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Tissue leptin and plasma insulin are associated with lipoprotein lipase activity in severely obese patients

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Abstract

The development of metabolic complications of obesity has been associated with the existence of depot-specific differences in the biochemical properties of adipocytes. The aim of this study was to investigate, in severely obese men and women, both gender- and depot-related differences in lipoprotein lipase (LPL) expression and activity, as well as the involvement of endocrine and biometric factors and their dependence on gender and/or fat depot. Morbidly obese, nondiabetic, subjects (9 men and 22 women) aged 41.1±1.9 years, with a body mass index (BMI) of 54.7±1.7 kg/m^2 who had undergone abdominal surgery were studied. Both expression and activity of LPL and leptin expression were determined in adipose samples from subcutaneous and visceral fat depots. In both men and women, visceral fat showed higher LPL mRNA levels as well as lower *ob* mRNA levels and tissue leptin content than the subcutaneous one. In both subcutaneous and visceral adipose depots, women exhibited higher protein content, decreased fat cell size and lower LPL activity than men. The gender-related differences found in abdominal fat LPL activity could contribute to the increased risk for developing obesity-associated diseases shown by men, even in morbid obesity, in which the massive fat accumulation could mask these differences. Furthermore, the leptin content of fat depots as well as plasma insulin concentrations appear in our population as the main determinants of adipose tissue LPL activity, adjusted by gender, depot and BMI.

Keywords: Abdominal fat depots; Lipoprotein lipase; Severe obesity; Gender-related effects; Leptin; Insulin

1. Introduction

Lipoprotein lipase (LPL) hydrolyzes the triglyceride component of circulating lipoproteins, allowing delivery of their constituent fatty acids to tissues. The relative levels of LPL activity in adipose tissue and muscle determine how dietary lipids are partitioned toward storage or utilization, and thus the amount of fat deposited. Given its ability to make free fatty acids available to adipose tissues, LPL and its tissue-specific regulation have been thought to be centrally involved in the pathogenesis of obesity. In this way, LPL activity has been seen to be markedly elevated in the adipose tissue of obese subjects. In addition, several novel functions of the enzyme have recently been identified, making it also an important factor in vascular disease [1,2]. In this way, a noncatalytic bridging function allows LPL to bind simultaneously to both lipoproteins and specific cell surface proteins, leading to the increased cellular uptake of lipoproteins [1].

The variation in LPL activity could be involved in the site differences in adipose tissue metabolism and could also account for the gender-dependent body fat distribution [3,4]. The accumulation of fat in the visceral region, which is much more common among men than women, has become an important predictor of obesity-related morbidity and mortality [5]. Visceral adipocytes have been shown to be more sensitive to catecholamine-induced lipolysis and less sensitive to the antilipolytic effects of insulin, as compared to the subcutaneous ones [6,7]. Moreover, both fat depots have shown a differential secretion of some bioactive factors [5] and also a differential mRNA expression for several

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genes, such as the *ob* gene [8–11] and LPL, for which some controversial results have been reported [9,11–13].

Leptin, one of the best characterized adipocyte secretory proteins, plays an important role in regulating food intake, energy expenditure and neuroendocrine function. Leptin is known to be associated with body weight control, and possibly to affect insulin sensitivity [14]. Expression and secretion of leptin are increased in obesity and a strong correlation exists between body fat stores and leptin plasma levels [15,16]. Moreover, a gender-dependent difference in plasma leptin concentration has also been described [10,17–19], which does not seem to be explained only by the different amount of body fat between genders but could be also related to the gender-based differences in circulating concentrations of gonadal steroids [20].

The aim of the present study was to investigate both gender- and depot-related differences in LPL expression and activity in severely obese subjects. Endocrine and biometric factors potentially affecting LPL activity, as well as their dependence on gender and/or fat depot, were further investigated.

2. Materials and methods

2.1. Subjects and adipose tissue biopsies

Thirty-one morbidly obese, nondiabetic subjects (9 men and 22 women) aged 41.1±1.9 years (23–60 years), with a body mass index (BMI) of 54.7±1.7 kg/m^2 (40–81 kg/m^2), undergoing weight reduction surgery were studied. None of the patients used any regular medication. The study protocol was approved by the Ethics Committee of the Son Dureta University Hospital (Palma de Mallorca, Spain) and all subjects gave their written consent to participate. Fat biopsies (15–30 g) were removed within 20 min after the start of general anesthesia from both abdominal subcutaneous and visceral (omental) adipose tissues. Tissue biopsies were immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis. Adipocytes were isolated from 6- to 10-g piece of fresh tissue using the method described by Rodbell [21]; fat cell size was determined by direct microscopy and the mean adipocyte diameter was calculated from the measurement of 50 cells in each tissue sample.

2.2. Plasma biochemical analysis

Fasting plasma glucose, insulin and lipid profile (triglycerides, total cholesterol and high- and low-density lipoprotein cholesterol) were measured by the hospital's routine laboratory.

Plasma leptin levels were measured in 6 men and 14 women (aged 40.7±2.6 years and BMI 49.8±1.5 kg/m^2) using a human leptin enzyme-linked immunosorbent assay kit. Given the previously reported relationship between leptin and testosterone levels, we also determined the concentration of this hormone in plasma samples from 9 men and 13 women (aged 42.0±2.6 years and BMI 52.6±1.6 kg/m^2) using a competitive immunoenzymatic method.

2.3. RT-PCR analysis of ob and LPL mRNA

Total RNA was isolated from fat biopsies using Tripure, quantified by spectrophotometry and checked for integrity by agarose gel electrophoresis.

Either 250 ng (*ob*) or 1 μg (LPL) of total RNA, in a final volume of 5 μl, was denatured at 90°C for 1 min and reverse transcribed to cDNA at 42°C for 1 hour with 25 U MuLV reverse transcriptase in a 10-μl volume of retrotranscriptase reaction mixture containing 1× buffer [10 mM Tris–HCl (pH 9.0), 50 mM KCl and 0.1% Triton X-100], 2.5 mM $MgCl_2$, 5 μM random hexamers, 10 U RNase inhibitor and 0.5 μM dNTP, with a final step at 99°C for 5 min in a DNA thermal cycler 2400 (Perkin Elmer, Madrid, Spain).

Semiquantitative PCR was carried out for *ob* and for beta-actin (reference gene) in a PCR mix containing 1.7 mM (*ob*) and 0.5 mM (beta-actin) of each primer (ob forward 5′-TTCACACACGCAGTCAGTCTCC-3′ and *ob* reverse 5′-ACAGAGTCCTGGATAAGGGGTG-3′; beta-actin forward 5′-GAGAAGATGACCCAGATCATGT-3′ and beta-actin reverse 5′-GTGGTGGTGAAGCTGTAGCC-3′). The amplification program consisted of a preincubation step for denaturation of the template cDNA (94°C for 3 min), followed by 23 cycles consisting of a denaturation step (95°C for 1 min), an annealing step (58°C for 1 min) and an extension step (72°C for 2 min), and then one final extension step (72°C for 10 min). PCR products were separated on a 2% agarose gel and analyzed by video-densitometric scanning using the Kodak 1D Image Analysis Software 3.5 for windows (Eastman Kodak, Rochester, NY). Levels of *ob* mRNA were expressed as the ratio of its signal intensity relative to that for beta-actin. Representative RT-PCR products are shown in Fig. 1.

mRNA LPL levels and beta-2 microglobulin (B2M, reference gene) were measured by real-time PCR (Light-Cycler, Roche Diagnostics, Barcelona, Spain) as previously described [22]. Each reaction contained 0.5 μM of the corresponding oligonucleotide primers (LPL forward

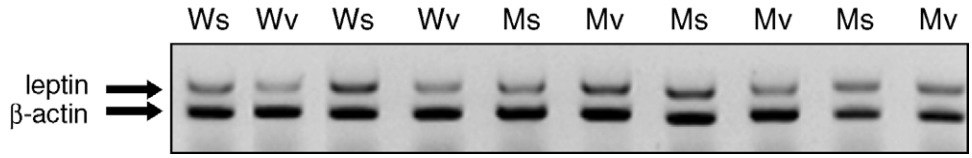

Fig. 1. RT-PCR analysis of *ob* and beta-actin mRNA levels in visceral (v) and subcutaneous (s) adipose tissue depots of severely obese men (M) and women (W). The resulting products were separated in 2% agarose gels and visualized by ethidium bromide staining.

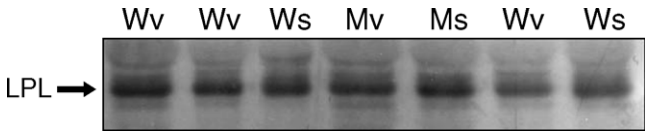

Fig. 2. Western blot analysis of LPL protein levels in visceral (v) and subcutaneous (s) adipose tissue depots of severely obese men (M) and women (W). Twenty micrograms of total tissue protein were loaded. Development of the immunoblots was performed using an enhanced chemiluminescence Western blotting analysis system.

5′-TGGCATTGCAGGAAGTCTGA-3′ and LPL reverse 5′-AGGAGAAAGACGACTCGGGG-3′; B2M forward 5′-CCTGAATTGCTATGTGTCTGGGTTTC-3′ and B2M reverse 5′-CTCCATGATGCTGCTTACATGTCTCG-3′), either 3 mM (LPL) or 4 mM (B2M) $MgCl_2$, 2 μl LightCycler l LightCycler FastStart DNA Master SYBR Green I mix (containing FastStart enzyme, dNTPs, $MgCl_2$ and SYBR Green I dye) and 5 μl of the cDNA dilution in a final volume of 20 μl. The amplification program consisted of a preincubation step for denaturation of the template cDNA (95°C for 10 min), followed by 45 cycles consisting of a denaturation step (95°C for 2 s for B2M or 0 s for LPL), an annealing step (58°C for 5 s) and an extension step (72°C for 18 s). After each cycle, fluorescence was measured at 72°C. PCR products were subjected to a melting curve analysis on the LightCycler and subsequently 1% agarose gel electrophoresis to confirm amplification specificity by Tm and amplicon size. Real-time PCR was performed in duplicate and a negative control without cDNA template was run in each assay. To calculate the real-time PCR efficiencies ($E=10^{(-1/\text{slope})}$), a relative standard curve using 1/25, 1/250 and 1/2500 cDNA dilution for one sample was performed in each assay. Crossing points (CP) were determined by the "Second Derivative Maximum Method" with the LightCycler analysis software version 3.5 (Roche Diagnostics) and were the mean value for each transcript of the 1/25 dilution. The normalized target concentrations (arbitrary units) of LPL in each tissue were calculated from the real-time PCR efficiencies and the CP of both the target and the reference gene [normalized target concentration $=E_{\text{LPL}}^{\text{CP LPL}}/E_{\text{B2M.}}^{\text{CP B2M}}$] using the mathematical model described by Pfaffl [23], but slightly modified.

2.4. LPL and leptin protein levels

For LPL immunoreactive mass determination, ~500 mg of frozen tissue was homogenized in phosphate-buffered saline buffer (137 mM NaCl, 3 mM KCl, 6.5 mM Na_2HPO_4 and 3.5 mM KH_2PO_4, adjusted to pH 7.4 with 2 M sodium hydroxide) with freshly added protease inhibitors (1 μg/ml aprotinin, 1 μg/ml leupeptin and 10 μg/ml phenylmethyl sulfonyl fluoride). The homogenate was centrifuged at 16,000×*g* (20 min, 4°C), and the infranatant was removed and stored at −20°C. Total tissue protein (20 μg) was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred on a nitrocellulose filter as described elsewhere [24]. Chicken polyclonal antibodies raised against bovine LPL, previously developed in our laboratory [25], were used as primary antibodies. Development of the immunoblots was performed using an enhanced chemiluminescence Western blotting analysis system. Bands in films (Fig. 2) were analyzed by scanner photodensitometry and quantified using the Kodak 1D Image Analysis Software 3.5 for Windows (Eastman Kodak).

Leptin levels were measured in the tissue homogenate used for LPL activity determination (see below) using the same human leptin enzyme-linked immunosorbent assay kit used for plasma samples.

2.5. LPL activity measurement

Adipose tissue samples were homogenized in a buffer containing 1 mM dithiothreitol, 1 mM EDTA, 0.25 M sucrose, 10 mM HEPES and 0.005% heparin (adjusted to pH 7.5 with 2 M potassium hydroxide) and then centrifuged (2000 rpm for 10 min at 4°C). The infranatant was used to determine the enzyme activity [26]. LPL activity was assayed using an emulsion of glycerol tri[9,10(n)-^{3}H]oleate containing normal human serum as a source of apoC-II. Samples were incubated with the substrate solution for 30 min at 37°C in a shaking bath and ^{3}H-labeled free fatty acids were separated by centrifugation using [^{14}C]oleic acid as internal standard for estimating recovery [27]. LPL activity was calculated by subtracting lipolytic activity determined in a final NaCl concentration of 0.5 M (non-LPL activity) from total lipolytic activity measured in the absence of NaCl. Enzyme activity was expressed as microunits (1 μU=1 μmol nonesterified fatty acids released per hour of incubation at 37°C).

To standardize LPL activity, the infranatant was also used to determine the protein content [28]. Tissue DNA levels and total lipid content were also determined by the methods of Hinegardner [29] and Folch et al. [30], respectively.

2.6. Materials

Assay kits for leptin and testosterone measurements were obtained from R&D Systems (Minneapolis, USA) and DIA.METRA (Segrate, Italy), respectively. Glycerol

Table 1
Biometric and plasma biochemical parameters of morbidly obese subjects

	Men	Women	*P*
Age (years)	41.9±3.4	40.7±2.3	NS
BMI (kg/m^2)	57.8±2.3	53.4±2.2	NS
Glucose (mmol/L)	6.11±0.48	5.75±0.21	NS
Insulin (μU/ml)	28.9±4.4	23.2±2.8	NS
Triglycerides (mmol/L)	1.59±0.12	1.54±0.16	NS
Cholesterol (mmol/L)	3.64±0.27	3.81±0.36	NS
HDL cholesterol (mmol/L)	1.08±0.09	1.30±0.10	NS
LDL cholesterol (mmol/L)	3.90±0.54	3.49±0.25	NS
Leptin (ng/ml)	37.2±5.1	57.2±4.3*	.02
Testosterone (ng/ml)	1.87±0.61	0.243±0.05*	.03

Results are the mean±S.E.M. Data were analyzed by Student's *t* test. NS, nonsignificant.

* $P<.05$ women vs. men.

Table 2
Tissue composition and fat cell size of subcutaneous and visceral fat depots from morbidly obese subjects

	Men		Women		ANOVA (P)
	Subcutaneous	Visceral	Subcutaneous	Visceral	
Protein (mg/g tissue)	7.02±1.04	10.2±0.8	8.69±0.71	12.5±0.9	G (.047) T (<.001)
Lipid (mg/g tissue)	538±27	528±36	552±15	482±21	T (.023)
DNA (mg/g tissue)	0.908±0.182	1.05±0.20	1.09±0.13	1.21±0.12	NS
Fat cell size (μm)	114±7	121±4	102±3	102±4	G (.003)

Results are the mean±S.E.M. The mean fat cell size was calculated from the measurement of the diameter of 50 adipocytes in each tissue sample. Data were analyzed by two-way ANOVA. Significant differences (P<.05): G, effect of gender; T, effect of adipose tissue depot; NS, nonsignificant.

tri[9,10(n)-^{3}H]oleate and [^{14}C]oleic acid were supplied by American Radiolabeled Chemicals (Madrid, Spain). Aprotinin, leupeptin, Tripure reagent and LightCycler FastStart DNA Master SYBR Green I mix were supplied by Roche Diagnostics (Barcelona, Spain). Retrotranscriptase reaction reagents were purchased from Applied Biosystems (Barcelona, Spain) and Life Technologies (Barcelona, Spain). PCR reagents were obtained from Promega (Madison, USA). Routine chemicals were provided by Amersham Pharmacia Biotech (Barcelona, Spain), Panreac (Barcelona, Spain) and Sigma-Aldrich (Madrid, Spain).

2.7. Statistical analysis

Statistical analysis was performed using the Windows SPSS version 11.5 software (SPSS, Chicago, USA). All data are expressed as means±S.E.M. Protein mass (integrated optical density units per microgram of total tissue protein) and mRNA (normalized by the reference gene) data are expressed relative to the mean value of the male subcutaneous group, which was set as 100%.

Differences between genders in biometric and plasma biochemical parameters were tested using Student's t test. Two-way analysis of variance (ANOVA) was used to determine main effects of tissue (subcutaneous and visceral) and gender, and their interactions. Differences between groups were also assessed by Student's t test as post hoc comparison, but only when an interactive effect of tissue and gender (T×G) was shown. A P value of less than .05 was considered statistically significant.

Multiple linear regression analysis was performed to investigate the relationship between LPL activity and plasma insulin, tissue leptin, lipid content, age, gender, tissue depot and BMI. LPL activity and plasma testosterone levels were logarithmically transformed to yield a normal distribution before parametric analyses.

3. Results

The biometric and plasma biochemical parameters of the subjects included in this study are described in Table 1. No statistical differences were found between men and women in any of the parameters considered, with the exception of both plasma leptin and testosterone levels. Fasting glucose and insulin levels were comparable to the ones reported for severely obese patients and were higher than those of control subjects [20,31]. Gender-based differences in serum leptin levels have been extensively reported [17–19], and our data confirm these observations. As was to be expected, testosterone levels were significantly higher in plasma from men than in that from women. Moreover, male testosterone levels were lower than those previously reported in normal-weight subjects [32], in agreement to what has been described to occur in severe obesity [33]. In a multiple linear regression analysis we found that plasma testosterone

Table 3
Leptin and LPL expression and lipase activity of subcutaneous and visceral adipose tissues from morbidly obese subjects

	Men		Women		ANOVA (P)
	Subcutaneous	Visceral	Subcutaneous	Visceral	
ob mRNA (AU)	100±9	89.0±16.0	108±10	65.1±7.0	T (.002)
Tissue leptin (ng/g total protein)	100±15	72.1±7.8	110±10	54.5±6.2	T (<.001)
LPL mRNA (AU)	100±16	141±20	116±11	140±12	T (.032)
LPL tissue mass (AU)	100±13	109±15	115±10	120±10	NS
LPL activity					
μU/g protein	133±35	99.5±28	80.4±11.5	55.6±8.1	G (.011)
μU/g lipid	1.36±0.22	1.79±0.55	1.10±0.10	1.24±0.18	NS
μU/g DNA	1307±415	1308±487	862±179	637±129	G (.044)

Results are the mean±S.E.M. mRNA and LPL protein (arbitrary units, or AU) and tissue leptin (ng/g total protein) mean values of subcutaneous depot from men were set as 100%. LPL activity was expressed as μU/g of tissue protein, lipid or DNA, and 1 μU was equal to 1 μmol nonesterified fatty acids released per hour of incubation at 37°C. Data were analyzed by two-way ANOVA. Significant differences (P<.05): G, effect of gender; T, effect of adipose tissue depot; NS, nonsignificant.

Table 4
Multiple linear regression analysis for adipose tissue LPL activity as a dependent variable, adjusted by gender, tissue, age and BMI

Nondependent variables	Standardized coefficient	*P* value
Plasma insulin (μU/ml)	.241	.045
Tissue leptin content (ng/g protein)	.625	<.001

LPL activity, expressed as microunits per gram of protein, was log-transformed.

levels (log-transformed) inversely correlated ($r=-.788$, $P=.012$) with plasma leptin levels after adjustment for gender, age and BMI.

Visceral and subcutaneous adipose tissue composition is shown in Table 2. In both adipose depots, women exhibited a higher protein concentration and decreased fat cell size than men. Protein content was higher in the visceral depot as compared to the subcutaneous one in both men and women, while only women showed differences as regards to lipid content, which was lower in the visceral tissue than in the subcutaneous one. No differences in fat cell size were found between adipose tissue depots either in men or women. In a linear regression analysis, fat cell size showed a significant relationship with plasma insulin levels (Pearson correlation coefficient $r=.355$, $P=.011$).

The relative *ob* mRNA and tissue leptin levels (Table 3) were lower in the visceral adipose tissue as compared to the subcutaneous one, in agreement with previous studies [8–11]. In women, depot-related differences were more marked than in men.

Visceral fat showed higher LPL mRNA levels (Table 3) than the subcutaneous one, in both men and women, while no statistical differences were observed in LPL mass (Fig. 2) either by gender or by tissue depot. Regarding LPL activity, a gender-dependent effect could be observed when the activity was standardized by either protein or DNA content, with greater values in men than in women.

A multiple linear regression analysis (Table 4) showed that LPL activity (standardized by total protein content and log-transformed) was associated to tissue leptin content (expressed as nanogram per gram of protein) and, to a minor extent, to plasma insulin levels, when adjusted for age, gender, tissue depot and BMI.

4. Discussion

The existence of depot-specific differences in the biochemical properties of adipocytes is well established, and it has been related with the association of visceral fat with the development of metabolic complications of obesity [5,34]. In our morbidly obese subjects, both *ob* mRNA and leptin tissue levels showed a one and a half- to two-fold increase in subcutaneous depot compared with the visceral one, which agrees with the lightly attenuated subcutaneous-to-visceral ratio for these parameters found in severely obese individuals [8–10] compared to lean or moderately obese subjects [9–11]. Moreover, the subcutaneous-to-visceral ratio tended to be higher in women with respect to men, although the values did not reach statistical significance, in contrast to what has been reported for non-obese and mildly obese individuals [10].

The gender-related difference in circulating leptin concentrations was not as great as that reported for normal-weight and moderately obese individuals [18,20,35], in which women have about threefold serum leptin levels with respect to men. The mechanism resulting in the attenuation of the gender difference in extremely obese subjects is not known, but could be partially due to the changes in the circulating levels of steroid hormones [36]. Testosterone has been proposed to be one of the most important factors contributing to the lower serum leptin levels in men compared with women [37]. Accordingly, in a multiple linear regression analysis we found that plasma testosterone levels inversely correlated with plasma leptin levels after adjustment for gender, age and BMI. Thus, the lower testosterone plasma levels shown by our male patients could explain, at least in part, the attenuation of the gender difference in serum leptin levels in severely obese subjects.

In both genders, visceral adipose tissue showed higher LPL mRNA levels than the subcutaneous one, results that differ from those of previously reported studies in which either no depot-related differences were found [9,11], or higher levels of LPL mRNA were shown in the subcutaneous compared to visceral adipose tissue [12,13]. Although the reasons for these discrepancies are not clear, one explanation for these contrasting results could be the different methods used to measure mRNA levels [13], which is, in our case, the highly sensitive real-time PCR technique. Furthermore, the lack of correlation we found between LPL activity, protein mass and mRNA levels is in agreement with the existence of posttranscriptional control mechanisms in the regulation of LPL gene expression. This represents the most widely used mechanism and includes regulation of LPL expression in response to changes in both nutritional and hormonal status. Such posttranscriptional regulation can potentially be mediated at a number of steps, including mRNA stability, translation, protein degradation, processing, secretion and translocation to the site of action [1,2].

Gender-dependent differences in LPL activity have been consistently published when fat from lower body regions — femoral and gluteal — or intra-abdominal non-omental, that is, mesenteric, depots have been considered [3,38]. In the present study, we show differences between men and women in LPL activity standardized by both tissue protein and DNA content in two fat depots from the abdominal region, that is, subcutaneous and visceral (omental) adipose tissues. These gender-related differences were also found when LPL-specific activity — that is, enzyme activity referred to LPL mass — was considered (results not shown). Moreover, the higher LPL activity exhibited by men's adipose tissues compared with those from women was accompanied by a greater adipocyte size. However, other aspects of adipose cell metabolism such as lipolysis,

reesterification, or insulin responsiveness [38] should not be forgotten as determinants of fat cell size. In this respect, we have found a significant relationship between fat cell diameter and plasma insulin levels in these morbidly obese, nondiabetic subjects.

Adipocytes from men were found to be more hypertrophied and exhibited a higher LPL activity than those from women, which would suggest an increased risk in men for developing obesity-associated diseases. Since hypertrophied visceral adipocytes are poorly insulin sensitive [6], free fatty acids released from lipoproteins by visceral adipose tissue LPL could be highly prone to enter the circulation, rather than being stored in the tissue [7], decreasing the efficiency of these adipocytes in buffering the flux of fatty acids in the circulation [39]. It could be suggested that the greater visceral fat LPL activity that we have found in men would contribute, together with the increased lipolytic activity described for visceral adipocytes [6,7], to an increase to the direct supply of free fatty acids to the liver. With respect to subcutaneous abdominal fat, although there is some controversy, it has been reported that the occurrence of larger adipocytes in this depot could be associated with further deterioration in the metabolic risk profile [40]. Therefore, the high LPL activity that we have found in both hypertrophied fat depots could also contribute to increasing the risk for developing health problems in morbidly obese men. However, in this study the metabolic profile of the men and women did not reach statistical significance despite gender- and depot-related abnormalities en LPL.

A multiple linear regression analysis has shown that, in our severely obese, nondiabetic patients, both tissue leptin content and plasma insulin levels are the main contributing factors to LPL activity of adipose tissues, after adjustment for gender, age, tissue depot and BMI. These results are in agreement with those from a previous study performed in middle-aged men, where plasma insulin levels were proposed to be, in addition to obesity and plasma leptin, the major determinants of adipose tissue LPL activity [41]. Obviously, the significant correlation seen between plasma insulin and adipose tissue LPL activity is not surprising since insulin is known to be one of the most potent regulators of LPL and, under most circumstances, adipose LPL activity increases with serum insulin levels and the degree of insulin sensitivity [2]. Furthermore, an additional multiple linear regression analysis pointed toward LPL activity as the only factor (r=.371, P=.017), among those considered, affecting adipose tissue lipid content (considered as a dependent variable), after adjustment for gender, age, tissue depot and BMI.

In conclusion, the gender-dependent differences seen in tissue hypertrophy and LPL activity in both subcutaneous and visceral abdominal depots could be responsible, at least in part, for an increased risk for developing obesity-associated diseases in obese men compared with women, even when the fat accumulation is massive as occurs in morbid obesity. As far as we are concerned, this is the first study where a relationship between LPL activity and the leptin content of the adipose tissues has been demonstrated. Our results indicate that in severe obesity the leptin content of fat depots as well as plasma insulin concentrations may be associated to adipose tissue LPL activity, after adjustment for gender, depot and BMI.

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References

[1] Mead JR, Irvine SA, Ramji DP. Lipoprotein lipase: structure, function, regulation, and role in disease. J Mol Med 2002;80:753–69.
[2] Preiss-Landl K, Zimmermann R, Hammerle G, et al. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. Curr Opin Lipidol 2002;13:471–81.
[3] Imbeault P, Almeras N, Richard D, et al. Effect of a moderate weight loss on adipose tissue lipoprotein lipase activity and expression: existence of sexual variation and regional differences. Int J Obes Relat Metab Disord 1999;23:957–65.
[4] Mauriege P, Marette A, Atgie C, et al. Regional variation in adipose tissue metabolism of severely obese premenopausal women. J Lipid Res 1995;36:672–84.
[5] Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000;21:697–738.
[6] McCarty MF. Modulation of adipocyte lipoprotein lipase expression as a strategy for preventing or treating visceral obesity. Med Hypotheses 2001;57:192–200.
[7] McCarty MF. A paradox resolved: the postprandial model of insulin resistance explains why gynoid adiposity appears to be protective. Med Hypotheses 2003;61:173–6.
[8] Hube F, Lietz U, Igel M, et al. Difference in leptin mRNA levels between omental and subcutaneous abdominal adipose tissue from obese humans. Horm Metab Res 1996;28:690–3.
[9] Lefebvre AM, Laville M, Vega N, et al. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 1998;47:98–103.
[10] Montague CT, Prins JB, Sanders L, et al. Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. Diabetes 1997;46:342–7.
[11] Montague CT, Prins JB, Sanders L, et al. Depot-related gene expression in human subcutaneous and omental adipocytes. Diabetes 1998;47:1384–91.
[12] Fried SK, Russell CD, Grauso NL, et al. Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. J Clin Invest 1993;92:2191–8.

[13] Panarotto D, Poisson J, Devroede G, et al. Lipoprotein lipase steady-state mRNA levels are lower in human omental versus subcutaneous abdominal adipose tissue. Metabolism 2000;49:1224–7.
[14] Margetic S, Gazzola C, Pegg GG, et al. Leptin: a review of its peripheral actions and interactions. Int J Obes Relat Metab Disord 2002;26:1407–33.
[15] Ostlund Jr RE, Yang JW, Klein S, et al. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 1996;81:3909–13.
[16] Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–5.
[17] Saad MF, Damani S, Gingerich RL, et al. Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab 1997;82:579–84.
[18] Staiger H, Tschritter O, Machann J, et al. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obes Res 2003;11:368–72.
[19] Williams LB, Fawcett RL, Waechter AS, et al. Leptin production in adipocytes from morbidly obese subjects: stimulation by dexamethasone, inhibition with troglitazone, and influence of gender. J Clin Endocrinol Metab 2000;85:2678–84.
[20] Rosenbaum M, Pietrobelli A, Vasselli JR, et al. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. Int J Obes Relat Metab Disord 2001;25:1365–71.
[21] Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 1964;239:375–80.
[22] Ramis JM, Franssen-van Hal NL, Kramer E, et al. Carboxypeptidase E and thrombospondin-1 are differently expressed in subcutaneous and visceral fat of obese subjects. Cell Mol Life Sci 2002;59:1960–71.
[23] Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:E45.
[24] Puigserver P, Llado I, Palou A, et al. Evidence for masking of brown adipose tissue mitochondrial GDP-binding sites in response to fasting in rats made obese by dietary manipulation. Effects of reversion to standard diet. Biochem J 1991;279(Pt 2):575–9.
[25] Llado I, Pons A, Palou A. Effects of fasting on lipoprotein lipase activity in different depots of white and brown adipose tissues in diet-induced overweight rats. J Nutr Biochem 1999;10:609–14.
[26] Ramirez I, Kryski AJ, Ben-Zeev O, et al. Characterization of triacylglycerol hydrolase activities in isolated myocardial cells from rat heart. Biochem J 1985;232:229–36.
[27] Belfrage P, Vaughan M. Simple liquid–liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. J Lipid Res 1969;10:341–4.
[28] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[29] Hinegardner RT. An improved fluorometric assay for DNA. Anal Biochem 1971;39:197–201.
[30] Folch J, Lees M, Sloane GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
[31] Bullo M, Garcia-Lorda P, Peinado-Onsurbe J, et al. TNFalpha expression of subcutaneous adipose tissue in obese and morbid obese females: relationship to adipocyte LPL activity and leptin synthesis. Int J Obes Relat Metab Disord 2002;26:652–8.
[32] Lima N, Cavaliere H, Knobel M, et al. Decreased androgen levels in massively obese men may be associated with impaired function of the gonadostat. Int J Obes Relat Metab Disord 2000;24:1433–7.
[33] Cohen PG. The hypogonadal-obesity cycle: role of aromatase in modulating the testosterone-estradiol shunt — a major factor in the genesis of morbid obesity. Med Hypotheses 1999;52:49–51.
[34] Larsson B, Bengtsson C, Bjorntorp P, et al. Is abdominal body fat distribution a major explanation for the sex difference in the incidence of myocardial infarction? The study of men born in 1913 and the study of women, Goteborg, Sweden. Am J Epidemiol 1992;135:266–73.
[35] Cnop M, Landchild MJ, Vidal J, et al. The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. Diabetes 2002;51:1005–15.
[36] Roemmich JN, Clark PA, Berr SS, et al. Gender differences in leptin levels during puberty are related to the subcutaneous fat depot and sex steroids. Am J Physiol 1998;275:E543–51.
[37] Luukkaa V, Pesonen U, Huhtaniemi I, et al. Inverse correlation between serum testosterone and leptin in men. J Clin Endocrinol Metab 1998;83:3243–6.
[38] Fried SK, Kral JG. Sex differences in regional distribution of fat cell size and lipoprotein lipase activity in morbidly obese patients. Int J Obes 1987;11:129–40.
[39] Frayn KN. Adipose tissue as a buffer for daily lipid flux. Diabetologia 2002;45:1201–10.
[40] Imbeault P, Lemieux S, Prud'homme D, et al. Relationship of visceral adipose tissue to metabolic risk factors for coronary heart disease: is there a contribution of subcutaneous fat cell hypertrophy? Metabolism 1999;48:355–62.
[41] Berman DM, Rogus EM, Busby-Whitehead MJ, et al. Predictors of adipose tissue lipoprotein lipase in middle-aged and older men: relationship to leptin and obesity, but not cardiovascular fitness. Metabolism 1999;48:183–9.