autre catégorie de lipoprotéines, les LDL. Les similitudes entre les voies métaboliques empruntées par les chylomicrons et les VLDL, vont jusqu'à la récupération des résidus de leur hydrolyse par le même récepteur hépatique, le récepteur à l'apo B/E. Il est donc important de bien comprendre ce partage, puisqu'il permettra de mieux saisir l'impact d'un métabolisme anormal des lipoprotéines riches en triglycérides, qu'elles soient d'origine endogène ou exogène, sur la lipémie post-prandiale [21].

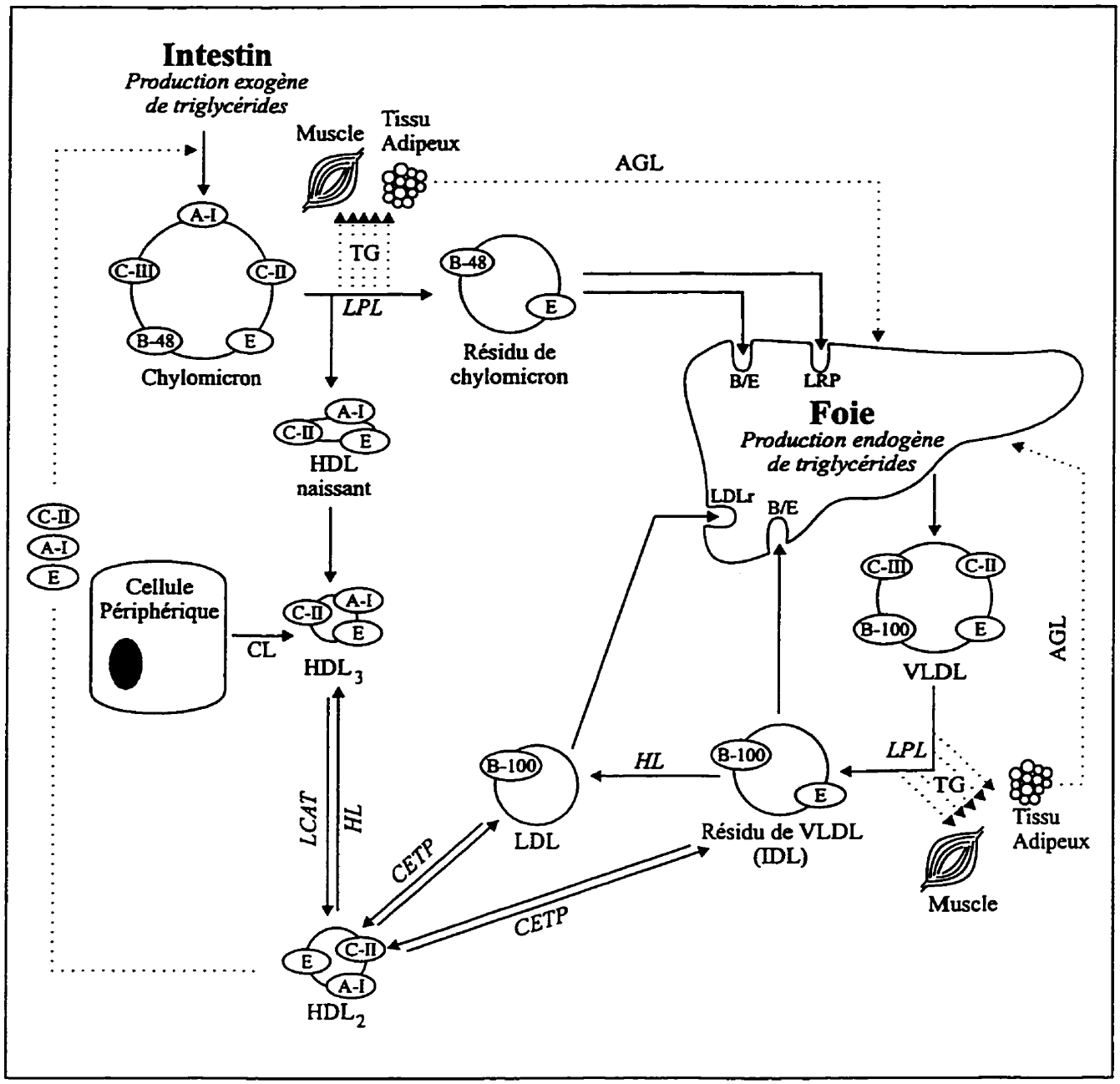

Figure 1 : Représentation schématique du métabolisme des lipoprotéines. TG: triglycérides; CL: cholestérol libre; AGL: acides gras libres; VLDL: lipoprotéine de très faible densité; IDL: lipoprotéine de densité intermédiaire; LDL: lipoprotéine de faible densité; HDL: lipoprotéine de densité élevée; A-I: apo A-I; B-48: apo B-48; B-100: apoB-100; C-II: apo C-II; C-III: apo C-III; E: apo E; LDLr: récepteur aux LDL; B/E: récepteur hépatique l'apo B/E; LRP: protéine apparentée au récepteur des LDL; LPL: lipoprotéine lipase; HL: lipase hépatique; CETP: protéine de transfert d'esters de cholestérol; LCAT: lecithine-cholestérol acyl transférase.

Tableau 2: Fonctions de différents éléments moléculaires impliqués dans le métabolisme des lipoprotéines riches en triglycérides

Molécules	*Fonctions*
Enzymes	
LPL	. Hydrolyse les triglycérides des chylomicrons et VLDL
	. Synthèse des LDL à partir des VLDL
	. Synthèse des HDL naissants à partir des chylomicrons
	. Liaison des résidus de chylomicrons aux récepteurs B/E hépatiques
HL	. Catabolisme des résidus de chylomicrons
	. Conversion des HDL_2 en HDL_3
CETP	. Échange de triglycérides et d'esters de cholestérol entre les lipoprotéines contenant l'apo B et les HDL (production de LDL denses et de résidus de chylomicrons enrichis en esters de cholestérol)
LCAT	. Estérification du cholestérol libre des membranes
Apolipoprotéines	
Apo B-48	. Structure des chylomicrons
Apo B-100	. Structure des LDL et VLDL
	. Liaison des lipoprotéines au récepteur des LDL
Apo C-II	. Activateur de l'activité hydrolytique de la LPL
Apo C-III	. Inhibiteur de l'activité hydrolytique de la LPL
	. Inhibiteur de la récupération hépatique des résidus de lipoprotéines
Apo E	. Liaison des lipoprotéines riches en triglycérides et de leurs résidus aux récepteurs des LDL et B/E
Récepteurs	
LDLr	. Catabolisme des LDL de même que des résidus de chylomicrons et de VLDL
B/E	. Élimination des lipoprotéines riches en triglycérides et de leurs résidus

Tiré de De Man et coll [25]

3.3 *Mesure des concentrations de lipoprotéines en période post-prandiale*

i) Test de tolérance aux lipides

Le but du test de tolérance aux lipides est de vérifier la capacité de l'organisme à disposer des lipides ingérés, lui permettant ainsi de rétablir son état d'homéostase relative. Dans l'étude du

métabolisme des lipoprotéines post-prandiales, certains préféreront utiliser l'injection par voie intraveineuse d'une solution lipidique tandis que d'autres favoriseront l'ingestion par voie orale d'un repas solide ou d'un mélange liquide enrichi en lipides [26]. Le choix d'un test par rapport à un autre sera dirigé par l'objectif poursuivi par l'expérimentateur. En effet, le test par voie intraveineuse a surtout été utilisé, jusqu'à maintenant, par ceux qui s'intéressent aux premières étapes du métabolisme des chylomicrons [27]. À la suite de l'injection de la solution lipidique, des prélèvements sanguins sont effectués à des intervalles de 5 minutes. À partir de ces mesures, il est possible de suivre les variations de triglycéridémie et d'apprécier l'efficacité de l'hydrolyse des lipides dans le sang [27]. Cependant, cette approche ne donne aucune information sur la vitesse avec laquelle les lipoprotéines riches en triglycérides entrent dans la circulation à la suite d'un repas, tout en générant des particules lipoprotéiques qui sont «artificielles» i.e. qui ne sont pas produites par les voies métaboliques naturelles (intestin ou le foie). Le test oral de tolérance aux lipides permettra cet examen car il permet de quantifier l'amplitude de la réponse en triglycérides provoquée par un repas habituellement riche en gras. L'interprétation des résultats du test oral de tolérance aux lipides repose également sur des mesures de triglycéridémie plasmatique, qui contrairement au test intraveineux, sont effectuées à des intervalles de 1 à 2 heures pour une période variant entre 4 et 24 heures. Cependant, la mesure de la triglycéridémie plasmatique lors du test de tolérance aux lipides, tant intraveineux qu'oral, ne donne aucune information quant à la nature et l'origine des lipoprotéines dans lesquelles sont contenus les triglycérides, et des protocoles expérimentaux plus élaborés sont requis afin d'atteindre ces objectifs.

ii) Marquage des lipoprotéines par les esters de rétinol

La marquage des lipoprotéines à l'aide d'esters de rétinol est une façon simple et couramment utilisée afin de différencier les lipoprotéines d'origine hépatique de celles produites par l'intestin. La vitamine A ajoutée au repas, sera incorporée, sous forme d'esters de rétinol, aux chylomicrons sécrétés par l'intestin. Les triglycérides contenus à l'intérieur des chylomicrons seront par la suite hydrolysés par la LPL et les résidus de chylomicrons, résultant de cette hydrolyse, seront finalement captés par le foie. Toutefois, les esters de rétinol contenus dans les chylomicrons ne sont pas hydrolysés par la LPL et demeurent dans le noyau lipidique de la lipoprotéine jusqu'à sa capture par le foie. La récupération des triglycérides des résidus de chylomicrons permettra au foie de synthétiser et de sécréter de nouvelles particules riches en triglycérides, les VLDL. Cependant, lorsque rendus au foie, les esters de rétinol ne sont pas réinsérés dans les VLDL [28] et ne retournent donc pas en circulation. En mesurant les concentrations plasmatiques d'esters de rétinol à différents laps de temps, il est possible d'évaluer la cinétique du catabolisme des lipoprotéines d'origine intestinale. C'est ainsi qu'il a été démontré que l'augmentation tardive des concentrations plasmatiques de triglycérides, en période post-prandiale, est en partie attribuable à la synthèse et à la sécrétion de VLDL par le foie, ces dernières venant s'ajouter aux lipoprotéines d'origine intestinale déjà en circulation [26].

Ce marquage des lipoprotéines avec les esters de rétinol repose sur deux principes : le premier étant l'incorporation uniforme de vitamine A dans tous les chylomicrons synthétisés et le second, qu'une fois incorporée, la vitamine A y demeure jusqu'à la capture des résidus de chylomicrons au foie. Cependant, il a été rapporté que le contenu en esters de rétinol du

chylomicron était proportionnel à sa taille [29]. De plus, des échanges de lipides et de composantes se produisent entre les différentes sous-classes de lipoprotéines au cours du temps [30,31]. C'est ainsi que les LDL de même que les HDL peuvent s'enrichir en esters de rétinol. Il a d'ailleurs été démontré qu'environ 9 heures après un repas auquel on a ajouté de la vitamine A, 33% des esters de rétinol plasmatiques se retrouvent dans les sous-classes de lipoprotéines HDL et LDL [30]. Le marquage des lipoprotéines aux esters de rétinol apparaît donc surtout efficace pour l'étude d'une période allant de 0 à 8 heures suivant un repas. La même technique de marquage est possible pour le test de tolérance aux lipides administré par voie intraveineuse, mais nécessite le mélange de la solution lipidique à injecter à une solution d'esters de rétinol avant l'administration. Par ailleurs, la dose de vitamine administrée varie grandement selon les études. Dans le passé, entre 10 000 et 150 000 UI de vitamine A ont été ajoutées aux repas, sous forme d'une préparation soluble de rétinol [32,33] ou encore dans une préparation huileuse de rétinyl palmitate [26,30,34-36]. À cet effet, il a été rapporté que la dose de vitamine A administrée est associée à la réponse post-prandiale en esters de rétinol [33]. Il semble donc important de tenir compte des limites de cette technique de marquage lors de l'interprétation des résultats.

iii) Mesure des concentrations en apolipoprotéines B-48 et B-100

La composition protéique des lipoprotéines renferme également beaucoup d'informations. L'apo B est, sans aucun doute, la protéine qui présente le plus d'intérêt. Cette protéine existe sous deux formes majeures soit la forme B-48 et la forme B-100. L'apo B-48 est en fait une portion (les premiers 48%) de la protéine complète B-100, et entre dans la composition des lipoprotéines

d'origine intestinale [37]. Par contre, l'apo B-100 est la principale protéine des lipoprotéines produites par le foie [38,39]. Contrairement aux esters de rétinol, ces protéines ne sont pas soumises aux échanges entre lipoprotéines. De plus, une caractéristique importante des apo B-48 et B-100, est que l'on en retrouve une seule par particule lipoprotéique. La mesure des concentrations tant d'apo B-48 que d'apo B-100, en plus de nous informer sur l'origine des lipoprotéines, nous permet également d'en estimer le nombre. Il s'agit sans aucun doute de la technique la plus adéquate pour quantifier la contribution des lipoprotéines riches en triglycérides d'origine intestinale et hépatique dans la lipémie post-prandiale. Cependant, peu d'études ont jusqu'à maintenant utilisé cette technique.

Tableau 3: Méthodes de quantification des lipoprotéines post-prandiales riches en triglycérides en période post-prandiale

Méthode	*Avantage*	*Désavantage ou inconvénient*
Triglycérides	. Facile à utiliser	. Non-spécifique sur l'origine des lipoprotéines (intestinale ou hépatique?)
Vitamine A et esters de rétinol	. Facile à utiliser	. Transfert entre lipoprotéines contenant l'apo B-100 . Pas de relation quantitative fixe avec l'apo B-48 . Délai entre le pic de concentration, et ceux de B-48 et de triglycérides (dans certaines études)
Apo B-48 et B-100 SDS-PAGE	. Mesure de la spécificité de l'origine . Mesure du nombre de particules	. Jusqu'à présent, peu d'études

Apo, apolipoprotéine ; SDS-PAGE, électrophorèse sur gel de polyacrylamide de dodécyl-sulfate de sodium

Tiré de Karpe et Hamsten [40]

L'avènement de ces différentes techniques de mesure a permis à ceux qui s'intéressent au métabolisme des lipoprotéines en période post-prandiale, d'améliorer les connaissances et de mieux comprendre les conséquences de divers changements métaboliques sur la tolérance aux lipides alimentaires.

3.4 *Effet de l'hypertriglycéridémie et de l'hypoalphalipoprotéinémie à jeun sur la lipémie post-prandiale*

Dans la plupart des études, la réponse triglycéridémique post-prandiale est proportionnelle à la concentration plasmatique de triglycérides à jeun [41]. De plus, de nombreux patients hypertriglycéridémiques à jeun sont également caractérisés par de faibles concentrations de HDL-cholestérol en période de jeûne. Certains auteurs ont été jusqu'à suggérer que la réduction des niveaux plasmatiques de HDL-cholestérol pourrait être un marqueur de l'accumulation de lipoprotéines riches en triglycérides dans le plasma en phase post-prandiale [42,43]. La comparaison des sujets normolipidémiques à d'autres caractérisés par des concentrations élevées en triglycérides et des niveaux réduits de HDL-cholestérol à jeun, révéla que les sujets hypertriglycéridémiques avaient une réponse lipémique post-prandiale plus importante que celle des individus ayant un profil lipidique normal [44,45]. Dans ces études, des concentrations élevées de triglycérides et faibles de HDL-cholestérol à jeun, étaient indicatrices d'une réponse exagérée en triglycérides à la suite d'un repas. Patsch et coll [42] ont démontré que la relation inverse observée entre les niveaux de HDL-cholestérol et la lipémie post-prandiale était due à une diminution de la quantité de HDL_2-cholestérol et non de HDL_3 [42,46,47]. Cependant, certains résultats indiquent plutôt que l'association observée entre les faibles concentrations de HDL-cholestérol et l'amplitude de la lipémie post-prandiale

requiert, au préalable, un état d'hypertriglycéridémie. En effet, l'examen de la réponse post-prandiale chez des sujets ayant une hypoalphalipoprotéinémie isolée, i.e. des concentrations faibles de HDL-cholestérol mais sans hypertriglycéridémie, révéla chez ces individus une réponse triglycéridémique post-prandiale comparable [44,48] et même plus faible [49] que celle des individus normolipidémiques. Une concentration faible en HDL-cholestérol a été identifiée comme un prédicteur indépendant du risque de développer des MCV [50-52]. En admettant que l'altération du métabolisme postprandial des lipoprotéines augmente l'incidence des pathologies coronariennes, il semble que les individus présentant des niveaux réduits de HDL-cholestérol ne montrent pas tous le même risque de développer des MCV. En effet, les résultats des expériences de tolérance aux lipides chez les sujets affichant de faibles concentrations de HDL-cholestérol sans autre dérèglement métabolique [44,48,49], laissent croire que les mécanismes physiologiques impliqués dans l'augmentation du risque de développer des pathologies coronariennes chez ces individus, affecteraient davantage le transport inverse du cholestérol que le métabolisme postprandial des triglycérides.

Par ailleurs, pourquoi devrait-on mesurer la réponse post-prandiale d'un individu, si cette information peut être prédite par la triglycéridémie à jeun? Il semble que, parmi les sujets normolipidémiques, certains répondent plus fortement à un repas riche en lipides malgré des niveaux normaux de triglycérides à jeun et non-annonciateurs d'une lipémie post-prandiale altérée. En effet, Schrezenmeir et coll [53] ont comparé les réponses post-prandiales d'individus normolipidémiques, mais ayant été subdivisés selon leur triglycéridémie. Les concentrations de triglycérides plasmatiques étaient distribuées de façon bimodale, ce qui permit de définir deux sous-groupes:

triglycéridémies normale et modérément élevée. Sans surprise, l'hypertriglycéridémie modérée était associée à une réponse post-prandiale en triglycérides plus élevée. Cependant, parmi les individus qui présentaient une réponse post-prandiale en triglycérides exagérée, certains avaient été classés, au départ, dans le sous-groupe de sujets avec une triglycéridémie normale (<1.7 mmol/l). De plus, ces individus étaient également caractérisés par des concentrations plasmatiques d'insuline plus élevées par rapport à celles des sujets montrant une lipémie post-prandiale moins prononcée [53]. Il semble donc que des anomalies du métabolisme postprandial des triglycérides existeraient, chez certains individus, avant même que les concentrations à jeun de triglycérides soient affectées. De plus, l'hyperinsulinémie à jeun, suggérant un état précoce de résistance à l'insuline, contribuerait à une lipémie post-prandiale exagérée chez ces mêmes individus [53].

3.5 *Effet de la résistance à l'insuline et du diabète non-insulino-dépendant (type II)sur la lipémie post-prandiale*

La maladie coronarienne est la première cause de morbidité et de mortalité chez les sujets souffrant d'un diabète de type II [54-60]. De plus, la plupart des facteurs de risque conventionnels sont plus fréquemment rencontrés chez les sujets diabétiques de type II [56-58]. Parmi ceux-ci, on note la résistance à l'insuline, l'hyperinsulinémie, l'obésité, l'hypertension, l'hypertriglycéridémie et les niveaux faibles de HDL-cholestérol [56-58]. Même si le diabète de type II semble être un facteur de risque indépendant pour les MCV, des anomalies du métabolisme des lipoprotéines contribueraient à leur développement prématuré. En effet, en plus de concentrations plasmatiques de triglycérides élevées et de niveaux faibles de HDL-cholestérol, les sujets diabétiques de type II sont fréquemment caractérisés par des particules LDL plus petites et plus denses [61-64], probablement le

résultat d'une augmentation de la concentration plasmatique d'apo B-100. Cependant, le risque accru de MCV chez certains patients diabétiques ne s'explique pas par les facteurs de risque conventionnels [55,59]. Serait-il possible que des altérations du métabolisme des lipoprotéines en période post-prandiale soient alors en cause?

Étant donné que l'hypertriglycéridémie est une caractéristique des patients diabétiques de type II [56-58], des anomalies dans l'élimination des lipoprotéines en phase post-prandiale sont prévisibles. Dans une étude menée par Lewis et coll [65], la tolérance aux lipides alimentaires de sujets diabétiques de type II mais normotriglycéridémiques à jeun, a été comparée à celle d'individus normaux. Les résultats de cette étude révélèrent que les sujets diabétiques présentaient une lipémie post-prandiale similaire à celle du groupe contrôle. Cette observation fut également rapportée par le groupe de Chen et coll [66]. Cependant, dans cette dernière étude, des profils différents dans les réponses ont été observés entre les individus diabétiques et les sujets contrôles et ce, malgré des valeurs de réponse identiques [66]. En effet, des augmentations plus grandes des niveaux de triglycérides et d'esters de rétinol ont été notées dans la fraction de résidus de chylomicrons des sujets diabétiques. Cette observation est en accord avec de récents résultats obtenus par Syvänne et coll [67], rapportant que les individus diabétiques de type II, mais affichant des concentrations normales de triglycérides à jeun, avaient une lipémie post-prandiale plus élevée que celle de sujets témoins d'âge, de sexe et d'adiposité comparables. Les discordances apparentes entre les résultats issus de ces différentes études trouvent peut-être une explication dans les méthodes utilisées pour le fractionnement des lipoprotéines, les techniques de Chen et coll [66] ainsi que de Syvänne et coll [67] étant les plus élaborées. Des différences dans la composition du repas administré aux sujets

peuvent également expliquer les écarts entre les études. Il est connu que le remplacement du contenu en gras d'un repas par des hydrates de carbone contribue à l'augmentation de la réponse post-prandiale en triglycérides [68,69]. Dans l'étude de Chen et coll [66], le contenu plus élevé en hydrates de carbone du repas, comparativement à celui de l'étude de Lewis et coll [65], a pu accentuer les différences entre les sujets diabétiques et contrôles. De plus, dans l'étude de Lewis et coll [65], le fait que les patients diabétiques soient également obèses a pu affecter le métabolisme postprandial des lipides. Toutefois, l'ensemble de ces résultats suggèrent une détérioration du métabolisme postprandial des lipoprotéines chez les sujets diabétiques de type II.

3.6 *Effet de l'obésité et de la distribution régionale du tissu adipeux sur la lipémie post-prandiale*

Peu d'études ont examiné les associations entre l'obésité et la lipémie post-prandiale. Les premiers à entreprendre ce genre de travaux furent Lewis et coll [70], tentant de vérifier l'hypothèse selon laquelle l'obésité affecterait spécifiquement cette partie du métabolisme des lipoprotéines. De plus, ces anomalies pourraient permettre d'expliquer, du moins en partie, l'état dyslipoprotéinémique observé à l'état de jeûne chez les individus obèses. Les résultats de cette étude, révélèrent que malgré une glycémie comparable à celle des sujets de poids normal, les individus obèses présentaient une hyperinsulinémie avant et après un repas riche en lipides. Les sujets obèses démontraient également une hypertriglycéridémie, des niveaux plus faibles de HDL-cholestérol et des niveaux plus élevés d'acides gras libres par rapport aux individus maigres durant la période post-prandiale. De plus, les concentrations d'esters de rétinol étaient plus importantes chez les individus obèses, suggérant que

les lipoprotéines d'origine intestinale restaient en circulation plus longtemps chez les sujets obèses que chez les individus de poids normal [70].

Par ailleurs, il est connu que la distribution régionale du tissu adipeux est plus étroitement associée à la détérioration du profil métabolique à l'état de jeûne que l'obésité en-soi [3,71,72]. Dans l'étude de Lewis et coll [70], l'impact de l'obésité abdominale sur le profil lipidique postprandial n'a pu être évalué puisqu'aucune mesure de distribution du tissu adipeux n'a été effectuée. Cependant, les résultats récents obtenus par Wideman et coll [73] ont démontré l'importance de l'obésité abdominale dans la détérioration de la lipémie post-prandiale, en comparant des hommes maigres à des individus caractérisés par une accumulation excessive de tissu adipeux au niveau de l'abdomen, telle que déterminée par l'utilisation du RTH. Ces auteurs rapportèrent que les individus souffrant d'obésité abdominale montraient une lipémie post-prandiale de plus grande amplitude que celle des sujets maigres. De plus, ceci était observé en dépit du fait que les individus obèses présentaient un profil lipidique à jeun identique et un niveau d'activité physique comparable à ceux des témoins [73].

Par ailleurs, les détériorations métaboliques, incluant l'hypertriglycéridémie, la résistance à l'insuline, les niveaux élevés d'apo B et les faibles concentrations de HDL-cholestérol, notées dans l'obésité abdominale seraient causées par l'accumulation de graisse viscérale [3,19]. Dans l'étude de Wideman et coll [73], aucune mesure de l'accumulation de tissu adipeux viscéral n'était disponible. Par contre, des résultats rapportés par Ryu et coll [74] permettent de penser que l'obésité viscérale jouerait un rôle important dans la détérioration du métabolisme des lipoprotéines post-prandiales. En effet, l'obésité viscérale s'est avérée être un prédicteur indépendant de la concentration plasmatique

maximale de triglycérides mesurée en période post-prandiale [74]. Les résultats de ces trois études réunies, suggèrent donc que l'obésité, et plus particulièrement une accumulation préférentielle de tissu adipeux viscéral, est associée à une lipémie post-prandiale plus importante.

3.4 *Autres facteurs influençant la tolérance aux lipides alimentaires*

Bien qu'elles y contribuent, il n'y a pas que les dyslipidémies, la résistance à l'insuline et l'obésité qui sont associées à la détérioration de la tolérance aux lipides alimentaires. D'autres facteurs physiologiques et environnementaux sont également connus pour affecter la lipémie post-prandiale d'un individu. Voici donc un bref résumé des principales variables ayant été étudiées au cours des dernières années.

i) Âge et sexe

Il est connu qu'avec l'âge, l'efficacité du catabolisme des lipoprotéines post-prandiales est réduite [75,76]. De plus, des différences dans l'amplitude de la lipémie post-prandiale ont été observées entre les hommes et les femmes [75,77], les hommes affichant une hypertriglycéridémie post-prandiale comparativement aux femmes. Les mécanismes physiologiques responsables de cette différence demeurent inconnus. Cependant, il a été démontré que l'administration d'oestrogènes diminuait la lipémie post-prandiale chez la femme [78] principalement par la récupération hépatique plus rapide des résidus de chylomicrons. Un dimorphisme sexuel dans l'activité de la LPL peut également être envisagé pour expliquer la lipémie post-prandiale plus faible chez la femme, mais rien n'est encore connu à ce sujet.

ii) Diète

La composition de la diète joue un rôle au niveau de la tolérance aux lipides et son effet se décompose en deux volets distincts. Tout d'abord il y a l'effet aigu, i.e. celui de la composition du repas ingéré, et ensuite, l'effet chronique de l'alimentation correspondant à la composition de la diète régulière d'un individu [79,80]. Bien que certains résultats soient contradictoires [81], plus le contenu en gras d'un repas est important, plus la réponse triglycéridémique est augmentée tant chez des sujets normaux que diabétiques [82,83]. De plus, il semble que la nature des acides gras, entrant dans la composition du repas, affecte également la lipémie post-prandiale. En effet, l'ingestion d'acides gras saturés provoque une élévation plus importante des triglycérides plasmatiques en période post-prandiale que celle qui résultait de l'ingestion d'acides gras insaturés [84,85]. Une quantité plus grande d'acides gras incorporés aux chylomicrons, ainsi qu'une absorption plus rapide des graisses saturées au niveau de l'intestin expliqueraient ces résultats [80]. Le remplacement du contenu en gras du repas par des hydrates de carbone entraîne une réponse triglycéridémique en période post-prandiale de plus grande amplitude [68,69]. Par ailleurs, il a été démontré que l'ingestion d'une quantité variable de protéines n'affecte pas la lipémie post-prandiale [86].

L'ingestion régulière de gras saturés est également associée à l'augmentation de la lipémie post-prandiale [21]. Dans ce cas, l'hypertriglycéridémie post-prandiale serait causée, en partie, par l'élévation des concentrations de triglycérides à jeun associée à la consommation régulière de gras saturés [21]. D'ailleurs, d'autres résultats viennent confirmer cette hypothèse en démontrant que la consommation d'un régime riche en acides gras saturés n'affecte pas la clairance des chylomicrons mais plutôt celle des VLDL [87]. Une diète riche en hydrates de carbone est associée à l'élévation de

la triglycéridémie à jeun [86]. De plus, il a été suggéré que l'augmentation de la lipémie post-prandiale observée chez les individus consommant une diète principalement composée de glucides, serait attribuable à une production accrue de VLDL par le foie [88,89].

iii) Exercice

L'exercice physique réduit la lipémie post-prandiale chez les sujets normotriglycéridémiques et hypertriglycéridémiques [80,90-93]. De plus, il est connu que l'activité physique augmente l'activité de la LPL [94-96], principal enzyme impliqué dans le catabolisme des lipoprototéines riches en triglycérides. C'est d'ailleurs le mécanisme proposé pour expliquer l'effet hypolipémiant de l'exercice. En effet, dans l'étude de Weintraub et coll [90], la baisse de lipémie post-prandiale chez les sujets actifs était également accompagnée d'une augmentation significative de l'activité de la LPL.

iv) Apolipoprotéines

L'apo E s'avère l'apolipoprotéine la plus étudiée. Il existe trois isoformes de l'apo E, soient l'apo E2, E3 et E4. Par sa liaison au récepteur hépatique B/E, l'apo E favorise l'élimination des lipoprotéines et de leurs résidus de la circulation. Cependant, les trois formes d'apo E montrent des affinités différentes pour le récepteur hépatique, l'apo E4 démontrant la plus forte, suivie dans l'ordre par l'apo E3 et E2. De plus, il a été démontré que la lipémie post-prandiale était d'amplitude moindre chez les individus caractérisés par l'allèle E4 de l'apo E, suivi de ceux présentant l'apo E3 et l'apo E2 [97,98]. Une récupération plus rapide des résidus de chylomicrons chez les individus

présentant l'apo E4 serait à l'origine de cette différence. Cependant, il a été rapportée que les individus avec un phénotype E4/E3 démontraient des réponses post-prandiales prolongées de l'apo B-48 et l'apo B-100 [99]. À cet effet, il a été proposé qu'un enlèvement plus lent des VLDL pourrait favoriser leur conversion en LDL et ainsi expliquer l'élévation des concentrations de particules LDL communément rencontrée chez les sujets présentant un allèle E4 [99].

v) Consommation d'alcool

La consommation d'alcool est associée à l'hypertiglycéridémie en période post-prandiale [100-102] . Cependant, cet effet est réversible par l'exercice physique. En effet, Hartung et coll [100] ont démontré que l'alcool retardait l'élimination des lipoprotéines riches en triglycérides en période post-prandiale chez des sujets sédentaires mais pas chez des individus entraînés. Bien que dans cette étude aucune mesure d'activité de LPL ne soit disponible, il est plus que probable que cet effet de suppression de l'influence de l'alcool sur la lipémie post-prandiale, soit médié par l'augmentation de l'activité de la LPL. En accord avec cette hypothèse, l'étude de Pownall [102] a démontré que la consommation d'alcool était associée à une réduction de la réponse lipolytique, probablement le fruit de l'inhibition de l'activité de la LPL.

vi) Pharmacologie

La complexité des mécanismes impliqués dans la synthèse et le métabolisme des lipoprotéines offre de nombreux niveaux d'intervention. Certaines étapes plus importantes dans le métabolisme des lipides peuvent donc représenter des cibles de choix pour l'action d'agents

pharmacologiques, favorisant ainsi un meilleur contrôle de la lipémie post-prandiale. Étant donné que la triglycéridémie à jeun est fortement associée à l'amplitude de la lipémie post-prandiale, les agents pharmacologiques permettant la réduction des niveaux de triglycérides à jeun semblent être indiqués pour l'amélioration de la tolérance aux lipides des individus. À cet effet, les fibrates sont sans aucun doute les plus efficaces dans la réduction des triglycérides. En effet, le traitement au gemfibrozil a été utilisé pour réduire la triglycéridémie post-prandiale [103]. Les fibrates possèdent un large champ d'action dans le métabolisme des lipoprotéines mais les mécanismes par lesquels ces composés agissent, restent nébuleux. La récente découverte que ces drogues agissent sur les récepteurs favorisant la prolifération des péroxisomes (PPARs) [104] permet donc de proposer certaines hypothèses mécanistiques. En effet, les PPARs sont impliqués dans la régulation d'enzymes telles que l'acyl-CoA carboxylase, la LPL et, d'apolipoprotéines comme les apo A-I et C-III. Il est connu que l'action des fibrates via les PPARs supprime la transcription du gène de l'apo C-III et induit la synthèse de LPL [103]. Ces changements métaboliques sont associés à l'augmentation de l'hydrolyse des chylomicrons ainsi qu'à une récupération plus efficace de leurs résidus par le foie.

Cependant, la variance commune entre les triglycéridémies à jeun et post-prandiale n'étant que de 50%, d'autres mécanismes semblent impliqués dans la détérioration du métabolisme postprandial, laissant place à l'effet potentiel d'autres produits pharmacologiques. À cet effet, les statines, l'acide nicotinique et la cholestyramine ont tous été étudiés mais le nombre restreint d'études [25,41] se rapportant aux effets de ces agents ne permet pas de conclure fermement sur les avantages d'utiliser de tels composés comme traitement.

CHAPITRE 4

LE PRODUIT DU GÈNE *obèse*: LA LEPTINE

De nombreux facteurs environnementaux sont connus pour favoriser le développement de l'obésité. De plus, certaines évidences permettent de croire à l'existence de prédispositions génétiques favorisant le développement de l'obésité. La découverte de modèles animaux d'obésité, ainsi que le progrès des techniques de biologie moléculaire ont favorisé l'étude des origines génétiques de l'obésité. À ce chapitre, la récente découverte de la leptine est remarquable, et permettra sans doute d'élargir les connaissances relatives aux mécanismes régulateurs de la prise alimentaire et de la dépense énergétique, dont les altérations sont associées à l'accumulation de tissu adipeux.

2.1 *Historique*

Il y a plus de 40 ans, un chercheur du nom de Kennedy émit l'hypothèse selon laquelle le contrôle du poids corporel et plus particulièrement celui de la quantité de tissu adipeux, pourrait être régulé hormonalement [105]. Selon cette hypothèse, l'hormone, produite par le tissu adipeux lui-même, agirait au niveau du cerveau en procurant de l'information sur l'état des réserves adipeuses de l'organisme. Ce signal serait alors comparé, dans le cerveau, à celui d'un poids préétabli pour l'individu (la consigne) et, en réaction à la différence entre ces signaux, des changements physiologiques et comportementaux se produiraient afin de respecter la consigne.

Ces changements incluraient des modifications métaboliques affectant la prise alimentaire et/ou la dépense énergétique, favorisant au besoin le gain ou la perte de poids.

Tableau 4: Caractéristiques physiques et métaboliques des principaux modèles d'obésité chez la souris

Nom	*Gène*	*Produit du gène*	*Caractéristiques physiologiques*
Obèse	*ob*	Leptine	. Obésité . Hyperphagie . Hyperinsulinémie . Diabète . Infertilité . Ralentissement de la croissance
Diabétique	*db*	Récepteur à la leptine	. Obésité . Hyperphagie . Hyperinsulinémie . Diabète . Infertilité . Ralentissement de la croissance
Agouti	*agouti*	Antagoniste de l'hormone stimulante des mélanocytes (MSH)	. Obésité . Pelage jaune . Accumulation progressive de masse grasse
Fat	*fat*	Carboxypeptidase E	. Obésité (adulte) . Hyperinsulinémie . Infertilité
Tubby	*tubby*	Protéine présente dans la rétine et dans le cerveau	. Obésité (adulte) . Cécité . Surdité

Tiré de Naggert et coll [106]

Vingt ans plus tard, des expériences de parabiose effectuées chez la souris [107], allaient confirmer les travaux de Kennedy [105]. La parabiose consiste à unir, suite à une intervention chirurgicale, les systèmes sanguins de deux animaux afin de ne former qu'un seul système circulatoire. Ces travaux ont été effectués sur des souches de souris caractérisées par un état d'obésité: la souris obèse *(ob/ob)* et la souris diabétique *(db/db)* découvertes quelques années auparavant [108,109]. Bien que partageant certains traits phénotypiques, dont l'obésité, les deux souris possèdent des patrimoines génétiques différents.

Au cours de ses études, Coleman [107] observa qu'en unifiant le système circulatoire d'une souris *ob/ob* à celui d'une souris normale (dite de type sauvage), la souris *ob/ob* perdait du poids tandis qu'aucun changement n'était observé chez la souris normale. Il proposa que le gène *ob* devait coder pour un facteur qui, lorsque sécrété dans le sang, signale au cerveau de l'animal l'état de ses réserves adipeuses. Ce facteur devait être inactif ou absent chez la souris *ob/ob*, mais présent chez la souris normale qui, en le partageant avec la souris *ob/ob*, assurait le contrôle du poids de cette dernière. Par ailleurs, l'union d'une souris *db/db* à une souris normale, amenait la souris normale à moins se nourrir et ultimement, à mourir. Coleman suggéra alors, que la souris *db/db* devait sécréter le produit du gène *ob* en abondance, tout en présentant une insensibilité face à cette même protéine. Le surplus de la protéine chez la souris normale, engendré par son union à la souris *db/db*, lui signifiait un état d'obésité inexistant. Lorsque Coleman croisa les systèmes sanguins des souris *ob/ob* et *db/db*, il observa que la souris *ob/ob* maigrissait jusqu'à un poids normal pendant que la souris *db/db* demeurait obèse et diabétique. Ces résultats confirmaient, en

quelque sorte, l'existence d'un facteur transporté dans le sang et qui contrôle le poids corporel chez la souris.

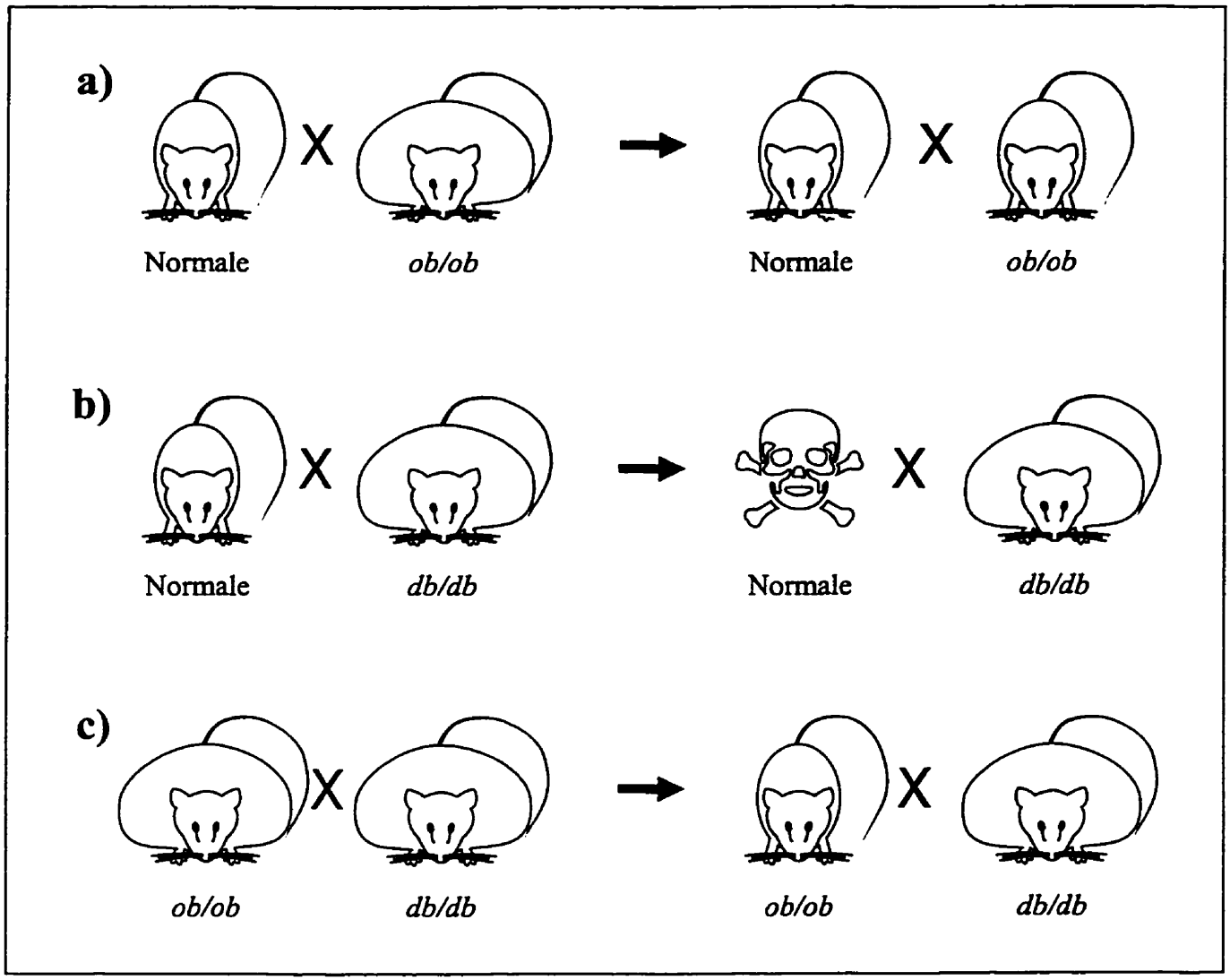

Figure 2: Expériences de parabiose (croisement des systèmes sanguins) chez des souris normales, obèses (*ob/ob*) et diabétiques (*db/db*). L'union des systèmes sanguins de différentes souches de souris entraîne des échanges de composés, présents dans le sang de chacune des souris, dont celui susceptible de contrôler le poids corporel. **a)** Le facteur sanguin actif de la souris normale agit chez la souris *ob/ob* entraînant une perte de poids chez cette dernière. **b)** La surabondance du facteur sanguin chez la souris *db/db* provoque une perte de poids excessive chez la souris normale et entraîne sa mort. **c)** Avec le croisement d'une souris *ob/ob* à une souris *db/db*, on note une perte de poids chez la souris *ob/ob* et aucun changement chez la souris *db/db*. Adapté de Coleman [107].

2.2 Découverte de la leptine

À la fin de 1994, un groupe de chercheurs de l'Université Rockefeller à New York, dirigé par le docteur Jeffrey M. Friedman [110], réussit à cloner le gène *ob* chez la souris, ainsi que son homologue chez l'humain. La protéine codée par ce gène, la leptine, fut rapidement synthétisée et, dans les mois qui suivirent, de nombreuses données furent publiées quant à l'effet résultant de l'administration de cette protéine à des souris *ob/ob*. Ce traitement entraîna une perte de poids chez la souris *ob/ob*, attribuable à une diminution de la prise alimentaire et à une augmentation de la dépense énergétique [111-116]. Par ailleurs, l'injection de leptine chez des souris *db/db* n'entraîna aucun changement de poids chez l'animal [111,112,116,117]. Il est à noter que chez la souris *db/db*, les concentrations de leptine sont très élevées [116]. À la lumière de ces résultats, il fut proposé qu'il y avait une déficience de leptine chez la souris *ob/ob* tandis que la souris *db/db* était caractérisée par une résistance à l'action de la leptine. Ces résultats étaient tout à fait en accord et complémentaires à ceux de Coleman recueillis 20 ans plus tôt. Il est maintenant connu que chez la souris *ob/ob*, la leptine est inactive puisqu'une mutation d'insertion dans le gène *ob*, situé sur le chromosome 6, introduit un codon d'arrêt prématuré dans la traduction de l'ARN messager (ARNm) du gène *ob* et qui provoque la synthèse d'une protéine tronquée et inactive [110]. De plus, il a été rapporté que le gène *db* code pour le récepteur à la leptine [118]. La mutation de ce gène résulte en la synthèse d'un récepteur inadéquat chez la souris *db/db* et ainsi, en l'incapacité à transmettre le signal véhiculé par la leptine. Chez l'humain, les individus obèses sont caractérisés par des niveaux élevés de leptine, suggérant que l'obésité humaine résulterait d'un état de résistance à la leptine similaire à celui rencontré chez la souris *db/db* [119-125].

Cependant, bien que l'on ait réussi à cloner les homologues humains des gènes *ob* et *db*, il ne semble pas que les mutations associées à ces gènes chez les souris *ob/ob* et *db/db* soient présentes chez l'humain [126,127]. Toutefois, l'absence de leptine chez l'humain due à la mutation du gène *ob* a été rapportée qu'à une seule reprise [128], tandis que récemment une famille française était identifiée comme étant caractérisée par une mutation dans le gène du récepteur à la leptine [129]. Dans les deux cas, la présence des mutations était associée au développement d'une obésité morbide en très bas âge [128,129].

2.3 *Caractéristiques et mécanismes d'action de la leptine*

La leptine, du mot grec *leptos* (mince), est une protéine de 167 acides aminés, qui circule dans le sang sous forme d'un polypeptide de 146 acides aminés, à la suite du clivage d'une séquence de 21 acides aminés lors de la sécrétion de la protéine [111]. La leptine est produite exclusivement par le tissu adipeux blanc [130-132]. De plus, la conservation du gène *ob* de la souris et son homologue humain, tel qu'indiqué par son homologie de séquence de 84% [110], suggère un rôle métabolique important de cette protéine. Les mécanismes par lesquels la leptine induit des changements de poids ne sont pas encore connus. Cependant, il a été rapporté que la leptine agit au niveau de l'hypothalamus, dans le noyau ventro-médian, où l'on retrouve une concentration importante de récepteurs à la leptine [133,134]. À cet endroit, la leptine inhiberait la sécrétion de neuropeptide Y (NPY), important régulateur de la prise alimentaire [134,135]. En effet, l'injection de leptine diminue les concentrations de NPY chez la souris *ob/ob* [134]. Par le biais de cette baisse des niveaux de NPY, il y aurait diminution de la prise alimentaire. En plus de

réduire la concentration de NPY dans l'hypothalamus, la leptine agirait également sur la liaison du NPY à son récepteur ou encore en interférant dans la cascade réactionnelle post-récepteur. C'est ce que suggèrent les résultats de Smith et coll [136], démontrant que l'injection de leptine dans le cerveau supprime la prise alimentaire anticipée par l'administration simultanée de NPY. Par ailleurs, les mécanismes sous-jacents à l'augmentation de la dépense énergétique chez la souris *ob/ob* lors du traitement à la leptine restent inconnus. Cependant, bien qu'une augmentation de la dépense énergétique soit observée avec le traitement à la leptine, il semble que l'effet de la leptine sur la balance énergétique se fasse principalement par la diminution de la prise alimentaire. L'augmentation de la dépense énergétique pourrait donc résulter, en bonne partie, de la hausse de mobilité entraînée par la perte de poids induite par la leptine. Jusqu'à présent, les résultats publiés sur les effets de traitement à la leptine, proviennent uniquement d'expériences effectuées chez la souris et sur des cultures de cellules adipeuses puisque les études d'intervention chez l'humain n'ont pas encore été entreprises.

2.4 *Régulation de l'expression du gène ob et de la sécrétion de leptine par le tissu adipeux*

La quantité de leptine, que ce soit sa concentration en ARNm ou son niveau plasmatique, est étroitement associée à l'adiposité [119-125]. À ce propos, il semble que chez l'humain, la leptine soit un meilleur indicateur de la quantité de tissu adipeux, donc reflète mieux la masse adipeuse totale plutôt que la distribution régionale de la graisse d'un individu [121,122,137]. De plus, de nombreuses hormones sont connues pour agir sur les cellules adipeuses et ces

observations laissent présager de l'implication de nombreux facteurs de régulation de synthèse et de sécrétion de la leptine par l'adipocyte.

i) Effet de l'insuline

L'effet de l'insuline est de loin, celui qui a été le plus étudié en rapport avec la régulation de l'expression et de la sécrétion de la leptine par le tissu adipeux. Cependant, les résultats obtenus chez l'animal et chez l'humain semblent contradictoires. Chez les rongeurs, il est très bien documenté que l'injection d'insuline augmente la quantité d'ARNm de la leptine [138-142]. Même si l'insuline affecte l'expression du gène de la leptine, peu d'études ont examiné l'effet de l'insuline sur les concentrations plasmatiques de leptine. À ce sujet, il a été démontré que l'administration d'insuline augmente les niveaux plasmatiques de leptine chez des rats normaux mais cet effet n'est pas observé chez des rats obèses (*fa/fa*) [143]. Chez l'humain, l'hyperinsulinémie provoquée n'a pas réussi à stimuler la sécrétion de leptine [144-152]. De plus, l'augmentation post-prandiale d'insuline ne provoque pas de hausse de leptinémie [119]. D'autres résultats obtenus chez l'humain de même qu'à partir d'expériences effectuées sur des cellules adipeuses en culture, laissent cependant présager d'un effet stimulant de l'administration d'insuline sur la production de leptine [153-160], mais cet effet n'est observable qu'après quelques heures d'exposition à des doses souvent très élevées d'insuline. Par contre, l'association positive entre les concentrations plasmatiques de leptine et d'insuline [153,155,161,162] indique qu'il existe une interrelation entre ces deux variables. Bien que l'importance physiologique de cette relation reste à être démontrée, il est probable qu'elle soit le reflet d'une régulation à long

terme de l'insuline sur la leptine. Certains ont même proposé que la relation entre la leptine et l'insuline pourrait être causée par des changements au niveau d'un troisième facteur commun à la leptine et à l'insuline [147]. Par exemple, les changements induits par l'insuline sur l'adipocyte pourraient indirectement affecter la sécrétion de leptine.

ii) Différence sexuelle

Le dimorphisme sexuel observé au niveau des concentrations plasmatiques de leptine [119,122,163-168] résiste à la correction pour les différences connues de composition corporelle entre les hommes et les femmes. En effet, même après ajustement pour une adiposité moins forte chez les hommes que chez les femmes, ces dernières présentent toujours des concentrations plus élevées de leptine comparativement aux hommes [122,163-168]. Cette observation a donc permis de suggérer que les hormones sexuelles pourraient réguler les concentrations de leptine [167]. Par ailleurs, même si la relation inverse entre les concentrations plasmatiques de testostérone et de leptine ne résiste pas à la correction pour l'adiposité chez l'homme [169], il a été proposé que la testostérone diminuerait [170-172] les niveaux de leptine dans le sang. Au contraire, les oestrogènes augmenteraient les concentrations de leptine en circulation [173]. En accord avec ces hypothèses, il a été démontré que le traitement à la testostérone d'hommes hypogonadiques diminue la concentration de leptine dans le plasma [174]. De plus, une étude de changement de sexe nous porte à croire que la testostérone serait plus étroitement liée à la leptinémie que les oestrogènes. En effet, le passage du sexe féminin à masculin (transsexuel femme-homme) par l'administration de testostérone, entraîne la diminution de la leptinémie, tandis que l'injection

d'oestrogènes, chez les transsexuels homme-femme, augmente les niveaux de leptine plasmatique [170]. Cependant, l'administration d'oestrogènes, chez les transsexuels homme-femme, n'a pas affecté les concentrations circulantes d'oestrogènes, mais a diminué celles de testostérone. Étant donné qu'il y avait une augmentation concomitante de l'adiposité chez les transsexuels homme-femme, les auteurs n'ont pas été en mesure d'évaluer les contributions respectives de la baisse de testostérone et de la hausse de l'adiposité dans l'élévation des niveaux de leptine. D'autres résultats suggèrent que les oestrogènes régulent positivement la leptinémie [173]. À la lumière de ces résultats, il semble évident qu'il existe un effet régulateur des hormones sexuelles sur la concentration de leptine en circulation et que cette régulation explique, du moins en partie, le dimorphisme sexuel noté dans les concentrations plasmatiques de leptine.

iii) Effet du jeûne et de la suralimentation

Chez les rongeurs, le jeûne entraîne une baisse de la concentration d'ARNm de la leptine, qui est réversible suite à une ré-alimentation [141,175-177]. Les expériences effectuées chez des sujets humains révèlent une baisse de leptinémie plasmatique pendant une période de jeûne [177-181]. De la même façon que chez les rongeurs, la ré-alimentation des individus restaure des niveaux plasmatiques normaux de leptine [177-180]. Il est à noter que tous ces changements surviennent même en l'absence de changement de poids corporel. Toutefois, contrairement aux rongeurs [182], la consommation d'un repas n'affecte pas la concentration de leptine chez l'humain [119,183]. Ces résultats indiquent donc que, chez l'humain, à l'encontre de ce qui est proposé chez l'animal [139], la leptine n'agirait pas comme un facteur de satiété.

iv) Effet site-spécifique de l'adipocyte

Des différences dans la production de leptine par des adipocytes provenant de dépôts adipeux de localisations anatomiques différentes ont été observées chez l'humain. En effet, bien que cette différence n'ait pas toujours été rapportée [132], l'expression du gène *ob* est plus importante dans les adipocytes d'origine sous-cutanée que dans ceux provenant de dépôts adipeux profonds [130,131]. Il a d'ailleurs été proposé que la différence sexuelle dans la distribution régionale du tissu adipeux [17,184] pourrait expliquer les concentrations de leptine plus élevées chez la femme que chez l'homme. En effet, les femmes présentent une distribution de la graisse principalement sous-cutanée tandis que les hommes accumulent préférentiellement du tissu adipeux viscéral [17,184]. Ainsi, une production accrue de leptine par le tissu adipeux sous-cutané serait tout à fait en accord avec le dimorphisme sexuel observé dans les concentrations de leptine.

De plus, la taille et le contenu en triglycérides des adipocytes ont également été proposés comme des régulateurs potentiels de l'expression et de la sécrétion de leptine par le tissu adipeux. En effet, il a été démontré que plus le volume de la cellule adipeuse est important, plus les concentrations d'ARNm de la leptine retrouvées dans l'adipocyte [185,186] ainsi que les niveaux plasmatiques de leptine [186] sont élevés. Bien que ces résultats suggèrent une régulation de l'expression et de la sécrétion de leptine par la taille et/ou le contenu en lipides de l'adipocyte, rien n'est cependant connu quant aux mécanismes par lesquels cette régulation s'appliquerait.

v) Effet des glucocorticoïdes

L'état d'obésité retrouvé chez les modèles animaux, comme les rongeurs, peut être éliminé par l'ablation des glandes surrénales et par la suite restauré suite à un traitement aux glucocorticoïdes [187-189]. Ces observations ont donc conduit certains groupes à proposer un effet inhibiteur des glucocorticoïdes sur l'action de la leptine. L'injection simultanée de dexaméthasone et de leptine dans le cerveau de rats normaux empêche la perte de poids causée normalement par l'administration de leptine seule [190]. Chez l'humain, les glucocorticoïdes augmentent également les concentrations plasmatiques de leptine [191-193] de même que la sécrétion de leptine par l'adipocyte [194]. De plus, des concentrations élevées de cortisol sont associées, tant chez l'animal que chez l'humain, à l'obésité, l'intolérance au glucose et la résistance à l'insuline [195]. Il a été démontré que la leptine inhibe la sécrétion de cortisol par les glandes surrénales [196]. Il semble que l'hormone de libération de la corticotropine (CRH) soit une des pièces importantes de la régulation du poids corporel par la leptine et les glucocorticoïdes. En effet, il est connu que le CRH inhibe la prise alimentaire chez les rongeurs [197,198], entraînant une perte de poids et une baisse des concentrations plasmatiques d'insuline. Étant donné que la leptine augmente les niveaux d'ARNm du CRH dans l'hypothalamus, il est plus que probable que l'action hypophagiante de la leptine résulte de l'élévation de la concentration de CRH [197]. Par contre, le CRH est un activateur important de l'axe hypothalamo-hypophysaire-surrénalien qui, lorsqu'activé, entraîne la sécrétion de glucocorticoïdes dont les effets sont associés au gain de poids. Cette dualité entre les effets des glucocorticoïdes et de la leptine est tout à fait en accord avec la régulation positive des glucocorticoïdes sur l'expression de leptine [199].

En effet, l'interaction entre les glucocorticoïdes et la leptine permettrait aux effets permissifs que les glucocorticoïdes exercent sur la prise de poids d'être ralentis et même supprimés par l'augmentation des concentrations de leptine.

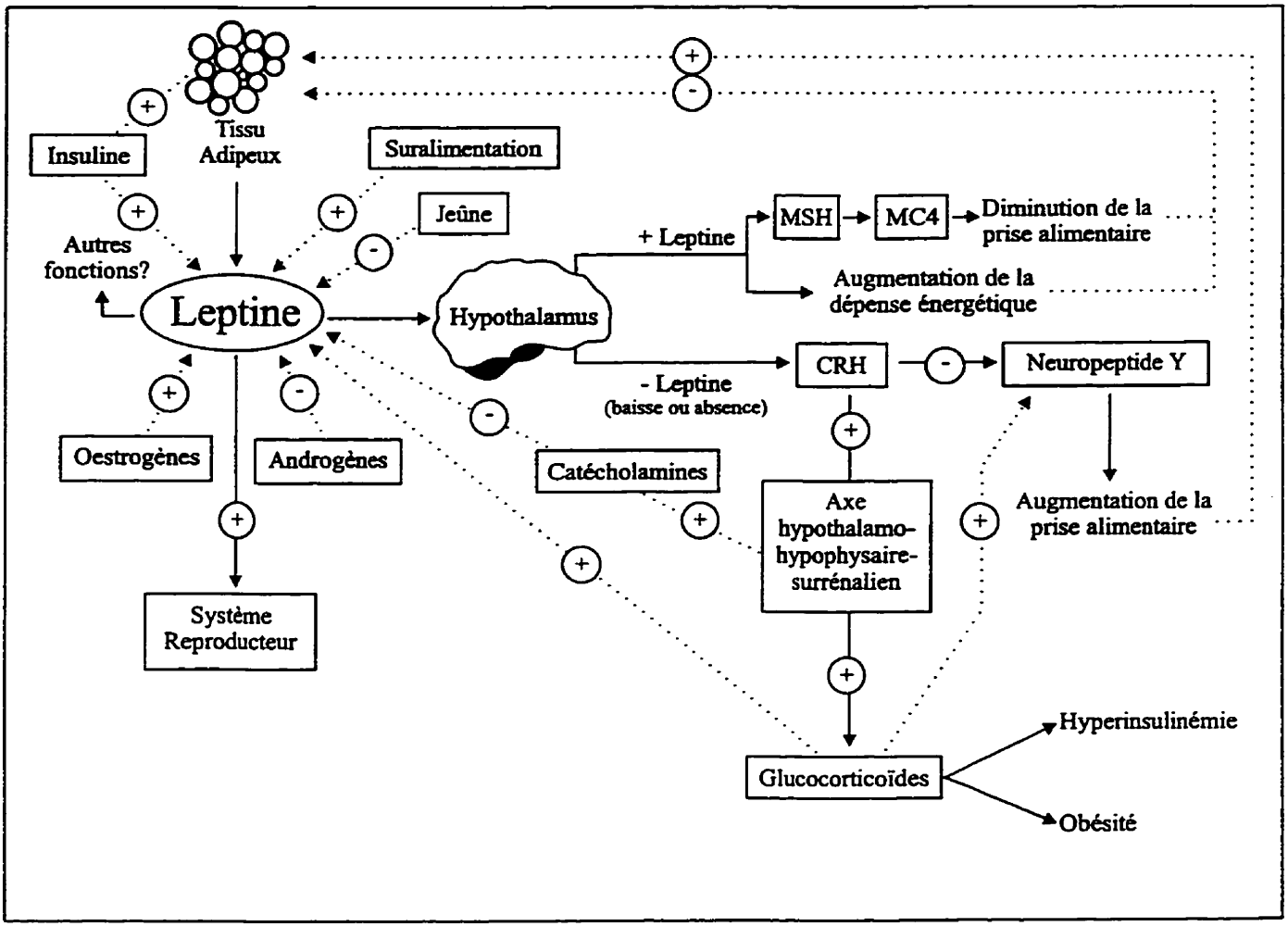

Figure 3 : Représentation schématique de la régulation de la leptine. Les renseignements présentés dans cette figure, tiennent compte à la fois des résultats recueillis chez l'animal et l'humain. CRH: hormone de libération de la corticotropine; MSH: hormone mélanotrope; MC4: récepteur à la mélanocortine.

2.5 *Autres fonctions physiologiques potentielles de la leptine*

L'importance de la leptine au niveau du système reproducteur intéresse de plus en plus les chercheurs. En effet, en plus de provoquer une perte de poids, le traitement à la leptine rétablit la fonction reproductrice déficiente des souris *ob/ob*. Ainsi, le système reproducteur se développe chez le mâle [200,201] tandis que la gestation et la lactation redeviennent possibles chez la femelle [201,202]. C'est pourquoi, il a été avancé que la leptine pourrait jouer un rôle important dans le contrôle de la fonction reproductrice. Cette hypothèse est d'ailleurs en accord avec les résultats issus d'expériences de surrénalectomie effectuées chez les rongeurs et montrant que l'ablation des glandes surrénales élimine l'obésité chez les souris *ob/ob* et *db/db*, de même que chez le rat *fa/fa* [187-189]. En effet, suite à la surrénalectomie, la prise alimentaire des rongeurs se normalise, la masse musculaire augmente, la croissance reprend et la résistance à l'insuline ainsi que l'hyperglycémie s'atténuent [195]. Cependant, malgré la perte importante de poids, l'infertilité caractéristique des animaux persiste. Ces résultats suggèrent donc que le développement de l'obésité ne dépendrait pas uniquement d'altérations relatives au métabolisme de la leptine, mais pourrait être causé par l'action des glucocorticoïdes. L'hypothèse de travail entourant le rôle de la leptine dans la reproduction est la suivante: la leptine, agissant comme un indicateur des réserves de tissu adipeux, indiquerait à l'organisme qu'il est en mesure de supporter une reproduction normale (Figure 4).

Des évidences recueillies chez l'humain permettent également de croire à une fonction de la leptine dans la reproduction. Chez les femmes entraînées en endurance (par exemple les marathoniennes) et chez les anorexiques, où la quantité de graisse corporelle atteint des niveaux

très bas, des dérèglements du cycle menstruel, allant même jusqu'à l'arrêt des menstruations, sont fréquemment observés [203-205]. De plus, des variations dans les concentrations de leptine ont également été notées durant le cycle menstruel chez la femme [206]. Ainsi, la concentration maximale de leptine, qui coïncide avec le pic de concentration de progestérone, est associée à la phase lutéale du cycle menstruel.

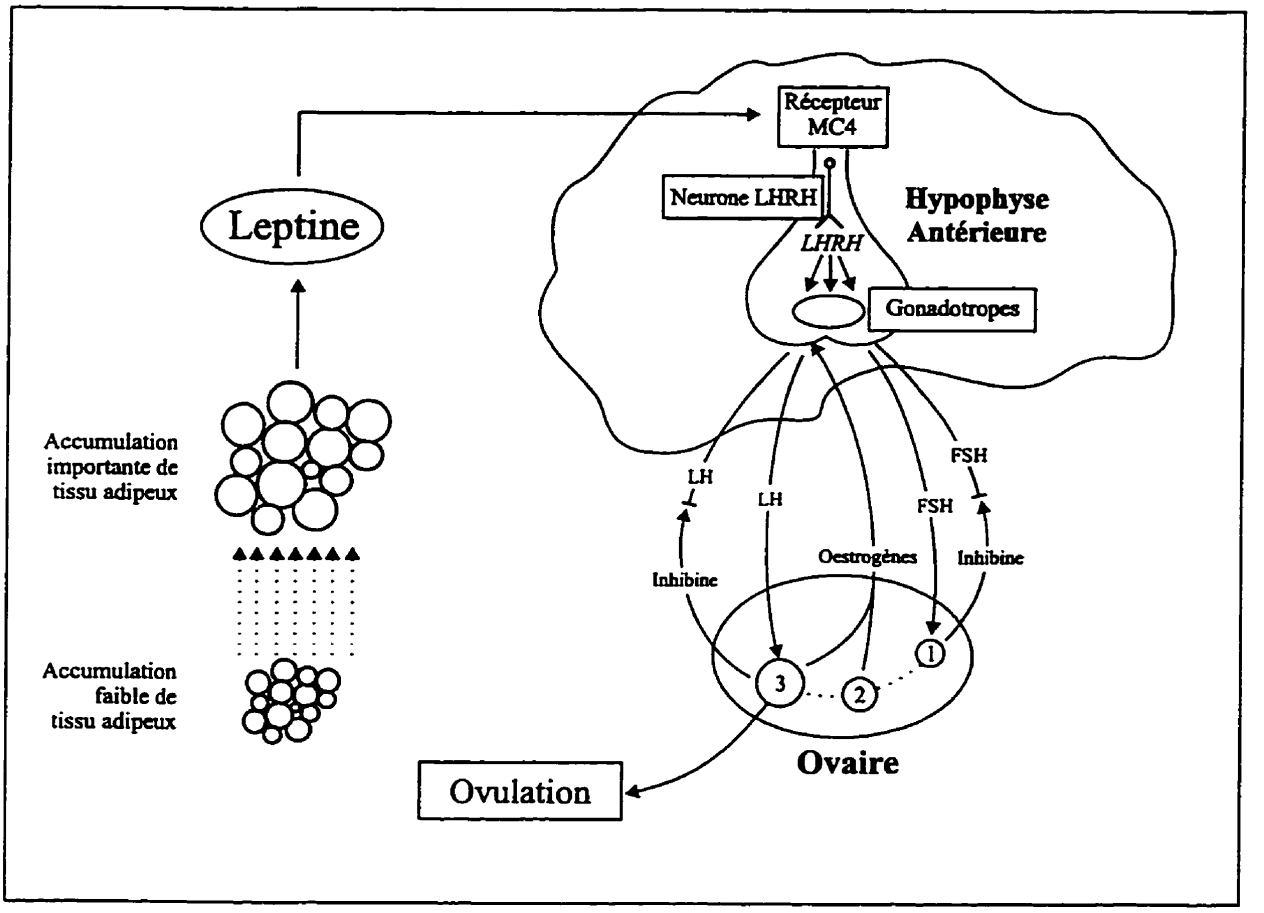

Figure 4 : Rôle hypothétique de la leptine dans le système reproducteur chez la femme

D'autres évidences laissent croire à l'importance de la leptine dans le développement du système reproducteur chez l'humain. En effet, chez les jeunes garçons, la chute des concentrations plasmatiques de leptine à l'adolescence pourrait agir en tant que signal du développement de la puberté puisqu'elle coïncide avec l'augmentation des niveaux de testostérone [206-208].

Finalement, les récentes découvertes de la présence de leptine dans le cordon ombilical [209] de même que de la production de leptine dans le placenta [210] chez l'humain, suggèrent que la leptine pourrait être impliquée dans la gestation bien que son rôle à ce niveau reste inconnu.

En résumé, le développement de l'obésité chez l'humain intrigue beaucoup la communauté scientifique et la découverte de la leptine a permis de franchir une nouvelle étape dans la compréhension de ce processus. Cependant, afin de déterminer avec justesse la fonction de cette hormone chez l'humain, il semble nécessaire d'étudier, au préalable, les associations potentielles entre la leptinémie et les nombreux variables métaboliques reconnues pour être affectées par une adiposité accrue. À cet effet, il semble de plus en plus évident que l'accumulation de tissu adipeux viscéral est une variable déterminante dans la détérioration métabolique liée à l'obésité. En effet, il est connu que l'obésité viscérale est associée à des altérations du profil lipidique à l'état de jeûne. Les prochains chapitres auront donc pour but de vérifier l'impact de l'accumulation excessive de tissu adipeux viscéral sur le métabolisme des lipoprotéines en période post-prandiale. Cette section de la thèse permettra également d'étudier les relations entre l'adiposité, la distribution du tissu adipeux de même que les complications métaboliques qui y sont rattachées, et les concentrations plasmatiques de leptine chez l'homme et la femme.

CHAPITRE 5

LES CONCENTRATIONS PLASMATIQUES DE HDL-CHOLESTÉROL À JEUN, ET NON CELLES D'APOLIPOPROTÉINE A-I, SONT ASSOCIÉES AU SYNDROME DYSLIPIDÉMIQUE LIÉ À L'OBÉSITÉ VISCÉRALE

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Plasma HDL-Cholesterol But Not apo A-I Is a Good Correlate of the Visceral Obesity-Insulin Resistance Dyslipidemic Syndrome

RUNNING TITLE : HDL-cholesterol and apo A-I in visceral obesity

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RÉSUMÉ

L'apolipoprotéine (apo) A-I est une composante majeure des lipoprotéines de densité élevée (HDL), et il a été suggéré que la mesure de sa concentration pourrait permettre d'obtenir des informations additionnelles quant au risque de développer des maladies coronariennes. Une population de 111 hommes (age moyen ● SD : 35.3 ± 6.6 ans), a été étudiée afin de savoir si des concentrations faibles d'apo A-I sont associées aux altérations métaboliques caractéristiques du syndrome dyslipidémique lié à l'obésité viscérale. Ainsi, nous avons comparé les hommes des 1er et 4^{e} quartiles de concentrations d'apo A-I et de HDL-cholestérol (HDL-C), pour les variables de distribution de la graisse, de tolérance au glucose et les niveaux de lipoprotéines plasmatiques. Les sujets du 1er quartile (en-dessous du 25^{e} percentile) de la distribution des valeurs de HDL-C, lorsque comparés à ceux du 4^{e} quartile (au-delà du 75^{e} percentile), étaient caractérisés par une accumulation plus importante de tissu adipeux viscéral ($p<0.05$), de même que par des concentrations plus élevées de triglycérides ($p<0.001$), d'apo B ($p<0.0005$) et d'insuline ($p<0.01$). Cependant, ces différences disparaissaient lorsque les individus des 1er et 4^{e} quartiles de concentrations d'apo A-I étaient comparés. Les résultats de la présente étude suggèrent que les concentrations de HDL-C sont associées plus étroitement, que les niveaux d'apo A-I, aux nombreuses altérations métaboliques du syndrome de résistance à l'insuline lié à l'obésité viscérale.

ABSTRACT

Apolipoprotein (apo) A-I is a major component of high density lipoproteins (HDL), and it has been suggested that its measurement may provide additional information in the assessment of coronary heart disease risk. In the present study, we have tested, in a sample of 111 men (mean age ± SD: 35.3 ± 6.6 years), whether a low apo A-I concentration is associated with the cluster of metabolic abnormalities which are characteristics of the visceral obesity-insulin resistance dyslipidemic syndrome. For that purpose, the first and fourth quartiles of apo A-I and HDL-cholesterol (C) concentrations were compared in relation to body fat distribution, glucose tolerance, plasma insulin and lipoprotein levels. Men in the 1st quartile (below the 25th percentile) of HDL-C, when compared to those in the 4th quartile (above the 75th percentile) were characterized by an elevated visceral adipose tissue accumulation ($p<0.05$), as well as by increased plasma levels of triglycerides ($p<0.0001$), apo B ($p<0.0005$), and insulin ($p<0.01$). These differences were not found, when the 1st and 4th quartiles of plasma apo A-I concentrations were compared. These results suggest that plasma levels of HDL-C are more closely associated with the various features of the visceral obesity-insulin resistance syndrome than plasma apo A-I.

INTRODUCTION

Obesity is usually associated with disturbances in lipid metabolism [1] and a dyslipidemic profile including hypertriglyceridemia [2,3,4,5], and hypoalphalipoproteinemia [6,7,8,9] is frequently observed among obese subjects. In fact, visceral adipose tissue (AT) accumulation shows stronger associations with various metabolic abnormalities than obesity per se [10,11]. Besides altered lipoprotein-lipid levels, an excess of visceral AT has been associated with an insulin resistant-hyperinsulinemic state in both men and women [10,11]. High plasma triglyceride (TG) and low plasma high-density lipoprotein cholesterol (HDL-C) concentrations are often simultaneously observed in insulin resistant-hyperinsulinemic subjects [12-14]. These alterations in plasma lipid profile and insulin levels found in visceral obesity are important risk factors in the etiology of diabetes and coronary heart disease (CHD) [2,8,15-18].

However, some CHD patients show nearly normal plasma lipid profiles. Therefore, emphasis has been given to the determination of plasma non-lipid variables, such as apolipoproteins (apo), for the assessment of the CHD risk profile in these subjects. In this regard, cross-sectional as well as prospective studies [19-26] have reported reduced plasma apo A-I levels in both men and women at risk for or with proven CHD. Thus, the measurement of plasma apo A-I concentration has been suggested to provide additional information besides HDL-C in the assessment of CHD risk. Since apo A-I is a major constituent of HDL particles, and considering that HDL-C levels are decreased in an insulin resistance state, we have tested whether low plasma apo A-I concentrations would also be affected as a part of the metabolic cluster found in the visceral obesity-insulin resistance syndrome. For that purpose, we have compared the 1st and 4th quartiles of plasma apo A-I and HDL-C

concentrations in a sample of 111 men (mean age ± SD: 35.3 ± 6.6 years), in relation to body fat distribution, glucose tolerance, plasma insulin levels, and plasma lipoprotein concentrations. Results of this study suggest that decreased plasma apo A-I concentrations, in contrast to HDL-C, are not a common feature of the cluster of metabolic abnormalities found in the visceral obesity-insulin resistance syndrome.

SUBJECTS AND METHODS

Subjects. One hundred and eleven men aged 20-53 years old were recruited through the media to participate in this study, which was approved by the medical ethics committee of Laval University and an informed consent document was signed by the participants. A complete physical examination was performed by a physician in charge of the medical supervision of the study, which also included medical history. All participants were nonsmokers and healthy. Exclusion criteria included diabetes, genetic dyslipidemias or evidence for the presence of coronary heart disease.

Anthropometric and body composition measurements. Weight, height, waist and hip circumferences were measured following the procedures recommended at the Airlie Conference [27], and the waist-to-hip ratio was calculated. Body density was measured by the hydrostatic weighing technique [28], and the mean of six measurements was used in the calculation of body density. Percentage body fat was obtained from body density using the equation of Siri [29].

Computed tomography. Computed tomography (CT) was performed on a Siemens Somatom DRH scanner (Erlagen, Germany) using previously described procedures [30,31]. Briefly, the subjects (n=97) were examined in the supine position with both arms stretched above the head. CT scan was performed at the abdominal level (between L4 and L5 vertebrae) with a radiograph of the skeleton as a reference to establish the position of the scan to the nearest millimeter. Total adipose tissue areas were calculated by delineating these areas with a graph pen and then computing the AT surfaces with attenuation range of -190 to -30 HU [30-32]. 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.

Oral glucose tolerance test. A 75-g oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast. Blood samples were collected under EDTA and Trasylol (Miles, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein at -15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes for the determination of plasma glucose and insulin concentrations. Plasma glucose was measured enzymatically [33], whereas plasma insulin was measured by radioimmunoassay (RIA) with polyethylene glycol separation [34]. However, the assay used for the measurement of plasma insulin showed some cross-reactivity with proinsulin. As diabetes was an exclusion criteria in our study, we beleive that such cross-reactivity did not have a significant impact on results obtained and their interpretation. The glucose and insulin areas under the curve during the OGTT were determined with the trapezoid method.

Plasma lipoprotein analyses. Blood samples were obtained in the morning after a 12-hour fast from an antecubital vein into vacutainer tubes containing EDTA. Cholesterol (CHOL) and triglyceride levels in plasma and in lipoprotein fractions were measured enzymatically on an RA-1000 Autoanalyzer (Technicon, Tarrytown, NY), as previously described [35]. Very-low-density lipoproteins (VLDL; $d<1.006$ g/ml) were isolated by ultracentrifugation, and the 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]. The cholesterol content of HDL_2 and HDL_3 subfractions was also determined after further precipitation of HDL_2 with dextran sulfate [37]. Total apo B concentration was measured in plasma whereas LDL-apo B and HDL-apo A-I were measured in the infranatant (d>1.006 g/ml) by the rocket immunoelectrophoretic method of Laurell, as previously described [38]. The lyophilized serum standards for apo measurements were prepared in our laboratory and calibrated with reference standards obtained from the Centers for Disease Control, Atlanta, GA. The cumulative coefficients of variation for the measurements of HDL-C and apo A-I were 3.3 % and 3.4% respectively.

Statistical analyses. Student's T-tests were used to compare the different quartiles of the apo A-I and HDL-C concentrations. Pearson's product-moment correlation coefficients were used to quantify associations between means. All statistical analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Figure 1 shows the relationship between plasma apo A-I and HDL-C levels. Although a significant correlation was noted, the shared variance only reached 32% which suggested that apo A-I measurements could not be used to estimate HDL-C levels.

Figure 2 shows that whereas plasma HDL-cholesterol levels were significantly correlated with visceral adipose tissue accumulation measured by computed tomography, apo A-I concentration was not a significant correlate of visceral adipose tissue deposition.

The characteristics of men in the 1st and 4th quartiles of the plasma apo A-I and HDL-C concentrations are presented in Table 1. Subjects in the 1st quartile of plasma apo A-I levels did not differ from those in the 4th quartile of apo A-I concentrations for body fatness variables. In contrast, comparisons related to HDL-C concentrations showed that subjects in the 1st quartile had increased body weight, BMI, %body fat, fat mass (FM), waist and hip circumferences, as well as a higher waist-to-hip ratio (WHR) compared to men in the 4th quartile. Men in the 1st quartile of HDL-C concentrations also had increased levels of visceral AT compared to those in the 4th quartile, and this difference in visceral AT was not found for apo A-I subgroups.

Table 2 compares plasma lipoprotein-lipid concentrations between subgroups characterized by low and high levels of either plasma apo A-I or HDL-C. Very few differences were noted between the 1st and 4th quartiles of apo A-I distribution as only plasma HDL-C, HDL_2-C and HDL_3-C levels were different among the groups. On the other hand, when subjects were subdivided on the basis of HDL-C concentrations, all metabolic variables were significantly different between the 1st and 4th quartiles, with the exception of plasma cholesterol and LDL-C levels.

Comparison of mean lipoprotein ratios presented in Table 3 revealed that subjects with low levels of plasma apo A-I were characterized by increased CHOL/HDL-C, LDL-C/HDL-C and HDL-TG/HDL-C ratios, whereas subjects with low HDL-C concentrations were significantly different from subjects with high HDL-C levels for all lipoprotein ratios considered.

Figure 3 shows the glycemic and insulinemic responses of subjects in the 1st and 4th quartiles of apo A-I and HDL-C concentrations to the oral glucose tolerance test (OGTT). When compared to those of the 4th quartile, subjects of the 1st quartile of apo A-I had identical glucose and insulin responses to the oral glucose challenge. However, although areas under the curves of glucose levels during the OGTT were not statistically different between both quartiles, subjects with low plasma HDL-C levels were characterized by significantly higher levels of insulin in the fasting state as well as during the OGTT compared to men with high concentrations of HDL-C. These differences resulted also in a significantly greater area under the curve of insulin for subjects with low HDL-C concentrations.

DISCUSSION

Visceral AT accumulation is an important correlate of the metabolic profile observed in obese men and women [1]. Indeed, increased plasma TG levels [2-5] and decreased HDL-C concentrations [6-9] are frequently observed in subjects with excess visceral AT. Furthermore, the insulin resistance state associated with visceral obesity [10,11] may lead to alterations in lipid metabolism [12,13,14]. This cluster of metabolic abnormalities increases the risk of CHD [2,8,15-18].

The relationship between reduced HDL-C levels and CHD is well documented. Since apo A-I is a major component of HDL particles, it has been suggested that measuring plasma apo A-I concentrations could provide information which would complement HDL-C levels in the assessment of CHD risk [19-26]. In fact, plasma apo A-I concentrations, especially in normolipidemic subjects, have been found to be a better predictor of coronary artery disease (CAD) than plasma lipid levels [38,39]. Furthermore, in case-control studies [40,41], men with CAD showed significantly lower plasma apo A-I concentrations compared to control subjects.

In the present study, although apo A-I concentrations were significantly correlated with HDL-C levels, low levels of apoA-I were not indicative of the alterations in adipose tissue distribution and anthropometric variables similar to those associated with reduced levels of HDL-C. Subjects with low levels of plasma HDL-C were characterized by increased body weight, BMI, FM, waist girth and WHR. Although a relationship between elevated BMI and low plasma apo A-I concentration has already been reported [42], it was not found in the present study. Furthermore, apo A-I levels were not associated with any measures of body fatness in contrast to low HDL-C.

A decreased plasma HDL-C concentration represents a plasma lipid abnormality frequently found in obese subjects. Our analyses showed that subjects with low levels of HDL-C had significant alterations in their plasma lipoprotein-lipid profile compared to men with high plasma HDL-C concentrations. With the exception of cholesterol and LDL-C, which are poor correlates of visceral obesity and insulin resistance [15,43], and HDL-TG, all other lipid parameters including apo B levels were significantly different between the two quartiles of plasma HDL-C concentrations. On the other hand, subjects with low levels of apo A-I did not show any major differences in their overall lipoprotein-lipid profile when compared to those with high plasma apo A-I concentrations.

Measurement of lipoprotein ratios revealed that men with low plasma HDL-C levels had lipoprotein ratios predictive of an increased CHD risk compared to those with high HDL-C concentrations. However, subjects in the 1st quartile of apo A-I showed differences compared to men of the 4th quartile that were not as marked as those that were observed when HDL-C lowest and highest quartiles were compared. These results suggest that measurement of apo A-I alone may be inadequate to appropriately assess CHD risk and the presence of the cluster of metabolic abnormalities (insulin resistance dyslipidemic syndrome) noted in visceral obesity. Indeed, differences in plasma insulin levels, were noted between the subgroups of low vs high HDL-C levels. Men with low HDL-C levels had significantly higher fasting insulin levels as well as following the glucose load compared to men with higher levels of HDL-C. These differences in the response may imply that our nondiabetic men with low HDL-C were characterized by a compensatory increase in insulin to a glucose challenge resulting from a state of insulin resistance.

No such difference in insulin levels was observed between subjects with low vs high plasma concentrations of apo A-I.

Since HDL-C concentrations have been reported to be decreased in the visceral obesity-insulin resistance syndrome [44,45], the lack of concomitant variation in apo A-I may reflect alterations in the composition of HDL. The estimation of the relative CHOL content of HDL particles, as crudely assessed by the HDL-C/apo A-I ratio, was not different between the 1st and 4th quartiles of the plasma apo A-I concentrations. Thus, low levels of apo A-I, that are believed to be indicative of a reduced number of HDL particles, were not associated with major changes in HDL composition. However, the complexity of HDL composition requires that more studies assessing HDL particle number and composition need to be conducted before a firm conclusion can be reached on this issue. Furthermore, the comparison of the 1st and 4th quartiles of plasma HDL-C revealed that both plasma apo A-I and the relative cholesterol content of HDL particles (as estimated by the HDL-C/apo A-I ratio) were significantly different between the two HDL-C subgroups. In this regard, it has been reported that obese women show reduced HDL-chol concentration per mole of HDL protein compared to leaner subjects [42]. In the present study, men in the 1st quartile of HDL-C levels had increased levels of body fat and a decreased HDL-C/apo A-I ratio, a finding which is concordant with these previous results. Furthermore, results from the Atherosclerosis Risk In Communities (ARIC) Study [46] also support this notion also, since it was reported that the reduction of HDL-C concentrations observed with obesity was primarly due to its associations with plasma TG levels, whereas changes in HDL-C concentrations attributable to a change in plasma apo A-I levels was more closely mediated by smoking and alcohol consumption. The elevated visceral

AT accumulation commonly observed in the insulin resistance syndrome, is associated with increased plasma TG levels, therefore providing substrate for lipid exchange with HDL by the action of lipid transfer proteins (i.e. CETP). This process leads to changes in the core composition of HDL particles, such as TG enrichment, rather than alterations in its protein composition. In our subjects, increased visceral AT accumulation and increased TG levels were observed among subjects in the 1st quartile of HDL-C compared to the 4th. These metabolic characteristics are in concordance with those reported in the ARIC Study. It is therefore proposed that the decreased plasma HDL-C concentrations found in subjects with the features of the visceral obesity-insulin resistance syndrome may be due to reductions in both HDL particle number and cholesterol content.

In summary, we have attempted to compare how low levels of apo A-I and HDL-C could identify individuals showing the features of the insulin resistance-dyslipidemic syndrome found in visceral obsese men. In this regard, it appears that low levels of apo A-I provide less information than low HDL-C levels, and that a reduced apoprotein A-I concentration does not appear to be a component of the prevalent metabolic cluster associated with the insulin resistance syndrome. Moreover, studies have shown that plasma apo A-I concentrations did not add to the predictive value of HDL-C and other conventionnal CHD risk factors [20,47,48]. Thus, results of the present study do not support the measurement of apo A-I in addition to conventionnal lipoprotein-lipid levels for a more precise assessment of CHD risk.

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FIGURE HEADINGS

Figure 1: Relationship between plasma high density lipoprotein cholesterol (HDL-C) and apo A-I concentrations in the sample of 111 men of the study.

Figure 2: Correlations between visceral adipose tissue accumulation and fasting plasma HDL-cholesterol (upper panel) as well as apo A-I concentrations (bottom panel) in the sample of men (n=97).

Figure 3: Plasma glucose and insulin responses following a 75g oral glucose load among men in the 1st , 2nd, 3rd and 4th quartiles of plasma apo A-I (panels A and C) and HDL-C (panels B and D) concentrations.

* AUC significantly different from quartiles 1 and 2 at the $p<0.01$ level.

3rd quartile of HDL-C: insulin levels are significantly different from the 1st and 2nd quartiles of HDL-C at 0, 15, 30, 45, 60, 90 minutes and from the 2nd quartile at 180 minutes.

4th quartile of HDL-C: insulin levels are significantly different from the 1st and 2nd quartiles of HDL-C at 0, 15, 30, 45, 60, 90, 120 minutes and from the 2nd quartile at 180 minutes.

Table 1: Physical characteristics of men in the 1st, 2nd, 3rd and 4th quartiles of the apo A-I and HDL-C concentrations

	Apo A-I				HDL-C			
	1st quartile n=26	**2nd quartile n=28**	**3rd quartile n=29**	**4th quartile n=28**	**1st quartile n=27**	**2nd quartile n=28**	**3rd quartile n=27**	**4th quartile n=29**
Age (years)	34.6 ± 5.0	34.7 ± 6.2	34.4 ± 7.1	37.3 ± 7.6	35.9 ± 6.5	37.8 ± 6.1	34.6 ± 5.5	32.8 ± 7.4 [2]
Weight (kg)	83.5 ± 11.1	78.5 ± 12.5	80.4 ± 14.9	81.7 ± 13.9	84.2 ± 13.2	83.9 ± 13.0	82.9 ± 13.2	73.4 ± 10.7 [1,2,3]
BMI (kg/m^2)	27.7 ± 3.8	25.9 ± 3.8	26.7 ± 4.6	26.8 ± 4.3	27.9 ± 3.9	28.2 ± 4.2	26.8 ± 3.6	24.3 ± 3.9 [1,2,3]
%Body fat	25.2 ± 7.5	24.1 ± 7.7	22.7 ± 9.6	23.3 ± 8.3	25.6 ± 7.7	26.5 ± 5.7	25.1 ± 7.7	18.2 ± 9.3 [1,2,3]
Fat mass (kg)	21.6 ± 8.2	19.6 ± 8.5	19.4 ± 10.9	20.1 ± 9.5	22.2 ± 9.5	23.0 ± 7.3	21.6 ± 9.0	14.1 ± 8.9 [1,2,3]
Waist girth (cm)	95.6 ± 10.8	92.1 ± 13.2	95.0 ± 24.0	95.6 ± 12.8	99.3 ± 22.5	99.1 ± 13.1	94.0 ± 11.8	86.3 ± 11.8 [1,2]
WHR	0.94 ± 0.06	0.91 ± 0.07	0.90 ± 0.08 [1]	0.94 ± 0.06 [3]	0.93 ± 0.07	0.95 ± 0.07	0.92 ± 0.06	0.89 ± 0.08 [1,2]
CT-DERIVED ABDOMINAL AT AREAS [a]								
Total (cm^2)	389 ± 118	347 ± 145	386 ± 157	394 ± 157	419 ± 132	411 ± 122	370 ± 151	306 ± 155 [1,2]
Visceral (cm^2)	129 ± 47	116 ± 47	131 ± 56	133 ± 57	142 ± 45	140 ± 46	121 ± 56	101 ± 52 [1,2]
Subcutaneous (cm^2)	260 ± 86	232 ± 103	255 ± 114	261 ± 115	277 ± 97	271 ± 83	249 ± 114	205 ± 116 [1,2]

[a] Apo A-I : 1st: n=23; 2nd: n=25; 3rd: n=23; 4th: n=26;
HDL-C : 1st: n=24; 2nd: n=27; 3rd: n=24; 4th: n=22;

1: significantly different from the 1st quartile
2: significantly different from the 2nd quartile
3: significantly different from the 3rd quartile

Table 2: Mean plasma lipoprotein-lipid concentrations of men in the 1st, 2nd, 3rd and 4th quartiles of the apoA-I and HDL-C concentrations

	Apo A-I				HDL-C			
	1st quartile n=26	2nd quartile n=28	3rd quartile n=29	4th quartile n=28	1st quartile n=27	2nd quartile n=28	3rd quartile n=27	4th quartile n=29
Cholesterol	4.83 ± 0.88	4.86 ± 1.04	4.88 ± 0.97	5.11 ± 0.67	4.91 ± 0.81	5.15 ± 0.90	4.95 ± 0.91	4.68 ± 0.94
Triglycerides	1.76 ± 0.88	1.66 ± 0.90	1.29 ± 1.02	1.52 ± 0.75	2.19 ± 1.08	1.77 ± 0.76 [1]	1.44 ± 0.67 [1]	0.84 ± 0.39 [1,2,3]
VLDL-C	0.65 ± 0.40	0.62 ± 0.43	0.45 ± 0.48	0.53 ± 0.31	0.86 ± 0.52	0.65 ± 0.37 [1]	0.50 ± 0.25 [1]	0.24 ± 0.15 [1,2,3]
VLDL-TG	1.22 ± 0.74	1.16 ± 0.76	0.86 ± 0.97	1.01 ± 0.64	1.63 ± 1.00	1.23 ± 0.67 [1]	0.97 ± 0.53 [1]	0.43 ± 0.30 [1,2,3]
LDL-C	3.30 ± 0.78	3.26 ± 0.98	3.38 ± 1.01	3.39 ± 0.63	3.31 ± 0.84	3.56 ± 0.87	3.37 ± 0.78	3.11 ± 0.91
LDL-TG	0.23 ± 0.13	0.22 ± 0.15	0.19 ± 0.10	0.24 ± 0.14	0.26 ± 0.14	0.26 ± 0.13	0.19 ± 0.14	0.17 ± 0.10 [1,2]
HDL-C	0.88 ± 0.17	0.97 ± 0.16	1.09 ± 0.18 [1,2]	1.19 ± 0.26 [1,2]	0.77 ± 0.06	0.93 ± 0.04 [1]	1.08 ± 0.04 [1,2]	1.33 ± 0.17 [1,2,3]
HDL-TG	0.31 ± 0.10	0.28 ± 0.09	0.24 ± 0.05 [1,2]	0.27 ± 0.07	0.30 ± 0.09	0.28 ± 0.07	0.28 ± 0.10	0.25 ± 0.06
HDL_2-C	0.29 ± 0.11	0.34 ± 0.12	0.40 ± 0.15 [1]	0.43 ± 0.18 [1,2]	0.22 ± 0.05	0.29 ± 0.07 [1]	0.39 ± 0.09 [1,2]	0.55 ± 0.12 [1,2,3]
HDL_3-C	0.58 ± 0.10	0.64 ± 0.09	0.69 ± 0.08 [1]	0.76 ± 0.14 [1,2,3]	0.55 ± 0.06	0.64 ± 0.06 [1]	0.70 ± 0.10 [1,2]	0.77 ± 0.13 [1,2,3]
Apo A-I	88.8 ± 4.7	101.7 ± 3.5 [1]	113.1 ± 3.3 [1,2]	135.8 ± 18.6 [1,2,3]	94.5 ± 9.4	109.0 ± 15.7	115.0 ± 19.8 [1]	121.7 ± 21.3 [1,2]
Apo B	95.3 ± 22.0	91.0 ± 24.9	85.9 ± 23.1	95.0 ± 23.0	100.6 ± 16.9	101.0 ± 23.0	88.0 ± 22.5 [1,2]	77.7 ± 22.5 [1,2]

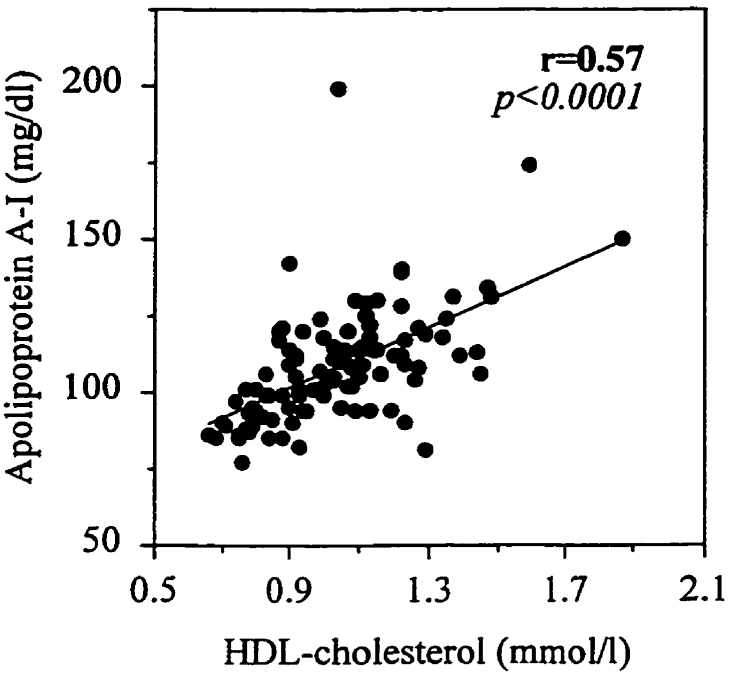
1: significantly different from the 1st quartile
2: significantly different from the 2nd quartile
3: significantly different from the 3rd quartile

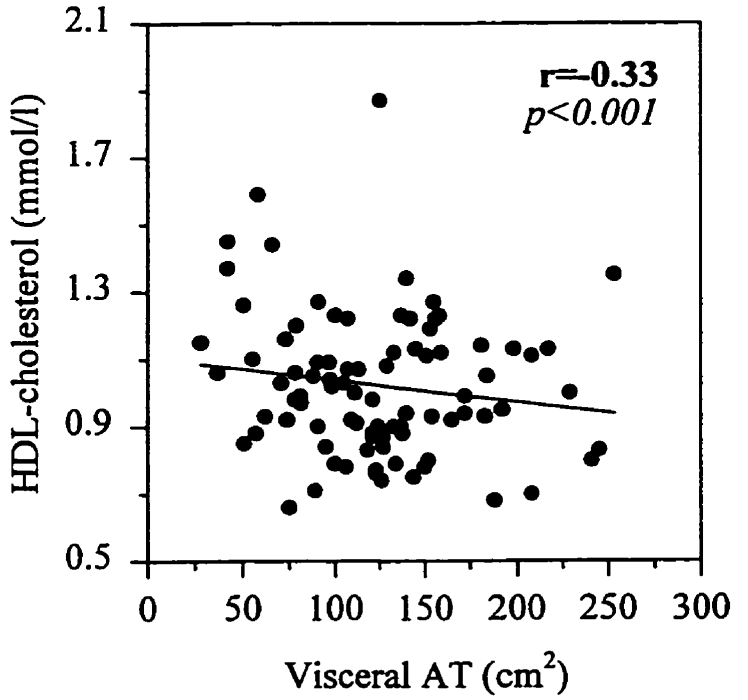
Concentrations are expressed in mmol/l excepted for apo A-I and apo B in mg/dl.
Values are expressed as mean ± SD

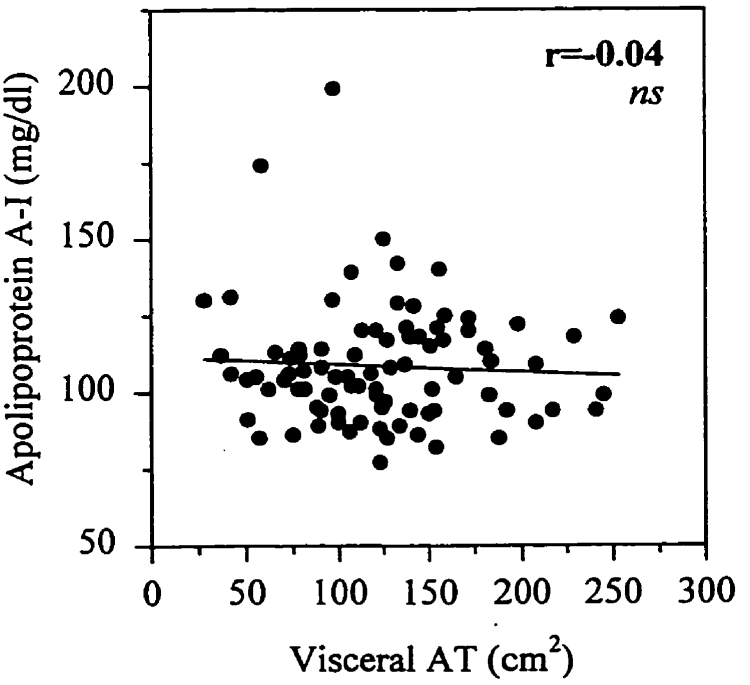
Table 3: Means of lipoprotein ratios of men in the 1st, 2nd, 3rd and 4th quartiles of the apo A-I and HDL-C concentrations

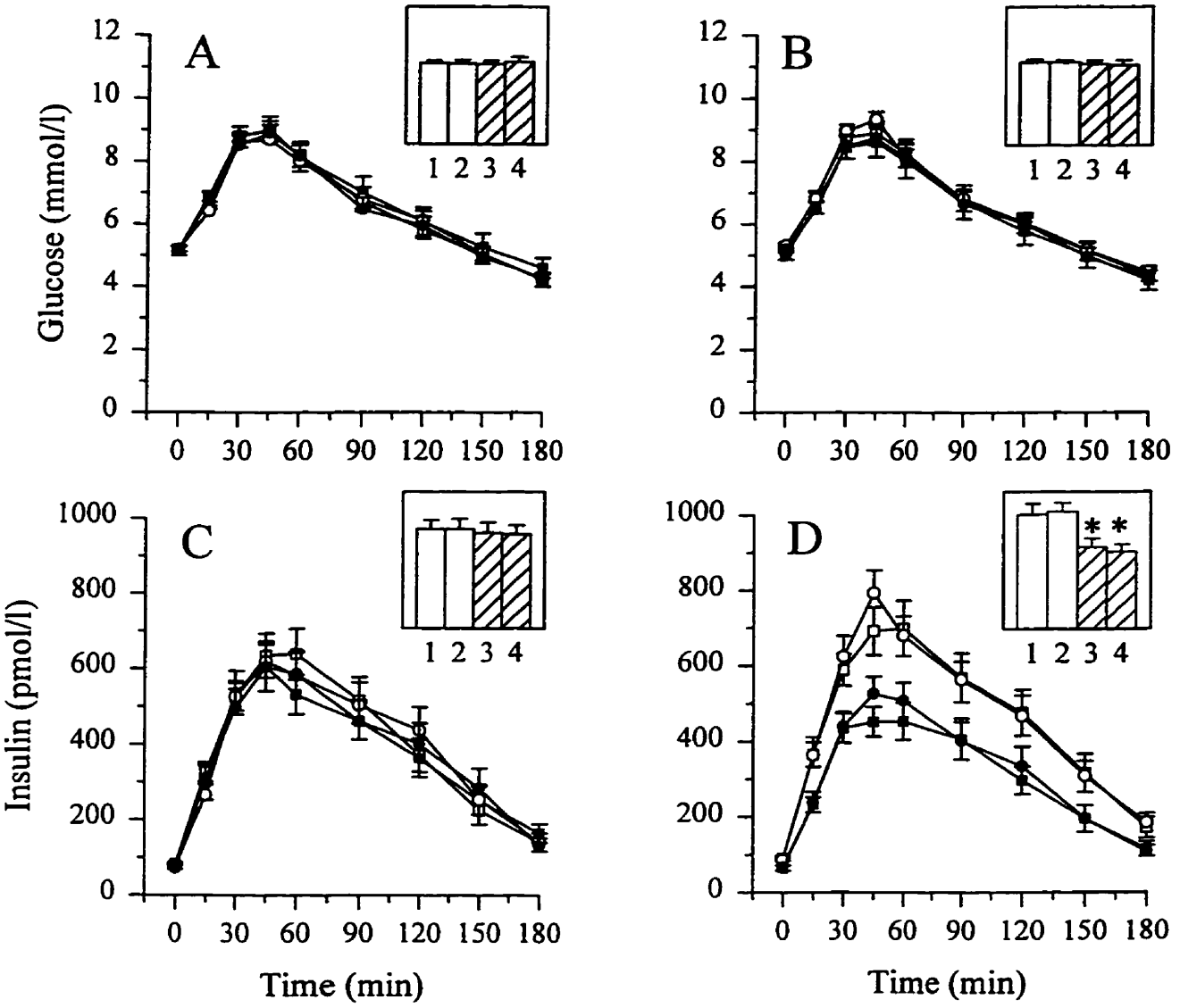
	Apo A-I				HDL-C			
	1st quartile n=26	2nd quartile n=28	3rd quartile n=29	4th quartile n=28	1st quartile n=27	2nd quartile n=28	3rd quartile n=27	4th quartile n=29
CHOL/HDL-C	5.70 ± 1.38	5.15 ± 1.45	4.66 ± 1.37 [1]	4.50 ± 1.16 [1]	6.36 ± 1.08	5.52 ± 0.98 [1]	4.59 ± 0.87 [1,2]	3.57 ± 0.82 [1,2,3]
LDL-C/HDL-C	3.88 ± 1.05	3.47 ± 1.21	3.21 ± 1.14 [1]	3.00 ± 0.89 [1]	4.26 ± 0.98	3.82 ± 0.92	3.12 ± 0.74 [1,2]	2.38 ± 0.79 [1,2,3]
LDL-apo B/LDL-C	26.4 ± 3.0	25.3 ± 3.3	23.9 ± 3.27 [1]	25.5 ± 3.5	27.7 ± 3.6	25.5 ± 3.0 [1]	24.3 ± 2.25 [1]	23.8 ± 3.1 [1,2]
HDL_2-C/HDL_3-C	0.51 ± 0.16	0.54 ± 0.20	0.58 ± 0.23	0.58 ± 0.29	0.42 ± 0.13	0.46 ± 0.14	0.58 ± 0.21 [1,2]	0.75 ± 0.24 [1,2,3]
HDL-TG/HDL-C	0.37 ± 0.13	0.30 ± 0.10	0.23 ± 0.08 [1,2]	0.24 ± 0.09 [1,2]	0.38 ± 0.13	0.31 ± 0.08 [1]	0.25 ± 0.09 [1,2]	0.19 ± 0.05 [1,2,3]
HDL-C/apoA-I	0.010 ± 0.002	0.010 ± 0.001	0.010 ± 0.002	0.009 ± 0.002	0.008 ± 0.001	0.009 ± 0.001	0.010 ± 0.001 [1,2]	0.011 ± 0.002 [1,2,3]

1: significantly different from the 1st quartile
2: significantly different from the 2nd quartile
3: significantly different from the 3rd quartile


r=0.57
p<0.0001
Apolipoprotein A-I (mg/dl)
200
150
100
50
0.5
0.9
1.3
1.7
2.1
HDL-cholesterol (mmol/l)


r=-0.33
p<0.001
HDL-cholesterol (mmol/l)
2.1
1.7
1.3
0.9
0.5
0
50
100
150
200
250
300
Visceral AT (cm2)


r=-0.04
ns
Apolipoprotein A-I (mg/dl)
200
150
100
50
0
50
100
150
200
250
300
Visceral AT (cm2)


A
B
C
D
Glucose (mmol/l)
Insulin (pmol/l)
Time (min)
Time (min)
0 30 60 90 120 150 180
1 2 3 4
* *

CHAPITRE 6

MÉTABOLISME POSTPRANDIAL DES TRIGLYCÉRIDES : EFFET DE L'ACCUMULATION DE TISSU ADIPEUX VISCÉRAL CHEZ L'HOMME

L'article composant ce chapitre est intitulé :

Postprandial Triglyceride Response in Visceral Obesity in Men

(Sous presse dans la revue Diabetes)

Postprandial Triglyceride Response in Visceral Obesity in Men

RUNNING TITLE: Postprandial lipemia in visceral obesity

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RÉSUMÉ

Même si des altérations métaboliques sont fréquemment rapportées chez les sujets obèses, une accumulation excessive de tissu adipeux viscéral est plus étroitement associée à des concentrations plasmatiques élevées de triglycérides et d'insuline, de même qu'à des niveaux plus faibles de HDL-cholestérol, que l'obésité en-soi. De plus, la concentration plasmatique de triglycérides à jeun est un puissant indicateur de l'amplitude et de la durée de la réponse triglycéridémique en période postprandiale. Toutefois, il n'existe que peu d'informations relatives aux contributions respectives de l'obésité et de la graisse viscérale dans les variations de clairance des triglycérides plasmatiques en période postprandiale. Dans la présente étude, nous avons observé des différences potentielles entre les réponses postprandiales de lipoprotéines riches en triglycérides (LRT) de sujets caractérisés par une accumulation faible vs élevée de tissu adipeux viscéral. Dans un échantillon de 43 hommes (âge moyen ± SD : 41.3 ● 9.6 ans), une adiposité importante et une accumulation excessive de graisse viscérale étaient associées à une réponse exagérée en triglycérides durant la période postprandiale (r compris entre 0.33 et 0.45). Nous avons également noté une forte association entre les concentrations de triglycérides à jeun et en période postprandiale ($r=0.79$, $p<0.0001$). Lorsque pairés pour la proportion de masse grasse, les individus présentant une accumulation élevée de tissu adipeux viscéral (>130cm^2 ; n=10), mesuré par tomographie axiale, étaient caractérisés par une réponse plus importante des fractions de MOYENNES et PETITES-LRT ($p<0.05$) comparativement à ceux montrant une accumulation plus faible de graisse viscérale (<130cm^2 ; n=10). De plus, cette réponse augmentée en triglycérides dans les PETITES-LRT chez les sujets ayant une importante accumulation de tissu adipeux viscéral, n'était pas accompagnée d'une plus grande réponse en

rétinyl plamitate dans cette fraction de LRT. Des réponses plus élevées en insuline et en acides gras libres ont également été observées en période postprandiale chez les sujets ayant une accumulation importante de graisse viscérale. Finalement, l'activité de la lipoprotéine lipase dans le plasma post-hépariné était corrélée négativement à la réponse postprandiale en triglycérides plasmatiques dans un sous-échantillon de 32 individus ($r=-0.37$, $p<0.05$) Les résultats recueillis dans la présente étude suggèrent que l'obésité viscérale est associée à la détérioration de la clairance des triglycérides en période postprandiale. De plus, une réponse postprandiale exagérée en acides gras libres chez les individus présentant une accumulation excessive de graisse viscérale suggère que l'obésité viscérale contribuerait à l'hypertriglycéridémie, à jeun et en phase postprandiale, en altérant le métabolisme des acides gras libres durant la période postprandiale.

ABSTRACT

Although metabolic disturbances are often observed in obese patients, an increased visceral adipose tissue (AT) accumulation has been shown to be more closely associated with high fasting triglyceride (TG) and insulin levels as well as with low high-density lipoprotein cholesterol concentrations than excess body fatness per se. Interestingly, the fasting concentration of plasma TG has been shown to be an important determinant of the magnitude and duration of the postprandial TG response. Yet, little is known about the respective contributions of obesity vs excess visceral AT to the variation in postprandial TG clearance. In the present study, we examined potential differences in postprandial TG-rich lipoprotein (TRL) responses in subjects characterized by high vs low levels of visceral AT. In a sample of 43 men (mean age ± SD: 41.3 ± 9.6 years), we found that excess body fatness as well as visceral obesity were both associated with increased postprandial TG responses in TOTAL-TRL (r between 0.33 and 0.45). We also found a strong relationship between fasting plasma TG levels and postprandial total TRL-triglyceride concentrations ($r=0.79$, $p<0.0001$). When matched for total body fat mass, individuals with high levels of visceral AT (>130 cm^2; n=10), assessed by computed tomography, were characterized by increased MEDIUM and SMALL TRL-triglyceride responses ($p<0.05$) compared with subjects displaying low visceral AT accumulation (<130 cm^2; n=10). Moreover, this elevated response of SMALL-TRL triglycerides noted in men with high levels of visceral AT was not accompanied by a concomitant increased retinyl palmitate response in this TRL fraction, suggesting that visceral obesity is accompanied by higher postprandial VLDL production compared to obese men with a lower accumulation of visceral AT. Increased postprandial insulin and free fatty acid (FFA) responses were also noted in men with high levels of

visceral AT. Finally, post-heparin plasma lipoprotein lipase activity was negatively correlated with the TOTAL-TRL triglyceride response in a subsample of 32 individuals ($r=-0.37$, $p<0.05$).The results of the present study suggest that visceral obesity is associated with an impaired postprandial triglyceride clearance. Furthermore, the exaggerated postprandial FFA response observed in subjects with high visceral AT suggests that visceral obesity may contribute to fasting and postprandial hypertriglyceridemia by altering FFA metabolism in the postprandial state.

Key Words: Postprandial lipemia, visceral fat, fat mass, insulin, lipoprotein lipase and free fatty acids

INTRODUCTION

Numerous alterations in plasma lipid and lipoprotein concentrations are found in obese patients [1,2]. Indeed, obese individuals, especially those with an increased abdominal adipose tissue (AT) accumulation, are characterized by higher fasting plasma triglyceride (TG) [3-6] and lower HDL-cholesterol [7-10] concentrations compared to lean individuals. These alterations are known to increase the risk of coronary heart disease. An increased visceral adipose tissue accumulation promoting an increased VLDL and apolipoprotein (apo) B secretion as well as a reduced lipoprotein lipase (LPL) activity measured in the plasma of obese subjects are believed to play a significant role among factors involved for the dyslipidemic state of abdominal obesity [11].

Most studies on the characterization of plasma lipoprotein levels in obesity have been done in the fasting state and postprandial lipoproteins have generally been neglected. The interest for postprandial studies grew when Zilversmit [12] hypothesized that the development of atherosclerosis could be a postprandial phenomenon. Since then, postprandial lipoproteins have received more attention and it has been reported that dietary fat tolerance is affected by numerous factors such as age [13,14], gender [13,15], diet [16], physical activity [17] and NIDDM [18,19]. Disturbances in dietary fat tolerance have also been related to anthropometric indices of body composition. Indeed, Lewis et al [20] reported a greater 24-hour postprandial response (area under the incremental curve; AUIC) for plasma TG in obese subjects (body-mass index; BMI~44 kg/m^2). However, because obesity was defined only on the basis of BMI, the relation between visceral AT accumulation and postprandial lipemia was not investigated. Visceral obesity has been related to alterations in the fasting lipoprotein profile [11,21], but little is known about the potential relation of visceral AT

accumulation to postprandial TG response. Whether the hypertriglyceridemic state that characterizes visceral obesity is an important determinant of the magnitude and duration of the postprandial TG response is also unknown.

Therefore, the aim of the present study was to examine the postprandial responses of various TG-rich lipoproteins (TRL) and their potential relationships with excess fatness in comparison to high levels of visceral AT. For this purpose, 43 adult men were investigated and plasma TRL responses measured over a period of 8 hours following a meal with a high fat content. Results from the present study suggest that visceral AT accumulation is associated with an impaired postprandial clearance of plasma TRL. Furthermore, alterations in postprandial free fatty acid (FFA) metabolism could contribute to the disturbances in postprandial TRL clearance in visceral obesity.

SUBJECTS AND METHODS

Subjects. Forty-three men, 22 to 56 years of age (mean age ± SD: 41.3 ● 9.6 years), were recruited through the media and selected on purpose to cover a wide range of body fatness values. Subjects gave their written consent to participate in the study which was approved by the Medical Ethics Committee of Laval University. Men with diabetes or with coronary heart disease were excluded from the present study. None of the subjects was on medication known to affect insulin action or plasma lipoprotein levels.

Anthropometric and Body Composition Measurements. Body weight, height, waist and hip ircumferences were measured following standardized procedures [22], and the waist-to-hip ratio (WHR) was calculated. Body density was measured by the hydrostatic weighing technique [23]. The mean of six measurements was used in the calculation of percent body fat from body density using the equation of Siri [24]. Fat mass was obtained by multiplying body weight by percent body fat.

Computed Tomography (CT). Visceral AT accumulation was assessed by CT, which was performed on a Siemens Somatom DRH scanner (Erlagen, Germany) using previously described procedures [25,26]. Briefly, the subjects were examined in the supine position with both arms stretched above the head. The scan was performed at the abdominal level (between L4 and L5 vertebrae) using an abdominal scout radiograph in order to standardize the position of the scan to the nearest millimeter. Total AT area was calculated by delineating the abdominal scan with a graph pen and then computing the AT surface with attenuation range of -190 to -30 HU [25-27]. The 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 (OLTT). After a 12-hour overnight fast, an intravenous catheter was inserted into a forearm vein for blood sampling. Each participant was given a test meal containing 60g fat/m^2 body surface area and 60 000 UI of vitamin A (Aquasol A, Astra Pharmaceuticals, Westborough, MA) [28]. The meal consisted of eggs, cheese, toasts, 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 hours but were given free access to water. Blood samples were drawn before the meal and every 2 hours after the meal over an 8-hour period; samples were handled in a dimmed light to avoid deterioration of vitamin A.

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 [29]. Each plasma sample (4ml) was then subjected to a 12-hour ultracentrifugation (50 000 rpm) in a Beckman 50.3Ti rotor (Palo Alto, CA) at 4°C, in 6ml 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 [30], 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 (100ml) 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 $MnCl_2$ [31]. 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 were measured at 0, 2, 4, 6 and 8 hours using a colorimetric method [32].

Post-Heparin Plasma Lipoprotein Lipase Activity (PH-LPL)

Plasma LPL activity was also measured on one occasion in subjects after a 12-hour overnight fast, 10 minutes after an intravenous injection of heparin (60 IU/kg body weight). The activity was measured using a modification of the method of Nilsson-Ehle and Ekman [33], as previously described [34], and expressed as nmoles of oleic acid released per ml of plasma per min.

Retinyl Palmitate (RP) Measurements. The RP content of TOTAL as well as LARGE, MEDIUM and SMALL-TRL fractions was analyzed using high-performance liquid chromatography (HPLC) as previously described [30]. Briefly, aliquots of 100 ml of TOTAL and LARGE-TRL as well as 500 ml of MEDIUM and SMALL-TRL were used for the analysis. The volume of total and LARGE-TRL fractions was adjusted to 500 ml with 0.15M NaCl. A volume of 200 ml 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 ml of mobile phase buffer prepared from 90 ml of hexane, 15ml of n-butyl chloride, 5ml 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 [30]:

RP (ng RA/ml) = (RP peak area/RA peak area) x (1/volume of sample used) x 40 ng RA

Glucose and Insulin Concentrations. Fasting and postprandial plasma glucose concentrations were determined using the glucose oxidase assay [35] (SIGMA, St-Louis, MO). Plasma insulin levels

were measured by a commercial double antibody radioimmunoassay (LINCO Research, St-Louis, MO) that shows little cross-reactivity (<0.02%) with pro-insulin [36].

Statistical Analyses. All analyses were conducted on the SAS statistical package (SAS Institute, Cary, NC). Pearson product-moment correlation coefficients were used to quantify associations between variables. Subjects matched for body fat mass, were also subclassified on the basis of visceral adipose tissue accumulation in accordance with previously proposed cutpoints [37]: 1) low visceral AT (less than 130 cm^2 ; n=10) and 2) high visceral AT (over 130 cm^2; n=10). Differences between these two subgroups were tested for significance using the Student t-test. ANOVA for repeated measures was performed within each subgroup of visceral AT accumulation in order to test overall differences in TOTAL, LARGE, MEDIUM and SMALL-TRL triglyceride levels over time. The same procedure was performed with plasma insulin, glucose and FFA concentrations. The different areas under the curve of triglyceride, FFA, insulin, glucose, and RP concentrations were determined by the trapezoid method. Statistical adjustment of data was performed with the General Linear Model (GLM) procedure with adjustments for age and/or fasting plasma TG concentrations.

RESULTS

Physical and metabolic variables were characterized by substantial variation among individuals (Table 1) as expected from the selection of subjects. Associations between body fatness and AT distribution variables with fasting and postprandial triglyceride concentrations in TOTAL-TRL are shown in Table 2. All adiposity indices showed positive correlations with TRL triglyceride levels assessed in the fasting or postprandial states. All adiposity variables were also significantly correlated with the postprandial triglyceride response in TOTAL-TRL (defined as the incremental area below the 0 to 8-hour TG curve; AUIC). Furthermore, although all adiposity indices showed comparable correlation coefficients, visceral AT cross-sectional area (in cm^2) and total body fat mass (in kg) were the best correlates of postprandial TRL concentrations.

Table 3 shows the relationships of fasting plasma and lipoprotein-lipid concentrations to fasting as well as postprandial TRL triglyceride levels. A strong correlation was found between fasting plasma TG levels and both the total (AUC) and incremental area under the 0 to 8-hour curve for TOTAL-TRL triglycerides. We also found significant correlations between fasting insulin levels and triglyceride concentrations of TOTAL-TRL measured during both the fasting and postprandial periods. On the other hand, fasting HDL-cholesterol concentrations were negatively correlated with fasting and postprandial total TRL triglyceride levels.

In an attempt to better isolate the contribution of visceral AT accumulation to the altered postprandial TRL responses in obesity, we matched subjects on the basis of total body fat mass and compared two groups with high vs low levels of visceral AT (Fig.1). Individuals with high levels of

visceral AT were older than those with low visceral AT (mean age ± SD: 48.0 ± 6.3 vs 36.0 ± 10.3 years, $p<0.01$)

Figure 2 illustrates triglyceride concentrations in TOTAL as well as in LARGE, MEDIUM and SMALL-TRL fractions before and following meal ingestion among subjects matched for fat mass but with different levels of visceral AT. No difference was found in fasting triglyceride concentrations contained in TOTAL as well as in LARGE, MEDIUM and SMALL-TRL. However, subjects characterized by a high visceral AT accumulation showed higher concentrations of TOTAL-TRL triglycerides at the 8-hour timepoint compared to subjects with low levels of visceral AT ($p<0.05$). Furthermore, individuals with high visceral AT accumulation tended to be characterized by an increased TOTAL-TRL triglyceride AUIC compared to those with low visceral AT deposition ($p=0.06$). Although no difference in postprandial LARGE-TRL triglyceride AUIC was found between the two subgroups, increased MEDIUM and SMALL-TRL triglycerides AUIC were noted in men with high levels of visceral AT (Fig.2) We also noted that triglyceride concentrations of TOTAL as well as LARGE, MEDIUM and SMALL-TRL at 8 hours were back to fasting values among men with low levels of visceral AT. However, among subjects with excess visceral AT accumulation, MEDIUM and SMALL-TRL triglycerides measured at the 8-hour timepoint remained significantly higher than fasting levels.

Postprandial changes in retinyl palmitate in TOTAL as well as in LARGE, MEDIUM and SMALL-TRL subfractions are illustrated in Figure 3. We found that subjects with elevated visceral AT were characterized by significantly increased RP area under the incremental 0 to 8-hour curve

for TOTAL, LARGE and MEDIUM-TRL. With the exception of the 6-hour timepoint, no difference in RP contained in SMALL-TRL was observed between men with low vs high levels of visceral AT.

We noted an increased postprandial insulin AUIC among men with high levels of visceral AT, while no difference was observed in postprandial glucose concentrations between the two subgroups (Figure 4). Although no difference was noted in postprandial FFA levels prior to 8 hours, we found an increased FFA area under the incremental 0 to 8-hour curve in individuals characterized by a high visceral AT accumulation. Furthermore, differences in the postprandial FFA profiles of the two subgroups were noted. We observed rather stable postprandial FFA concentrations in the low visceral AT subgroup, whereas FFA levels progressively increased following the meal among men characterized by a high visceral AT accumulation. An analysis of variance on repeated measures revealed no time-related difference in FFA concentrations among subjects with low levels of visceral AT whereas subjects with a high visceral AT accumulation had FFA concentrations at 8 hours which were significantly higher than fasting levels. In addition, postprandial plasma FFA response was associated positively with TOTAL-TRL triglyceride response (Figure 5).

Finally, Figure 6 illustrates the relationship of TOTAL-TRL triglyceride response to PH-LPL activity. We found that PH-LPL activity was negatively associated with the TOTAL-TRL triglyceride response. Although visceral AT accumulation was not correlated to PH-LPL activity in this subsample of 32 men (data not shown), men with high visceral AT tended to be characterized by lower PH-LPL activity compared to those with low visceral AT (mean ● SD : 29.6 ± 23.2 vs 57.8 ± 40.1 nmol/min/ml, respectively). However, this difference did not reach statistical significance (p=0.09).

DISCUSSION

Obesity, especially when associated with high levels of adipose tissue in the abdominal cavity, is recognized to have detrimental effects on the metabolic profile [1,2,11]. In accordance with this notion, we found that men with high visceral AT accumulation (above 130 cm^2) were characterized by elevated insulin levels. Men with high visceral AT accumulation were also characterized by increased TG and decreased HDL-cholesterol concentrations, although when compared to men with low visceral AT, these differences were not statistically significant. These are well known metabolic abnormalities found among obese subjects, particularly among those with high levels of visceral AT [1-11]. In addition to the altered fasting metabolic profile, an excess of body fat was also associated with an increased postprandial TRL triglyceride response to the meal. Such conclusions were also reached by Lewis and colleagues [20] who studied postprandial TG metabolism in obese individuals, and reported an exaggerated postprandial TG response in obese patients compared to lean controls. However, they studied massively obese individuals as reflected by an average body-mass index (BMI) reaching 44 kg/m^2. In our study, we also found associations between increased BMI and alterations of postprandial TRL metabolism. Furthermore, we found significant correlations between increased visceral AT accumulation and delayed postprandial TRL clearance. Similar observations were reported in a sample middle-aged individuals (mean age around 62 years) [38]. Alterations in postprandial lipemia were also reported in a small sample of men with increased waist-to-hip ratio [39]. However, in that study, abdominal obesity was only measured by anthropometry and the importance of visceral AT in postprandial TG clearance disturbances was not

investigated. The present study extends those previous observations to moderately obese, and younger subjects with increased visceral AT accumulation.

In the present study, although significant associations were found between adiposity indices and postprandial TRL triglyceride concentrations, fasting plasma TG, insulin and HDL-cholesterol levels showed stronger correlations with postprandial TRL triglyceride concentrations than body fatness and AT distribution variables, a finding which is concordant with previous observations. Indeed, high fasting TG and low HDL-cholesterol concentrations have been reported to be associated with increased postprandial TRL levels [19,40-43]. Moreover, alterations of postprandial TG metabolism have been observed in NIDDM patients who are insulin resistant and dyslipidemic in the fasting state [18,19,44].

We also found that features of the insulin resistance syndrome, namely fasting hypertriglyceridemia, hyperinsulinemia and low HDL-cholesterol concentrations as well as increased visceral AT accumulation, were all significant correlates of an impaired postprandial TRL clearance. In this regard, we have further examined the importance of visceral AT accumulation as a potential modulator of postprandial metabolic alterations. For that purpose, we have compared two subgroups of men matched for their level of total body fat but with either a low or a high visceral AT accumulation. Comparison of postprandial TRL triglyceride concentrations in these two groups revealed that men characterized by high levels of visceral AT presented increased MEDIUM and SMALL-TRL triglyceride responses after the meal compared to those with a low visceral AT accumulation. These increased postprandial MEDIUM and SMALL-TRL triglyceride responses in subjects with high levels of visceral AT did not appear to be the influenced by the quantity of larger

TRL particle as no difference was found in LARGE-TRL triglycerides between both subgroups of men. Competition for LPL between chylomicrons, chylomicron-remnants and VLDL during the postprandial period could be responsible, at least in part, for the delayed clearance TRL [45]. Delayed uptake of lipoproteins by the liver could also be a cause of the retarded clearance of TRL particles. In our study, postprandial SMALL-TRL triglyceride concentrations were higher in visceral obese men. This observation is in accordance with previously published results that underlined the importance of hepatic TG-rich particles in altered postprandial TG clearance [40].

Fasting hypertriglyceridemia is a common feature of visceral obesity [1,2]. This metabolic alteration is believed to be the result of an increased flux of FFA to the liver. Indeed, visceral adipocytes are characterized by a lively lipolytic activity which is poorly inhibited by insulin, resulting in the elevation of FFA in the portal circulation and in the plasma [46]. In response to this increased FFA availability, an increased esterification of FFA and a reduced hepatic degradation of apolipoprotein B lead to an increased synthesis and secretion of VLDL particles. In the present study, the two subgroups of subjects (classified on the basis of visceral AT accumulation) showed different FFA response patterns to the fat load. Indeed, we noted a slight but nonsignificant decrease in FFA levels 2 hours after the meal test in men with low levels of visceral AT which also corresponded to the peak in postprandial insulin concentrations, a finding largely explained by the antilipolytic effect of insulin on adipose tissue [47,48]. In fact, our results indicated that postprandial FFA levels were not different from fasting concentrations in men with a low visceral AT accumulation. However, the decrease in FFA concentrations at peak insulin levels and the maintenance of FFA concentrations throughout the postprandial period were not observed in men

with high levels of visceral AT. Indeed, plasma FFA levels increased progressively throughout the entire postprandial period, which resulted in a significantly increased FFA response in visceral obese subjects, even in the presence of a marked postprandial hyperinsulinemic state in these individuals. These results are concordant with previously published observations which reported an impaired postprandial plasma FFA metabolism in upper-body obesity [49]. Furthermore, while FFA concentrations measured at the end of the test returned to near fasting values in men with low levels of visceral AT, men with excess visceral AT had FFA concentrations that were significantly higher than fasting values even 8 hours after meal ingestion. These altered postprandial FFA levels in individuals with visceral obesity may contribute to the elevation of fasting TG through the stimulation of hepatic VLDL-TG secretion long after meal ingestion. This interpretation is supported by results obtained from the measurement of TRL retinyl palmitate concentrations. Indeed, the increased triglyceride response in SMALL-TRL noted among men with high levels of visceral AT, was not accompanied by a higher retinyl palmitate response in that TRL fraction in comparison with obese men with lower levels of visceral AT. This observation supports the notion that triglycerides from endogenous TRL, presumably VLDL particles, account for most of the increase in SMALL-TRL triglyceride levels observed late through the postprandial period. A possible contribution of the raised postprandial FFA to the increased production of VLDL in visceral obesity is also supported by the positive correlation that we found between postprandial FFA levels and the TOTAL-TRL triglyceride response to the meal. Furthermore, when we compared subgroups of subjects matched on the basis of visceral AT but showing either low vs high body fat mass, no difference was found in postprandial TRL metabolism (data not shown).

Other factors such as decreased lipoprotein lipase activity are thought to play a major role in an altered postprandial TRL metabolism [50,51]. In the present study, PH-LPL activity was measured in 32 men. We found a negative relationship between PH-LPL and TOTAL-TRL triglyceride response, but not with visceral AT accumulation. However, men with high visceral AT were characterized by lower PH-LPL activity compared to men matched for body fat mass but showing low visceral AT levels. Although this difference was not significant ($p<0.09$), it suggests that reduced PH-LPL activity may be implicated in the altered postprandial TRL metabolism among viscerally obese subjects. Further studies are required in order to validate this observation.

In summary, the present results indicate that excess visceral AT accumulation is associated with an impaired postprandial TRL triglyceride clearance which is largely determined by phenomena that can also lead, to some extent, to fasting hypertriglyceridemia. Results of the present study also suggest that increased visceral AT accumulation could contribute to this fasting hypertriglyceridemic state by altering FFA metabolism, particularly in the postprandial state.

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FIGURE HEADINGS

Figure 1: A) Body fat mass, B) abdominal subcutaneous and C) visceral adipose tissue accumulation in two subgroups of men matched for fat mass (within a 1 kg difference) but with visceral AT below 130 cm^2 (Low; 91 ± 8 cm^2; n=10; white bars) and above 130 cm^2 (High; 173 ● 10 cm^2; n=10; black bars). Values are means ± SEM. * significantly different from men with low levels of visceral AT at the $p<0.0001$ level.

Figure 2: Postprandial triglyceride responses of A) TOTAL triglyceride-rich lipoproteins (TRL;) as well as B) LARGE, C) MEDIUM and D) SMALL-TRL in two subgroups of men matched for total body fat mass (within a 1 kg difference) but with low (n=10; white squares and bars) versus high (n=10; black squares and bars) levels of visceral AT. Bars respresent the responses (area under the incremental curve) of each subgroup. Values are means ± SEM. *, † significantly different from men with low levels of visceral AT, at the $p<0.05$ and $p<0.005$ level respectively.

Figure 3: Postprandial retinyl palmitate (RP) responses of A) TOTAL triglyceride-rich lipoproteins (TRL) as well as B) LARGE, C) MEDIUM and D) SMALL-TRL in two subgroups of men matched for total body fat mass (within a 1 kg difference) but with low (n=10; white squares and bars) versus high (n=10; black squares and bars) levels of visceral AT. Bars represent the responses (area under the incremental curve) of each subgroup. Values are means ± SEM. * significantly different from men with low levels of visceral AT, at the $p<0.05$ level.

Figure 4: Postprandial plasma A) glucose, B) insulin and C) free fatty acid (FFA) responses in two subgroups of men matched for total body fat mass (within a 1 kg difference) but with low (n=10; white squares and bars) versus high (n=10; black squares and bars) levels of visceral AT. Bars represent the responses (area under the incremental curve) of each subgroup. Values are means ± SEM. *, † significantly different from men with low levels of visceral AT, at the $p<0.05$ and $p<0.01$ level respectively.

Figure 5: Association between postprandial plasma free fatty acid (FFA) and TOTAL-TRL triglyceride responses in the whole sample of 43 men.

Figure 6: Association between post-heparin plasma lipoprotein lipase (PH-LPL) activity and TOTAL-TRL triglyceride response in a subsample of 32 men.

Table 1: Physical characteristics and fasting metabolic profile of the sample of 43 men

Variables	*Mean ± SD*	*Range*
Age (years)	41 ● 10	22 - 56
BMI (kg/m^2)	29.3 ± 4.6	20 - 41
%Body Fat	26.2 ± 7.1	11 - 41
Fat Mass (kg)	24.3 ± 9.3	8 - 46
Waist Girth (cm)	101.7 ± 12.2	76 - 129
WHR	0.97 ± 0.07	0.76 - 1.15
Abdominal Adipose Tissue Areas (cm^2)		
Subcutaneous	282 ± 117	35 - 525
Visceral	154 ± 73	38 - 357
Plasma Cholesterol (mmol/l)	5.15 ± 0.84	3.55 - 6.81
Plasma Triglycerides (mmol/l)	2.09 ± 0.98	0.68 - 4.37
HDL-cholesterol (mmol/l)	0.90 ± 0.22	0.55 - 1.63
Free Fatty Acids (mmol/l)	0.65 ± 0.25	0.14 - 1.43
Insulin (pmol/l)	101 ± 51	35 - 250
Glucose (mmol/l)	5.2 ± 0.6	3.6 - 6.9

Table 2: Correlations between body fatness and adipose tissue distribution variables vs fasting as well as postprandial plasma triglyceride concentrations in total TRL in the sample of 43 men.

Variables	*Total TRL-Triglycerides*		
	Fasting (mmol/l)	***AUC (mmol/l/8h)***	***AUIC (mmol/l/8h)***
Age	0.21	0.27	0.28
BMI	0.40 †	0.41 †	0.37 *
%Body fat	0.43 ‡	0.40 †	0.33 *
FM	0.46 ‡	0.46 ‡	0.41 *
Waist girth	0.38 *	0.42 ‡	0.40 *
WHR	0.31 *	0.34 *	0.33 *
Abdominal Adipose Tissue Areas			
Visceral	0.39 *	0.45 ‡	0.45 ‡
Subcutaneous	0.38 *	0.34 *	0.28

AUC: Total area under the curve
AUIC: Area under the incremental 0 to 8-hour curve
* $p<0.05$ † $p<0.01$ ‡ $p<0.005$

Table 3: Correlations between fasting lipid profile vs fasting as well as postprandial triglyceride concentrations in total TRL in the sample of 43 men.

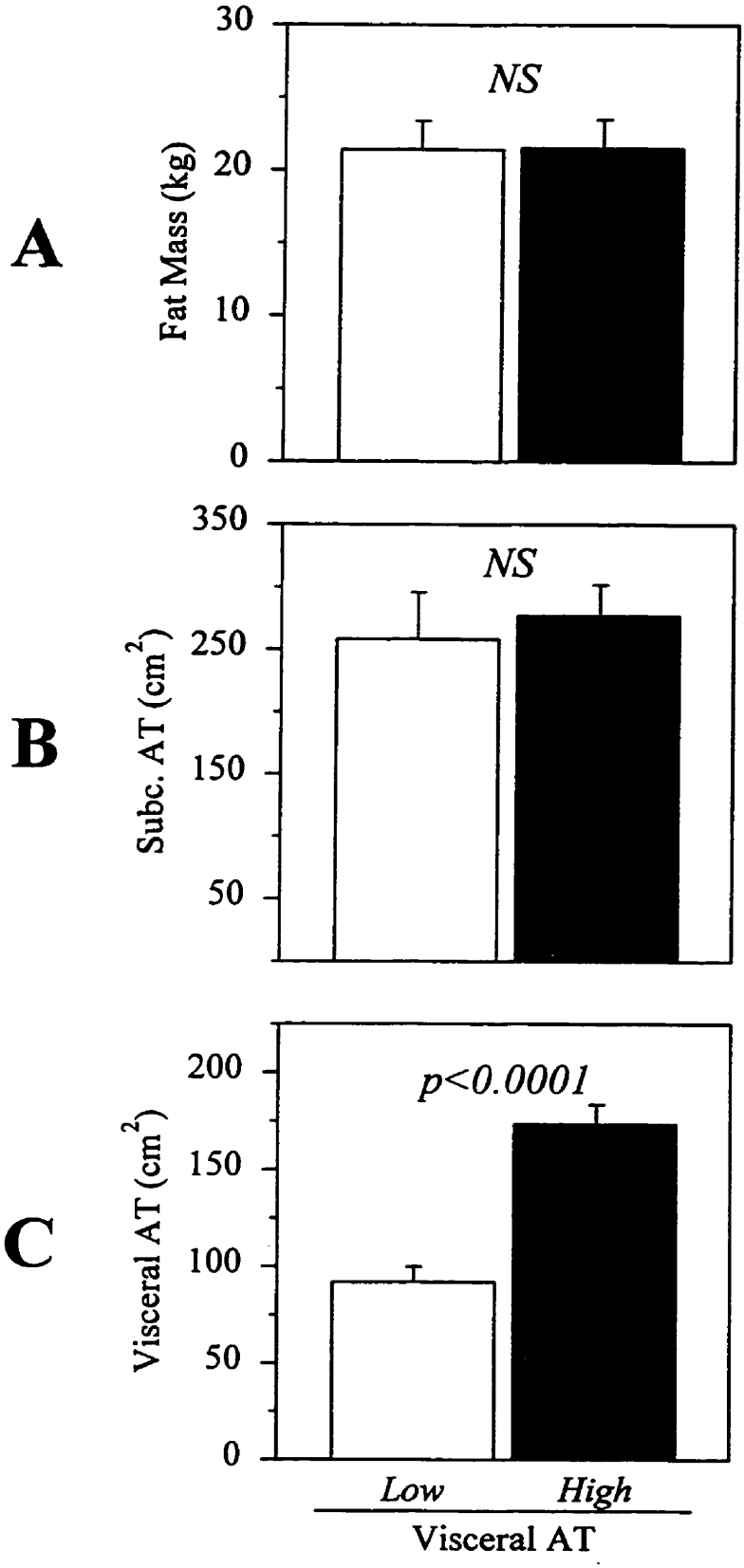
Fasting Variables	*Total TRL-Triglycerides*		
	Fasting (mmol/l)	***AUC (mmol/l/8h)***	***AUIC (mmol/l/8h)***
Plasma Cholesterol	0.37 *	0.39 †	0.36 *
Plasma Triglycerides	0.98 #	0.93 #	0.79 #
HDL-cholesterol	-0.52 ¶	-0.50 §	-0.43 ‡
Free Fatty Acids	-0.04	-0.07	-0.09
Insulin	0.40 †	0.50 §	0.52 ¶
Glucose	0.15	0.12	0.11

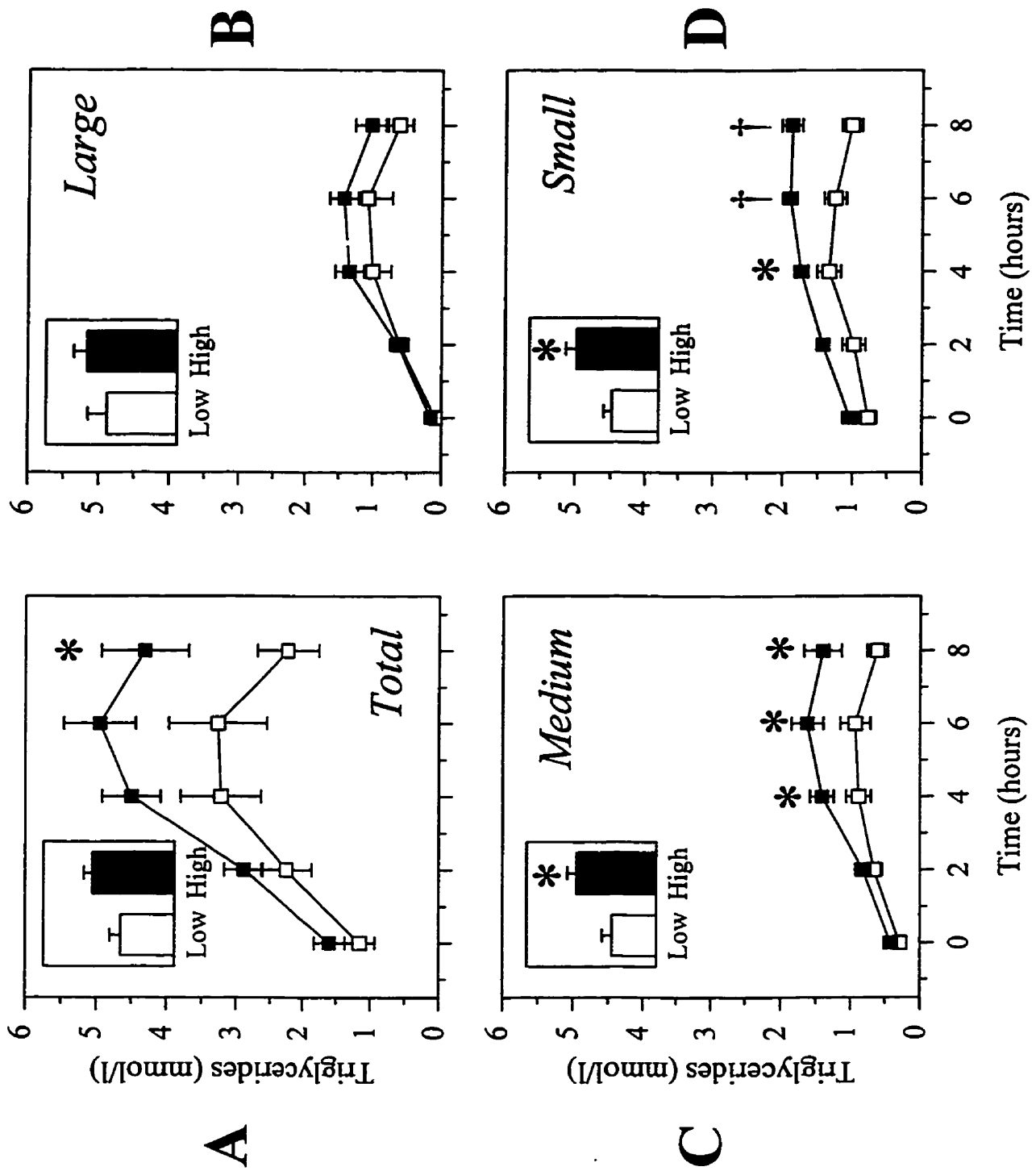
AUC : Total area under the curve
AUIC: Area under the incremental 0 to 8-hour curve
* $p<0.05$ † $p<0.01$ ‡ $p<0.005$ § $p<0.001$ ¶ $p<0.0005$ # $p<0.0001$

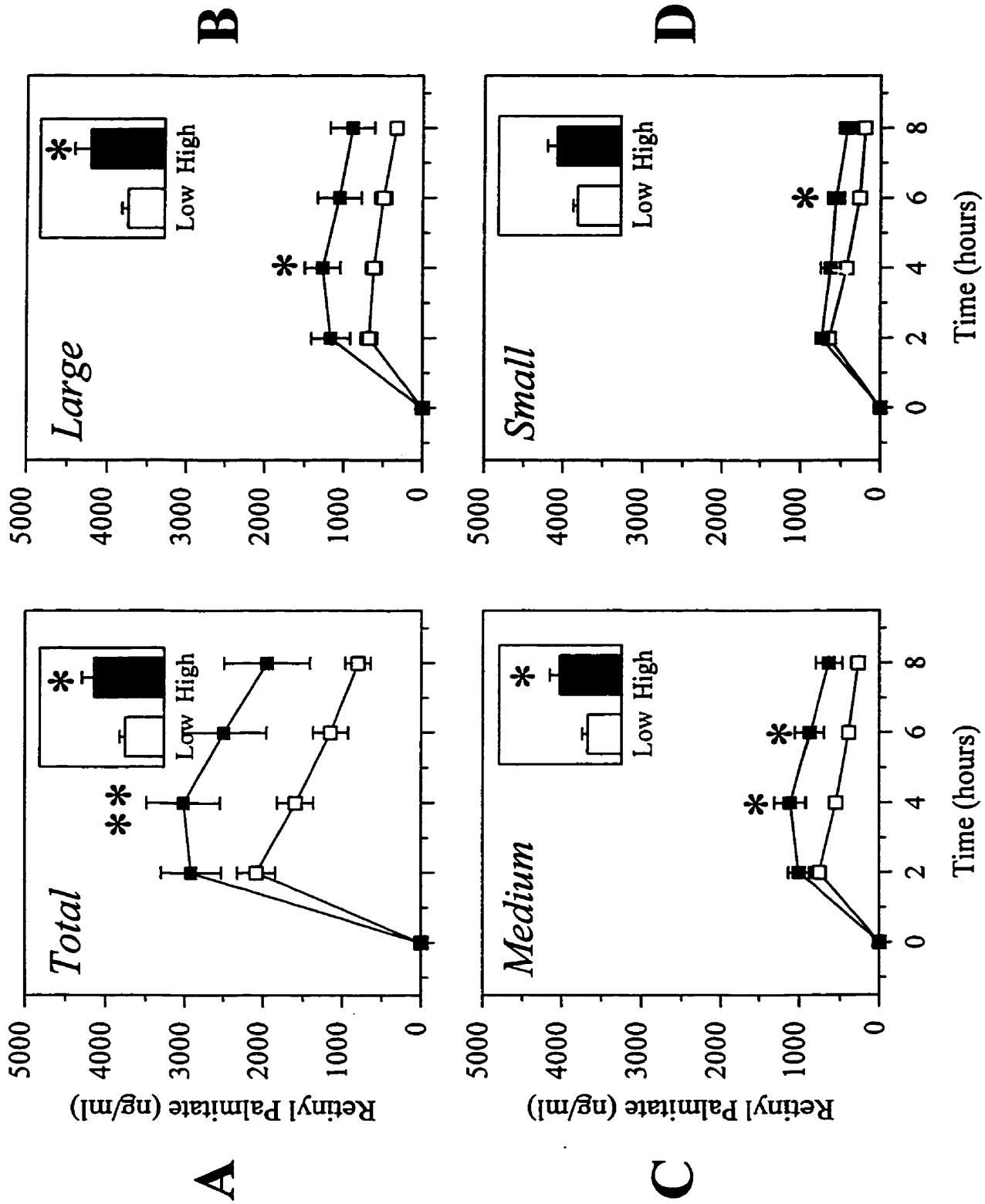
Table 4: Fasting metabolic profile of men matched on the basis of total body fat mass but with low vs high visceral adipose tissue accumulation

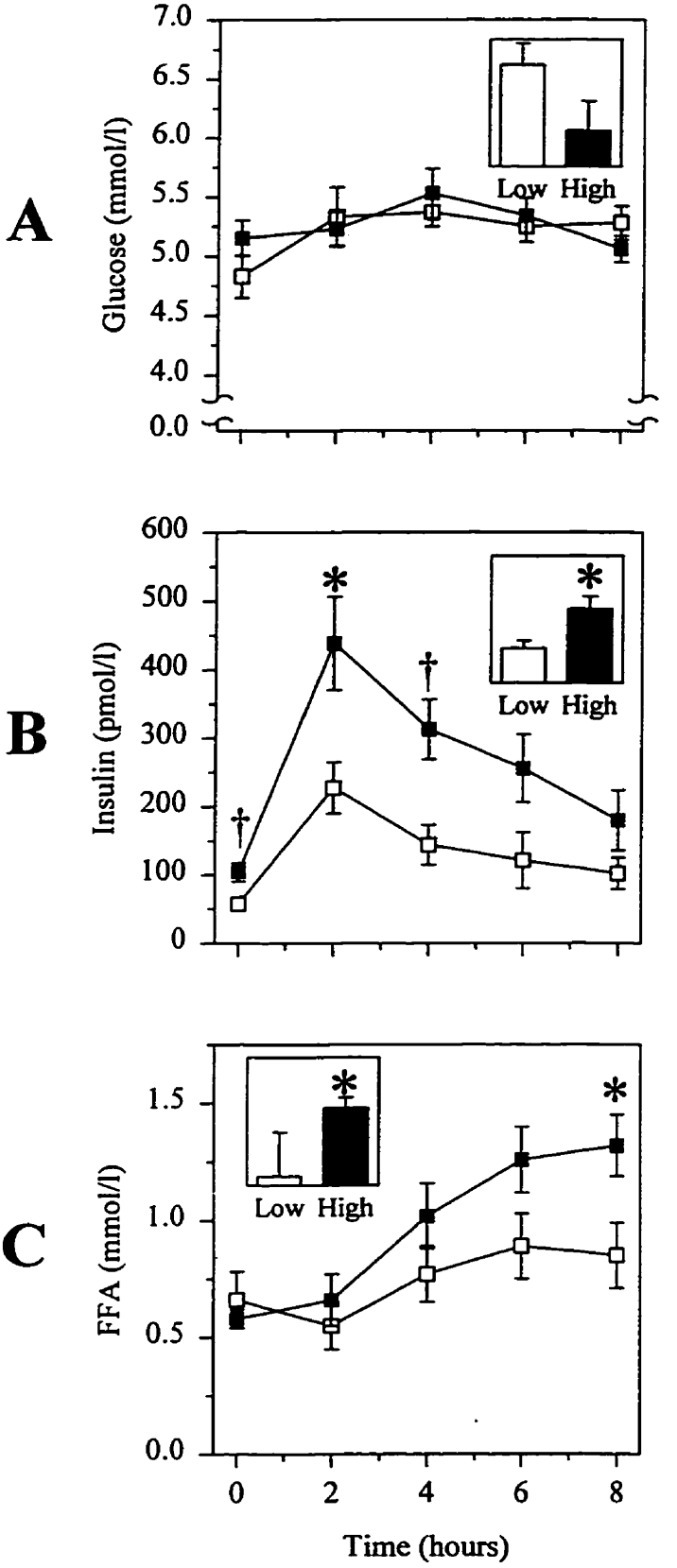
Variables	*Low Visceral AT*	*High Visceral AT*
Plasma Cholesterol (mmol/l)	4.85 ± 0.90	5.59 ± 0.50 *
Plasma Triglycerides (mmol/l)	1.75 ± 0.87	2.39 ● 0.80
HDL-cholesterol (mmol/l)	0.98 ± 0.28	0.85 ± 0.15
Free Fatty Acids (mmol/l)	0.66 ± 0.39	0.58 ± 0.11
Insulin (pmol/l)	57 ± 18	105 ± 42 †
Glucose (mmol/l)	4.8 ± 0.6	5.2 ± 0.5

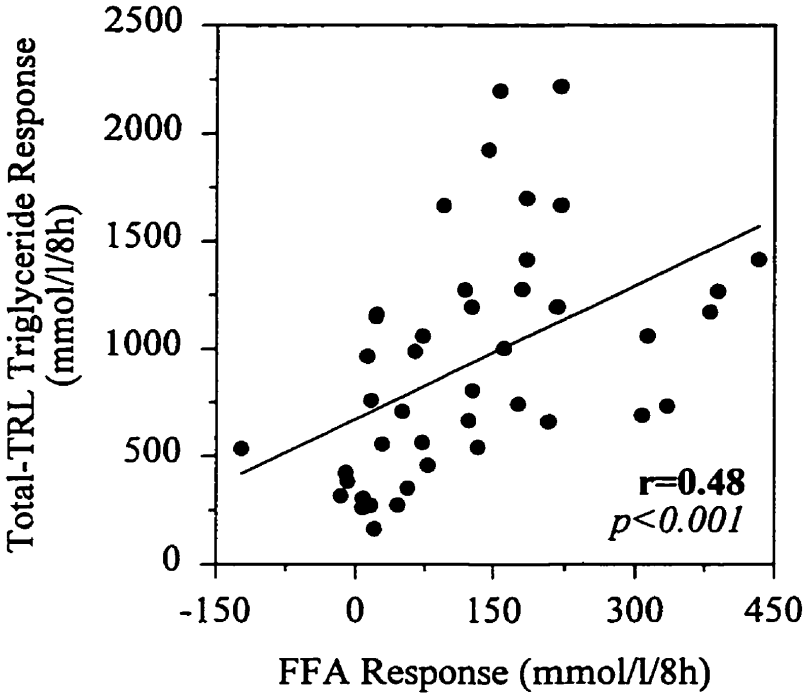
* significantly different from the low visceral AT subgroup, $p<0.05$
† significantly different from the low visceral AT subgroup, $p<0.005$

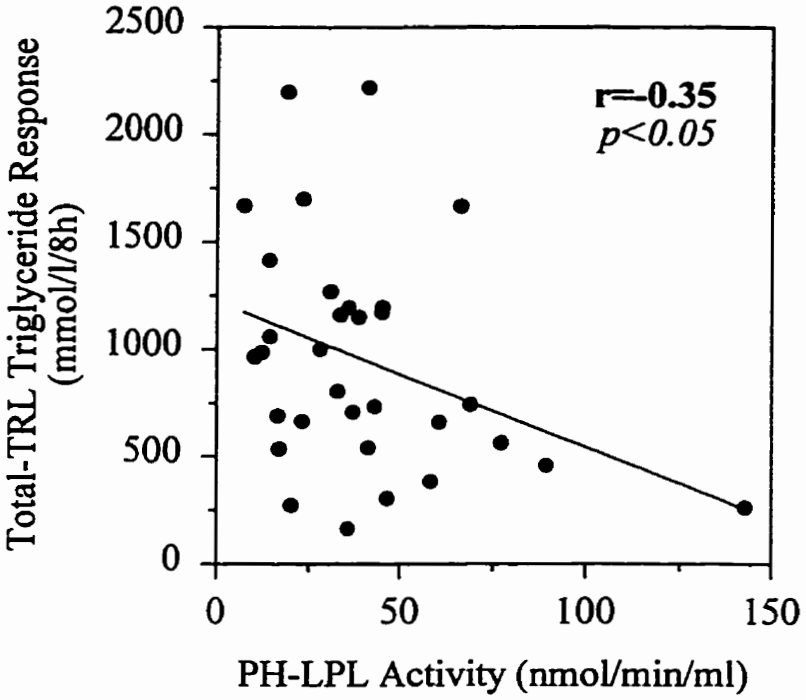

A
30
20
10
0
Fat Mass (kg)
NS
B
350
250
150
50
Subc. AT (cm2)
NS
C
200
150
100
50
0
Visceral AT (cm2)
p<0.0001
Low
High
Visceral AT


A
B
C
D
Total
Large
Medium
Small
Low High
Triglycerides (mmol/l)
Time (hours)
0
1
2
3
4
5
6
0
2
4
6
8
*
†


A
B
C
D
Total
Large
Medium
Small
Low High
Retinyl Palmitate (ng/ml)
Time (hours)
0
1000
2000
3000
4000
5000
0
2
4
6
8
*
**


A
Glucose (mmol/l)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0.0
Low High
B
Insulin (pmol/l)
600
500
400
300
200
100
0
*
†
Low High
C
FFA (mmol/l)
1.5
1.0
0.5
0.0
*
Low High
0
2
4
6
8
Time (hours)


Total-TRL Triglyceride Response (mmol/l/8h)
2500
2000
1500
1000
500
0
-150
0
150
300
450
FFA Response (mmol/l/8h)
r=0.48
p<0.001


Total-TRL Triglyceride Response (mmol/l/8h)
2500
2000
1500
1000
500
0
0
50
100
150
PH-LPL Activity (nmol/min/ml)
r=-0.35
p<0.05

CHAPITRE 7

DIFFÉRENCE SEXUELLE DANS LA RÉPONSE LIPÉMIQUE EN PÉRIODE POSTPRANDIALE : IMPORTANCE DE L'OBÉSITÉ VISCÉRALE

L'article composant ce chapitre est intitulé:

Gender Difference in Postprandial Lipemia: Importance of Visceral Obesity

Gender Difference in Postprandial Lipemia
Importance of Visceral Obesity

RUNNING TITLE: *Postprandial lipemia in men and women*

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RÉSUMÉ

Les altérations métaboliques, incluant les concentrations élevées de triglycérides et faibles de HDL-cholestérol, ont été identifiées comme facteurs de risque de maladies cardio-vasculaires, tant chez l'homme que chez la femme. Bien qu'il ait été proposé que le développement de l'athérosclérose par le biais d'altérations concernant les concentrations de lipides plasmatiques, puisse être un phénomène postprandial, la plupart des études ayant examiné la différence sexuelle dans les concentrations de lipides l'ont fait en utilisant les niveaux à jeun. Le but de notre étude était donc de comparer les réponses triglycéridémiques en période postprandiale chez 63 hommes et 25 femmes adultes. Pour ce faire, de nombreuses mesures morphologiques et métaboliques ont été effectuées incluant la pesée hydrostatique et la tomographie axiale. Malgré une adiposité comparable à celle des femmes, les hommes étaient caractérisés par une accumulation préférentielle de graisse au niveau abdominal tel que démontré par une circonférence de la taille et un rapport taille-hanche plus élevés ($p<0.001$). Ils montraient également une accumulation de graisse viscérale plus importante que les femmes ($p<0.001$) et présentaient une réponse triglycéridémique de plus grande amplitude ($p<0.005$) de même que des réponses plus élevées en insuline et en acides gras libres ($p<0.01$) en période postprandiale comparativement aux femmes. La quantité de tissu adipeux viscéral était positivement corrélée à la réponse postprandiale en triglycérides tant chez l'homme ($r=0.50$, $p<0.0001$) que chez la femme ($r=0.45$, $p<0.05$). Les résultats de la présente étude suggèrent que le dimorphisme sexuel observé dans la distribution du tissu adipeux contribue à la réponse triglycéridémique exagérée en période postprandiale retrouvée chez l'homme.

ABSTRACT

Insulin resistance, hyperinsulinemia, hypertriglyceridemia and low HDL-cholesterol concentrations are common features of a plurimetabolic syndrome which increases the risk of cardiovascular disease (CVD). Although it has been proposed that the development of atherosclerosis through alterations in plasma lipid levels, could be a postprandial phenomenon, most studies on gender differences in plasma lipoprotein-lipid concentrations have reported fasting levels. Therefore, the aim of our study was to examine the postprandial triglyceride-rich lipoprotein (TRL) triglyceride response to a standardized meal in 63 men and 25 women. In addition to the measurement of fasting and postprandial plasma lipid levels, numerous physical and metabolic variables were assessed including body composition by underwater weighing and body fat distribution by computed tomography. Although no gender difference was noted in total body fat mass, expressed in kg, men were characterized by a preferential accumulation of abdominal adipose tissue (AT) as revealed by an increased waist circumference and a greater visceral AT accumulation (50% difference) compared to women ($p<0.001$). Men also showed a greater TRL triglyceride response ($p<0.005$) as well as increased postprandial insulin and free fatty acid levels compared to women ($p<0.01$). Visceral AT was significantly associated with the TRL triglyceride response in both genders (men: $r=0.50$, $p<0.0001$ and women: $r=0.45$, $p<0.05$). Finally, when men and women were matched for visceral AT accumulation, the gender difference in TRL triglyceride response was eliminated. Thus, results of the present study suggest that the well known gender difference in visceral AT accumulation is an important contributing factor involved in the exaggerated postprandial TRL triglyceride response noted in men compared to women.

INTRODUCTION

Alterations in plasma lipoprotein-lipid concentrations are known to increase the risk of coronary heart disease (CAD) in both men and women [1,2]. However, at any age, the incidence of CAD in women is lower than in men, and the sex difference in plasma lipoprotein-lipid levels and prevalence of diabetes are believed to be responsible, at least in part, for the higher CAD risk observed in men. Indeed, men are characterized by an overall less favorable plasma lipid profile, which includes high fasting triglyceride and low HDL-cholesterol concentrations, compared to women [3]. Men and women also show marked differences in indices of plasma glucose-insulin homeostasis [4,5]. It has also been reported that an increased visceral adipose tissue (AT) accumulation in men compared to women could be responsible for the gender difference in the metabolic risk profile [6,7].

Although the contribution of altered plasma lipoprotein-lipid levels to the increased risk of CAD is well known, most studies reporting such a relationship have examined fasting concentrations. However, Zilversmit [8] has suggested that the development of atherosclerosis could be a postprandial phenomenon, and the renewal of the interest for postprandial studies has allowed the identification of various physiological conditions that influence postprandial lipoprotein metabolism. Indeed, it has been reported that age [9,10], diet [11], physical activity [12-14], NIDDM [15,16] as well as obesity [17] and body fat distribution [18,19,20] are all affecting dietary fat clearance. In addition, a sex dimorphism has been reported in postprandial lipoprotein-lipid metabolism as women generally show a less marked postprandial triglyceride response to a dietary

fat challenge compared to men [10,21]. However, little is known about the physiologic mechanisms responsible for this sexual dimorphism.

Therefore, the aim of the present study was to examine the postprandial triglyceride responses of various TG-rich lipoproteins (TRL) in both men and women. For that purpose, 63 men and 25 women were investigated and their plasma TRL responses measured over a period of 8 hours following ingestion of a standardized meal. Results of the present study show that women are characterized by a lower TRL triglyceride response to the meal compared to men. Our results also suggest that increased visceral AT accumulation in men may contribute to the alterations of postprandial TRL metabolism.

SUBJECTS AND METHODS

Subjects. Sixty-three men (mean age ± SD: 45.0 ± 10.0 years) and twenty-five women (41.6 ± 10.9 years), were recruited through the media and selected on purpose to cover a wide range of body fatness values. Subjects gave their written consent to participate in the study which was approved by the Medical Ethics Committee of Laval University. Subjects with diabetes or with coronary heart disease were excluded from the present study. None of the subjects was on medication known to affect insulin action or plasma lipoprotein levels.

Anthropometric and Body Composition Measurements. Body weight, height, waist and hip circumferences were measured following standardized procedures [22], and the waist-to-hip ratio (WHR) was calculated. Body density was measured by the hydrostatic weighing technique [23]. The mean of six measurements was used in the calculation of percent body fat from body density using the equation of Siri [24]. Fat mass was obtained by multiplying body weight by percent body fat.

Computed Tomography (CT). Visceral AT accumulation was assessed by CT, which was performed on a Siemens Somatom DRH scanner (Erlagen, Germany) using previously described procedures [25,26]. Briefly, the subjects were examined in the supine position with both arms stretched above the head. The scan was performed at the abdominal level (between L4 and L5 vertebrae) using an abdominal scout radiograph in order to standardize the position of the scan to the nearest millimeter. The total AT area was calculated by delineating the abdominal scan with a graph pen and then computing the AT surface with attenuation range of -190 to -30 HU [25-27]. The 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 (OLTT). After a 12-hour overnight fast, an intravenous catheter was inserted into a forearm vein for blood sampling. Each participant was given a test meal containing 60g fat/m^2 body surface area [28]. The meal consisted of eggs, cheese, toasts, 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 hours but were given free access to water. Blood samples were drawn before the meal and every 2 hours after the meal over an 8-hour period.

Fasting and Postprandial Plasma Lipoprotein Concentrations. Plasma was separated immediately after blood collection by centrifugation at 3000 rpm for 10 minutes at 4°C. Triglyceride and cholesterol concentrations in total plasma were determined enzymatically on a RA-1000 Auto-Analyzer (Technicon Instruments Corporation, Tarrytown, NY), as previously described [29]. Each plasma sample (4ml) was then subjected to a 12-hour ultracentrifugation (50 000 rpm) in a Beckman 50.3Ti rotor (Palo Alto, CA) at 4°C, in 6ml 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 [30], 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 (100ml) 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 $MnCl_2$ [31]. The triglyceride and cholesterol contents of each fraction, i.e. 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. Plasma FFA levels were also measured at 0, 2, 4, 6 and 8 hours using a colorimetric method [32].

Glucose and Insulin Concentrations. Fasting and postprandial plasma glucose concentrations were determined using the glucose oxidase assay [35] (SIGMA, St-Louis, MO). Plasma insulin levels were measured by a commercial double antibody radioimmunoassay (LINCO Research, St-Louis, MO) that shows little cross-reactivity (<0.02%) with pro-insulin [36].

Statistical Analyses. Pearson product-moment correlation coefficients were used to quantify associations between variables. Differences between men and women were tested for significance using the Student t-test. The different areas under the curve of triglyceride, FFA, insulin and glucose concentrations were determined by the trapezoid method. Multiple regression analyses were

performed to quantify the independent contributions of age, gender, fat mass, abdominal visceral and subcutaneous AT to the variance of postprandial TOTAL-TRL triglyceride response. Fasting triglyceride, HDL-cholesterol, insulin and FFA levels, as well as postprandial insulin and FFA responses were also included in the statistical model. All analyses were conducted on the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Physical characteristics and fasting metabolic profiles of men and women are presented in Table 1. Although both genders had the same amount of total body fat, there were significant differences in body fat distribution. Indeed, men were characterized by increased abdominal AT accumulation as expressed by higher waist circumference and waist-to-hip ratio compared to women. Furthermore, men displayed a greater amount of visceral AT than women. In contrast, significantly higher abdominal subcutaneous AT was noted in women compared to men. Gender differences in the fasting metabolic risk profile were also noted. Indeed, men were characterized by increased plasma cholesterol, triglyceride and glucose levels as well as by decreased plasma HDL-cholesterol concentrations compared to women. Men also showed higher fasting plasma insulin levels than women, but this difference did not reach statistical significance.

Figure 1 illustrates triglyceride concentrations in TOTAL as well as in LARGE, MEDIUM and SMALL-TRL fractions of men and women throughout the entire postprandial period. In each TRL fraction, men showed significantly higher triglyceride levels at all time compared to women, with the exception of the triglyceride content of LARGE-TRL at the 2-hour time-point. These higher triglyceride levels noted during the postprandial period, resulted in greater triglyceride responses in all TRL fractions in men compared to women. Furthermore, gender differences were also observed in postprandial insulin and FFA concentrations (Figure 2), as men displayed higher insulin and FFA levels compared to women.

In both genders, increased adiposity was associated with a greater postprandial TOTAL-TRL triglyceride response, as body fat mass as well as visceral and subcutaneous AT were positively

correlated with TOTAL-TRL triglyceride response (Figure 3). Associations between abdominal visceral and subcutaneous AT accumulations, and the postprandial triglyceride response in LARGE, MEDIUM and SMALL-TRL fractions are presented in Figure 4. A sex difference was observed in the relationship of body fat distribution to the triglyceride response in the different TRL fractions. Indeed, in men, visceral AT was significantly associated with all the TRL subfractions while in women, only the association between visceral AT and LARGE-TRL triglyceride response was significant. On the other hand, in women, significant associations were found between the LARGE, MEDIUM as well as SMALL-TRL triglyceride responses and subcutaneous AT accumulation, which was not the case in men as only the correlation between subcutaneous AT and MEDIUM-TRL triglyceride response was found to be significant. We also found that in men, abdominal visceral AT, but not subcutaneous AT, was positively associated with the postprandial FFA response (Figure 5). However, this association was not observed in women.

As shown in Table 2, fasting metabolic profile variables were associated more closely with the TOTAL as well as LARGE, MEDIUM and SMALL-TRL triglyceride responses than adiposity indices. Indeed, increased fasting plasma triglyceride, insulin levels were predictive of a greater triglyceride responses in all TRL fractions in both men and women. We also found that low fasting HDL-cholesterol levels were associated with increased TOTAL as well as LARGE, MEDIUM and SMALL-TRL triglyceride responses, and that elevated fasting apo B concentrations were correlated with higher TOTAL, LARGE and MEDIUM-TRL triglyceride responses. However, these relationships were only noted in men.

In order to further examine the importance of visceral AT accumulation to the sex difference in postprandial lipemia, we matched men and women on the basis of visceral AT regardless of total body fat mass and examined their respective postprandial triglyceride concentrations in each of the TRL fractions (Figure 6). After this matching procedure, differences in TOTAL, LARGE AND MEDIUM-TRL triglyceride responses were no longer significant between men and women. However, the matching procedure did not affect SMALL-TRL levels as women were still characterized by a lower triglyceride response in that subfraction compared to men.

Finally, we conducted multiple regression analyses in order to quantify the contribution of age, gender, adiposity indices as well as fasting and postprandial metabolic profile variables to the variance of the TOTAL-TRL triglyceride response (Table 3). Fasting triglyceride level was by far the best predictor of TOTAL-TRL triglyceride response, accounting for more than 60% of its variance (Model 1). However, when fasting TG level was removed from the model (Model 2), fasting apo B level showed the greatest contribution to the TOTAL-TRL triglyceride response (37%). In Model 3, both fasting TG and apo B levels were taken out on purpose from the statistical model. In this restricted model, 29% of the variance of the TOTAL-TRL triglyceride response was attributed to visceral AT accumulation. It seems important to point out that, in all models, postprandial FFA response and fasting insulin concentration were significant predictors of the TOTAL-TRL triglyceride response.

DISCUSSION

Gender differences in fasting plasma lipoprotein-lipid concentrations have already been reported [3]. Similar conclusions were reached in the present study as men were characterized by increased fasting plasma cholesterol and triglyceride levels as well as by decreased HDL-cholesterol concentrations compared to women. In addition, men also displayed higher fasting plasma glucose and insulin levels, although the gender difference in fasting insulinemia did not reach statistical significance. These metabolic characteristics have all been identified as features of the insulin-resistance syndrome [37]. On the other hand, it has been suggested that differences in adiposity, especially in body fat distribution, between men and women may be responsible for the sex dimorphism noted in plasma lipoprotein-lipid levels. Indeed, men are known to present a preferential accumulation of AT in the abdominal visceral depot while women are characterized by a more peripheral AT distribution [38]. In the present study, we found that despite having similar levels of total body fat in kg compared to women, men were characterized by an increased abdominal fat accumulation as indicated by higher waist circumference and visceral AT accumulation.

Significant differences were noted in the postprandial triglyceride clearance of men and women. Indeed, men were characterized by greater TOTAL as well as LARGE, MEDIUM and SMALL-TRL triglyceride responses compared to women. These results are concordant with previous observations that reported a higher postprandial triglyceride levels in men than in women. [10,21]. In the present study, sex differences in postprandial triglyceride response profiles of the different TRL fractions were also noted. Indeed in women, the triglyceride content of each TRL fraction peaked earlier in the postprandial period than in men indicating that, in addition to having an

increased postprandial TRL triglyceride response to the meal, men also showed a delayed clearance of postprandial TRL compared to women.

The strong association between fasting TG and triglyceride response of all TRL fractions, indicates that fasting triglyceridemia is an important factor in the gender difference in postprandial lipemia. Indeed, upon their entry into the circulation, both newly synthesized and endogenous TRL compete for lipoprotein lipase (LPL) in order to be hydrolyzed [39]. Thus, in men, the presence of TRL before the meal as indicated by their fasting hypertriglyceridemic state, may contribute to the delayed clearance of postprandial triglyceride from the plasma. This accumulation of TRL caused by the saturation of LPL activity would also postpone the postprandial peak plasma triglyceride concentration. The gender difference in TRL clearance after a meal, could also be the result of an increase in the contribution of hepatic TRL to TOTAL-TRL at the late stages of the postprandial period [40]. Under insulin resistant conditions, the anti-lipolytic effect of insulin on adipose tissue is not adequate, thus increasing FFA levels in the plasma. This increased flux of FFA to the liver would promote the synthesis and secretion of VLDL. This model is supported by our results presented in Figures 4 and 5. Indeed, we noted that, in men, there was a progressive increase in plasma FFA levels which resulted in 8-hour plasma FFA concentrations that remained well above fasting value. However, in women, plasma FFA levels at the end of the postprandial period were near fasting concentrations.

Multiple regression analyses revealed that fasting triglyceride was by far the best predictor of the TOTAL-TRL triglyceride response to the fat load (Model 1). The contribution of apo B-containing lipoproteins to postprandial lipemia was also highlighted as fasting apo B concentration

became the strongest predictor of TOTAL-TRL response after fasting triglyceridemia was eliminated from the statistical model. Since apo B, found in the fasting plasma, is secreted through lipoproteins of hepatic origin, our results provide further support to the concept of an hepatic contribution to the delayed clearance of TRL in men. However, further studies are needed to further support this concept as we have only used fasting apo B-48 and apo B-100 concentrations which are respectively used as markers of TRL of intestinal and hepatic origin respectively.

It is known that visceral obesity is associated with metabolic abnormalities such as fasting hypertriglyceridemia, hyperinsulinemia and increased apo B concentrations as well as lower HDL-cholesterol levels [6,41]. Recently, we have reported that visceral obese men are characterized by an altered TRL clearance in the postprandial period compared to obese men with low levels of visceral AT [20]. In the present study, when both fasting TG and apo B concentrations were eliminated from the multiple regression analyses, the amount of visceral AT was found to be the best predictor of TRL triglyceride response. As women have less visceral AT than men [38], it appears that their preferential accumulation of subcutaneous AT could be associated with a more favorable postprandial TRL metabolism. The contribution of subcutaneous AT to the clearance of TRL will require further studies.

Our results suggest that visceral AT accumulation plays a major role in the gender difference in postprandial lipemia. Indeed, we found no difference in TOTAL as well as LARGE and MEDIUM-TRL triglyceride responses between men and women matched for visceral AT accumulation. However, despite the matching procedure, a gender difference persisted in the SMALL-TRL triglyceride response as men had higher 2, 4 and 6-hour SMALL-TRL triglyceride

concentrations compared to women with similar levels of visceral AT. It is suggested that the increased postprandial FFA response noted in men may have contributed to their higher SMALL triglyceride response compared to women.

Although matching men and women for the level of visceral AT eliminated the gender difference in TOTAL-TRL triglyceride response, there still was a tendency for men to display an exaggerated TOTAL-TRL triglyceride response compared to women. Other factors have been proposed in the explain the reduced postprandial lipemia in women. In that sense, it seems that estrogens may have a favorable impact on postprandial triglyceridemia [42]. A potential difference in LPL activity between men and women [43,44] could also be implicated in the gender difference in postprandial lipemia. Once again, the greater accumulation of subcutaneous fat in women than in men could play a role in the gender difference noted in the clearance of TRL following a dietary fat challenge.

In summary, the results of the present study indicate that there is a gender difference in postprandial lipemia as men show a greater postprandial triglyceridemic response to a meal than women. Although this difference is likely to result from the influence of several factors, our results suggest that in men, an increased visceral AT accumulation may contribute to a delayed dietary fat clearance. Concomitant impairment of postprandial FFA metabolism following a meal and a reduced ability to store lipids in subcutaneous adipose tissue may be responsible, at least in part, for this exaggerated TRL triglyceride response observed in men compared to men.

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FIGURE HEADINGS

Figure 1 : Postprandial triglyceride concentrations of A) TOTAL as well as B) LARGE, C) MEDIUM and D) SMALL-TRL in 63 men (black circles and bars) and 25 women (white circles and bars). Bars represent the areas under the incremental curves (responses). Values are expressed as means ± SEM. *,**, †, †† significantly different from women at $p<0.05$, <0.01, <0.005 and <0.001 respectively.

Figure 2 : Postprandial concentrations of A) glucose, B) insulin and C) free fatty acids (FFA) in 63 men (black circles and bars) and 25 women (white circles and bars). Bars represent the areas under the incremental curves (responses). Values are expressed as means ± SEM. *,**, † significantly different from women at $p<0.05$, <0.01 and <0.005 respectively.

Figure 3 : Associations between A) fat mass as well as B) abdominal visceral and C) subcutaneous AT, and the TOTAL-TRL triglyceride response to the meal in 63 men (black circles and lines) and 25 women (white circles and dotted lines).

Figure 4 : Associations between abdominal visceral (left panels) and subcutaneous AT (right panels), and postprandial LARGE (panels A and D), MEDIUM (panels B and E) as well as SMALL-TRL (panels C and F) triglyceride responses in 63 men (black circles and lines) and 25 women (white circles and dotted lines).

Figure 5: Associations between A) abdominal visceral as well as B) subcutaneous AT accumulation and postprandial FFA response in 63 men (black circles and lines) and 25 women (white circles and dotted lines).

Figure 6: Postprandial triglyceride concentrations of A) TOTAL as well as B) LARGE, C) MEDIUM and D) SMALL-TRL in 19 pairs of men (black circles and bars) and women (white circles and bars) matched for visceral AT accumulation. Bars represent the areas under the incremental curves (responses). Values are expressed as means ± SEM. *,**, significantly different from women at $p<0.05$ and <0.01 respectively.

Table 1: Physical characteristics and fasting metabolic profile of the subjects

Variables	**Men** *(n=63)*	**Women** *(n=25)*
Age (years)	45.0 ± 10.0	41.6 ± 10.9
BMI (kg/m^2)	29.0 ± 4.1	26.9 ± 6.0
%Body Fat	27.4 ● 6.3	26.4 ● 11.4
FM (kg)	24.5 ● 8.4	26.8 ± 14.3
Waist girth (cm)	98.3 ± 10.2	82.6 ± 13.1 §§
Waist-to-Hip Ratio	0.95 ± 0.06	0.81 ± 0.06 §§
Abdominal Adipose Tissue Areas (cm^2)		
Visceral	148 ± 63	99 ± 57 **
Subcutaneous	275 ● 108	348 ± 194 *
Cholesterol (mmol/l)	5.09 ± 0.80	4.39 ± 0.75 §
Triglycerides (mmol/l)	1.87 ± 0.93	1.24 ± 0.61 *
HDL-cholesterol (mmol/l)	0.91 ± 0.21	1.14 ± 0.28 **
Cholesterol/HDL-C Ratio	5.84 ± 1.55	4.07 ± 1.20 §§
Free Fatty Acids (mmol/l)	0.64 ± 0.24	0.71 ± 0.24
Apolipoprotein B (g/l)	1.07 ± 0.21	0.90 ± 0.19 §
Fasting Glucose (mmol/l)	5.09 ± 0.61	4.70 ± 0.45 *
Fasting Insulin (pmol/l)	96.2 ± 52.6	78.1 ± 30.8

*, **, §, §§ significantly different from the men at $p<0.005$, <0.001, <0.0005 and <0.0001 respectively.

Table 2: Associations between fasting metabolic profile variables and the postprandial triglyceride response of the different triglyceride-rich lipoprotein (TRL) fractions in men and women

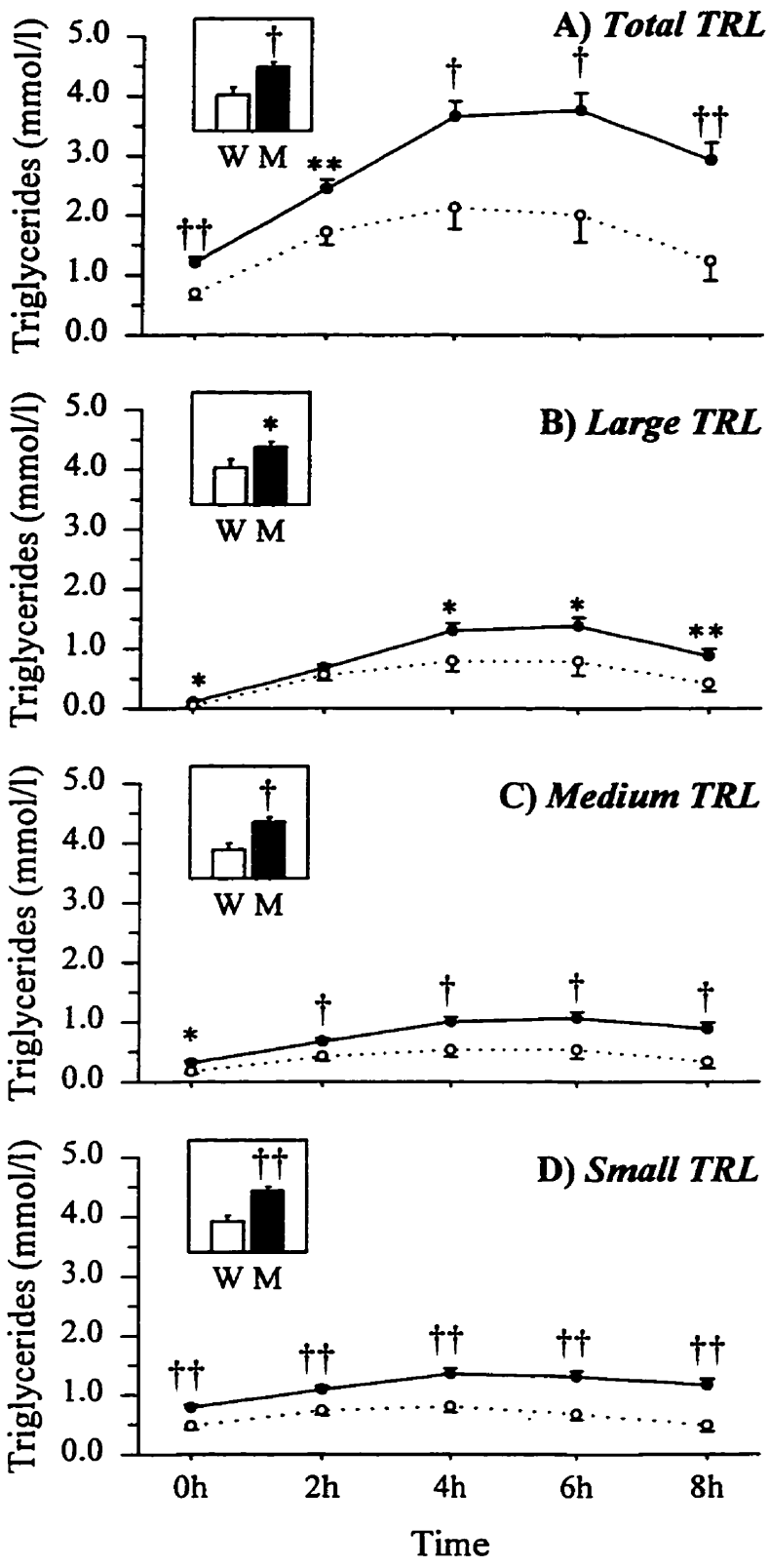
Variables	Men (n=63)				Women (n=25)			
	TRL Triglycerides				TRL Triglycerides			
	Total	*Large*	*Medium*	*Small*	*Total*	*Large*	*Medium*	*Small*
Age	0.20	0.20	0.20	0.13	0.36	0.37	0.30	0.28
Triglycerides	0.78 §§	0.70 §§	0.81 §§	0.42 ††	0.74 §§	0.77 §§	0.69 §	0.46 *
HDL-cholesterol	- 0.43 §	- 0.39 †	- 0.46 §§	- 0.22	- 0.10	- 0.11	- 0.06	- 0.08
Free Fatty Acids	- 0.08	- 0.13	- 0.01	- 0.01	0.02	0.02	0.03	0.03
Apolipoprotein B	0.56 §§	0.55 §§	0.55 §§	0.24	0.39	0.38	0.33	0.40
Fasting Insulin	0.43 §	0.38 †	0.42 ††	0.30 *	0.77 §§	0.76 §	0.77 §§	0.58 **

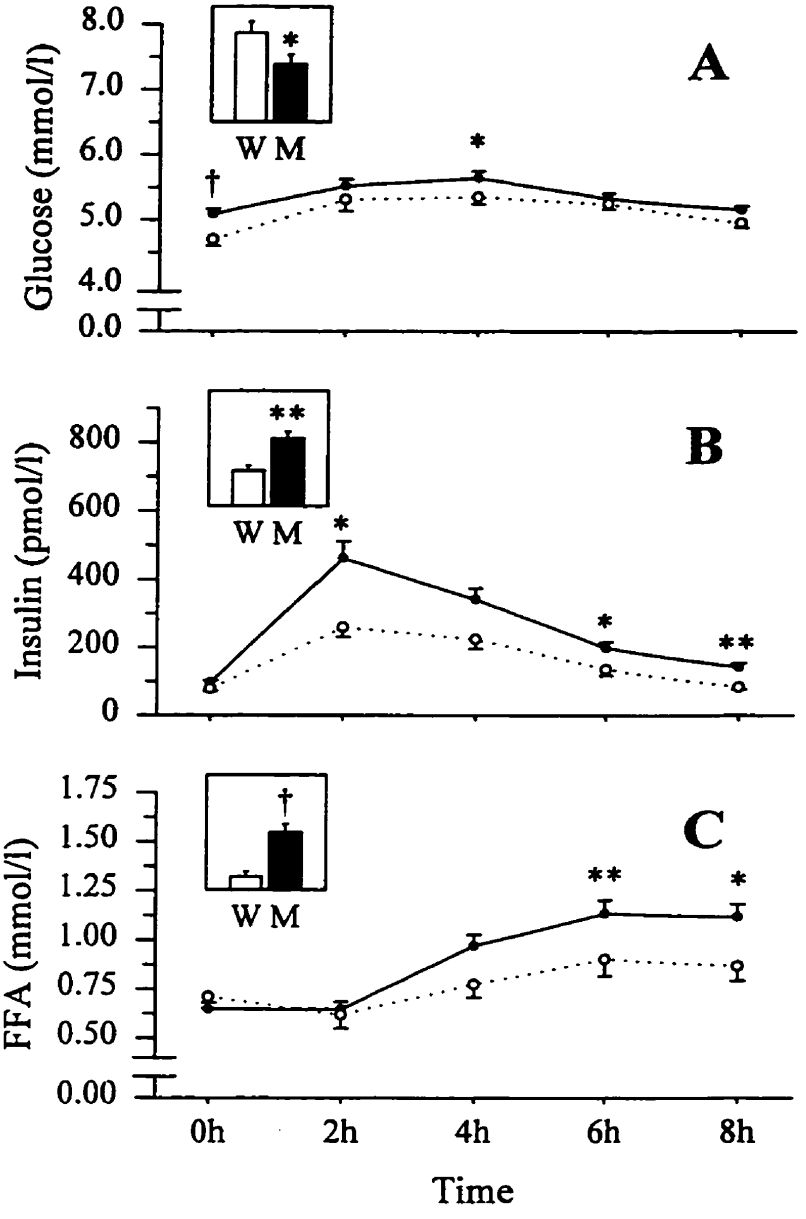
*p<0.05 ; **p<0.01 ; † p<0.005 ; †† p<0.001 ; § p<0.0005 and §§ p<0.0001.

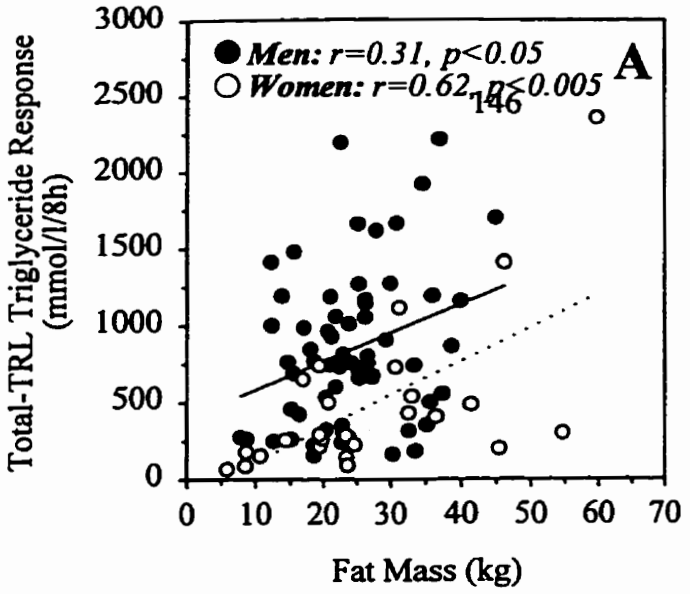
Table 3: Multivariate regression analyses showing the independent contributions of physical and metabolic characteristics to the postprandial TOTAL-TRL triglyceride response.

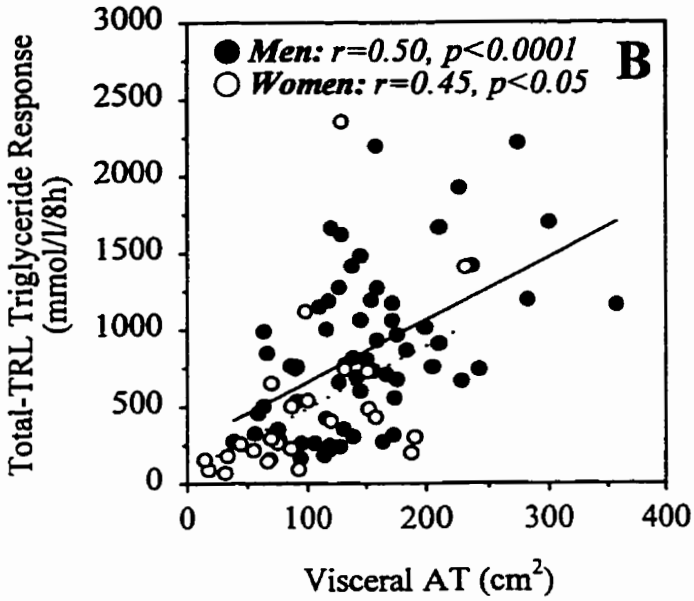
Dependent Variable	*Independent Variable*	*Partial (R^2 X 100)*	*Total (R^2 X 100)*	*P*
Model 1*				
TOTAL-TRL triglyceride response	Fasting TG	60.7	66.2	0.0001
	FFA Response	3.2		0.0130
	Fasting Insulin	2.3		0.0292
Model 2 (excluding fasting TG)				
TOTAL-TRL triglyceride response	Apo B	37.2	55.9	0.0001
	Fasting Insulin	9.8		0.0004
	FFA Response	5.4		0.0052
	Fat Mass	3.5		0.0204
Model 3 (excluding fasting TG and apo B)				
TOTAL-TRL triglyceride response	Visceral AT	29.0	44.4	0.0001
	FFA Response	11.9		0.0002
	Fasting Insulin	3.5		0.0358

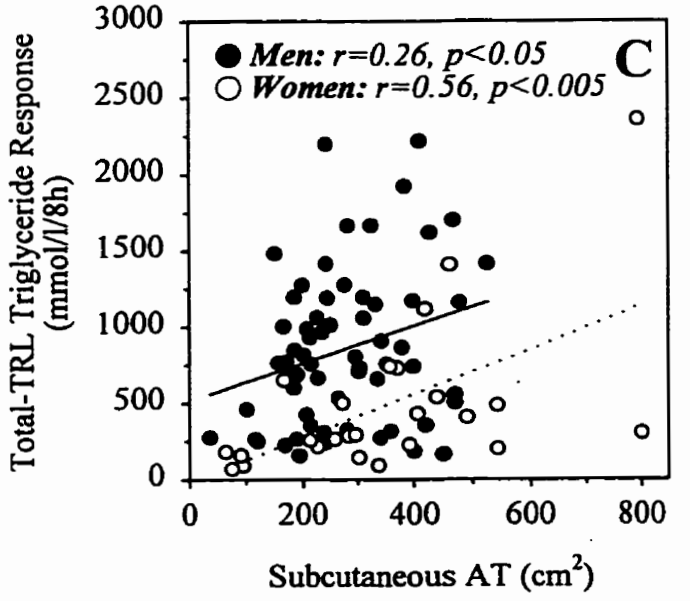
* Model 1 included age, gender, visceral AT, subcutaneous AT as well as fasting triglyceride, HDL-cholesterol,insulin, apoB
as well as postprandial insulin and FFA responses.

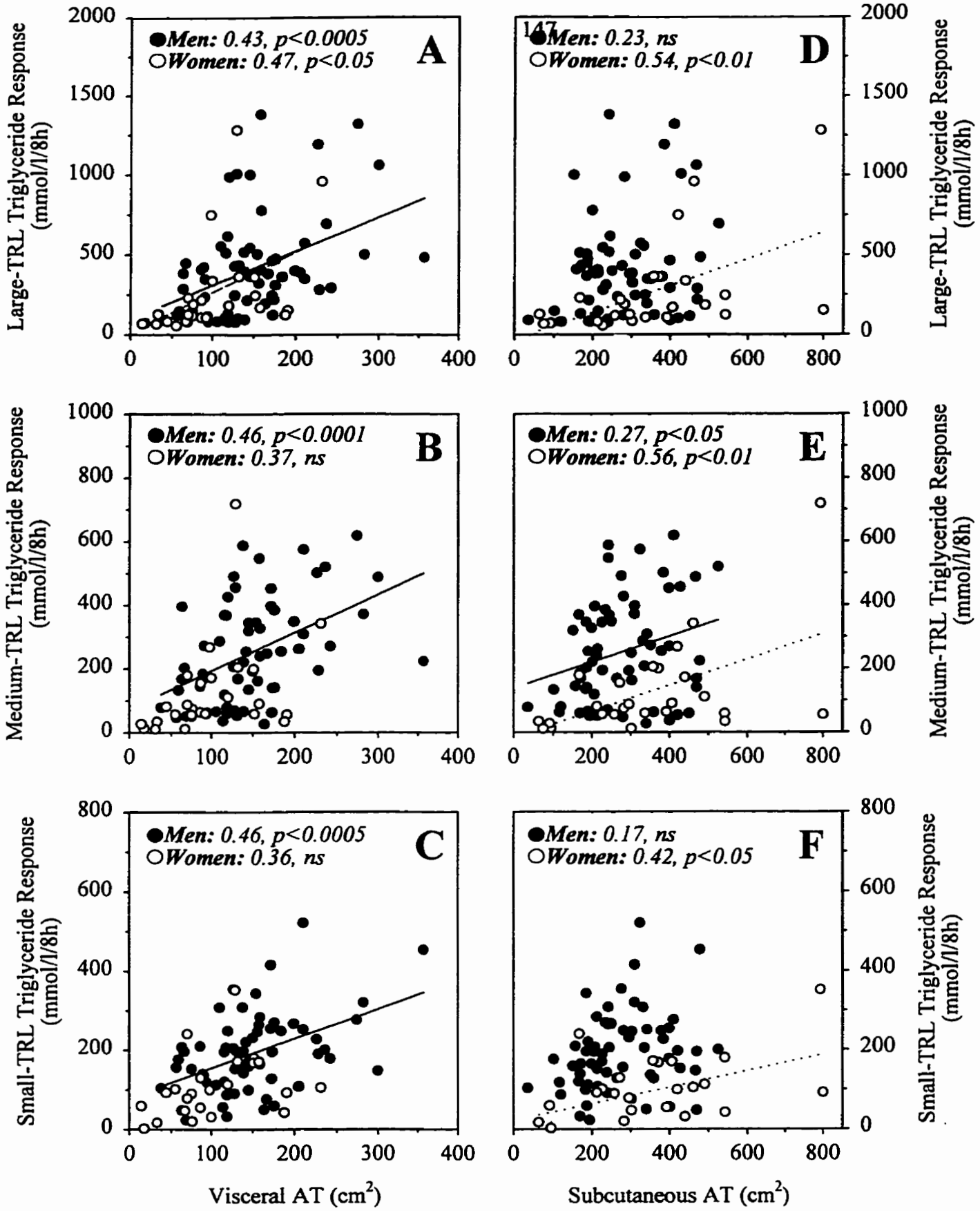

A) Total TRL
B) Large TRL
C) Medium TRL
D) Small TRL
Triglycerides (mmol/l)
5.0
4.0
3.0
2.0
1.0
0.0
W M
0h
2h
4h
6h
8h
Time

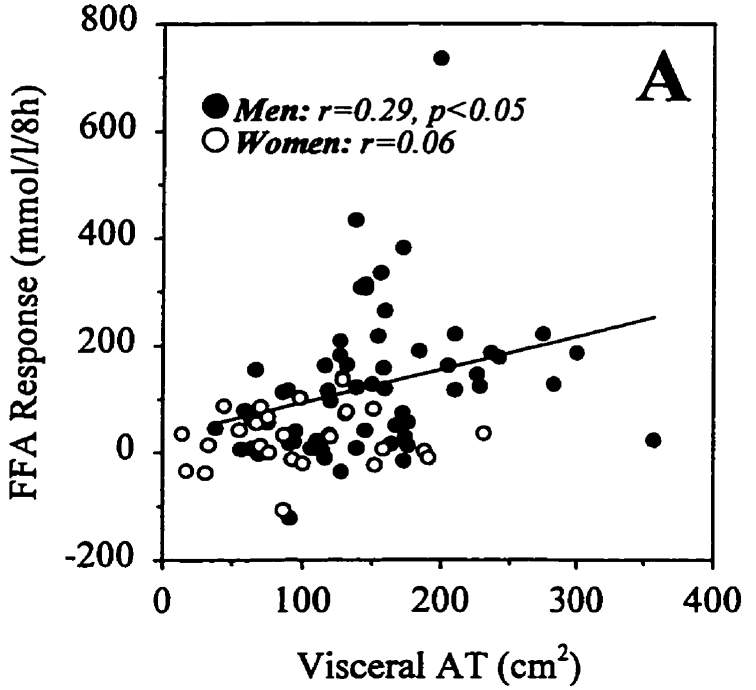

A
B
C
Glucose (mmol/l)
8.0
7.0
6.0
5.0
4.0
0.0
Insulin (pmol/l)
800
600
400
200
0
FFA (mmol/l)
1.75
1.50
1.25
1.00
0.75
0.50
0.00
W M
0h
2h
4h
6h
8h
Time

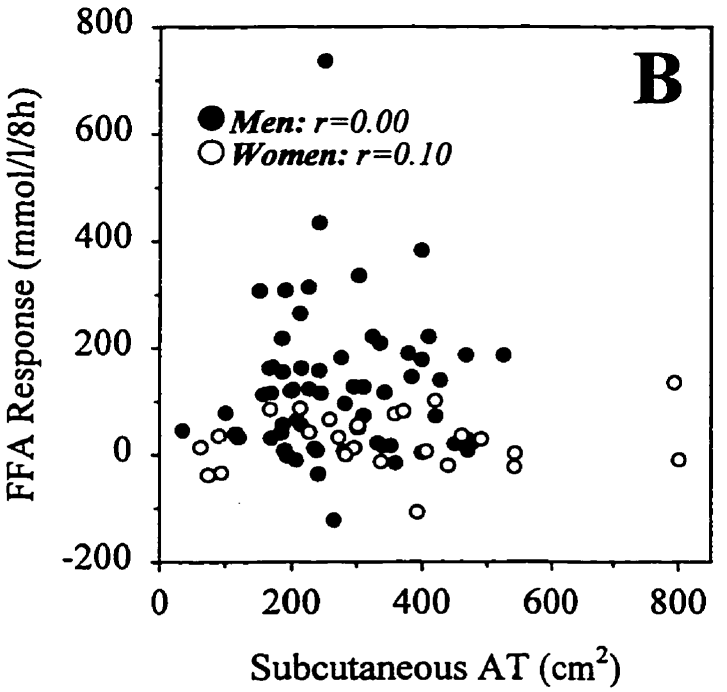

Men: r=0.31, p<0.05
Women: r=0.62, p<0.005
A
Total-TRL Triglyceride Response (mmol/l/8h)
Fat Mass (kg)
0
500
1000
1500
2000
2500
3000
0
10
20
30
40
50
60
70

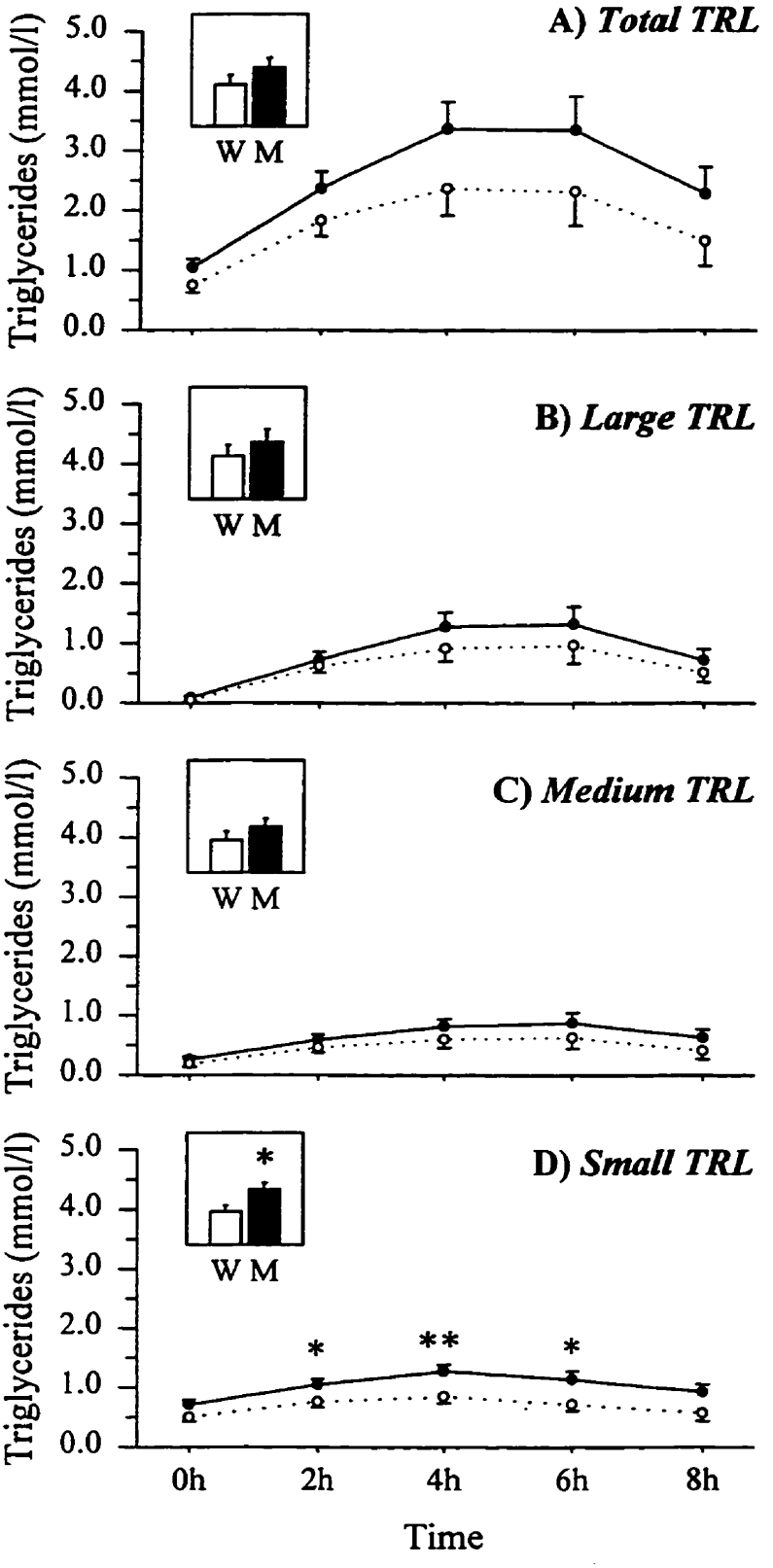

Men: r=0.50, p<0.0001
Women: r=0.45, p<0.05
B
Total-TRL Triglyceride Response (mmol/l/8h)
Visceral AT (cm2)
0
500
1000
1500
2000
2500
3000
0
100
200
300
400


Men: r=0.26, p<0.05
Women: r=0.56, p<0.005
C
Total-TRL Triglyceride Response (mmol/l/8h)
Subcutaneous AT (cm2)
0
500
1000
1500
2000
2500
3000
0
200
400
600
800


●Men: 0.43, p<0.0005
○Women: 0.47, p<0.05
A
●Men: 0.23, ns
○Women: 0.54, p<0.01
D
●Men: 0.46, p<0.0001
○Women: 0.37, ns
B
●Men: 0.27, p<0.05
○Women: 0.56, p<0.01
E
●Men: 0.46, p<0.0005
○Women: 0.36, ns
C
●Men: 0.17, ns
○Women: 0.42, p<0.05
F
Large-TRL Triglyceride Response (mmol/l/8h)
Medium-TRL Triglyceride Response (mmol/l/8h)
Small-TRL Triglyceride Response (mmol/l/8h)
Visceral AT (cm²)
Subcutaneous AT (cm²)


A
Men: r=0.29, p<0.05
Women: r=0.06
FFA Response (mmol/l/8h)
800
600
400
200
0
-200
0
100
200
300
400
Visceral AT (cm2)


B
Men: r=0.00
Women: r=0.10
FFA Response (mmol/l/8h)
800
600
400
200
0
-200
0
200
400
600
800
Subcutaneous AT (cm2)


A) Total TRL
B) Large TRL
C) Medium TRL
D) Small TRL
Triglycerides (mmol/l)
5.0
4.0
3.0
2.0
1.0
0.0
W M
*
**
0h
2h
4h
6h
8h
Time

CHAPITRE 8

DIFFÉRENCES SEXUELLES DANS LES CONCENTRATIONS PLASMATIQUES DE LEPTINE ET SES ASSOCIATIONS AVEC LES FACTEURS DE RISQUES POUR LES MALADIES CARDIO-VASCULAIRES

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Plasma Leptin Concentrations : Gender Differences and Associations With Metabolic Risk Factors for Cardiovascular Disease

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Plasma Leptin Concentrations

Gender Differences and Associations With Metabolic Risk Factors for Cardiovascular Disease

RUNNING TITLE: *Gender differences in leptinemia*

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RÉSUMÉ

Le clonage du gène *obèse* et de la caractérisation de sa protéine, la leptine, ont permis l'étude d'une nouvelle hormone probablement impliquée dans la régulation de la masse adipeuse. Dans la présente étude, les différences sexuelles dans les concentrations plasmatiques de leptine et ses relations avec l'adiposité, la distribution du tissu adipeux ainsi que le profil métabolique ont été examinées chez 91 hommes (âge moyen ● SD : 37.3 ± 4.8 ans) et 48 femmes (38.5 ± 6.8 ans). Les concentrations de leptine étaient fortement associées à la masse grasse mesurée par pesée hydrostatique (hommes :r=0.80, p<0.0001 ; femmes : r=0.85, p<0.0001). Tant chez l'homme que chez la femme, les niveaux de leptine étaient corrélés à la circonférence de la taille ainsi qu'aux aires de tissu adipeux abdominal sous-cutané et viscéral mesurées par tomographie axiale. Les femmes avaient en moyenne, une leptinémie trois fois plus élevée que celle des hommes. De plus, cette différence sexuelle demeurait significative lors de la comparaison d'hommes et de femmes pairés pour une même masse grasse. Les associations entre les concentrations plasmatiques de leptine et celles de lipoprotéines étaient dépendantes du degré d'adiposité. Des niveaux élevés de leptine étaient corrélés à de hautes concentrations d'insuline mais l'association est restée significative seulement chez la femme, après correction pour l'adiposité. Les résultats de la présente étude démontrent donc que les femmes ont des concentrations plasmatiques de leptine plus élevés que les hommes et ce, indépendamment de la variation concomitante de l'adiposité. De plus, ces résultats suggèrent que, du moins chez la femme, l'association entre la leptine et l'insuline est indépendante de l'adiposité, une observation qui confirme le rôle de l'insuline dans la régulation de la sécrétion de leptine par le tissu adipeux.

SUMMARY

The cloning of the *obese* gene and the characterization of its protein product, leptin, has allowed the study of a new hormone potentially involved in the regulation of adipose tissue mass. The present study examined the gender differences in fasting plasma leptin concentration and its relationship to body fatness, adipose tissue distribution and the metabolic profile in samples of 91 men (mean age ● SD: 37.3 ± 4.8 years) and 48 women (38.5 ● 6.8 years). Plasma leptin concentrations were strongly associated with body fat mass measured by underwater weighing [men: $r=0.80$, $p<0.0001$; women: $r=0.85$, $p<0.0001$]. In both genders, plasma leptin levels were also strongly correlated with waist girth as well as cross-sectional areas of abdominal subcutaneous and visceral adipose tissue measured by computed tomography. Women had, on average, plasma leptin concentration that were three-fold higher than men. Furthermore, this gender difference remained significant when comparing men and women matched for similar levels of body fat mass. The associations between plasma leptin and lipoprotein concentrations were dependent of adiposity. In both men and women, elevated fasting plasma leptin levels were associated with higher plasma insulin concentrations, but only in women was the association maintained after correction for fat mass. Thus, results of the present study show that women have higher plasma leptin levels compared to men, independently from the concomitant variation in total body fat mass. Furthermore, our results also suggest that, in women, the association between plasma leptin and insulin concentrations is independent of adiposity, a finding which provide further support to the observation that adipose tissue leptin secretion may be upregulated by insulin.

Key Words: *Leptin, gender differences, insulin, lipoproteins*

Abbreviations:

CVD:	cardiovascular disease
AT:	adipose tissue
FM:	fat mass
CT:	computed tomography
OGTT:	oral glucose tolerance test
CHOL:	plasma cholesterol
TG:	plasma triglycerides
apo:	apolipoprotein
FFM:	fat-free mass
WHR:	waist-to-hip ratio

INTRODUCTION

Obesity results from an imbalance between energy intake and expenditure. Furthermore, obesity has long been recognized to have detrimental effects on health including an increased risk of CVD [1]. In this regard, the recent cloning of the mouse *(ob)* and human *(OB) obese* genes and the characterization of its protein product, leptin [2], has been a breakthrough of potentially great importance for the understanding of the pathophysiology of obesity.

Leptin has been shown to lower body weight by reducing food intake and increasing energy expenditure in leptin-deficient *obese* mice *(ob/ob)* and also to normalize blood glucose levels in the same animals [2-8]. Leptin is secreted by white adipose cells and is exclusively expressed in AT [2,9-13]. In this regard, numerous studies have reported a strong relationship between adiposity and plasma leptin concentrations or its adipose tissue mRNA levels [14-21]. Furthermore, expression of the *obese* gene is believed to be regulated by insulin both *in vivo* and *in vitro* [3,13,22,23].

Thus, the objectives of the present study were: 1) to examine the potential relationships of body FM assessed by underwater weighing, plasma lipid, insulin as well as glucose concentrations, with plasma leptin levels in both men and women, and 2) to test the potential gender difference in plasma leptin levels when adjusting for the well known gender difference in total adiposity. For this purpose, morphological and metabolic variables were measured on 91 men (mean age ± SD: 37.3 ● 4.8 years) and 48 women (38.5 ± 6.8 years), and associations with fasting plasma leptin examined.

SUBJECTS AND METHODS

Subjects. Ninety-one men (mean age ± SD: 37.3 ● 4.8 years) and 48 women (38.5 ± 6.8 years) were recruited through the media to participate in this study, which was approved by the Medical Ethics Committee of Laval University and an informed consent document was signed by all participants. A complete physical examination, which also included medical history, was performed by a physician. All participants were nonsmokers and free from diseases requiring treatment. Exclusion criteria included diabetes, monogenic dyslipidemias or evidence for the presence of coronary heart disease.

Anthropometric measurements. Weight, height, waist and hip circumferences were measured following the procedures recommended at the Airlie Conference [24], and the waist-to-hip ratio was calculated. Body density was measured by the hydrostatic weighing technique [25], and the mean of six measurements was used in the calculation of body density. Percentage body fat was obtained from body density using the equation of Siri [26].

Computed tomography. CT was performed on a Siemens Somatom DRH scanner (Erlagen, GERMANY) using previously described procedures [27,28]. Briefly, the subjects were examined in the supine position with both arms stretched above the head. A single CT scan was performed at the abdominal level (between L4 and L5 vertebrae) with a scout abdominal radiograph used as a reference to establish the position of the scan to the nearest millimeter. Total AT area was calculated by delineating the area with a graph pen and then computing the AT surface with an attenuation range of -190 to -30 Hounsfield Units [27-29]. The 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 glucose tolerance test. A 75-g OGTT was performed in the morning after an overnight fast. Blood samples were collected under EDTA and Trasylol (Miles, Rexdale, Ontario, CANADA) through a venous catheter from an antecubital vein at -15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes for the determination of plasma glucose and insulin concentrations. Plasma glucose was measured enzymatically [30], whereas plasma insulin was measured by RIA with polyethylene glycol separation [31]. However, the assay used for the measurement of plasma insulin showed some cross-reactivity with proinsulin. As diabetes was an exclusion criteria in our study, we believe that such cross-reactivity did not have a significant impact on results obtained and their interpretation.

Plasma lipoprotein analyses. Blood samples were obtained in the morning after a 12-hour fast from an antecubital vein into vacutainer tubes containing EDTA. CHOL and TG levels in plasma and in lipoprotein fractions were measured enzymatically on an RA-1000 Autoanalyzer (Technicon, Tarrytown, New York, USA), as previously described [32]. VLDL ($d<1.006$ g/ml) were isolated by ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d>1.006$ g/ml) with heparin and $MnCl_2$ [33]. The cholesterol content of HDL_2 and HDL_3 subfractions was also determined after further precipitation of HDL_2 with dextran sulfate [34]. Total apo B concentration was measured in plasma by the rocket immunoelectrophoretic method of

Laurell, as previously described [35]. The lyophilized serum standard for apo B measurement was prepared in our laboratory and calibrated with reference standards obtained from the Centers for Disease Control (Atlanta, Georgia, USA).

Plasma leptin concentrations. Fasting plasma leptin concentrations were determined with a highly sensitive commercial double-antibody RIA (Human Leptin Specific RIA Kit, LINCO Research, St-Louis, Missouri, USA) which detects relatively low leptin levels of 0.5 ng/ml and which does not crossreact with human insulin, proinsulin, glucagon, pancreatic polypeptide or somatostatin. Our coefficients of variation for the repeated assays ranged from 4.0 to 5.5% for lower leptin concentrations and from 6.5 to 8.5% for higher plasma leptin concentrations.

Statistical analyses. Student t-tests were used to examine gender differences. The same procedure was also used for the comparison of subgroups matched on the basis of body FM. In these analyses, we individually paired men (n=26) and women (n=26) for total body FM (within a maximal difference of 2 kg) and compared their respective fasting plasma leptin and insulin concentrations as well as their abdominal subcutaneous and visceral AT accumulation. Pearson product-moment correlation coefficients were used to examine associations among variables. All analyses were performed with the SAS statistical package (SAS Institute, Cary, North Carolina, USA).

RESULTS

Physical and metabolic characteristics of subjects are shown in Table 1. Indices of body fatness showed significant gender differences as %body fat and body FM (in kg) were higher in women compared to men. Women also showed higher levels of subcutaneous AT measured by CT compared to men. However, in spite of the fact that women displayed higher levels of total body fat than men, no significant gender difference was found in visceral AT accumulation. With the exception of lower plasma HDL-, HDL_2- and HDL_3-cholesterol concentrations and an increased CHOL/HDL-cholesterol ratio in men, no significant gender difference was noted in the remaining variables of the plasma lipid profile. Furthermore, men were characterized by higher insulin and glucose concentrations in the fasting state compared to women. However, plasma leptin concentrations were more than 3-times higher in women than in men.

In both men and women, plasma leptin levels showed strong correlations with adiposity. Figures 1 and 2 illustrate the relationships of plasma leptin concentrations to body FM and waist circumference (Figure 1) as well as to abdominal subcutaneous and visceral AT accumulation (Figure 2). For a given body FM (or any adiposity variables studied), women showed higher plasma leptin concentrations compared to men. We also observed substantial individual variation in plasma leptin levels among subjects with increased adiposity, whether it was evaluated by total body FM, waist girth, subcutaneous or visceral AT accumulation.

Although we observed significant associations between plasma leptin concentrations and lipid levels, statistical adjustment for body FM eliminated those relationships in both genders (Table 2). The associations of plasma leptin concentrations with plasma insulin and glucose levels, either in

the fasting state or following the oral glucose load are depicted in Table 3. As opposed to men, correction of the relationships with FM did not eliminate the significance of the association between plasma leptin and insulin concentrations in women.

To better examine the potential gender difference in plasma leptin concentrations, we have also compared men (n=26) and women (n=26) who were matched for their total body FM within a 2 kg difference. Figure 3 indicates that although subjects were closely matched for total body FM (mean ± SEM; men: 20.2 ± 1.8 kg vs women: 19.9 ± 1.8 kg), women showed plasma leptin levels which were approximately twice the concentrations found in men. Moreover, men displayed significantly higher fasting insulin levels and visceral AT accumulation compared to women. However, no gender difference was found in subcutaneous AT accumulation.

DISCUSSION

The relationship of abdominal obesity to numerous metabolic disturbances identified as risk factors for the development of CVD, such as dyslipidemias and NIDDM is well documented [1]. Moreover, although women have on average higher levels of body fat than men, their metabolic profile seems to be less affected by obesity. This is concordant with the results of the present study as women were characterized by a higher body fat content than men. However, in spite of the fact that women of the present study were fatter than the sample of men examined, they tended to show more favorable fasting plasma lipoprotein, insulin and glucose concentrations than men. This situation could perhaps be partly explained by the fact that women had more subcutaneous fat than men but showed no difference in visceral AT accumulation, this latter depot having been shown to be more closely related with alterations in the lipid profile as well as in the insulin-glucose homeostasis than excess fatness per se [36]. These results further emphasize the role of visceral fat as a correlate of the metabolic complications of obesity. In this regard, as a set of morphometric and metabolic variables was assessed in both men and women, the present study offered an opportunity to examine the potential associations of total body FM as well as of AT distribution indices to plasma leptin levels, and to test for potential gender differences in the magnitude of these relationships.

The *obese* gene product, leptin, is secreted and exclusively expressed by AT [2,9-13]. Numerous studies have now reported strong positive correlations between total adiposity and fasting plasma leptin concentrations or its AT mRNA levels [14-21]. In the present study, an increased body FM was associated with higher plasma leptin concentrations in men and women. However, the slope

of the relationship was steeper in women compared to men. This observation indicates that for a given total body FM, women show elevated plasma leptin concentrations compared to men.

In our study, the associations between plasma leptin and lipid concentrations were dependent of the degree of obesity as statistical adjustment for body FM did eliminate the associations. This is concordant with previous studies that reported no relationships between plasma leptin and lipid concentrations after statistical adjustment for adiposity [37,38]. We also found highly significant correlations between plasma leptin concentrations and insulin levels measured in the fasting state or following an oral glucose challenge. However, this relationship was only independent of adiposity in women. These results are rather concordant with several previous studies where it had been reported that insulin administration *in vivo* or insulin *in vitro* could increase AT *obese* gene mRNA levels [3,13,22,23].

We also found a substantial difference in leptinemia between men and women. Previous studies had suggested that this difference could be the result of the increased body fat content of women compared to men [14,17,20,37-42]. However, this gender difference remained after adjustment of leptin for adiposity, mostly BMI [14,17,20,37,38,40-42]. In the present study, when women and men were matched for FM, a two-fold difference in leptinemia remained, women having higher levels than men. It is also relevant to point out that women matched with men on the basis of body FM had lower plasma insulin levels than men. The understanding of the mechanism responsible for this phenomenon is beyond the scope of the present study and will require further investigation. However, gender differences in visceral AT could be suspected in men and women matched for total body fatness, lower values being reported in women [43]. Indeed, in addition to

higher plasma insulin concentrations, men also showed increased visceral AT accumulation compared to women. Thus, this elevated visceral AT depot, which is closely related to alterations in glucose-homeostasis [44], may contribute to the hyperinsulinemic state in men.

Increased leptin production has been reported in subcutaneous vs intraabdominal AT depots [9,45]. The well known gender difference in AT distribution may account for some of the difference in leptin concentrations between men and women. However, in our study, men and women matched for FM displayed similar average levels of subcutaneous AT. Thus, it does not appear that increased subcutaneous AT in women is responsible for the difference in circulating leptin concentrations between men and women.

Recently, Rosenbaum *et al* have also reported a gender difference in leptin concentrations [39]. They also observed a decrease in plasma leptin levels at menopause in women, but leptin concentrations in postmenopausal women remained higher than those found in men. In this regard, they proposed that estrogen and/or progesterone could affect leptin levels resulting in elevated leptin concentrations in premenopausal women. Furthermore, they also proposed that the lower leptin levels that are reported in men, compared to women, could be due to elevated androgen concentrations. This hypothesis requires further investigation.

Another issue that will have to be examined is whether the proportion of free vs bound leptin in the plasma could be different between men and women. Indeed, Sinha *et al* have reported a difference in the proportion of free vs bound (i.e. associated to binding proteins) leptin between lean and obese subjects [46]. In this study, it was also noted that obese subjects had higher leptin concentrations compared to lean individuals, and that a majority of the leptin in circulation was in

the free form. Since free leptin is believed to be the bioactive form, these observations provide further support to the leptin resistance theory in human obesity. In the present study, women had increased adiposity and higher plasma leptin concentrations compared to men. However, matching subjects on the basis of FM did not eliminate the gender difference in leptin levels. In our study, we were not able to quantify the contributions of the two forms of leptin (free vs bound) to the total circulating concentration. However, in the event that a gender difference in the proportion of free vs bound leptin contributed to elevated plasma leptin levels in women, results of the present study suggest that this phenomenon is unlikely to be the result of increased adiposity.

In summary, the results of the present study clearly indicate that women have increased plasma leptin levels compared to FM-matched men. Since plasma leptin concentrations were not related to alterations of the plasma lipoprotein concentrations which are known risk factors for CVD, it does not seem relevant to include leptinemia in the set of metabolic risk factors for CVD. Although this study was obviously not designed to examine the mechanisms that are involved in the regulation of the *obese* gene expression in AT, the present results reinforce the notion that, in women, leptin production in AT is related to insulinemia, and that this relationship is independent of FM. However, further studies are needed to identify factors responsible for the marked difference in leptinemia among men and women with similar levels of total body fat.

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FIGURE HEADINGS

Figure 1: Associations between fasting plasma leptin concentrations and total body FM (upper panel) as well as waist girth (bottom panel) in 91 men (n) and 48 women (¡).

Figure 2: Associations between fasting plasma leptin concentrations and abdominal subcutaneous (upper panel) as well as visceral AT (bottom panel) in 91 men (n) and 48 women (¡).

Figure 3: Fasting plasma leptin and insulin concentrations, as well as visceral and subcutaneous AT accumulation in men (white bars; n=26) and women (black bars; n=26) matched on the basis of total body FM (mean ± SEM; men: 20.2 ● 1.8 vs women: 19.9 ± 1.8 kg). Values in figure are expressed as means ± standard error of the mean (SEM).

Table 1: Physical and metabolic characteristics of subjects

Variables	Men (n=91)	Women (n=48)
Age (years)	37.3 ± 4.8	38.5 ● 6.8
Weight (kg)	82.7 ± 12.9	75.4 ± 18.9 [b]
BMI (kg/m^2)	27.3 ± 4.0	29.0 ± 7.1
%Body fat	25.8 ± 6.8	37.6 ± 12.1 [f]
FM (kg)	22.1 ± 8.4	30.3 ± 15.6 [d]
FFM (kg)	60.9 ± 6.2	45.2 ± 5.6 [f]
Waist girth (cm)	96.1 ± 12.0	87.1 ● 15.6 [e]
WHR	0.94 ± 0.06	0.81 ± 0.05 [f]
CT-DERIVED ABDOMINAL AT AREAS (cm^2)		
Subcutaneous	251 ± 106	379 ± 213 [f]
Visceral	126 ± 52	108 ± 61
METABOLIC PROFILE		
Cholesterol (mmol/l)	5.02 ± 0.78	5.08 ± 0.92
Triglycerides (mmol/l)	1.67 ± 0.91	1.40 ± 0.68
HDL-chol (mmol/l)	1.02 ± 0.22	1.18 ± 0.32 [d]
HDL$_2$-chol (mmol/l)	0.35 ± 0.15	0.44 ± 0.21 [c]
HDL$_3$-chol (mmol/l)	0.68 ± 0.12	0.74 ± 0.17 [b]
Apo B (mg/dl)	94.4 ± 21.8	95.3 ± 23.2
CHOL/HDL-C	5.17 ± 1.31	4.60 ± 1.43 [a]
Leptin (ng/ml)	6.2 ± 3.5	19.9 ± 15.0 [f]
Insulin (pmol/l)	77.9 ± 30.9	64.9 ± 43.9 [a]
Glucose (mmol/l)	5.16 ± 0.51	4.92 ± 0.42 [c]
Insulin Area (10^{-3}pmol/l/min)	75.8 ± 36.9	74.6 ± 37.7
Glucose Area (10^{-3}mmol/l/min)	1.18 ± 0.23	1.12 ● 0.21

Values are expressed as means ± SD.
Gender differences: [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.005$, [d] $p<0.001$, [e] $p<0.0005$ and [f] $p<0.0001$

Table 2: Associations between lipid profile variables versus fasting plasma leptin concentrations in the 91 men and 48 women of the study.

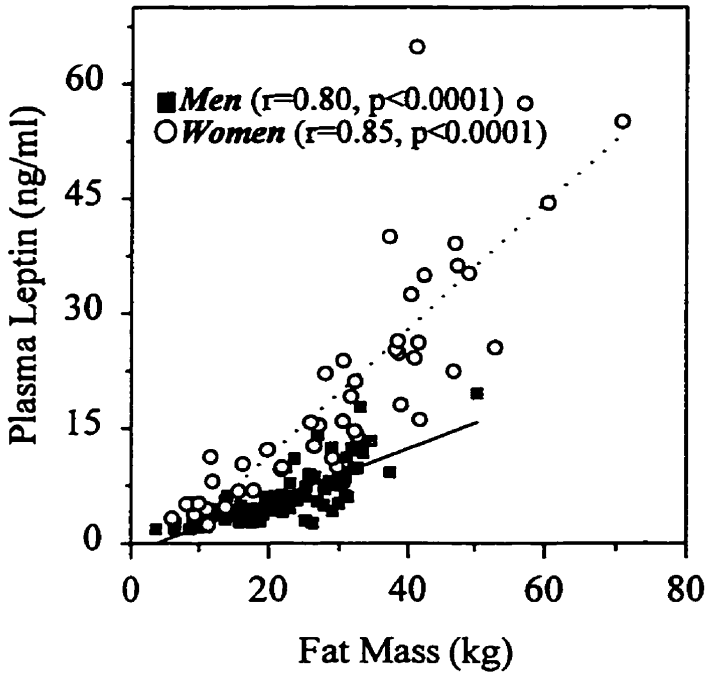
Variables	Plasma Leptin (ng/ml)			
	Not Adjusted		Adjusted for FM	
	Men	Women	Men	Women
Cholesterol (mmol/l)	0.15	0.48 [c]	0.04	0.15
Triglycerides (mmol/l)	0.15	0.41 [c]	-0.08	-0.07
HDL-C (mmol/l)	-0.16	-0.36 [a]	0.15	0.08
HDL_2-C (mmol/l)	-0.21 [a]	-0.40 [b]	0.09	0.02
HDL_3-C (mmol/l)	-0.04	-0.18	0.16	0.12
Apo B (mg/dl)	0.24 [a]	0.59 [c]	0.03	0.07
Cholesterol/HDL-C	0.19	0.56 [c]	-0.09	0.00

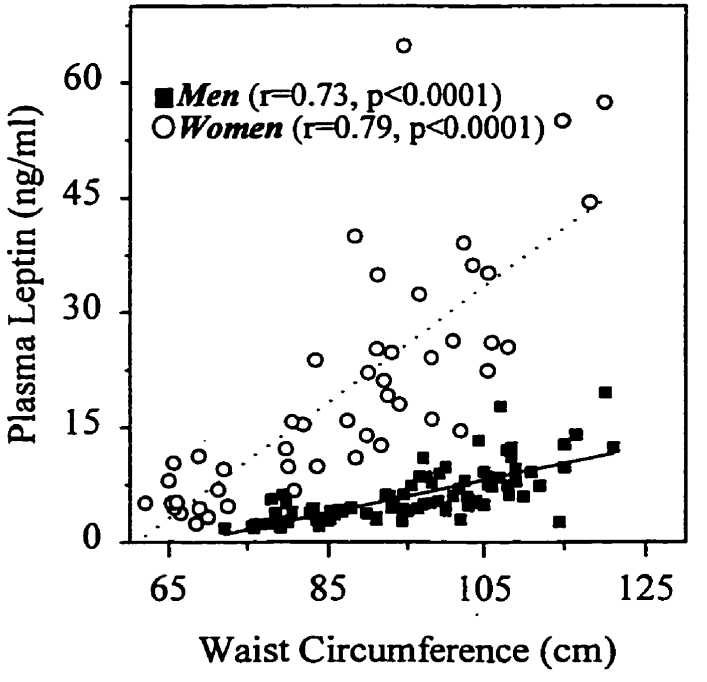
[a] $p<0.05$, [b] $p<0.01$, and [c] $p<0.0005$

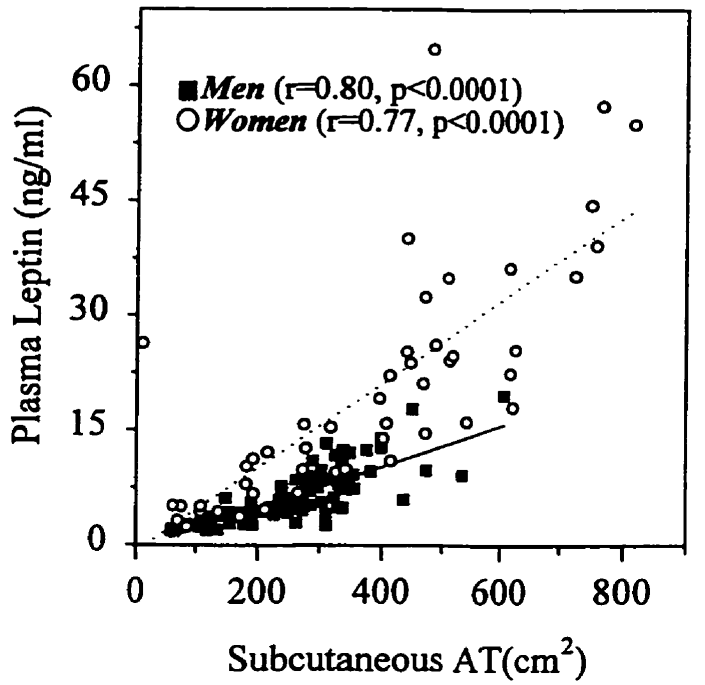
Table 3: Correlation coefficients for the associations between plasma glucose and insulin levels measured in the fasting state and following a 75g oral glucose load (OGTT) versus plasma leptin levels in the 91 men and 48 women.

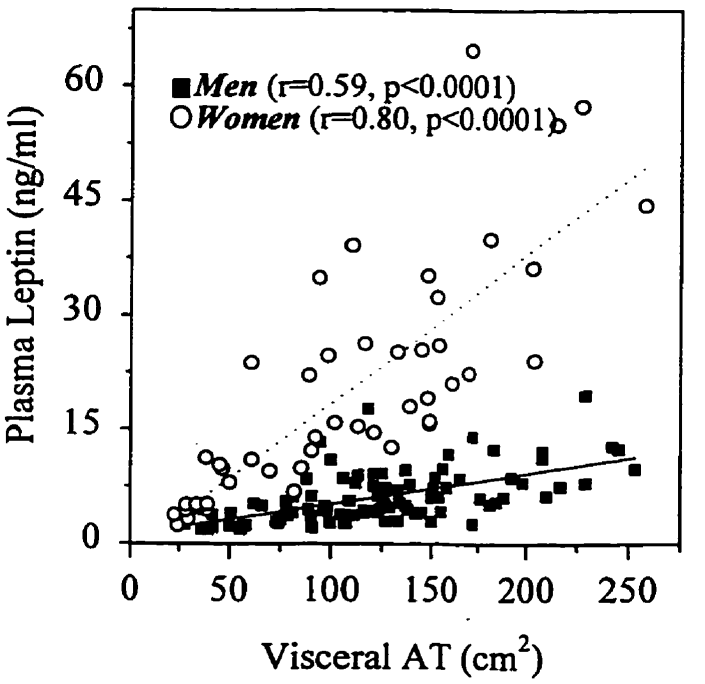
Variables	Plasma Leptin			
	Not adjusted		Adjusted for FM	
	Men	Women	Men	Women
FASTING				
Glucose	0.44 [b]	0.61 [c]	0.18	0.32 [a]
Insulin	0.47 [b]	0.66 [c]	0.18	0.31 [a]
OGTT				
Glucose Area	0.53 [b]	0.55 [b]	0.29	0.35 [a]
Insulin Area	0.47 [b]	0.64 [b]	0.13	0.26

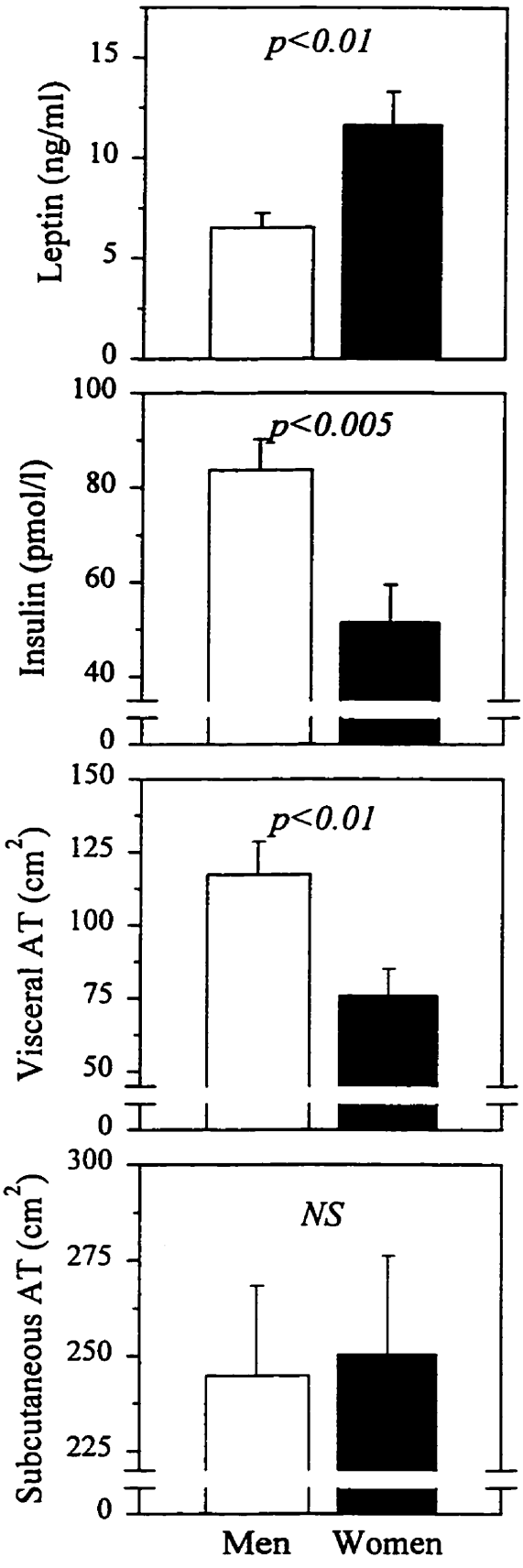
[a] $p<0.05$, [b] $p<0.01$ and [c] $p<0.001$


Men (r=0.80, p<0.0001)
Women (r=0.85, p<0.0001)
Plasma Leptin (ng/ml)
0
15
30
45
60
0
20
40
60
80
Fat Mass (kg)


Men (r=0.73, p<0.0001)
Women (r=0.79, p<0.0001)
Plasma Leptin (ng/ml)
0
15
30
45
60
65
85
105
125
Waist Circumference (cm)


Men (r=0.80, p<0.0001)
Women (r=0.77, p<0.0001)
Plasma Leptin (ng/ml)
0
15
30
45
60
0
200
400
600
800
Subcutaneous AT(cm2)


Men (r=0.59, p<0.0001)
Women (r=0.80, p<0.0001)
Plasma Leptin (ng/ml)
0
15
30
45
60
0
50
100
150
200
250
Visceral AT (cm2)


p<0.01
Leptin (ng/ml)
15
10
5
0
p<0.005
Insulin (pmol/l)
100
80
60
40
0
p<0.01
Visceral AT (cm2)
150
125
100
75
50
0
NS
Subcutaneous AT (cm2)
300
275
250
225
0
Men
Women

CHAPITRE 9

ASSOCIATIONS ENTRE LES CONCENTRATIONS PLASMATIQUES DE LEPTINE, HORMONES STÉROÏDIENNES ET DE GLOBULINE DE LIAISON DES STÉROÏDES (SHBG) CHEZ L'HOMME

L'article composant ce chapitre est intitulé :

Reduced Plasma Sex Hormone-Binding Globulin Levels Are Associated With Increased Plasma Leptin Concentrations in Men

(Soumis pour publication à la revue Journal of Endocrinology)

Reduced Plasma Sex Hormone-Binding Globulin Levels Are Associated With Increased Plasma Leptin Concentrations in Men

RUNNING TITLE : Plasma leptin and SHBG levels in men

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RÉSUMÉ

L'obésité, qui résulte du déséquilibre entre la prise alimentaire et la dépense énergétique, a depuis longtemps été rapportée pour avoir des effets néfastes sur la santé. À cet effet, la découverte de la leptine, hormone capable de réduire la prise alimentaire et d'augmenter la dépense énergétique chez les rongeurs, a permis d'élaborer de nouvelles avenues de recherche visant l'étude de l'étiologie de l'obésité. Chez l'humain, une différence sexuelle dans les niveaux plasmatiques de leptine a été consignée, et il a été suggéré que les hormones sexuelles pourrait être impliquées dans la régulation de la leptinémie. Le but de la présente étude était d'examiner les associations entre la leptinémie à jeun et les concentrations d'hormones stéroïdiennes endogènes de même que celles de la globuline de liaison des hormones stéroïdiennes (SHBG) chez 69 hommes adultes d'âge moyen. Nous avons trouvé que les niveaux de leptine étaient corrélés négativement aux concentrations plasmatiques de testostérone (T ; $r=-0.38$, $p<0.005$), de déhydroépiandrostérone (DHEA ; $r=-0.36, p<0.005$) et d'androstenedione (Δ^4-DIONE ; $p=-0.25, p<0.05$) de même qu'aux rapports oestradiol:T ($r=0.41, p<0.0005$) et d'oestrone: Δ^4-DIONE ($r=0.26, p<0.05$). L'ajustement statistique pour la masse grasse a éliminé toutes les associations entre les niveaux plasmatiques de leptine et d'hormones stéroïdiennes. Toutefois, cette procédure de correction n'a pas réussi à éliminer la corrélation négative observée entre les concentrations de SHBG et de leptine (avant ajustement: $r=-0.39, p<0.001$; après: $r=0.26, p<0.05$). Les résultats provenant de l'analyse de régression multiple de même que la comparaison de sujets appariés pour la quantité de masse grasse mais affichant des concentrations faibles ou élevées d'insuline ont suggéré que l'état hyperinsulinémique retrouvé fréquemment chez les sujets ayant des niveaux élevés de leptine, est associé à la réduction des

niveaux de SHBG observée chez les individus hyperleptinémiques, ces deux conditions étant probablement associées à une activité androgénique faible chez les individus modérément obèses.

ABSTRACT

Obesity, which results from an imbalance between food intake and energy expenditure, has long been recognized to have detrimental effects on health. In this regard, the discovery of leptin, a hormone known to reduce food intake and increase energy expenditure in rodents, has provided some insights in the etiology of obesity. In humans, as a gender difference in plasma leptin concentrations has been reported, it has been suggested that sex steroid hormones could be involved in the regulation of leptinemia. The aim of the present study was to examine the associations between fasting plasma leptinemia and steroid hormone as well as sex-hormone binding globulin (SHBG) concentrations in a sample of 69 middle-aged men. We found that leptin concentrations were negatively associated with testosterone (T; $r=-0.38$, $p<0.005$), dehydroepiandrosterone (DHEA; $r=-0.36$, $p<0.005$) and androstenedione (Δ^4-DIONE; $r=-0.25$, $p<0.05$) concentrations and positively correlated with DHEA-sulfate ($r=0.34$, $p<0.05$) levels as well as with the estradiol:T ($r=0.41$, $p<0005$) and estrone: Δ^4-DIONE ($r=0.26$, $p<0.05$) ratios. Statistical adjustment for body fat mass (FM) eliminated all the relationships between leptin and steroid levels. However, statistical adjustment for FM failed to eliminate the significant negative correlation noted between SHBG and leptin concentrations (before adjustment: $r=-0.39$, $p<0.001$; after $r=-0.26$, $p<0.05$). Results from the multiple regression analyses as well as comparison of subjects matched on the basis of fat mass, but with either low vs high insulin levels, suggested that the hyperinsulinemic state which is commonly found among subjects with increased leptin levels, is correlated with the reduced plasma SHBG concentrations found among « hyperleptinemic » individuals, both conditions being possibly associated with low androgenic activity in those relatively obese subjects.

INTRODUCTION

Chronic positive energy balance resulting from increased food intake and decreased energy expenditure contributes to body fat accumulation and obesity, leading to possible complications having detrimental effects on health [1]. The cloning of the mouse (Lep) and human (LEP) *ob* gene and the characterization of its protein product, leptin [2], has provided a new impetus to the efforts aimed at understanding the pathophysiological aspects of obesity. Leptin has been shown to lower body weight by reducing food intake and increasing energy expenditure in leptin-deficient *ob/ob* mice [2-8]. In both rodents and humans, the leptin gene is expressed only in the adipose tissue (AT) [2,9-13]. Numerous studies have reported a highly significant relationship between adiposity and plasma leptin concentrations or adipose tissue mRNA levels [14-21].

A gender difference in plasma leptin levels, which is thought to be partly independent of body fatness, is commonly observed. Indeed, women have higher levels of leptin compared to men [22-27] and it has been proposed that sex hormones could be involved in this sex dimorphism [27].

On the other hand, associations between obesity and plasma steroid hormone concentrations have also been reported. Obese men are characterized by reduced plasma testosterone (T) and sex hormone-binding globulin (SHBG) levels and by increased estrogen concentrations [28]. It has also been shown that abdominal obese men have decreased C_{19} steroid levels e.g. reduced T, dehydroepiandrosterone (DHEA), androstenedione (Δ^4-DIONE) and androst-5-ene-3β, 17β-diol (Δ5-DIOL) [28,29] concentrations compared to lean controls.

Thus, the aim of the present study was to examine the relationship between fasting leptinemia and plasma steroid and SHBG levels in middle-aged men. The results reported herein indicate that

fasting plasma leptin concentrations are associated with plasma steroid levels but that these relationships are largely mediated by the concomitant variation in body fatness. However, even after statistical adjustment for body fat mass (FM), high plasma SHBG levels remained significantly associated with low leptin concentrations.

SUBJECTS AND METHODS

Subjects. Sixty-nine men (mean age ± SD: 36.6 ± 3.2 years) were recruited by sollicitation through the media to participate in this study, which was approved by the Medical Ethics Committee of Laval University. An informed consent document was signed by all participants. A complete physical examination, which also included medical history, was performed by a physician. All participants were nonsmokers and free from diseases requiring treatment. Exclusion criteria included diabetes, monogenic dyslipidemias or evidence for the presence of coronary heart disease.

Anthropometric Measurements. Weight and height were measured following standardized procedures [30]. Body density was measured by the hydrostatic weighing technique [31], and the mean of six measurements was used in the calculation of body density. Pulmonary residual volume was measured with the helium dilution technique. Percentage body fat was obtained from body density using the equation of Siri [32]. Fat mass was determined by multiplying percent body fat by body weight.

Plasma Steroid and SHBG Levels. After a 12-hour fast, blood was collected between 07h00 and 09h00 in the morning and plasma was obtained after centrifugation at 1500 rpm for 15 minutes. The samples were frozen at -80°C until steroid measurements were performed [33]. Five milliliters of ethanol were added to 1 ml plasma and centrifuged. The resulting pellet was further washed with 5 ml ethanol, and the two ethanol extracts were combined. After evaporation under nitrogen, the residue was suspended in water/methanol (95:5) and chromatographed on C_{18} columns (Amersham,

Oakville, Canada). Unconjugated steroids were isolated by elution with water/methanol (15:85). The solvolysis of sulfated steroids was performed as follows: 0.1 ml of 12 M HCl was added to the water phase and 5 ml of ethyl ether saturated with HCl were added and the mixture was left to stand at room temperature overnight. The organic phase was then evaporated and the residue solubilized with 1 ml of 0.2 M phosphate buffer (pH 7.0) and further extracted twice with 5 ml of ethyl ether. The organic phase was evaporated and the residue kept for separation of non-conjugated steroids. To separate non-conjugated as well as hydrolyzed sulfate derivative steroids, chromatography on LH-20 was performed [34]. After solubilization in 1 ml isooctane/toluene/methanol (90:5:5), the steroids were deposited on Sephadex LH-20 columns (Pharmacia, Uppsala, Sweden). Elution was performed by increasing the polarity of the organic solvent mixture, and four fractions were collected. After deposition of steroids, 15 ml isooctane/toluene/methanol (90:5:5) were eluted and discarded. After addition of 20 ml isooctane/toluene/methanol (90:5:5), Δ^4-DIONE and DHEA were collected. Isolation of testosterone was achieved by elution of another 20 ml of the solvent. The mixture isooctane/toluene/methanol (70:15:15) caused the elution of Δ^5-DIOL and estrone (E_1). Estradiol (E_2) was obtained after elution with isooctane/toluene/methanol (60:20:20). Steroid levels were measured by radioimmunoassay (RIA) using specific antibodies as previously described [33,34]. Immunoreactive SHBG concentrations were measured using the IRMA-count SHBG kit (Diagnostic Products Corporation, Los Angeles, CA).

Plasma Leptin Concentrations. Leptin levels were determined with a highly sensitive commercial double-antibody RIA (Human Leptin Specific RIA Kit, LINCO Research, St-Louis, MO) which

detects relatively low leptin levels of 0.5 ng/ml and which does not crossreact with human insulin, proinsulin, glucagon, pancreatic polypeptide or somatostatin. Our coefficients of variation for the repeated assays ranged from 4.0 to 5.5% for lower leptin concentrations and from 6.5 to 8.5% for higher plasma leptin concentrations.

Plasma Insulin Levels. Blood samples were collected under EDTA and Trasylol (Miles, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein after an overnight fast. Plasma insulin was measured by radioimmunoassay (RIA) with polyethylene glycol separation [35].

Statistical Analyses. Pearson product-moment correlation coefficients were used to quantify the relationships among variables. Multiple regression analyses were performed to estimate the independent contributions of each variable to the variation in fasting plasma leptin concentrations. Body FM as well as plasma insulin, T, DHEA, DHEA-SO_4, Δ^4-DIONE and SHBG levels were considered as independent variables. We also included the E_2:T and E_1:Δ^4-DIONE ratios as independent variables in these analyses. Student t-tests were used to investigate differences between subgroups of subjects matched for total body fat mass but with either low (n=13) or high (n=13) fasting plasma leptin levels using the 50th percentile of the leptin distribution as the cutpoint between the low and high leptin subgroups. The same matching procedure was used for the comparison of subjects with low (n=19) vs high (n=19) fasting plasma insulin levels, again using the 50th percentile of the insulin distribution to separate the low and high insulin subgroups. All analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

As shown in Table 1, subjects were quit heterogeneous in terms of body fatness, as revealed by body FM values ranging from 6 to 37 kg. As expected from the previously reported relationships between plasma steroid levels and adiposity, the men of the present study had relatively low levels of testosterone and SHBG in the plasma (12.4 ± 3.2 nmol/l and 30.5 ± 11.0 nmol/l, respectively).

The associations between plasma steroid and leptin levels are shown in Table 2. High plasma T, DHEA and Δ^4-DIONE concentrations were associated with low plasma leptin levels. Furthermore, DHEA-SO_4 levels as well as E_2:T and E_1:Δ^4-DIONE ratios were positively correlated to fasting plasma leptin concentrations. However, statistical adjustment for body FM, measured by hydrostatic weighing, eliminated those significant relationships.

Figure 1 illustrates the relationship of plasma leptin levels to SHBG concentrations. We found that high plasma leptin levels were associated with low SHBG concentrations ($r=-0.39$, $p<0.001$), the relationship remaining significant after statistical adjustment for body FM ($r=-0.26$, $p<0.05$).

We then performed multiple regression analyses in order to quantify the individual contribution of steroid hormone, SHBG and insulin levels as well as FM to the variation in fasting plasma leptin concentrations (Table 3). When only plasma steroid hormone and SHBG levels were included in the statistical model *(Model 1)*, SHBG concentrations explained 15.2% ($p<0.001$) of the variance in plasma leptin levels. Addition of plasma insulin level as an independent variable *(Model 2)* attenuated the contribution of SHBG concentration to the variation of leptinemia, although it remained statistically significant ($p<0.05$). In *Model 2*, insulin levels explained 21.7% of the variance in leptin concentrations ($p<0.0001$). However, FM was by far the best predictor of plasma

leptin concentrations (72.9%; *Model 3*). Although body FM was a strong contributor to the variation in plasma leptin levels, insulin concentration remained a significant predictor of plasma leptinemia. Furthermore, although the variance in leptinemia explained by SHBG was almost similar to the contribution of insulin in the latter model (1.2% vs 1.9% respectively), it did not reach statistical significance ($p=0.08$).

Finally, in an attempt to provide further insight into the relationships between leptin, insulin and SHBG levels, we matched subjects on the basis of body FM and classified them according to fasting plasma leptin concentration (low vs high; Figure 2). No difference in SHBG levels was noted between those with low vs high fasting leptin concentrations. We conducted similar analyses in men matched for body FM but with low vs high insulin levels. In this latter case, men with increased insulin concentrations had significantly lower SHBG levels compared to those with low insulin concentrations.

DISCUSSION

Leptin, the *ob* gene product, is a hormone which increases energy expenditure and decreases food intake in selected rodent models [2-8]. The *ob* gene is exclusively expressed in AT [2,9-13] and plasma leptin concentrations have been closely associated with body fatness [14-21]. Furthermore, obesity is related to alterations in plasma steroid hormone levels. Indeed, reduced plasma T, DHEA, Δ^4-DIONE and Δ^5-DIOL as well as SHBG concentrations have been reported in obese men [28,29]. Obesity is also associated with high plasma estrogen levels in men [28].

Gender differences in plasma leptin levels, independent of the expected sex dimorphism in body fatness, have been reported with women are having higher leptin concentrations than men [22-27]. Thus, it has been proposed that differences in sex steroid concentrations between men and women could explain, at least in part, this sexual dimorphism in plasma leptin levels [27]. Indeed, it was suggested that estrogen and/or progesterone could increase while androgens could decrease the LEP gene expression and leptin secretion. In contrast, Saad et al [36] have suggested that sex steroid hormones did not seem to play a major role in the explanation of the gender difference in fasting leptinemia. In the present study, we found significant associations between leptin concentrations and plasma steroid hormone levels as low T, DHEA and Δ^4-DIONE levels but high DHEA-SO_4 concentrations, E_2:T and E_1:Δ^4-DIONE ratios were found in men with high plasma leptin levels. However, these associations were strongly dependent upon the degree of adiposity as statistical adjustment for body fat mass eliminated all these relationships. A recent report by Haffner et al [37] also indicated that the significant relationships between leptin and sex hormone concentrations were dependent upon adiposity. Thus, these results taken together do not suggest an independent

contribution of testosterone and more generally of C_{19} steroids in the gender difference observed in leptin concentrations.

On the other hand, we found a significant negative correlation between plasma leptin and SHBG levels with high leptin concentrations being associated with low SHBG levels. In contrast to steroid hormones, this relationship did not depend upon the degree of adiposity of the subjects as statistical adjustment for body FM had little effect on the association. Since SHBG levels are increased by intracellular androgens [38] and DHEA, Δ^5-diol and Δ^4-dione are transformed by an intracrine mechanism [39,40] without parallel changes in the circulation, the statistically significant correlation between SHBG and leptin could reflect this mechanism.

Although the study by Haffner and colleagues [37] reached similar conclusions, one limitation of their study was that no direct measurement of body fatness had been performed. In our study, total body FM was assessed by underwater weighing. Results from the multiple regression analyses revealed that when body FM and insulin were used as independent variables in the prediction of fasting plasma leptin level, SHBG concentration was no longer a significant contributor to fasting leptinemia. In order to further investigate this issue, we have compared subjects matched for FM but with either low vs high fasting plasma insulin levels. We found that subjects with elevated insulin concentrations also displayed significantly decreased plasma SHBG levels compared to those with low plasma insulin levels. This difference in SHBG levels was not found when subjects matched for body FM but characterized by low vs high fasting plasma leptin levels were compared. Although the physiological relevance of this observation is not clear at this time, a possible mechanism can be proposed. Insulin has been reported to reduce the production of SHBG by HepG2 cells [41]. Hence,

insulin could be an important modulator of SHBG metabolism [42-44]. Such an effect could also be explained by the high fasting insulin levels observed with hypoandrogenecity. Low androgens, high insulin as well as obesity and high leptin concentrations are associated with hyperinsulinemia [17,20,25,45], the relationship between leptin and SHBG levels noted in the present study could reflect, at least in part, the known effect of insulin on SHBG production or may result from hypoandrogenecity. This possibility is supported by the suggestion that SHBG levels may better reflect plasma levels of free and active steroid concentrations [38] and especially the intracellular concentration of steroids made locally by intracrine mechanism [39,40,47].

In summary, the results of the present study indicate that sex steroid hormone levels in men are associated with fasting leptin concentrations. These associations are largely explained by the concomitant variation in the level of total body fat which, in turn, may result from changes on androgenic activity. Hyperleptinemia is associated with reduced SHBG levels before and after statistical adjustment for body fat mass. Although more studies are needed to fully understand the physiological relevance of this phenomenon, it appears that the hyperinsulinemic state often found in subjects with increased leptin concentrations could explain, at least in part, the lower SHBG plasma concentrations found in obese men.

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FIGURE HEADINGS

Figure 1: Relationship between fasting plasma leptin (log_{10}) and SHBG concentrations in the 69 men of the study.

Figure 2. Body fat mass as well as plasma leptin, SHBG and insulin concentrations of men matched on the basis of fat mass (within a difference of 1 kg) but with low (white bars) vs high (black bars) leptin (panel A; 13 pairs) or insulin levels (panel B; 19 pairs). Values are expressed as mean ± SEM.

Table 1: Physical, metabolic and hormonal characteristics of the sample of 69 men

Variables	*Mean ± SD*	*Range*
Age (years)	36.6 ± 3.2	30 - 42
BMI (kg/m^2)	27.2 ± 3.7	20 - 34
%Body Fat	26.0 ± 6.6	10 - 39
Fat Mass (kg)	21.9 ± 8.0	6 - 37
Fasting Leptin (ng/ml)	5.88 ± 2.95	1.82 - 13.32
Fasting Insulin (pmol/l)	79.5 ± 31.1	29.5 - 181.5
Plasma Steroids		
Testosterone (nmol/l)	12.4 ± 3.2	6.8 - 22.2
DHEA (nmol/l)	13.0 ± 5.9	4.5 - 33.3
DHEA-SO_4 (nmol/l)	5571 ± 3671	891 - 21 694
Δ^4-DIONE (nmol/l)	1.7 ± 0.6	0.9 - 3.5
E_1 (pmol/l)	492.6 ± 125.4	201.1 - 928.7
E_2 (pmol/l)	134.6 ± 38.4	61.0 - 247.1
E_2:Testosterone	11.5 ± 4.2	4.1 - 24.3
E_1:Δ^4-DIONE	318.7 ± 137.8	78.0 - 787.0
SHBG (nmol/l)	30.5 ± 11.0	12.0 - 61.1

Table 2: Pearson correlation coefficients between plasma steroid and fasting plasma leptin concentrations, before and after adjustment for body fat mass.

Plasma Steroids	*Plasma Leptin*[1]	
	Unadjusted	*Adjusted for FM*
Testosterone	-0.38 **	-0.18
DHEA	-0.36 **	-0.01
DHEA-SO$_4$	0.34 **	-0.10
Δ^4-DIONE	-0.25 *	-0.09
E_1	0.21	-0.04
E_2	0.19	-0.09
E_2/Testosterone	0.41 ***	0.05
E_1/Δ^4-DIONE	0.26 *	0.05

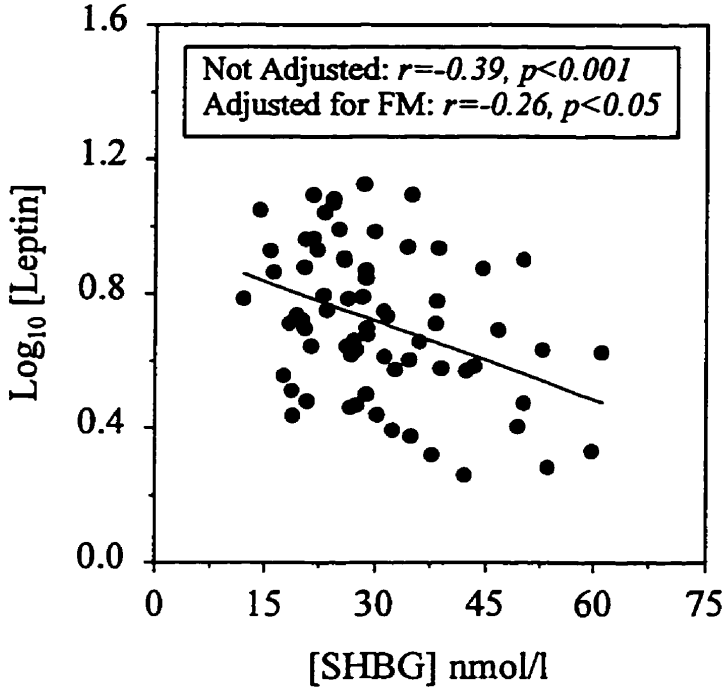
[1] Leptin concentrations are $\log_{10}$ transformed
* $P<0.05$, ** $P<0.005$ and *** $P<0.0005$

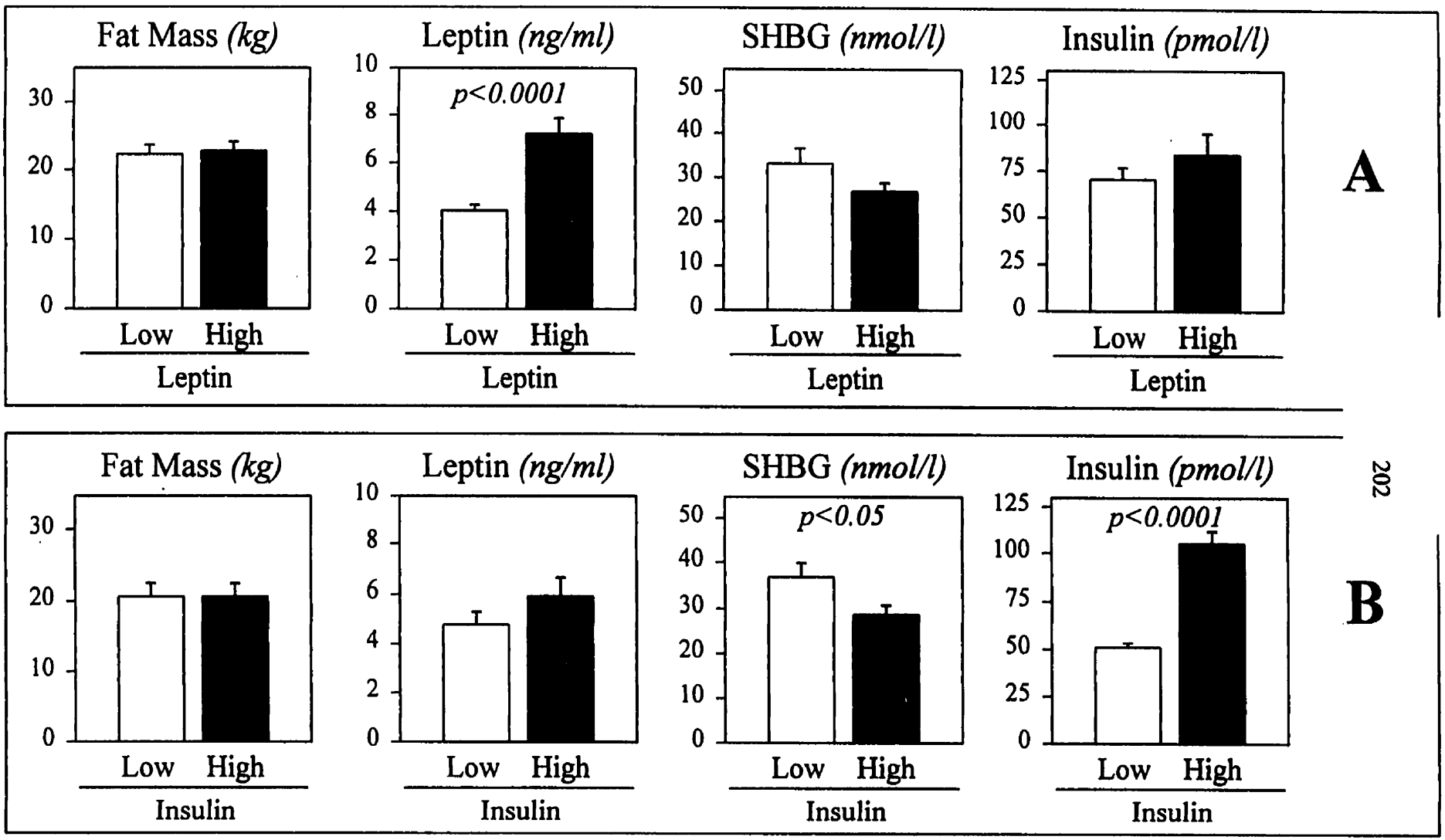
Table 3: Multivariate regression analyses showing the independent contributions of body fat mass, plasma steroids (testosterone, DHEA, DHEA-SO_4, Δ^4-DIONE, E_1:Testosterone, E_2:Δ^4-DIONE), insulin and SHBG to the variation in fasting leptin levels.

Dependent Variable	*Independent Variable*	*Partial (R^2 X 100)*	*Total (R^2 X 100)*	*P <*
Model 1				
Fasting Leptin[1]	SHBG	15.2	32.9	0.001
	DHEA	9.0		0.01
	DHEA-SO_4	8.7		0.01
Model 2				
Fasting Leptin	Fasting Insulin	21.7	43.1	0.0001
	DHEA	8.7		0.01
	DHEA-SO_4	8.1		0.01
	SHBG	4.7		0.05
Model 3				
Fasting Leptin	Fat mass	72.9	74.8	0.0001
	Fasting Insulin	1.9		0.05

Model 1: Included T, DHEA, DHEA-SO_4, Δ^4-DIONE, E_1:Testosterone, E_2:Δ^4-DIONE and SHBG
Model 2: All variables of model 1 with addition of fasting insulin
Model 3: All the variables of model 2 with addition of fat mass

[1] Plasma leptin concentrations were $\log_{10}$ transformed for all analyses


Not Adjusted: r=-0.39, p<0.001
Adjusted for FM: r=-0.26, p<0.05
Log10 [Leptin]
1.6
1.2
0.8
0.4
0.0
0
15
30
45
60
75
[SHBG] nmol/l


Fat Mass (kg)
30
20
10
0
Low High
Leptin
Leptin (ng/ml)
10
8
6
4
2
0
p<0.0001
Low High
Leptin
SHBG (nmol/l)
50
40
30
20
10
0
Low High
Leptin
Insulin (pmol/l)
125
100
75
50
25
0
Low High
Leptin
A
Fat Mass (kg)
30
20
10
0
Low High
Insulin
Leptin (ng/ml)
10
8
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4
2
0
Low High
Insulin
SHBG (nmol/l)
50
40
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10
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p<0.05
Low High
Insulin
Insulin (pmol/l)
125
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25
0
p<0.0001
Low High
Insulin
B

CHAPITRE 10

L'HYPERLEPTINÉMIE EST ASSOCIÉE PLUS ÉTROITEMENT À L'HYPERTROPHIE DES CELLULES ADIPEUSES QU'À L'HYPERPLASIE DU TISSU ADIPEUX

L'article composant ce chapitre est intiulé :

Hyperleptinemia is more closely associated with adipose cell hypertrophy than with adipose tissue hyperplasia

Hyperleptinemia is More Closely Associated With Adipose Cell Hypertrophy Than With Adipose Tissue Hyperplasia

RUNNING TITLE : *Hypertrophy of Adipose Tissue and Hyperleptinemia*

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RÉSUMÉ

Le clonage du gène obèse et la caractérisation de sa protéine, la leptine, a permis l'étude d'une nouvelle hormone possiblement impliquée dans la régulation de la masse adipeuse. Cependant, les mécanismes physiologiques par lesquels la production de leptine par le tissu adipeux est régulée demeurent méconnus. La présente étude a pour but d'examiner les associations entre le poids adipocytaire de même que l'estimation du nombre de cellules adipeuses, et la concentration plasmatique de leptine chez 63 hommes (âge moyen ± déviation standard : 36 ± 4 années) et 42 femmes (35 ± 5 années). Tant chez l'homme que chez la femme, les poids adicpocytaires moyens abdominal et fémoral de même que l'estimé du nombre total d'adipocytes étaient positivement corrélés aux indices d'adiposité corporelle et de distribution du tissu adipeux (r compris entre 0.28 et 0.84). En moyenne, les femmes avaient un poids adipocytaire abdominal et fémoral plus élevé que les hommes. De plus, cette différence sexuelle dans l'adiposité était associée à des niveaux de leptine plus élevés chez la femme que chez l'homme. Tant chez l'homme que chez la femme, l'augmentation du poids adipocytaire abdominal était associée à des concentrations plasmatiques de leptine et d'insuline plus élevées. Toutefois, les associations entre le poids adipocytaire fémoral et la leptine de même que l'insuline n'ont été retrouvées que chez la femme. Chez l'homme, l'insuline et la leptine étaient corrélées à l'estimation du nombre d'adipocytes tandis que chez la femme, une telle association n'était observée qu'avec l'insuline plasmatique. La correction statistique pour l'accumulation de masse grasse a éliminé toutes les associations significatives, à l'exception de la relation entre le poids adipocytaire abdominal et la leptine chez la femme ($r=0.35, p<0.05$). De plus, lorsque des groupes d'hommes et de femmes étaient

appariés pour la concentration plasmatique de leptine, aucune différence n'était observée au niveau du poids adipocytaire tant abdominal que fémoral et ce, malgré un nombre réduit d'adipocyte chez les femmes comparativement aux hommes. Les résultats de la présente étude indiquent que, tant chez l'homme que chez la femme, le poids adipocytaire est positivement corrélé aux concentrations plasmatiques de leptine. De plus, nos résultats démontrent que la relation entre le poids adipocytaire abdominal et la leptinémie est indépendante de la quantité totale de masse grasse. Ces observations confirment le rôle de la grosseur de la cellule adipeuse dans la régulation de la production de leptine par le TA. Étant donné que les femmes sont caractérisées par des adipocytes plus gros que les hommes, nos résultats laissent entrevoir une explication pour la différence sexuelle des niveaux plasmatiques de leptine chez l'humain.

ABSTRACT

The cloning of the *obese* gene and the characterization of its protein product, leptin, has allowed the study of a new hormone produced by the adipose tissue and potentially involved in the regulation of energy balance and adipose tissue (AT) mass. Furthermore, the physiological mechanisms implicated in the regulation of leptin production by AT in humans are not fully understood. The present study examined the relationships of fat cell weight (FCW) as well as of estimated total adipose cell number, to fasting plasma leptin concentration in samples of 63 men (mean age ± SD: 36.2 ● 3.8 years) and 42 women (34.7 ● 4.6 years). In both genders, mean abdominal and femoral FCW as well as the estimated adipose cell number were positively correlated with body fatness and AT distribution variables ($0.28 \leq r \leq 0.84$). Larger abdominal and femoral FCW were found in women than in men. This gender difference in adipose cell size was associated with increased leptin levels in women compared to men. In both men and women, increased abdominal FCW was associated with higher plasma leptin concentrations. However, the association between femoral FCW and leptinemia was only significant in women. Contrary to women, plasma leptin levels were associated with the estimated adipose cell number in men. Results from multiple regression analyses revealed that gender (38%), abdominal FCW (15.8%) and the estimated adipoce cell number (4.4%) were all significant predictors of fasting leptinemia. Thus, results of the present study indicate that in men and women, both the abdominal and femoral FCW are positively associated with plasma leptin concentrations. Furthermore, results from the multiple regression analyses suggest that the association between plasma leptin and abdominal FCW is independent of the expected concomitant variation in the level of total body fat. These findings provide further

support to the observation that adipose tissue leptin secretion may be upregulated by adipocyte size. The present study suggest that the higher plasma leptin levels found in women than in men could be explained, at least to a certain extent, by the gender difference in adipose cell size and number.

INTRODUCTION

The discovery of leptin [1] has been an important breakthrough in the understanding of the pathophysiology of obesity [2]. Indeed, when administered to obese leptin-deficient mice *(ob/ob)*, leptin has been shown to lower body weight by reducing food intake and increasing energy expenditure [1,3-8]. Concordant with the exclusive secretion of leptin in adipose cells and expression of the *ob* gene in adipose tissue (AT) [1,9-13], numerous studies have reported a strong relationship between adiposity and plasma leptin concentrations or adipose tissue mRNA levels [14-22].

Furthermore, in humans, a gender difference, which is independent of adiposity, has been noted in plasma leptin concentrations as women are characterized by increased leptin levels compared to men [22-27]. Although it has been suggested that sex steroid hormones may be implicated in this gender difference [28], androgens tending to decrease leptin levels [29-31] while estrogens would increase leptinemia [32], they do not seem to be the only factors responsible for this discrepancy. With that in mind, a regional difference in *ob* gene expression has been reported i.e. that adipocytes from subcutaneous depots produce more leptin than fat cells obtained from intraabdominal depots [33,34]. In addition, it has been suggested that fat cell size could be an important contributor to leptin secretion by the AT. Indeed, smaller adipocytes are characterized by lower expression of the *ob* gene and secretion of leptin compared to larger fat cells [35,36].

As women are genrally characterized by larger adipocytes and by greater estimated total number of adipose cells compared to men [37-40], we examined the potential relationships between subcutaneous abdominal as well as femoral fat cell weight (FCW), the estimated adipose cell

number, and plasma leptin levels in both men and women. Results of the present study indicate that fat cell size is more closely associated with plasma leptin levels than the estimated total number of adipose cells. Thus, our results suggest that adipose cell hypertrophy and hyperplasia both contribute to the higher plasma leptin levels found in women compared to men. However, other factors are involved in the well-documented sex dimorphism in leptinemia.

Key Words: *Leptin, gender differences, adipocyte size*

SUBJECTS AND METHODS

Subjects. Sixty-three men (mean age ± SD: 36.2 ± 3.8 years) and forty-two women (34.7 ± 4.6 years) were recruited through the media to participate in this study, which was approved by the Medical Ethics Committee of Laval University. An informed consent document was signed by all participants. A complete physical examination, which also included medical history, was performed by a physician. All participants were nonsmokers and free from diseases requiring treatment. Exclusion criteria included diabetes, monogenic dyslipidemias, evidence for the presence of coronary heart disease as well as use of medication known to affect carbohydrate and lipid metabolism.

Anthropometric measurements. Weight and height were measured following the procedures recommended at the Airlie Conference [41]. Body density was measured by the hydrostatic weighing technique [42], and the mean of six measurements was used in the calculation of body density. Percentage body fat was obtained from body density using the equation of Siri [43].

Computed tomography. CT was performed on a Siemens Somatom DRH scanner (Erlagen, Germany) using previously described procedures [44,45]. Briefly, the subjects were examined in the supine position with both arms stretched above the head. A single CT scan was performed at the abdominal level (between L4 and L5 vertebrae) with a scout abdominal radiograph used as a reference to establish the position of the scan to the nearest millimeter. Total AT area was calculated by delineating the area with a graph pen and then computing the AT surface with an attenuation

range of -190 to -30 Hounsfield Units [44-46]. The 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 biopsies and determination od adipose cell size and number. Following skin anesthesia with 1% xylocaine, 1 cm incisions were performed in the abdominal and femoral regions, and adipose tissue was surgically removed. After collection, adipose tissue was quickly transferred to the laboratory, in saline (0.9% NaCl)-HEPES (5mM) (pH 7.4). Adipocytes were isolated according to the method of Rodbell [47] in Krebs-Ringer bicabonate buffer (pH 7.4) containing 4% bovine serum albumin (KRBA) and 5mM glucose, plus 1 mg/ml collagenase as already described [48]. Digestion took place in shaking water bath under a gas phase of 95% O_2 and 5% CO_2, for 40 minutes at 37°C. The suspension was then filtered and the cellular filtrate obtained was rinsed 3 times with 5 ml KRBA. Isolated adipocytes were finally resuspended in KRBA, to obtain a final concentration of approximately 500 cells per 50 µl. Adipose cell diameters were determined using a Leitz microscope equipped with a graduated ocular (Rockleigh, NJ). Mean fat cell diameter was assessed from the measurement of >500 cells, and the density of triolein was used to transform adipose cell volume into fat cell weight, aspreviously described [48]. Total adipose cell number was calculated using the following equation:

$$\frac{\text{Total body fat mass}}{(\text{Abdominal FCW} + \text{Femoral FCW})*0.5}$$

Although we are aware that this value does not fully take into account the regional differences in fat cell sizes, e.g. intraabdominal cells are generally smaller than subcutaneous adipocytes, we believe that it offers a fairly useful estimation of the total number of adipocytes.

Plasma insulin and leptin concentrations. Blood samples were collected under EDTA (Miles, Rexdale, Ontario, CANADA) through a venous catheter from an antecubital vein in the morning after a 12-hour fast, and plasma insulin was measured by RIA with polyethylene glycol separation [49]. Plasma leptin concentrations were determined with a highly sensitive commercial double-antibody RIA (Human Leptin Specific RIA Kit, LINCO Research, St-Louis, MO) which detects relatively low leptin levels of 0.5 ng/ml and which does not crossreact with human insulin, proinsulin, glucagon, pancreatic polypeptide or somatostatin. Our coefficients of variation for the repeated assays ranged from 4.0 to 5.5% for lower leptin concentrations and from 6.5 to 8.5% for higher plasma leptin concentrations.

Statistical analyses. Student t-tests were used to examine gender differences in abdominal and femoral FCW as well as the total body estimated adipose cell number. Pearson product-moment correlation coefficients were used to examine associations among variables. Multiple regression analyses were also performed to estimate the independent contribution of gender, body fat mass, abdominal and femoral FCW as well as the estimated adipose cell number to the variation in fasting plasma leptin concentrations. All analyses were performed with the SAS statistical package (SAS Institute, Cary, North Carolina, USA).

RESULTS

Table 1 shows the subjects' physical characteristics. There was a significant gender difference in body fatness as women had higher BMI, percentage of body fat and total body fat mass values compared to men. We also observed differences in body fat distribution between men and women. Indeed, men were characterized by an increased abdominal visceral AT accumulation, while women displayed higher levels of subcutaneous fat in the abdominal region. This increased adiposity found in women was also accompanied by significantly higher abdominal and femoral FCW compared to men. A trend for an increased estimated number of adipocytes was noted in women, but this difference did not reach statistical significance ($p=0.07$). As body fatness and plasma leptin concentrations are closely related, we also noted a significant and expected gender difference in leptinemia. Indeed, women showed plasma leptin levels that were 3-times as high as those noted in men.

Relationships of FCW as well as the estimated adipose cell number to total body fatness and AT distribution variables are presented in Table 2. The estimated adipose cell number as well as both the abdominal and femoral FCW were significantly correlated with all body fatness and AT distribution indices in both men and women.

Figures 1 illustrates the relationships of abdominal and femoral FCW to fasting plasma insulin as well as leptin concentrations. In both genders, abdominal FCW was positively associated with plasma insulin and leptin levels, although the associations were stronger in women than in men. Furthermore, in women, increased femoral FCW was associated with higher plasma insulin and leptin concentrations while in men, neither plasma insulin nor leptin levels were associated with

femoral FCW. As shown in Figure 2, elevated plasma insulin was correlated with an increased estimated adipose cell number in both genders. However, while men showed a significant association between the estimated fat cell number and leptinemia, this association was not found in women.

We also performed multiple regression analyses in order to quantify the independent contributions of gender, body fat mass, abdominal and femoral FCW as well as the estimated adipose cell number to the variation in fasting plasma leptin concentrations (Table 3). In all these analyses, total body fat mass was not a significant predictor of leptinemia after including gender, abdominal FCW and estimated fat cell number in the model. Furthermore, abdominal FCW (15.8%) and the estimated adipose cell number (4.4%) contributed significantly to the variance in leptinemia. However, in our study, gender was by far the best predictor of plasma leptin concentrations explaining 38% of its variation. It is also relevant to note that after control for the above variables, fasting insulin concentration did not contribute significantly to the variance in leptinemia.

DISCUSSION

Gender differences in body fatness, AT distribution and in the metabolic risk profile reported in the present study have been previously well documented [22]. In the present study, a gender difference in adipocyte size was found as women had increased FCW both in the abdominal and femoral regions compared to men. This observation is also concordant with previous results from our laboratory and from other groups [37-40].

Although significant associations between FCW and body fatness were found in both genders, the relationship of adiposity to femoral FCW was weaker in men compared to women. This is not surprising, considering that men are generally characterized by abdominal obesity and by less subcutaneous fat than women [2,50,51]. Furthermore, much smaller in men than in women, which may explain the weaker relationship between body fatness and femoral FCW in male subjects of the present study.

Abdominal obesity has also been associated with hyperinsulinemia [2,51]. Accordingly, in the present study, increased abdominal FCW was associated with higher plasma insulin and leptin concentrations in both men and women. However, associations between femoral FCW and plasma insulin as well as leptin levels were only noted in women. Since plasma leptin concentrations have not been reported to be associated with regional AT distribution [22,52-54], we believe that the reduced AT accumulation in the femoral region in men may account for the absence of relationship found in our male subjects. Overall, our observations support the notion that fat cell size is an important correlate of leptinemia [35,36]. On the other hand, AT hyperplasia was also associated with hyperleptinemia, but this association was only noted in men. Since adipocytes are generally

smaller in men than in women [37-40], our results suggest that, in men, varaition in estimated adipose cell number have a gerater impact on leptinemia in men than in women. Further studies will be necessary to better explain this phenomenon.

The mechanism by which an enlarged adipocyte would produce more leptin is not well known. In the present study, plasma insulin concentrations were associated with abdominal FCW in both men and women. In addition, insulin has been reported to up-regulate *ob* gene expression [3,13,55,56] and be positively associated with plasma leptin concentration [14-21]. On the other hand, in our study, men were characterized by increased visceral AT accumulation while women had a greater abdominal subcutaneous fat deposition. Visceral adipose cells have been shown to be more insulin resistant than subcutaneous adipocytes. Thus, despite hyperinsulinemia, it is possible that the insulin resistant visceral fat depot produces less leptin than the insulin sensitive subcutaneous fat. Indeed, in vitro studies have suggested that leptin production in the adipose cell may be related more to its metabolic state than to its size. Thus, activation of lipolysis would down regulate the *ob* gene expression (energy mobilization) whereas stimulation of fat accumulation and inhibition of lipolysis by insulin would incresae leptin production, sending a signal to the brain that there is accumulation of fat.

The present study suggest that fat cell weight a better correlate of plasma leptin concentration than fat cell number. However, the present study was not designed to identify which mechanism(s) is(are) responsible for such relationship. Furthermore, in the present study, despite significant contributions of abdominal FCW and estimated adipose tissue cell number to fasting leptinemia, gender was by far the best predictor of plasma leptin concentrations explaining 38% of

its variance. This suggests that, in addition to FCW and adipose cell number, other factors are likely to be implicated in the regulation of leptin prodcution and secretion by AT. Exclusion of gender from the multiple regression analyses revealed that adipose tissue mass was the major factor responsible for the contribution of gender to leptinemia (data not shown). Thus, results of the present study suggest that irrespective of the type of fat accumulation found (hypertrophic or hyperplastic), excess fatness per se is associated with higher leptin levels. In addition, further studies on the role of insulin and steroid hormones in the regulation of leptin will be needed, as they have both shown to influence *ob* gene expression [3,13,29-32,55,56].

In summary, results of the present study indicate that increased abdominal as well as femoral FCW are associated with hyperleptinemia in both genders. These results suggest that variation in fat cell size may have a significant impact of adipose tissue leptin production and secretion. As women are characterized by larger adipocytes than men, this phenomenon may explain, at least in part, the gender difference in leptinemia. Furthermore, our results also suggest that plasma leptin concentration is more closely associated with abdominal FCW than with the estimated number of adipocytes of an individual. However, as the hyperplastic/hypertrophic nature of adipose tissue only has a small contribution to the variation in leptinemia, additional studies are needed to better understand the physiological mechanism(s) responsible for higher leptin levels noted in women compared to men.

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FIGURE HEADINGS

Figure 1: Associations between abdominal (panels A and C) as well as femoral (panels B and D) adipose cell weight, expressed in μg lipid/cell, and fasting plasma insulin (panels A and B) and leptin (panels C and D) concentrations in 63 men (●) and 42 women (○).

Figure 2: Associations between the estimated total number of adipose cells and fasting plasma insulin (upper panel) and leptin (bottom panel) concentrations in 63 men (●) and 42 women (○).

Table 1: Physical and metabolic characteristics of the subjects

Variables	Men (n=63)	Women (n=42)
Age (years)	36.2 ± 3.8	34.7 ± 4.6
Weight (kg)	81.8 ● 12.2	77.8 ± 19.6
BMI (kg/m²)	27.2 ± 3.8	30.3 ± 8.4 *
%Body fat	26.4 ● 6.3	39.5 ± 10.4 ***
FM (kg)	22.1 ± 7.6	32.4 ± 15.4 ***
CT-Derived Abdominal Adipose Tissue Areas (cm²)		
Subcutaneous	246 ± 101	411 ± 189 ***
Visceral	121 ± 47	101 ± 48 *
Adipose Cell Weight (μg lipid/cell)		
Abdominal	0.52 ● 0.13	0.64 ± 0.24 **
Femoral	0.55 ± 0.12	0.72 ± 0.21 ***
Adipose Cell Number (x10⁹ cells)[1]	4.14 ± 1.23	4.66 ± 1.58 #
Fasting Leptin (ng/ml)	6.3 ± 4.1	18.5 ± 11.2 ***
Fasting Insulin (pmol/l)	79.3 ● 36.4	70.6 ± 47.0

Values are expressed as means ± SD.
[1] Estimated adipose cell number *(please refer to the Subjects and methods section)*
Gender differences: # p=0.07, * p<0.05, ** p<0.005 and *** p<0.0001
BMI: Body-Mass Index; FM: Fat Mass; CT: Computed Tomography

Table 2: Associations between mean abdominal and femoral fat cell weight (FCW) as well as estimated total body adipose cell number versus adiposity indices in the 63 men and 42 women of the study.

Variables	Abdominal FCW		Femoral FCW		Adipose Cell Number[1]	
	Men	Women	Men	Women	Men	Women
Weight	0.37 **	0.67 £	0.36 **	0.55 £	0.81 £	0.82 £
BMI	0.50 £	0.64 £	0.37 **	0.53 §§	0.72 £	0.84 £
% Body Fat	0.60 £	0.78 £	0.47 £	0.61 £	0.75 £	0.72 £
Fat Mass	0.52 £	0.72 £	0.44 £	0.59 £	0.84 £	0.82 £
CT-Derived Adipose Tissue Areas						
Visceral	0.61 £	0.72 £	0.28 *	0.49 **	0.43 §	0.69 £
Subcutaneous	0.56 £	0.78 £	0.40 £	0.62 £	0.74 £	0.60 £

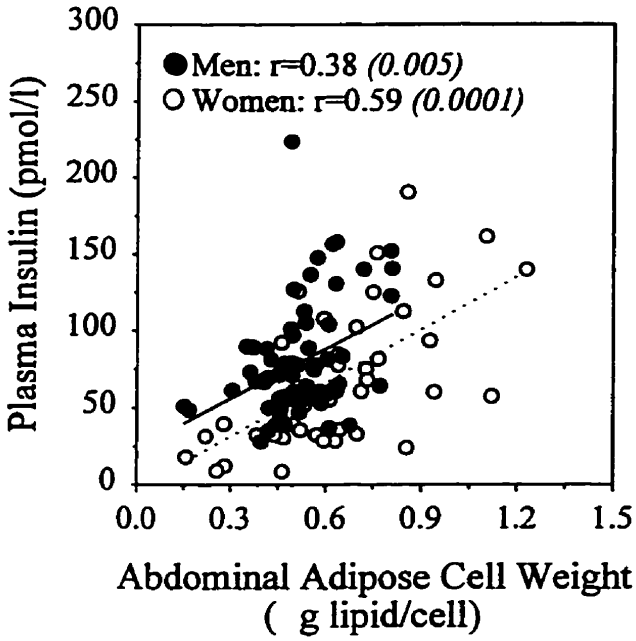
* $p<0.05$, ** $p<0.005$, § $p<0.001$, §§ $p<0.0005$ and £ $p<0.0001$

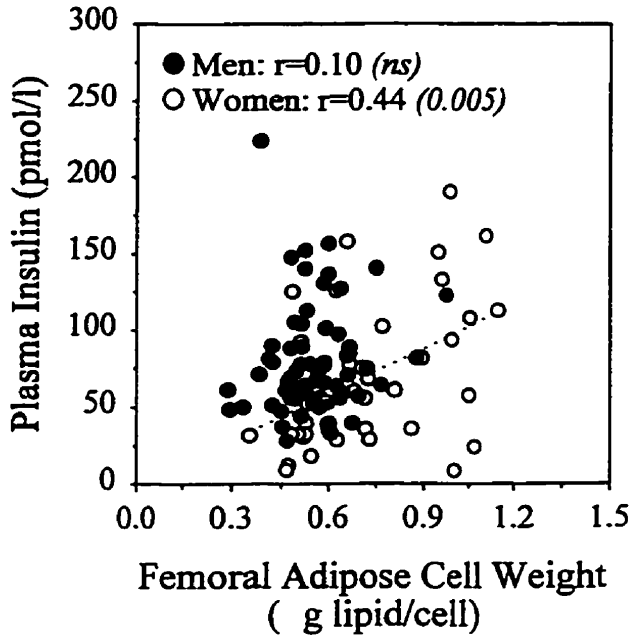
[1] Estimated adipose cell number *(please refer to the Subjects and methods section)*

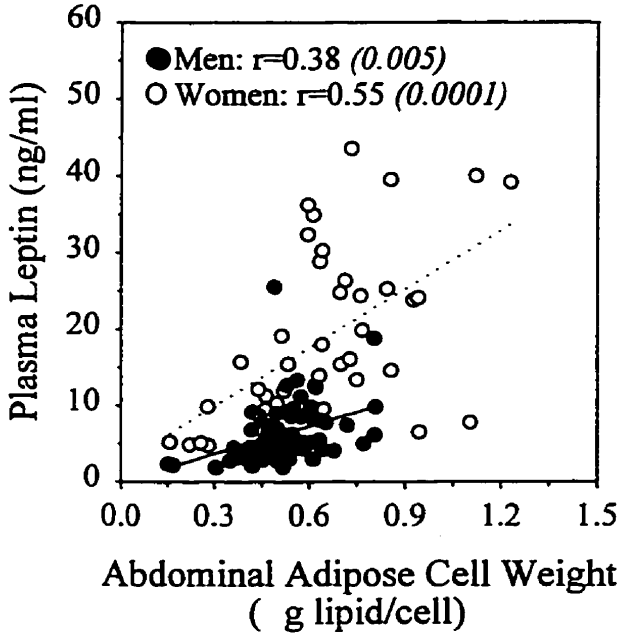
Table 3: Multivariate regression analyses showing independent contributions of gender, fasting insulin, adipose cell weight and estimated number to the variance of fasting plasma leptin concentrations

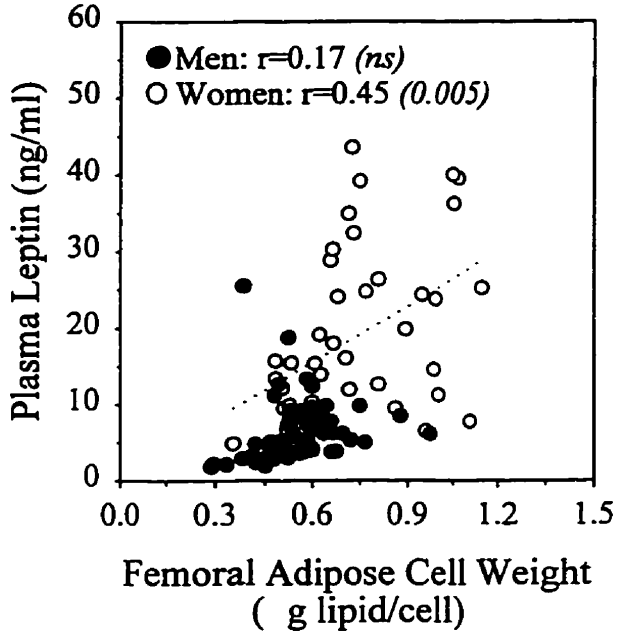
Dependent Variable	*Independent Variable*	*Partial (R^2 X 100)*	*Total (R^2 X 100)*	*P <*
Fasting Leptin	Gender	38.0	58.2	0.0001
	Abdominal FCW	15.8		0.0001
	Estimated Adipose Cell Number	4.4		0.0015

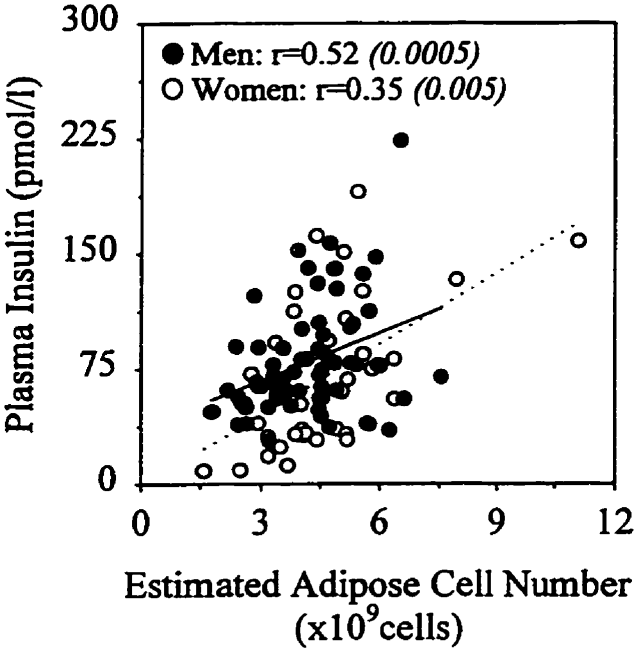
Variables included gender, fasting insulin total body fat mass, abdominal and femoral adipose cell weight (FCW) as well as estimated adipose cell number

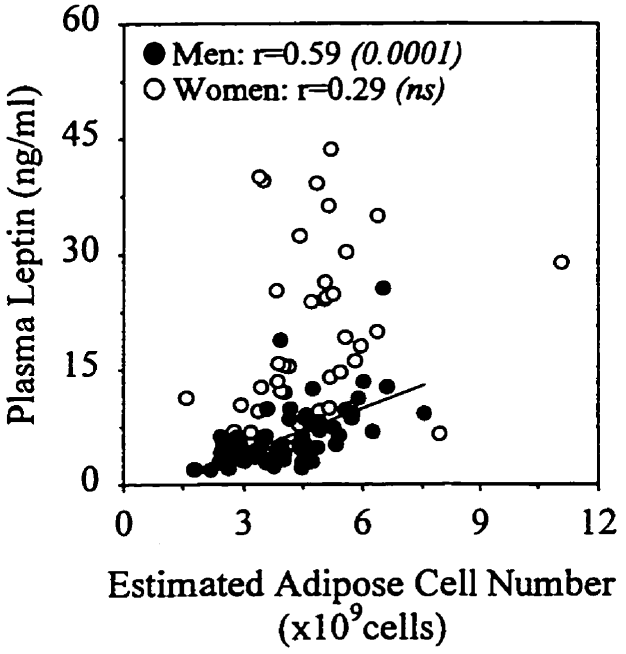

Plasma Insulin (pmol/l)
300
250
200
150
100
50
0
Men: r=0.38 (0.005)
Women: r=0.59 (0.0001)
0.0
0.3
0.6
0.9
1.2
1.5
Abdominal Adipose Cell Weight
(g lipid/cell)


Plasma Insulin (pmol/l)
300
250
200
150
100
50
0
Men: r=0.10 (ns)
Women: r=0.44 (0.005)
0.0
0.3
0.6
0.9
1.2
1.5
Femoral Adipose Cell Weight
(g lipid/cell)


Plasma Leptin (ng/ml)
60
50
40
30
20
10
0
Men: r=0.38 (0.005)
Women: r=0.55 (0.0001)
0.0
0.3
0.6
0.9
1.2
1.5
Abdominal Adipose Cell Weight
(g lipid/cell)


Plasma Leptin (ng/ml)
60
50
40
30
20
10
0
Men: r=0.17 (ns)
Women: r=0.45 (0.005)
0.0
0.3
0.6
0.9
1.2
1.5
Femoral Adipose Cell Weight
(g lipid/cell)


300
225
150
75
0
Plasma Insulin (pmol/l)
Men: r=0.52 (0.0005)
Women: r=0.35 (0.005)
0
3
6
9
12
Estimated Adipose Cell Number
(x10^9cells)


60
45
30
15
0
Plasma Leptin (ng/ml)
Men: r=0.59 (0.0001)
Women: r=0.29 (ns)
0
3
6
9
12
Estimated Adipose Cell Number
(x10^9cells)

CHAPITRE 11

LA CONCENTRATION PLASMATIQUE DE LEPTINE N'EST PAS UN FACTEUR DE RISQUE POUR LA CARDIOPATHIE ISCHÉMIQUE : RÉSULTATS PROSPECTIFS DE L'ÉTUDE CARDIO-VASCULAIRE DE QUÉBEC

L'article composant ce chapitre est intitulé :

Leptinemia is Not a Risk Factor for Ischemic Heart Disease in Men: Prospective Results From the Québec Cardiovascular Study

(Sous presse dans la revue Diabetes Care)

Leptinemia is Not a Risk Factor for Ischemic Heart Disease in Men

Prospective Results From the Québec Cardiovascular Study

RUNNING TITLE : *Leptin and Ischemic Heart Disease*

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Key Words: Leptin, ischemic heart disease, Québec Cardiovascular Study

RÉSUMÉ

BUT DE L'ÉTUDE: Étudier la possibilité que les niveaux plasmatiques de leptine soient en mesure de prédire le risque de cardiopathie ischémique par son association avec l'adiposité corporelle.

MÉTHODOLOGIE: La cohorte de l'Étude Cardio-vasculaire de Québec comprend 2103 hommes canadiens français dépourvus de maladies cardiaques en 1985, suivis jusqu'en 1990, et parmi lesquels 114 ont développé la maladie. Ces individus ayant subi un épisode de cardiopathie ischémique, ont été subséquemment appariés pour l'âge, l'indice de masse corporelle (IMC), la consommation de cigarettes et d'alcool, à 114 individus ne démontrant aucun symptôme de maladie cardiaque lors de la période de suivi. Après exclusion des sujets diabétiques et de ceux chez qui la leptine n'a pu être mesurée, nous avons été en mesure de comparer le profil métabolique, en 1985, de 86 hommes avec cardiopathie ischémique et 95 témoins.

RÉSULTATS : Les concentrations de leptine étaient corrélés positivement à l'IMC ($r=0.67, p<0.0001$) et aux niveaux plasmatiques d'insuline ($r=0.46, p<0.0001$) dans l'échantillon total. Ces relations significatives étaient également observées lorsque les hommes ayant ou non des maladies cardiaques étaient étudiés séparément (Témoins: IMC: $r=0.68$; Insuline: $r=0.45$ vs Malades: IMC: $r=0.65$; Insuline: $r=0.50$). Aucune différence dans les concentrations de leptine n'a été notée entre les deux sous-groupes d'hommes en 1985 (Malades: 5.56 ± 3.12 vs Témoins: 5.36 ± 2.90 ng/ml). Donc, même si elle était corrélée à l'IMC et l'hyperinsulinémie à jeun, la concentration plasmatique de leptine n'était pas un prédicteur de l'incidence de cardiopathie ischémique sur une période de suivi de 5 ans. Cette absence d'association avec l'incidence cardiaque a été notée lorsque les niveaux de leptine ont été analysés en tant que tertiles ou encore en tant que variable continue.

CONCLUSIONS : Ces résultats longitudinaux suggèrent que la leptinémie, en dépit de sa forte association à l'obésité, ne semble pas être un facteur de risque indépendant pour la cardiopathie ischémique chez l'homme.

ABSTRACT

OBJECTIVE: To investigate the possibility that leptin levels may predict the risk of ischemic heart disease (IHD) through its relationship with body fatness.

RESEARCH DESIGN AND METHODS: The Québec Cardiovascular Study cohort includes 2103 French-Canadian men without IHD in 1985 and followed until 1990, from which 114 developed an IHD event. These IHD men were then individually matched for age, body-mass index (BMI), cigarette smoking and alcohol intake with 114 subjects free of IHD at follow-up. After exclusion of diabetic patients and those in whom leptin levels could not be measured, we were able to compare the initial metabolic profile of 86 IHD men and 95 controls.

RESULTS: Plasma leptin concentrations were positively correlated with the BMI [$r=0.67$, $p<0.0001$] and with fasting insulin concentrations [$r=0.46$, $p<0.0001$] in the overall sample. These significant associations were also observed when examined separately in men with and without IHD [Controls: BMI: $r=0.68$; insulin: $r=0.45$ vs IHD cases: BMI: $r=0.65$; insulin: $r=0.50$]. With the exception of plasma TG [$r=0.25$, $p<0.001$], no significant association was found between leptin and plasma lipoprotein and lipid concentrations. Furthermore, plasma insulin remained significantly associated with leptin levels even after adjustment for the BMI ($r=0.22$, $p<0.005$). There was no difference in baseline leptin levels among men who developed IHD vs men who remained IHD-free over the 5-year follow-up (5.56 ● 3.12 vs 5.36 ± 2.90 ng/ml respectively). Thus, although significantly correlated with the BMI and fasting insulin levels, plasma leptin concentration was not a significant predictor of the 5-year incidence of IHD. This lack of relationship with IHD was noted

when leptin levels were analyzed as tertiles or when leptin concentration was analyzed as a continuous variable.

CONCLUSIONS: These prospective results suggest that leptinemia, despite being a strong correlate of obesity, does not appear to be an independent risk factor for the development of IHD in men.

INTRODUCTION

Obesity has long been recognized to have detrimental effects on health and a higher rate of metabolic complications are found in overweight compared to lean individuals [1]. These complications contribute to increase the risk of non-insulin dependent diabetes mellitus and of ischemic heart disease (IHD) in obese patients [1]. In this regard, the recent cloning of the human *obese* gene, and the characterization of its protein product, leptin [2], have been major breakthroughs leading to a new series of investigation aiming at the etiology of at least some forms of obesity. These studies have shown that leptin is essentially produced by adipose tissue and that plasma leptin levels are strongly and positively correlated with the amount of body fat [3-9]. Furthermore, insulin, a risk factor for IHD, has also been reported to up-regulate the expression of the *obese* gene in adipose cells [3-5,9,10-14]. Accordingly, plasma insulin concentration is an independent correlate of leptinemia, even after control for the effect of body fatness [6,10]. In the present study, we test the hypothesis that this newly discovered hormone may be related to IHD through its relationship with obesity and hyperinsulinemia. The 5-year follow-up results of the Québec Cardiovascular Study have allowed us to examine this question.

SUBJECTS AND METHODS

Study subjects. A complete and extensive description of the Québec Cardiovascular Study cohort has already been published elsewhere [15-17]. Briefly in 1985, a sample of 2103 men aged 47 to 76 years old, were characterized as free of IHD and underwent a complete IHD risk-factor profile evaluation. Over a five-year follow-up period, 114 of these men developed an IHD event. These 114 IHD men were then individually matched with men, who remained healthy over the 5-year follow-up, for the following variables: age, body-mass index (BMI), smoking and alcohol consumption [16,17]. After exclusion of IHD men who could not be matched due to extreme smoking habits, of subjects with diabetes, and of those with unavailable plasma for leptin assays, we were able to obtained data on 86 IHD cases and 95 controls.

Definition of events. The diagnosis of a first IHD event included typical effort angina, coronary insufficiency, nonfatal myocardial infarction and coronary death. Myocardial infarction was diagnosed according to the electrocardiographic criteria 1.1 of the Minnesota code or in the presence of 2 of the following 3 criteria: prolonged typical chest pain, ischemic electrocardiogram (ECG) changes or creatine phosphokinase (CPK) levels twice above the upper limit. The diagnosis of effort angina was based on typical symptoms of retrosternal squeezing or pressure-type discomfort of less than 5 minutes duration, occurring on exertion and relieved by rest and/or nitroglycerin. This diagnosis was always confirmed by a cardiologist. Coronary insufficiency was considered if typical retrosternal chest pain of 5 to 15 min duration was associated with transient ischemic changes on ECG (Minnesota codes 5.1 or 5.2) but without significant elevation in levels of CPK. All ECG were

read by the same cardiologist, who was unaware of the participants' risk profile. Criteria for the diagnoses of coronary deaths included: confirmation from death certificate, or autopsy report confirming the presence of coronary disease and without evidence for non-cardiac or atherosclerosis disease that could explain death. Myocardial infarction was considered fatal if death occurred within 4 weeks of myocardial infarction, or if it was diagnosed at autopsy. Informed consents were obtained to review relevant hospital files. Autopsies were performed in about a third of deaths.

Evaluation of risk factors. In 1985, data on demographic and lifestyle variables as well as medical history and medication were obtained through a standardized questionnaire, administered by trained nurses and further reviewed by a physician. Body weight and height were recorded. Resting blood pressure measurements were performed after a 5 min rest in a sitting position using phases I and V of Korotkoff sounds for systolic and diastolic blood pressure respectively. The mean of two blood pressure measures taken 5 min apart was used. The following information was compiled from the questionnaire: family and personal history of diabetes, smoking habits, alcohol consumption and medication use. Use of hypolipidemic drugs was not prevalent in 1985, as it only reached about 1% in men with and without IHD. Regular use of ß-blockers, mainly propranolol and metoprolol, was observed in 11% of men with IHD and in 6.5% of those without IHD. Proportion of men using diuretics on a regular basis was 7.5% and 2.8% in IHD cases and controls respectively. Alcohol intake was computed from the type of beverage (beer, wine and spirits) consumed in ounces per week and then standardized as absolute quantity, one once of absolute alcohol being equivalent to

22.5g of alcohol [18]. Family history of IHD was considered positive when at least one parent and/or one sibling had a previous history of IHD.

Laboratory analyses. In 1985, 12-hour fasting blood samples were obtained from an antecubital vein while participants were in a sitting posture. A tourniquet was used but released before blood withdrawal into Vaccutainer tubes (Becton Dickinson, Mountain View, CA) containing EDTA. Plasma was separated from blood cells by centrifugation and immediately used for lipid and apolipoprotein measurements. Aliquots of fasting plasma were frozen at -80°C at time of collection for subsequent analyses. Plasma cholesterol (CHOL) and triglyceride (TG) levels were determined on an Auto Analyzer II (Technicon Instruments, Tarrytown, NY) as previously described [19]. High-density lipoprotein cholesterol (HDL-chol) was measured in the supernatant fraction after precipitation of apolipoprotein (apo) B-containing lipoproteins with heparin-$MnCl_2$ [20]. Plasma apo B levels were measured by the rocket immunoelectrophoresis method of Laurell [21] as previously described [19]. Serum standards for apo B determination were prepared in our laboratory and calibrated against sera from the Centers for Disease Control (Atlanta, GA). Peak heights between 15mm and 35mm yielded linear and reliable results. The cumulative coefficients of variation for cholesterol, HDL-chol, triglyceride and apo B measurements were all < 3%. Fasting insulin concentrations were measured by a commercial double antibody radioimmunoassay (RIA) (Human Insulin Specific RIA Method, LINCO Research, St-Louis, MO). This assay shows little cross-reactivity (<0.2%) with human pro-insulin [22]. Finally, plasma leptin concentrations were determined with a highly sensitive commercial double antibody RIA (Human Leptin Specific RIA

Kit, LINCO Research, St-Louis, MO) which detects relatively low leptin levels of 0.5 ng/ml and does not cross-react with human insulin, proinsulin, glucagon, pancreatic polypeptide or somatostatin. Our coefficients of variation ranged from 4.0 to 5.5% for lower leptin concentrations and from 6.5 to 8.5% for higher plasma leptin concentrations.

Statistical analyses. All analyses were performed on the SAS statistical package (SAS Institute, Cary, NC). Student t-tests were used to compare means between men with and without IHD. Associations among variables were assessed using Spearman correlation coefficients. Odds ratios (RO) of IHD were calculated by use of the coefficients (b) obtained from the various logistic models. Odds ratios of the continuous variables were computed as the increase or decrease in risk of IHD associated with an elevation of 1 standard deviation (SD) in the concentration of the different risk factors. Men were also subdivided into tertiles of plasma leptin and CHOL/HDL-chol ratio, and the risk of IHD among the tertiles was assessed by logistic regression analyses using the tertile with the lowest value as reference, which, by definition, was assigned a risk of 1.0. Relative odds of developing IHD were adjusted for potential confounders other than those used in the matching procedures, namely systolic blood pressure, medication use, and family history of IHD. Adding the matching factors to the logistic models had essentially no impact on the parameter estimates.

RESULTS

Baseline characteristics of men who developed a first IHD event during the follow-up period, and those who remained free of IHD are shown in Table 1. Men with IHD presented significantly higher fasting plasma cholesterol, triglyceride, apolipoprotein B and insulin concentrations compared to controls. Men who developed IHD also had a higher initial average CHOL/HDL-chol ratio compared to men who remained IHD-free. However, the two groups showed no difference in plasma leptin concentrations.

Multiple logistic regression analyses were performed to examine the association between plasma leptin concentration and the risk of developing IHD. No significant change in the risk of IHD was associated with elevated plasma leptin concentration (odds ratio for IHD for every 1 SD increase in leptin concentrations, 1.0; 95% confidence interval, 0.8 to 1.4). Figure 1 compares the risk of developing IHD according to tertiles of leptin levels and the risk associated with a well known risk factor for IHD, i.e. the CHOL/HDL-chol ratio in the same sample of men.

Figure 2 illustrates the highly significant relationship between plasma leptin levels and BMI in the overall sample. This relationship was also observed when men with IHD ($r=0.65$, $p<0.0001$) and without IHD ($r=0.67$, $p<0.0001$) were examined separately.

Finally, plasma leptin concentrations were positively associated with plasma TG and insulin levels, all other metabolic variables studied showing no association with leptinemia (Figure 3). When plasma insulin levels were adjusted for concomitant variation in adiposity (i.e. BMI) its association with leptin levels remained significant ($r=0.22$, $p<0.005$).

DISCUSSION

Leptin, the protein product of the *obese* gene, is a hormone expressed and secreted by adipose cells [2,12]. Results of the present study in which the BMI (a crude correlate of subjects' adiposity) was positively correlated with leptin levels, are in accordance with the frequently reported close relationship of plasma leptin levels to adiposity [3-9]. This association remained significant when we analyzed men with and without IHD separately. Furthermore, the slope of the regression of leptinemia over the BMI was similar in controls vs IHD cases. Thus, for a given BMI, men with IHD had plasma leptin levels that were fully comparable to men without IHD.

Despite significant differences in plasma cholesterol, triglyceride, apolipoprotein B and insulin levels between men with and without IHD, leptinemia was not associated with plasma lipoprotein and lipid concentrations excepted for plasma triglyceride levels. Thus, plasma leptin, in addition to not being an independent risk factor for IHD, is not a good correlate of known lipid risk factors for IHD with the exception of TG. However, we found an association between leptin and fasting insulin concentrations, this relationship with insulin levels being independent of adiposity (BMI). These results support the notion that hyperinsulinemia is related to increased leptin concentrations, a finding concordant with previously published observations [6,10]. However, this relationship with insulin does not appear to cause prejudice regarding IHD risk.

Thus, results of the present study suggest that fasting leptinemia is not an independent risk factor for IHD in men. Obesity is considered by many investigators to be a risk factor for the development of IHD through numerous metabolic disturbances resulting from excess adipose tissue accumulation [1]. The present matched design did not allow us to examine the contribution of the

BMI as a risk factor for IHD in the present study. When analyses were conducted in the whole cohort of the Québec Cardiovascular Study, the BMI was not found to be associated with IHD risk [23]. However, as BMI is not necessarily a good correlate of body composition [24] whereas leptin, being selectively produced by AT, appears to be fairly closely associated with the level of body fat [3-9], the possibility remained that leptinemia (through its association with body fat mass) would be predictive of IHD risk. On the other hand, it has been suggested that abdominal visceral adipose tissue may represent the critical factor associated with an increased risk of IHD among obese subjects [25]. The lack of association between fasting plasma leptin concentrations and the 5-year incidence of IHD observed in the present study would tend to support that notion. Indeed, it has been reported that abdominal subcutaneous adipose cells show an increased expression of the *obese* gene compared to intraabdominal adipocytes [12]. In the present prospective study, computed tomography was not used, and we were thus not able to obtain measurements of total abdominal visceral vs subcutaneous adipose tissue accumulation. However, hyperinsulinemia resulting from insulin resistance has been shown to be strongly correlated with an increased visceral adipose tissue accumulation [26]. In the present study, men who developed IHD had significantly higher fasting plasma insulin concentrations compared to controls ($p<0.001$). The fasting hyperinsulinemic state of IHD patients may suggest that they also had a higher visceral adipose tissue accumulation than men who remained healthy over the 5-year follow-up.

Because we report no association between plasma leptin levels and the risk of IHD, the question of study power is a very important issue that requires discussion. We have performed additional analyses to determine the power of the present study to detect a significant and possibly

clinically relevant difference in plasma leptin concentrations between IHD cases and controls. As mentioned above, the discovery of leptin remains fairly recent. For that reason and given the exploratory nature of the study, it is difficult at this point to determine a clinically and biologically relevant difference between the two study groups. Based on the relative case-control difference in plasma cholesterol (10%), triglyceride (19%), and apolipoprotein B (16%) concentrations- which were all statistically significant in the Québec Cardiovascular Study [23]-, we can speculate that a 15% relative case-control difference in plasma leptin concentrations would indeed represent a clinically and biologically relevant difference. Additional analyses revealed that the difference in leptinemia between IHD cases and controls observed in the present study would reach statistical significance ($p=0.05$, with a power of 80%) only if the sample size exceeded 40,000 subjects. Finally, the analysis of risk across tertiles of leptin levels presented in this report, which essentially eliminates the potential negative impact of the broad distribution of leptin levels on the study power, shows no increase in the risk of IHD in the group of men with the highest concentrations (OR=1.0, 95% confidence interval from 0.8 to 1.4) compared with men in the lowest tertile of the distribution. For these reasons, we therefore believe that the lack of association between plasma leptin concentrations and the risk of IHD reported herein is not likely to be attributable to insufficient statistical power.

In summary, results of the present study indicate that plasma leptinemia is unlikely to be a major independent risk factor for the development of IHD in men. Moreover, the lack of association between leptin and plasma lipoprotein-lipid levels exclude a major contribution of leptin as a

modulator or a correlate of dyslipidemic state predictive of an increased IHD risk. However, a significant association was found between increased leptin levels and hyperinsulinemia which was independent from the concomitant variation in the BMI. Thus, although the discovery of leptin will allow us to obtain new insights regarding factors involved in the regulation of energy balance, results of the present study do not support the measurement of fasting leptinemia in the evaluation of IHD risk.

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FIGURE HEADINGS

FIGURE 1: Odds ratios of developing IHD among tertiles of leptinemia and of the CHOL/HDL-cholesterol (CHOL/HDL-chol) ratio in the overall sample of 181 men. Adjustment of risk for systolic blood pressure, medication use and family history of IHD failed to alter these results.
* $p<0.0003$ for the CHOL/HDL-chol model.

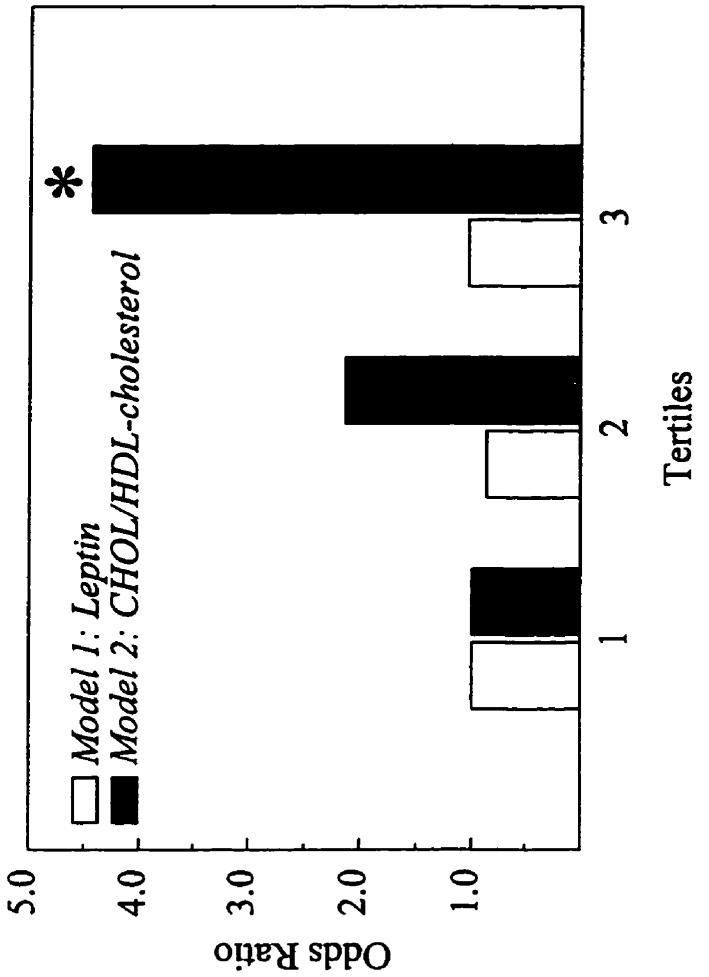
FIGURE 2: Relationship between plasma leptin concentrations and the body-mass index (BMI) in the overall sample of 181 men.

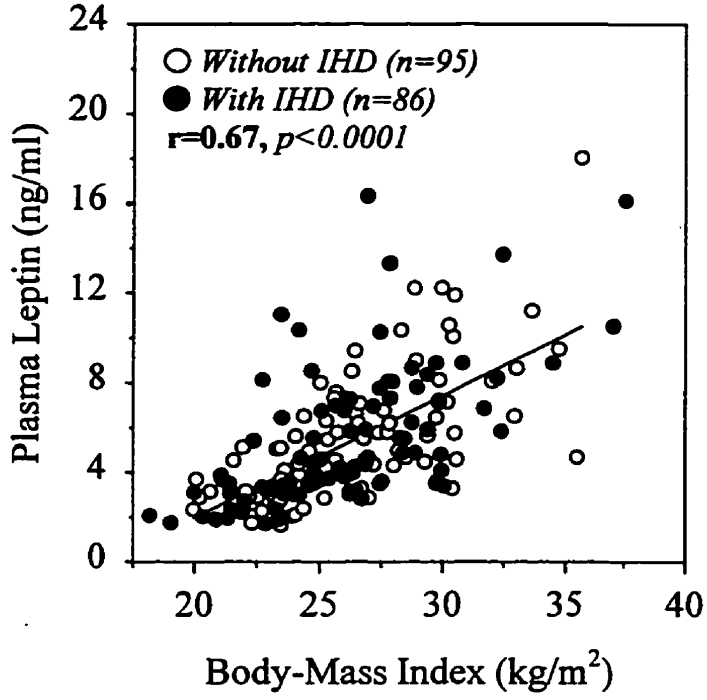
FIGURE 3: Relationships between plasma leptin concentrations and metabolic variables in the overall sample of 181 men. Men with IHD: ●; Men without IHD: O.

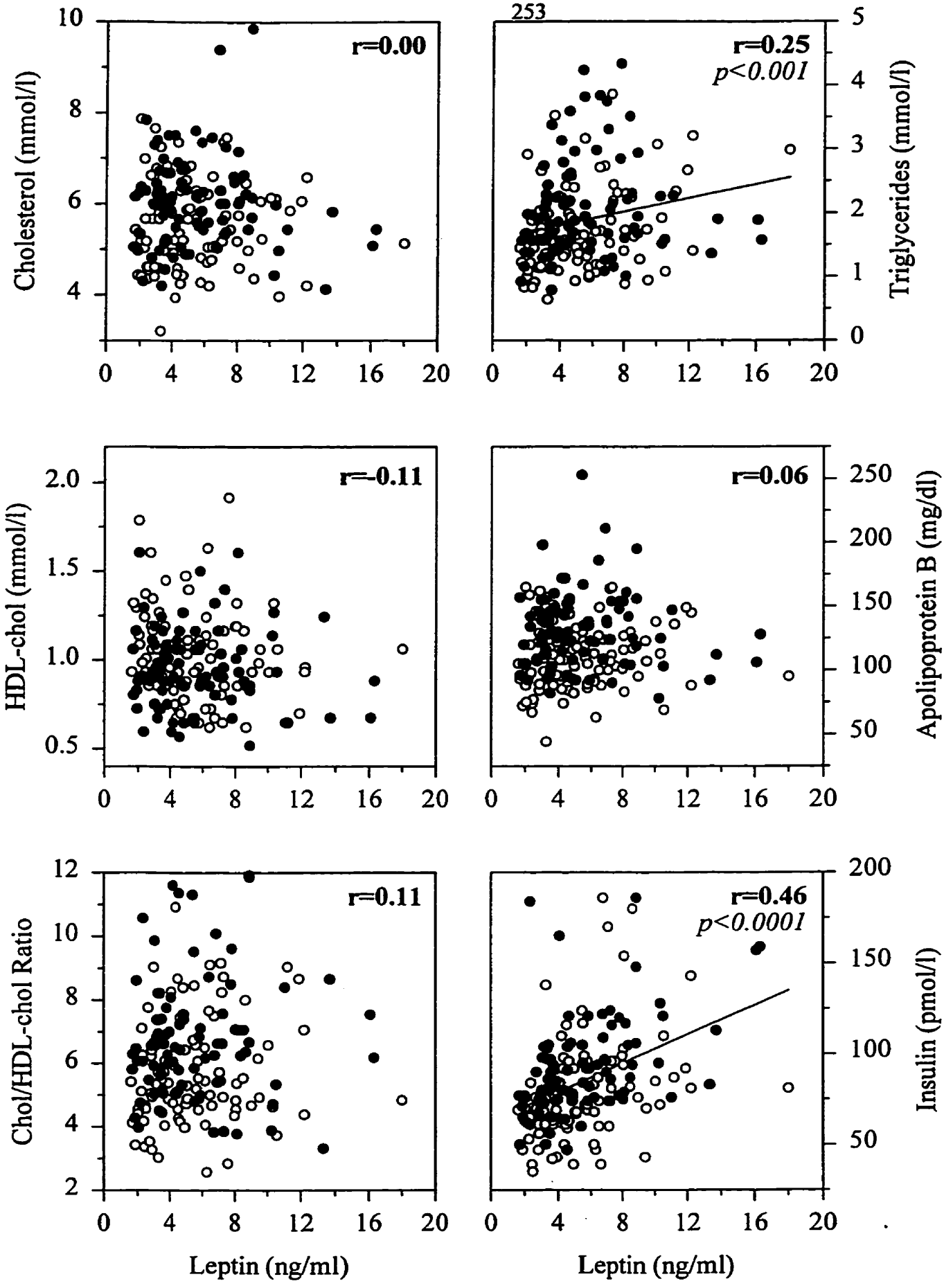
Table 1: Baseline characteristics of 86 men who developed a first ischemic heart disease event (IHD cases) vs 95 subjects from the Québec Cardiovascular Study who remained free from IHD over the 5-year follow-up period of the study.

Characteristics	*Controls (n=95)*	*Cases (n=86)*	*P Value*
Age (years)	58.9 ± 6.9	59.3 ± 7.8	-
Weight (kg)	75.8 ± 11.1	74.0 ± 12.3	-
Smoking (cigarettes/day)	10.0 ± 14.0	10.4 ± 15.1	-
Alcohol Consumption (ounces/week)	5.25 ± 8.30	5.27 ± 7.98	-
BMI (kg/m^2)	26.2 ± 3.5	26.1 ± 3.7	-
Systolic blood pressure (mm Hg)	133 ± 18	136 ± 17	0.3
Cholesterol (mmol/l)	5.51 ± 0.94	6.07 ± 1.00	<0.001
Triglycerides (mmol/l)	1.70 ± 0.66	2.02 ± 0.78	<0.005
HDL-chol (mmol/l)	1.02 ± 0.25	0.95 ± 0.22	0.06
Apolipoprotein B (mg/dl)	112 ± 27	130 ± 30	<0.001
CHOL/HDL-chol ratio	5.73 ± 1.66	6.73 ± 1.93	<0.001
Fasting insulin (pmol/l)	78.1 ± 29.1	92.3 ± 27.6	<0.001
Fasting leptin (ng/ml)	5.36 ± 2.90	5.56 ± 3.12	0.7

Values are expressed as mean ± SD.
The variables used in matching cases and controls were age, BMI, smoking status, and alcohol consumption.


Odds Ratio
5.0
4.0
3.0
2.0
1.0
Model 1: Leptin
Model 2: CHOL/HDL-cholesterol
*
1
2
3
Tertiles


Without IHD (n=95)
With IHD (n=86)
r=0.67, p<0.0001
Plasma Leptin (ng/ml)
Body-Mass Index (kg/m2)
0
4
8
12
16
20
24
20
25
30
35
40


Cholesterol (mmol/l)
r=0.00
Triglycerides (mmol/l)
r=0.25
p<0.001
HDL-chol (mmol/l)
r=-0.11
Apolipoprotein B (mg/dl)
r=0.06
Chol/HDL-chol Ratio
r=0.11
Insulin (pmol/l)
r=0.46
p<0.0001
Leptin (ng/ml)
Leptin (ng/ml)

CHAPITRE 12

CONCLUSIONS ET PERSPECTIVES D'ÉTUDES

La rédaction des différents manuscrits de cette thèse a permis d'émettre de nombreuses conclusions quant aux associations entre l'obésité, et plus particulièrement de la distribution du tissu adipeux, et ses conséquences sur les concentrations de leptine plasmatiques ainsi que sur le métabolisme des lipides en période post-prandiale. À cet effet, le présent chapitre ne se veut pas une simple révision des conclusions principales de chacun des manuscrits, mais bien leur intégration dans un contexte plus global, tout en énonçant certaines propositions quant aux avenues de recherches futures.

La première étude a permis de comparer la justesse avec laquelle les niveaux plasmatiques d'apo A-I et de HDL-cholestérol peuvent identifier les individus montrant les caractéristiques du syndrome plurimétabolique associé à l'obésité viscérale. En effet, les individus affichant une accumulation excessive de tissu adipeux viscéral sont caractérisés par des niveaux élevés de triglycérides et d'insuline de même que par des particules LDL plus petites et plus denses [15-17]. Ces personnes sont atteintes également d'hypoalphalipoprotéinémie [16,17] mais les causes de cette réduction des concentrations de HDL-cholestérol restent nébuleuses. Cependant, nos résultats indiquent que les concentrations faibles d'apo A-I sont moins précises dans l'identification des individus à risque de MCV que les niveaux réduits de HDL-cholestérol. De plus, une concentration faible d'apo A-I ne semble pas faire partie des altérations métaboliques observées dans le syndrome de résistance à l'insuline liée à l'obésité viscérale. En admettant que, par analogie avec l'apo B et les LDL, les concentrations d'apo A-I indiquent le nombre de particules HDL, nos résultats suggèrent que la baisse des niveaux de HDL-cholestérol serait conséquente à la réduction du contenu en

cholestérol plutôt que du nombre de particules. Par ailleurs, de nombreuses études ont démontré que les concentrations d'apo A-I ne permettent pas d'augmenter la puissance prédictive des niveaux de HDL-cholestérol quant au risque de développer des maladies coronariennes [211-213]. D'un point de vue clinique, les résultats obtenus lors de notre étude suggèrent de ne pas prendre en compte la mesure de la concentration d'apo A-I dans les tests de dépistage des individus à risque de maladies coronariennes, particulièrement les sujets caractérisés par le syndrome plurimétabolique.

La seconde étude présentée dans cette thèse, a porté sur l'impact de l'obésité viscérale sur la tolérance aux lipides alimentaires chez l'homme. Les résultats obtenus indiquent que l'obésité et plus particulièrement, une accumulation préférentielle de tissu adipeux au niveau de l'abdomen sont associées à la détérioration du métabolisme des lipoprotéines riches en triglycérides en période post-prandiale. Cette conclusion est non seulement en accord avec des observations précédentes [70,73,74], mais les résultats issus de notre étude ont permis de dissocier la contribution de l'accumulation de tissu adipeux viscéral de celle de l'obésité en-soi dans l'exagération de la lipémie post-prandiale. Les résultats obtenus permettent également de proposer un mécanisme physiologique par lequel une accumulation importante de tissu adipeux viscéral contribuerait à l'hypertriglycéridémie à jeun et en période post-prandiale. En effet, l'action anti-lipolytique de l'insuline sur le tissu adipeux est altérée chez les individus caractérisés par une obésité viscérale [214]. Ainsi, il en résulte un flux continu d'acides gras au foie en période post-prandiale, contribuant à la synthèse et à la sécrétion de VLDL par le foie, processus qui devraient être normalement ralentis après les repas. Dans notre étude, les individus affichant une obésité viscérale étaient caractérisés par

une réponse plus importante en acides gras libres en période post-prandiale comparativement aux sujets obèses mais présentant peu de graisse viscérale. Cette réponse plus élevée en acides gras, chez les individus ayant une importante accumulation de tissu adipeux viscéral, était également accompagnée d'une l'augmentation tardive des concentrations de lipoprotéines riches en triglycérides de petite taille, vraisemblablement des VLDL. Étant donné la compétition pour la LPL entre les lipoprotéines riches en triglycérides, tant d'origine intestinale qu'hépatique, cette arrivée importante et constante de VLDL dans la circulation en période post-prandiale nuirait au catabolisme des chylomicrons et de leurs résidus. C'est d'ailleurs par l'enrichissement en cholestérol de ces dernières sous-classes de lipoprotéines, favorisé par un séjour prolongé en circulation, qu'il a été proposé que le développement de l'athérosclérose serait un processus postprandial [20].

Un dimorphisme sexuel dans la tolérance aux lipides alimentaires a déjà été rapporté [75,77]. Cette observation a également été consignée dans notre étude. En effet, nos résultats montrent que les hommes présentent une lipémie post-prandiale de plus grande amplitude que les femmes. Même si plusieurs facteurs métaboliques peuvent être envisagés pour expliquer une telle différence, l'accumulation préférentielle de tissu adipeux viscéral chez l'homme contribuerait à la détérioration du métabolisme postprandial. En effet, lorsqu'appariés pour une même accumulation de graisse viscérale, les hommes montrent une réponse triglycéridémique en période post-prandiale qui est similaire à celle des femmes. Cependant, malgré l'absence de différence significative dans la lipémie post-prandiale, une tendance à l'exagération de la réponse persiste chez l'homme. Cette observation suggère donc que d'autres facteurs sont impliqués dans la détérioration du métabolisme des

lipoprotéines riches en triglycérides chez l'homme ou à l'augmentation de son efficacité chez la femme.

Cette première partie des travaux a permis d'évaluer l'impact de l'obésité viscérale sur les concentrations de HDL-cholestérol à jeun de même que sur la tolérance aux lipides. Étant donné que ces changements métaboliques ont été examinés dans des études transversales, il serait intéressant de valider nos observations dans le cadre d'études longitudinales, afin de mieux comprendre comment se développent les différentes altérations métaboliques observées. De plus, les études d'associations comme celles effectuées dans la présente thèse sont limitées quant à l'approche mécanistique entourant les différents changements métaboliques. Des études portant sur l'activité lipolytique du tissu adipeux viscéral, la synthèse hépatique des VLDL, et leurs contributions dans la détérioration du métabolisme des lipoprotéines riches en triglycérides en période post-prandiale s'avéreraient tout aussi intéressantes. Pour ce qui est de la différence sexuelle dans la tolérance aux lipides, nos résultats démontrent une fois de plus, la dichotomie qui existe entre le métabolisme des lipoprotéines des hommes et celui des femmes. Cependant, bien que l'accumulation plus importante de graisse viscérale chez l'homme ait été proposée pour expliquer, du moins en partie, la lipémie post-prandiale exagérée, il semble qu'elle ne soit pas le seul facteur responsable pour cette différence sexuelle. En effet, malgré la procédure de pairage d'hommes et de femmes pour l'accumulation de graisse viscérale, une différence persiste dans la réponse des lipoprotéines riches en triglycérides de petite taille. Des études, plus approfondies sur les causes de l'efficacité du catabolisme des lipoprotéines riches en triglycérides semblent donc nécessaires. À cet effet, il serait intéressant

d'étudier la contribution des lipoprotéines riches en triglycérides d'origine intestinale et hépatique à la lipémie post-prandiale tant chez l'homme que chez la femme. Le potentiel d'oxydation des lipides, estimé par la mesure du métabolisme énergétique durant la période post-prandiale, pourrait également être étudié afin de mieux comprendre de quelle façon les lipides sont utilisés en période post-prandiale chez l'homme et chez la femme.

La seconde partie des travaux de cette thèse a porté sur les associations entre l'obésité, la distribution de la graisse et ses complications métaboliques, et les concentrations plasmatiques de leptine. Nous avons également examiné les relations entre la leptinémie et certaines caractéristiques métaboliques susceptibles d'être impliquées dans la régulation des niveaux circulants de leptine.

Nos résultats indiquent clairement que les femmes sont caractérisées par des concentrations plasmatiques de leptine plus élevées comparativement aux hommes et ce, bien que les hommes et les femmes soient appariés pour une même quantité de masse grasse. Cette observation est tout à fait en accord avec les données de la littérature [122,163-168]. Étant donné que les niveaux de leptine n'étaient pas associés à l'altération du profil lipidique, il ne semble pas nécessaire d'inclure la leptinémie dans l'ensemble des facteurs de risque pour la maladie cardio-vasculaire ordinairement rapportée chez les individus obèses. De plus, même si ce n'était pas le but premier de cette étude, nos résultats confirment le fait que, du moins chez la femme, la production de leptine soit régulée par l'insuline [153,155,161,162] et ce, indépendamment de l'accumulation de masse grasse. Toutefois,

des études supplémentaires seront nécessaires afin d'identifier d'autres facteurs contribuant à la différence marquée de leptinémie entre les hommes et les femmes.

Dans le but de mieux comprendre la régulation de la sécrétion de leptine par le tissu adipeux chez l'homme, nous avons également exploré les associations entre les concentrations d'hormones sexuelles et les niveaux plasmatiques de leptine. À cet effet, les associations significatives notées entre les niveaux de stéroïdes et de leptine, se sont avérées presque entièrement expliquées par la variation concomitante de l'adiposité, ce qui est en accord avec les récents résultats de Haffner et coll [169]. Cependant, dans notre étude, l'hyperleptinémie était associée à des niveaux réduits de globuline de liaison des hormones stéroïdiennes endogènes (SHBG) et ce, avant et après l'ajustement statistique des concentrations de leptine pour la quantité de masse grasse. Par ailleurs, il est connu que l'insuline inhibe la synthèse de SHBG dans les cellules hépatiques en culture [215]. Même si des expériences complémentaires seront nécessaires pour bien comprendre l'importance physiologique de nos observations, les résultats obtenus lors de notre étude suggèrent que l'état hyperinsulinémique souvent rencontré chez les sujets présentant des concentrations élevées de leptine pourrait expliquer, du moins en partie, la réduction des niveaux plasmatiques de SHBG observée chez les hommes obèses. En effet, l'augmentation des concentrations d'insuline caractéristique des individus obèses, pourrait réduire la production de SHBG par le foie.

Par ailleurs, des évidences suggèrent que la taille de la cellule adipeuse puisse être un facteur de régulation de l'expression et de la sécrétion de leptine par le tissu adipeux. En effet, le volume de

l'adipocyte est associé positivement aux concentrations d'ARNm du gène de la leptine [185,186]. Les résultats obtenus lors de notre étude confirment cette observation, puisque la concentration plasmatique de leptine est associée au poids de la cellule adipeuse. De plus, la différence de poids adipocytaire entre les hommes et les femmes pourrait expliquer du moins en partie le dimorphisme sexuel des concentrations de leptine chez l'humain. En effet, les femmes ayant participé à notre étude affichaient un poids adipocytaire moyen plus élevé que les hommes et ce, tant au niveau du tissu adipeux abdominal que fémoral. D'autres résultats issus de notre étude, confirment l'importance de la taille adipocytaire dans la différence sexuelle de leptinémie. En effet, lorsque les hommes et les femmes étaient appariés pour les concentrations plasmatiques de leptine, la différence sexuelle notée dans le poids adipocytaire disparaissait et ce, même si le nombre estimé de cellules adipeuses était plus élevé chez l'homme. Nos résultats suggèrent donc que l'hypertrophie du tissu adipeux est plus étroitement liée à l'hyperleptinémie que l'hyperplasie (nombre d'adipocytes). Cependant, l'ajustement statistique des niveaux de leptine pour le poids adipocytaire n'a pas réussi à éliminer la différence sexuelle dans la leptinémie, les femmes présentant toujours des niveaux plus élevés de leptine que les hommes. Il est donc possible que d'autres facteurs contribuent au dimorphisme sexuel des concentrations de leptine. Bien que la taille cellulaire soit potentiellement impliquée dans la régulation de la production de leptine, nous n'étions pas en mesure d'identifier si le contenu en lipides de l'adipocyte est directement lié à la production accrue de leptine ou si des changements membranaires de l'adipocyte était responsable de la hausse de la sécrétion.

Finalement, les résultats de l'étude sur le risque de développer une cardiopathie ischémique indiquent que la leptinémie n'est pas un facteur de risque indépendant majeur dans le développement d'une telle pathologie. De plus, l'absence d'association entre la leptine et le profil lipidique, exclut la contribution de la leptine en tant que modulateur de l'état dyslipidémique chez les individus obèses et annonciateur de l'élévation du risque de cardiopathie ischémique. Cependant, l'association significative notée entre les niveaux plasmatiques élevés de leptine et l'hyperinsulinémie, l'était de façon indépendante de la variation concomitante dans l'indice de masse corporelle. Cette relation est connue [153,155,161,162] et nos résultats renforcent l'hypothèse selon laquelle l'insuline serait un régulateur des niveaux plasmatiques de leptine. Même si la découverte de la leptine permettra donc d'obtenir de nouvelles informations quant aux facteurs impliqués dans la régulation de la balance énergétique, les résultats issus de nos travaux ne favorisent pas la mesure de la leptinémie à jeun dans l'évaluation du risque de cardiopathie ischémique chez l'homme. De plus, il semble qu'une mesure précise de l'adiposité totale n'est peut-être pas requise afin de mieux estimer le risque de cardiopathie ischémique.

Les études d'intervention n'ayant pas encore été entreprises, il est difficile et hasardeux de prévoir quels seront les effets de l'administration de leptine chez l'humain. À cet effet, il semble improbable que le traitement à la leptine puisse provoquer des changements métaboliques semblables à ceux engendrés par l'administration de leptine à des souris *ob/ob* puisqu'il a été proposé que l'obésité humaine résulterait plutôt d'un état de résistance à la leptine identique à celui noté chez la souris *db/db* [119-125]. Par ailleurs, l'étude des mécanismes d'action et de

régulation de la leptine sont probablement les domaines les plus susceptibles de connaître de l'expansion dans les prochaines années. En effet, les voies métaboliques par lesquelles l'action de la leptine est transmise, sont jusqu'à présent méconnues. De plus, bien que l'insuline soit pressentie comme un régulateur potentiel de la concentration de leptine, le rôle de cette régulation de même que les mécanismes par lesquels elle s'exercerait, demeurent vagues. Par ailleurs, de plus en plus d'évidences sont en faveur d'un rôle de la leptine dans le système reproducteur [203-208] et il semble plus qu'intéressant de mieux comprendre l'importance de cet effet sur la capacité reproductrice chez l'humain.

CHAPITRE 13

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IMAGE EVALUATION
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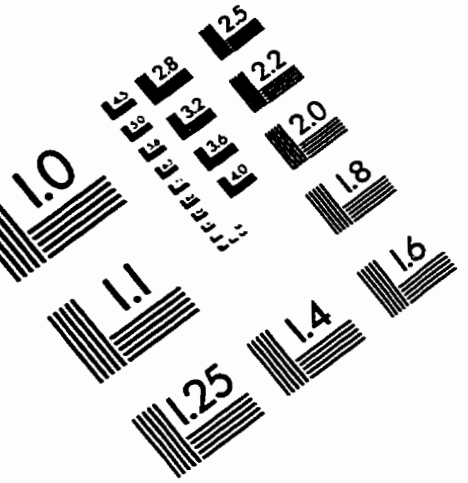

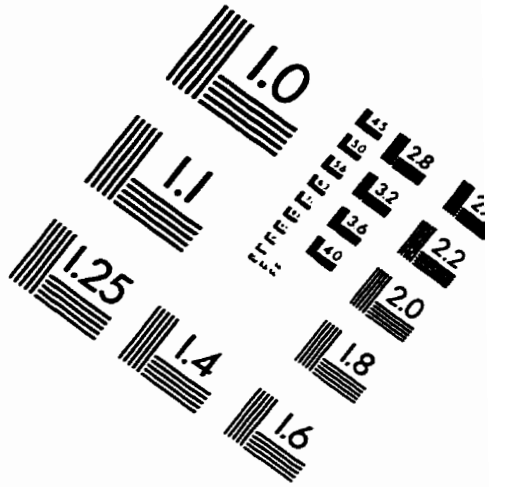

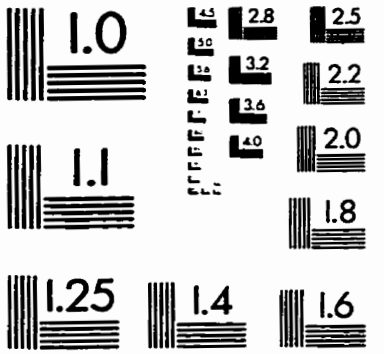

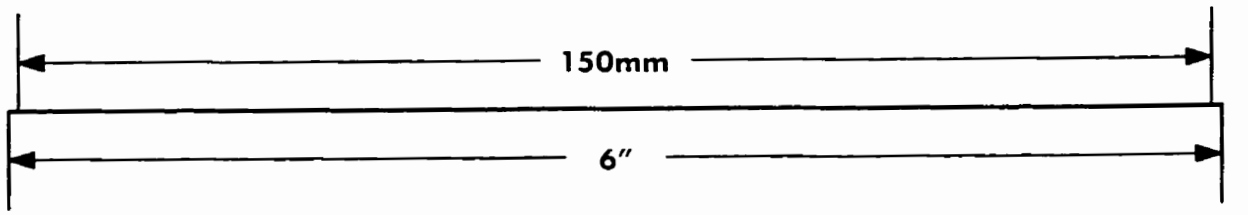

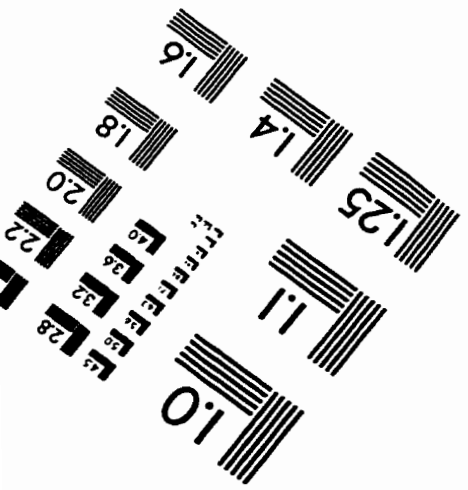

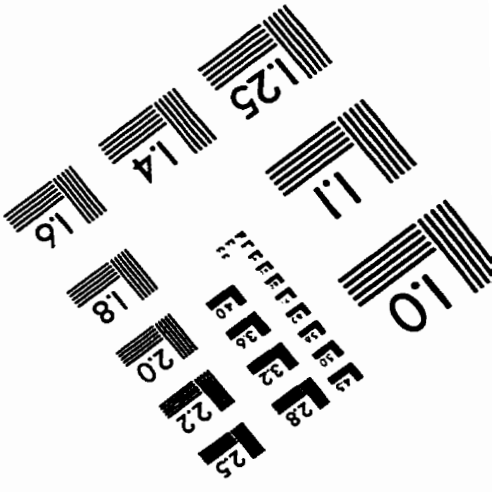

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