Lipoprotein Lipase Steady-State mRNA Levels Are Lower in Human Omental Versus Subcutaneous Abdominal Adipose Tissue

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Adipose tissue synthesizes lipoprotein lipase (LPL), which helps in the postprandial clearance of triglyceride-rich lipoproteins. Because visceral adipose tissue is generally accepted as the most important metabolic tissue, we sought to verify whether there are regional differences in the expression of LPL. Samples of adipose tissue from subcutaneous and omental fat deposits were obtained from 20 adults undergoing surgery. Total adipose tissue LPL activity was measured using a conventional radioactive substrate assay. Steady-state levels of LPL mRNA were assessed using the very sensitive RNase protection assay technique with 18S ribosomal RNA as an internal control. A correlation was demonstrated between LPL activity levels in subcutaneous and omental tissue ($r = .72$; $P < .01$) and between mRNA levels at both sites ($r = .47$, $P = .04$). LPL mRNA levels were significantly lower in omental compared with subcutaneous depots (omental *v* subcutaneous, 1.7 ± 0.7 *v* 2.1 ± 0.7 arbitrary units [AU] over 18S, $P < .05$). In paired comparisons, LPL mRNA levels in omental adipose tissue were, on average, 20% ± 7% (range, −57% to +9.0%) lower than the levels measured in subcutaneous adipose tissue ($P < .05$). In conclusion, these data suggest that subcutaneous adipose tissue is a reliable surrogate of the expression (activity and mRNA) of LPL in omental adipose tissue, even though omental depots express proportionally less LPL than subcutaneous depots.

BECAUSE IT SYNTHESIZES lipoprotein lipase (LPL), adipose tissue is closely linked to the peripheral metabolism of circulating lipoproteins.[1] The anatomic distribution of adipose tissue has attracted much attention during recent years, having been clearly linked to various metabolic markers[2-4] and a disposition to develop coronary artery disease.[5-8] These cross-sectional data indicating a link between the accumulation of visceral fat tissue and cardiovascular risk have prompted numerous hypotheses on the physiologic role of this fat depot. Visceral adipose tissue, in comparison to the subcutaneous depot, has been shown to produce more plasminogen activator inhibitor-1,[9] more uncoupling protein-2,[10] and more type 3 17β-hydroxysteroid deshydrogenase,[11] but less leptin.[12] All of these findings suggest that visceral adipose tissue is uniquely regulated and that it has intrinsic characteristics possibly involved in the pathogenesis of obesity, as well as cardiovascular disease.[2,8] As a consequence, changes in the visceral expression of LPL could affect both the rate of fat accumulation and the metabolism of triglyceride-rich lipoproteins.

Studies to ascertain if the expression of LPL is different in omental and subcutaneous adipose tissue have produced inconsistent results.[12-18] Since adipose tissue LPL plays a pivotal role in lipid metabolism and is a major determinant of the amount of capillary-anchored enzyme available for the clearance of triglyceride-rich lipoproteins,[19] it is highly desirable to define how LPL is regulated in the two adipose tissue depots. An important corollary of such uncertainty is that while subcutaneous adipose tissue is usually the most accessible fat depot in clinical studies, changes in LPL in that depot might not be representative of events in intraabdominal adipose tissue.

Our study addresses these issues and shows that (1) LPL between omental and subcutaneous adipose tissue is well correlated and (2) there is a significant reduction of LPL expression in intraabdominal adipose tissue.

SUBJECTS AND METHODS

Study Population

Adipose tissue biopsies were obtained from 20 patients (12 men and 8 women) undergoing elective open-abdominal surgery at the Centre universitaire de santé de l'Estrie (Sherbrooke, Quebec, Canada). All patients fasted for at least 6 hours preoperatively. The surgical procedures included 16 laparotomies for bowel disease, 1 for gynecologic disease, 2 for lysis of abdominal adherence, and 1 for insertion of a dialysis catheter. One patient had chronic renal insufficiency. A posteriori, we found that the inclusion of this particular subject did not affect the strength of our analyses; therefore, we decided to leave this patient in the final analysis. All women were postmenopausal, and 5 of the 8 women who participated reported the use of estrogen replacement therapy. Besides this, no patient was using steroids or any medication known to affect LPL or lipid metabolism. The study was approved by the local hospital ethics committee, and all patients provided informed written consent.

Adipose Tissue Sampling

Biopsies of omental and subcutaneous adipose tissue (2 to 3 g each) were collected at the start of surgery. To avoid destruction of tissue and degradation of RNA, cauterization was not used on the biopsy site prior to sampling. After sampling, specimens were immediately frozen on dry ice and stored at −80°C until analysis for steady-state mRNA levels and total LPL tissue activity.

Measurement of Steady-State LPL mRNA Levels

The concentration of human LPL mRNA was determined with a RNase protection assay as described previously.[20] Total cellular RNA from adipose tissue samples was isolated using a guanidinium thiocyanate-phenol technique. Briefly, approximately 100 mg adipose tissue was homogenized in 1 mL TRIzol Reagent (GIBCO BRL Life

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Technologies, Burlington, Ontario, Canada), and the fatty cake resulting from centrifugation at 4°C and 12,000 × *g* was discarded before proceeding with the other steps of the procedure. RNA was subsequently hybridized overnight with a molar excess of [^{32}P]-labeled antisense LPL cRNA transcripts and the housekeeping gene 18S ribosomal cRNA. The mixture was then digested with RNases A and T1, and the protected fragments were separated on a 6% acrylamide urea denaturing gel. After electrophoresis, the gels were quantified using a PhosphorImager system (Molecular Dynamics, Sunnyvale, CA). The LPL mRNA content was normalized per unit of 18S ribosomal RNA and expressed as arbitrary units (AU) of 18S ribosomal cRNA.[20]

Measurement of Total Tissue LPL Activity

The total extractable LPL activity was measured as described previously.[20] Briefly, approximately 150 mg frozen adipose tissue was homogenized with a polytron in 300 µL extraction buffer (0.5% deoxycholate, 0.2 mol/L Tris, 0.25 mol/L sucrose, 15% bovine serum albumin, 10 U/mL heparin, and 0.02% Nonidet P-40, pH 8.6) and centrifuged for 15 minutes. Samples of the infranatant below the fatty cake were diluted 1:15 with detergent-free extraction buffer. Aliquots (100 and 150 µL) were then mixed with 150 µL glycerol-stabilized [^{3}H]-triolein substrate containing heated human serum, and the mixture was incubated for 1 hour at 37°C. The results are expressed as micromoles of nonesterified fatty acid released per hour (or unit) per 1 mg frozen adipose tissue.

Statistical Analyses

Data are the mean ± SD unless otherwise indicated. Because of the relatively small sample size, nonparametric tests were used in all analyses unless otherwise indicated. A Wilcoxon signed rank test was used to compare continuous variables on the same individual, and a Mann-Whitney *U* rank test was used to compare continuous variables between sexes. The Spearman test was used to verify correlations. A *P* value less than .05 was considered significant. All statistical analyses were performed with the software StatView 5.0 for Windows (SAS Institute, Chicago, IL).

RESULTS

Table 1 shows characteristics of the patients according to gender. No statistically significant differences were apparent between men and women according to age or body mass index (BMI), nor were there any differences in LPL activity or steady-state mRNA between omental and subcutaneous adipose tissue.

Table 1. Characteristics of the Subjects by Gender

Characteristic	Men	Women	All	*P**
No. of subjects	12	8	20	
Age (yr)	58 ± 10	62 ± 9	59 ± 9	NS
BMI (kg/m^2)	27.6 ± 5.6	25.9 ± 6.4	26.9 ± 5.8	NS
Subcutaneous adipose tissue				
Total LPL activity (U/mg)	4.04 ± 0.90	4.67 ± 2.35	4.29 ± 1.61	NS
mRNA level (AU)	2.01 ± 0.65	2.33 ± 0.86	2.14 ± 0.74	NS
Omental adipose tissue				
Total LPL activity (U/mg)	4.34 ± 0.95	4.59 ± 1.63	4.44 ± 1.23	NS
mRNA level (AU)	1.71 ± 0.84	1.71 ± 0.71	1.71 ± 0.77	NS

*Men *v* women.

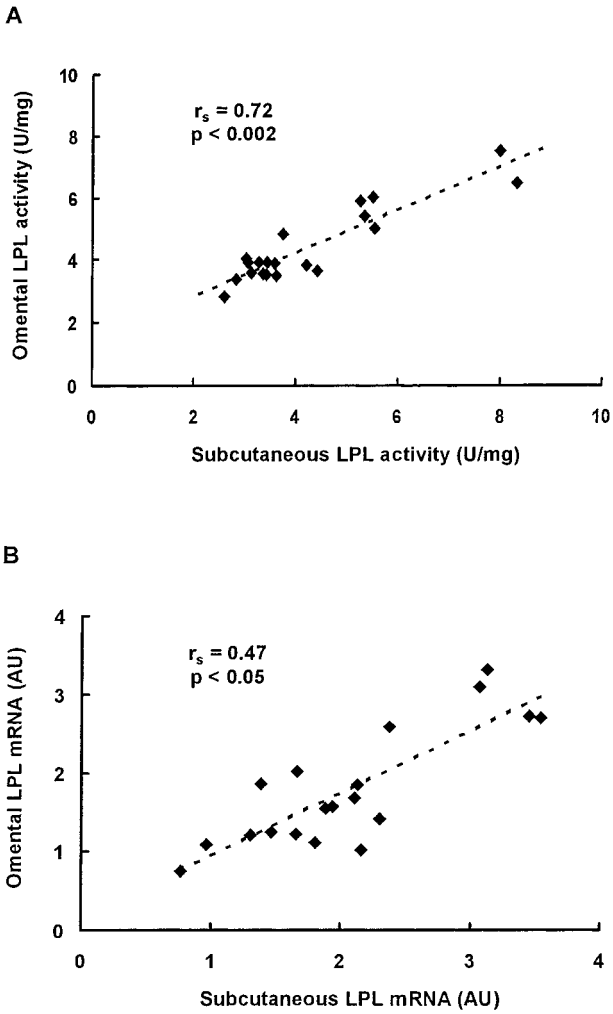

Fig 1. Relationship between total tissue LPL activity (A) and steady-state LPL mRNA concentrations (B) within subcutaneous or omental adipose tissue depots.

LPL activity and steady-state levels of LPL mRNA were well correlated between subcutaneous and omental adipose tissue (Fig 1). The correlation coefficient was .72 and .47 for LPL activity and LPL mRNA levels, respectively. LPL activity was similar in both depots (4.3 ± 1.6 U/mg in subcutaneous tissue *v* 4.4 ± 1.2 U/mg in omental tissue, P = NS by Mann-Whitney *U* rank sum test), but mRNA levels were significantly reduced in omental adipose tissue (2.1 ± 0.7 AU) compared with subcutaneous tissue (1.7 ± 0.7 AU, $P = .008$ by Mann-Whitney *U* rank sum test) (Fig 2). This difference in LPL mRNA levels was present in both genders (data not shown). However, in this gender-stratified analysis, the statistical significance was lost in men but maintained in women ($P = .13$ for men and $P < .05$ for women).

Finally, we explored the relationship between total tissue LPL activity and mRNA levels in both subcutaneous and

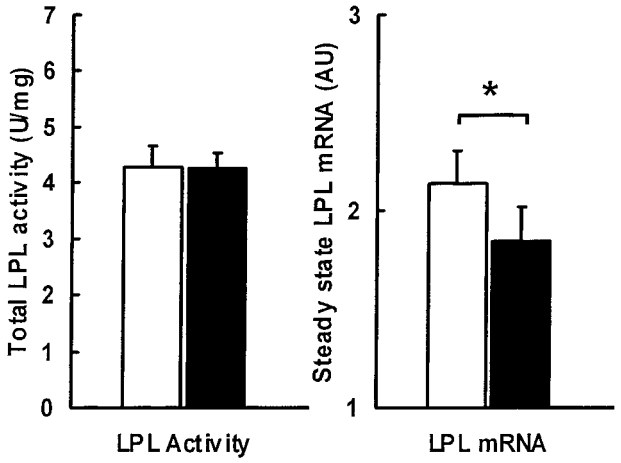

Fig 2. Difference in total tissue LPL activity and steady-state LPL mRNA levels in subcutaneous (□) and omental (■) adipose tissue. *$P = .008$. Error bars are the SEM.

omental adipose tissue. There was no correlation between these variables in either adipose tissue depot ($P > .05$ by Spearman test; data not shown).

DISCUSSION

There have been few published studies on the relationship of variables such as metabolic activity or mRNA levels between the omental and subcutaneous fat depots for the purpose of establishing whether subcutaneous tissue is a dependable surrogate for the less accessible visceral tissue. The fact that subcutaneous adipose tissue is usually not considered as important metabolically as visceral tissue has probably contributed to this situation.[2-4] However, there are indications that some metabolic variables in subcutaneous adipose tissue correlate with plasma lipid and insulin concentrations,[21] as well as with some variables of visceral obesity as measured by computed tomography.[22] Our study supports these data by demonstrating that abdominal subcutaneous adipose tissue expresses LPL and that there are clear correlations for LPL between the subcutaneous and omental fat depots. If we do not take into account the relative volume of both fat depots, it is therefore not excluded that subcutaneous tissue can also theoretically contribute, via its LPL synthesis, to the overall metabolism of lipoproteins.

In this study, total extractable LPL activity did not differ between the two adipose tissue regions, but steady-state LPL mRNA levels were lower in omental versus subcutaneous adipose tissue. While some studies have compared LPL between subcutaneous and omental adipose tissue, the majority have measured either the activity[15-18] or the mRNA level of LPL alone.[12,14] Only the study by Fried et al[13] measured both variables in the two adipose tissue regions. However, in this study, adipose tissue from morbidly obese individuals (mean BMI, 48 kg/m^2) was placed in organ culture for 7 to 8 days. Under these conditions, there was less LPL mRNA and less LPL activity in response to insulin in omental adipose tissue; the basal LPL activity in each depot was not compared, but was shown to be unchanged in a prior study from the same group.[17] In the other studies that measured LPL activity alone, there was either higher[15,16] or lower[18] LPL activity in visceral compared with subcutaneous adipose tissue. The reasons for these discrepancies are not clear, but could be related to the fact that (1) it is sometimes the total extractable or heparin-releasable fraction that is assayed, and (2) LPL activity is expressed using various denominators such as cell surface area or tissue weight. While none of these denominators are perfect, we decided to express our activity data per whole tissue weight unit, the commonest denominator used in the literature. However, we are well aware that omental fat cells of women tend to be smaller and that the LPL activity per cell can actually be lower.[17] This argument would concur with lower levels of LPL mRNA in the omental adipose tissue of women.

Despite the similar LPL activity, we found lower steady-state LPL mRNA levels in omental versus subcutaneous adipose tissue. Two other studies besides the one from Fried et al[13] have examined this question, but none were able to detect any difference for LPL mRNA levels between visceral and subcutaneous adipose tissue.[12,14] The reasons for these contrasting results are not clear, but could be due to the fact that mRNA was measured using a semiquantitative reverse transcriptase–polymerase chain reaction technique that can be less exact under certain circumstances[23] or that the size of their study sample was too small. The fact that Fried et al[13] found a significant difference with a less sensitive Northern blot analysis suggests that the observed differences might have been underlined by the fact that the study sample included only morbidly obese individuals. Eckel[24] had indeed previously shown that the higher the BMI, the higher the adipose tissue activity. It is of interest that in our study and that of Fried et al[13] the difference in LPL mRNA levels was apparent in women only. The pathophysiologic relevance of such a finding is slightly beyond the scope of this report, but may indicate that visceral adipose tissue LPL in women is less responsive to physiologic stimuli such as insulin and glucocorticoids.

Although LPL mRNA levels were lower in omental adipose tissue, LPL activity was essentially the same at both sites. Furthermore, there was no correlation between total tissue LPL activity and steady-state mRNA levels in either of the adipose tissue depots. Given the variety of opportunities for posttranscriptional regulation of LPL,[25-28] it is perhaps not surprising that mRNA concentrations have little to do with the total tissue LPL activity.[29] Our group[20] and others[30] have previously reported a similar lack of correlation. We assessed neither the changes in the intracellular partition of LPL nor the exact proportion of active to nonactive LPL molecules in the two fat depots. It remains plausible, therefore, that a reduced transcription of LPL in visceral adipose tissue may result in an altered intracellular partitioning of LPL, as well as changes in active LPL anchored on capillaries in the vicinity of this intraabdominal adipose tissue.

The fact that our study population included subjects from both genders, one patient with renal insufficiency, and some postmenopausal women should not preclude us from generalizing our findings to a larger population. As mentioned earlier, the inclusion of one patient with renal insufficiency did not impact our analyses in any way. More specifically, our sample has

given us the opportunity to study correlations over a 3- to 4-fold range of variation for any of the LPL variables (activity or mRNA). Despite this fact, we were still able to discern clear differences for LPL mRNA between the two fat depots, a finding corroborated by one other group.[13]

In conclusion, our data demonstrate that subcutaneous and omental LPL are positively correlated, and a subcutaneous abdominal fat biopsy can be a reasonably good surrogate for omental adipose tissue. Alternatively, we have also shown that steady-state mRNA levels for LPL were significantly reduced in omental compared with subcutaneous fat of nondiabetic individuals. The physiologic relevance of this finding in conditions associated with insulin resistance remains to be elucidated, but the regional difference in LPL mRNA abundance indicates that adipose tissue of different depots may not contribute to serum LPL in the same proportion.

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