Expression of lipoprotein lipase in different human subcutaneous adipose tissue regions

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Abstract Steady state expression of lipoprotein lipase was compared in abdominal and gluteal subcutaneous adipose tissue of nonobese men and women. In both regions enzyme activity and lipoprotein lipase mRNA levels were significantly higher in women than in men. In men the enzyme activity was higher in abdominal than in gluteal adipose tissue ($P<0.01$) whereas the opposite was observed in women ($P<0.05$). In both sexes, however, lipoprotein lipase mRNA levels were threefold higher in the abdominal as compared to the gluteal site, whether they were determined in isolated fat cells or in fat segments ($P<0.01$). This regional difference persisted when the mRNA values were expressed as a function of the mRNA concentration for beta-actin. There was a correlation between the two adipose tissue regions as regards the values for enzyme activity and mRNA level (r = 0.6–0.8). Northern blot analysis revealed two mRNA species of 3.5 and 3.7 kilobases, respectively. It is concluded that there are regional variations in the steady state expression of lipoprotein lipase in human subcutaneous adipose tissue. This involves site variations in gene expression as well as posttranslational modification of lipoprotein lipase enzyme activity and may contribute to the characteristic variations in adipose tissue mass and distribution between men and women. —**Arner, P., H. Lithell, H. Wahrenberg, and M. Brönnegård.** Expression of lipoprotein lipase in different human subcutaneous adipose tissue regions. *J. Lipid Res.* 1991. **32:** 423–429.

Supplementary key words fat cell • messenger RNA • abdominal adipose tissue • gluteal adipose tissue

It is well recognized that the distribution of body fat differs between the sexes. Men accumulate fat predominantly in the abdominal region whereas women are prone to deposit fat in the femoral and gluteal areas. In addition, the proportion of body fat is higher in nonobese women than in nonobese men. The characteristic difference in body fat distribution between the sexes is observed in nonobese subjects and is maintained in grossly obese individuals although males sometimes can develop a female type of obesity and vice versa (1). The classification of obesity into different subtypes is of general importance to clinical medicine. Only the male type of fatness (android obesity) is associated with metabolic and cardiovascular complications to obesity; this association is observed even in moderate forms of android obesity (2–4). The female type of obesity (gynoid fatness) has no such association and becomes dangerous only in grossly obese subjects (2–4).

The mechanisms responsible for regional differences in the adipose distribution are only partly elucidated. It appears that site variations in fat cell metabolism play an important role in this respect (see references 5–7 for recent reviews). Over 95% of the fat cell volume constitutes of triacylglycerols. Thus, regional changes in the rate of synthesis and breakdown of triacylglycerols in adipocytes may influence the net deposition of fat in various adipose areas. Lipoprotein lipase (LPL) in adipose tissue may play an important role in this respect, since this enzyme is involved in the regulation of triacylglycerol formation. It is repeatedly documented that there are site differences in the adipose tissue activity of LPL. Studies on nonobese and obese women have shown higher activity of this enzyme in femoral as compared to the abdominal subcutaneous area (8–11).

LPL is synthesized within the fat cell and then transported to its site of action on the capillary endothelium of adipose tissue. The activity of the adipose tissue LPL can be regulated at multiple levels including transcription, translation, glycosylation, and release from fat cells (12). Gene expression may be of importance in this respect, since there is a correlation between the content of mRNA for lipoprotein lipase in rat fat cells and the protein synthesis rate for LPL in these cells (13).

In the present study we have investigated whether there is a difference in steady-state LPL activity between gluteal and abdominal subcutaneous adipose tissue in nonobese subjects and whether such a variation can be explained at the level of LPL gene expression of fat cells from these regions. This was done by comparing LPL activity and

Abbreviations: LPL, lipoprotein lipase; TNA, total nucleic acid.

mRNA levels encoding for the LPL gene in adipose tissue obtained after an overnight fast.

MATERIALS AND METHODS

Subjects

Eleven nonobese men and 8 women, all nonobese, participated in the investigation. The age was 37 ± 3 years (mean ± SE) in men and 37 ± 4 years in women. All were healthy and drug-free. Among women, none took oral contraceptives during the 6 months preceeding the study. The women were investigated in the middle of the menstrual cycle. One woman was post-menopausal. The remaining women had regular menstruations. Body mass index ranged from 21.9 to 25.8 in men and from 19.1 to 24.7 in women. These values did not differ significantly between the sexes. Waist to hip ratio was 0.95 ± 0.1 in men and 0.90 ± 0.01 in women ($P < 0.05$ using Student's unpaired *t*-test). Total body fat, determined as described previously using a caliper method (14), was 22 ± 1% in men and 34 ± 2% in women ($P < 0.01$). After the subjects had an overnight fast, one subcutaneous specimen (about 0.5 g) was excised from the upper lateral quadrant of the gluteal region and the other from the umbilicus region (abdominal site). Local anesthesia was given in such a way that it did not interfere with the excised adipose tissue (15). One part of the tissue was immediately frozen in liquid nitrogen and stored at −70°C for subsequent molecular biology studies. One part was cut into fragments weighing 5–10 mg each and stored at −70°C for later analysis of LPL activity. The remaining part was used for the preparation of isolated fat cells (14), and was stored at −70°C for subsequent molecular biology experiments. For methodological experiments two nonobese male subjects (age 47 and 50 years, respectively) undergoing cholecystectomy were investigated; they were otherwise healthy. At the beginning of the operation, adipose tissue (1–2 g) was obtained from the surgical wound and a liver biopsy (0.5–1 g) was obtained from the left lobule. The tissue was stored as above. The study was approved by the Ethics Committee of the Karolinska Institute.

LPL activity

The activity of LPL was determined as described in detail (16). In brief, adipose tissue was incubated in a glycine buffer (2.1 mol/l, pH 8.4) containing heparin (1 g/l), bovine serum albumin (0.11 mmol/l), and serum (3%). A[^{3}H]triolein emulsion (5.7 mmol/l) with purified egg lecithin as an emulsifier was used as substrate. One nmol of fatty acid released per min was equal to 1 mU enzyme activity. Three determinations were made on each tissue sample. Separate samples were used for lipid extraction or determination of mean fat cell volume and cell volume and mean fat cell lipid weight; the latter two parameters were determined as described by Hirsch and Gallian (17). LPL activity was expressed either per g of lipid weight or per 10^7 fat cells. The number of fat cells incubated was obtained by dividing the total lipid weight of the sample by the mean cellular lipid weight. Previous methodological studies (16) have shown that there is no difference in LPL activity between fresh adipose tissue and adipose tissue that has been frozen and stored at −70°C.

Preparation of hybridization probes

For construction of the human LPL probe, a 50-base pair oligonucleotide corresponding to nucleotides 258–308 (or amino acids 1–17) in the human complementary cDNA LPL sequence (18) was synthesized according to a procedure described by Melton et al. (19) and cloned into the PstI/HindIII sites in pGEM TM I. The sequences of the insertions were confirmed by DNA sequencing using the dideoxy chain-termination method (20). Vectors and reagents required for in vitro synthesis of cRNA and mRNA using the Sp6/T7 riboprobe system were obtained from Promega Biotech (Madison, WI). For solution hybridization analysis, the probes were radiolabeled with [^{35}S]UTP and for Northern blot analysis with [^{32}P]UTP. As a methodological control we used a ^{35}S-labeled cDNA probe encoding the beta-actin (21).

Solution hybridization procedure

Total LPL mRNA was determined using solution hybridization, which was carried out essentially as described previously (22, 23).

For preparation of total nucleic acids (TNA), adipose tissue or liver (250–500 mg) was homogenized in a sodium dodecyl sulfate (SDS) buffer (1% of SDS; 10 mmol/l of Tris-HCl, pH 7.5, and 5 mmol/l of EDTA), digested with 100 mg of proteinase K for 45 min at 45°C, and subsequently extracted with phenol–chloroform after the addition of isoamyl alcohol (vol 24:1). TNA concentrations were determined spectrophotometrically. For solution hybridization, TNA samples were hybridized to approximately 20,000 cpm/sample of the [^{35}]UTP RNA probe RNA. Hybrids were allowed to form in 40 μl of a buffer consisting of 0.6 mol/l of NaCl, 30 mmol/l of Tris-HCl, pH 7.5, 5 mmol/l EDTA, 0.1% of SDS, 10 mmol/l of dithiothreitol, and 25% formamide at 68°C. After an overnight incubation, samples were digested with RNase by adding 1 ml of a solution containing 40 μg RNAse A and 2 μg RNase T_1 (both from Boehringer-Mannheim, Mannheim, Germany). Digestion was performed for 45 min at 45°C after which RNAse-resistant RNA was precipitated by the addition of 0.1 ml of 6 mol/l of trichloroacetic acid. Precipitates were collected for scintillation counting by filtration on glass fiber filters (Whatman GF/Clifton, NJ).

The amount of LPL mRNA of a sample was determined in duplicate and calculated from a linear standard

curve constructed from incubations with known amounts of in vitro synthesized mRNA, (0.5–30 × 10^{-8} mol/incubation), complementary to the ^{35}S-labeled probe. The between-assay variation and the variation between different identical samples were less than 10%, as estimated by analyzing the same sample in triplicate from three different experiments. RNase-resistant counts per min were less than 3% of input counts per min. The amount of LPL mRNA was related to the amount of TNA in the sample.

Northern blot analysis

Northern blot analysis was performed using total RNA from adipose tissue. This analysis required about 1 g of subcutaneous adipose tissue, which could be obtained from the abdominal and gluteal regions of one of the female subjects. The tissue was extracted as above. Total RNA was prepared by the acid guanidinium thiocyanate method combined with the $CsCl_2$ centrifugation procedure (24). The yield of RNA samples was estimated from the absorbance at 260/280 nm. Northern blot hybridization was performed essentially as described by Maniatis, Fritsch, and Sambrook (24). RNA samples (20–30 μg) were denaturated for 10 min at 65°C in dimethylsulfoxide (50%), formaldehyde (2.2 mol/l), sodium phosphate (10 mmol/l, pH 7.5), EDTA (0.5 mmol/l), and then electrophoresed through an agarose gel (1.2%) containing formaldehyde (2.2 mol/l) in sodium phosphate (10 mmol/l, pH 7.5) for 500 volt-hours in a running buffer containing morpholinopropane sulfonic acid (MOPS, 0.04 mmol/l, pH 7.4), sodium acetate (10 mmol/l), and EDTA (1 mmol/l). The integrity of RNA samples was verified by gel electrophoresis and ethidium bromide staining. After two 30-min equilibrations in 20 × SSC (3 mol/l of NaCl, 0.3 mol/l of sodium citrate) the RNA was transferred by the Southern technique to Hybond-N filters (Amersham Corp., Buckinghamshire, UK). Nylon membranes to which RNA had been transferred were then prehybridized for 2 h at 55°C in 50% formamide, 5 × SSC (1.0 mol/l NaCl, 0.1 mol/l of sodium citrate), 5 × Denhardt's solution (0.02% of Ficoll, 0.02% of polyvinyl pyrrolidine, and 0.02% of bovine serum albumin), 5 mmol/l of phosphate buffer (pH 6.5), 5 mmol/l of SDS, and 200 μg/ml of salmon testis DNA. Hybridization was carried out for 40 h at 55°C in an identical solution containing 4–6 × 10^2 cpm/ml of ^{32}P-labeled cRNA probe. After hybridization, the filter was washed serially with 0.1 × SSC and 0.1% of SDS at 68°C to eliminate nonspecific binding of the probes to the filter and to ribosomal RNA. Autoradiographs were obtained by exposure to Kodak XAR-5 film with an intensifying screen for 48 h at 70°C. Molecular weight standards (0.65–0.00 kilobases) were purchased from Boehringer Mannheim (Mannheim, Germany).

Statistics

Values are mean ± SE. Significance of differences was tested by Student's *t*-test or by analysis of variance. Linear regression analysis was performed using the method of least squares.

RESULTS

Measurements of LPL activity

The activity of LPL in the different fat depots is shown in **Fig. 1**. In men the LPL activity was 30% higher in abdominal than in gluteal adipose tissue ($P<0.01$) when expressed per g lipid weight. When, however, the activity was expressed per fat cell number, no regional differences were observed. In women there were no regional differences in LPL activity when it was expressed per g lipid weight. When, however, it was expressed per fat cell number, the activity was 35% higher in gluteal as compared to abdominal adipose tissue ($P<0.05$).

In both regions the LPL activity was higher in women as compared to men. This difference was 45–90% when LPL was expressed per lipid weight ($P<0.05$) and two- to threefold when the enzyme activity was expressed per cell number ($P<0.01$).

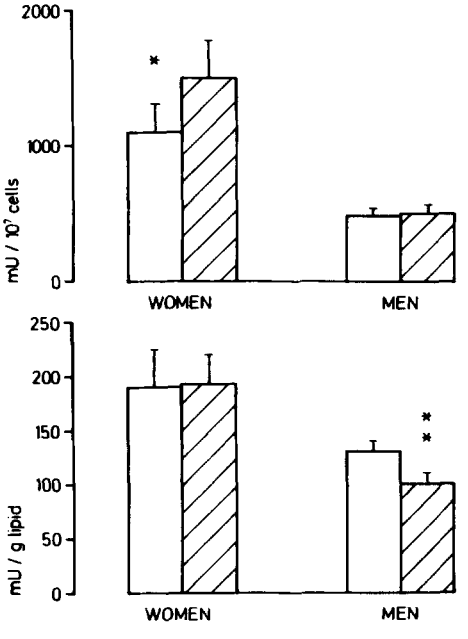

Fig. 1. Expression of lipoprotein lipase activity in abdominal (open bars) and gluteal (hatched bars) subcutaneous adipose tissue of 11 men and 8 women. All subjects were nonobese. Enzyme activity was expressed per cell (upper graphs) of per lipid weight (lower graphs). Values are mean ± SE. Statistical difference was tested using Student's *t*-test; * = $P<0.05$; ** = $P<0.01$.

Measurements of mRNA for LPL

To ensure that the probe used for measuring LPL mRNA detected the correct transcript, experiments using Northern blot analysis were performed on RNA extracts from adipose tissue (**Fig. 2**). In both samples the probe hybridized to mRNA species of 3.5 and 3.7 kilobases, respectively. However, the band corresponding to 3.5 kilobases was much weaker than the 3.7-kilobase band. For technical reasons, it was not possible to completely visualize the 3.5-kilobase band on the photographic print. The data suggest that the probe hybridized to mRNA species similar in size to those previously reported for human LPL (19).

The mRNA levels for LPL in TNA extracts from isolated fat cells are shown in **Fig. 3**. In both men and women, the levels were almost three times higher in abdominal compared to gluteal cells ($P<0.01$). In addition, the level of mRNA was higher in women as compared to men ($P<0.05$); the latter was observed in abdominal (80%) as well as in gluteal fat cells (40%). The absolute values for LPL mRNA (amol/μg TNA) in abdominal cells were 75 ± 9 in women and 43 ± 6 in men. Similar results were obtained whether Student's *t*-test or analysis of variance was used as the statistical method.

Beta-actin mRNA was measured in gluteal and abdominal adipose tissue of six men and six women. No regional differences were observed. The values in women (amol/μg TNA) were 245 ± 19 in the abdominal region and 249 ± 10 in the gluteal region. The corresponding values for men were 243 ± 12 and 247 ± 18. The ratio between mRNA for LPL and beta-actin was also calculated. For women these values were 0.27 ± 0.05 in the abdominal site and 0.12 ± 0.03 in the gluteal site ($P<0.01$). The corresponding values for men were 0.15 ± 0.03 and 0.04 ± 0.01 ($P<0.005$).

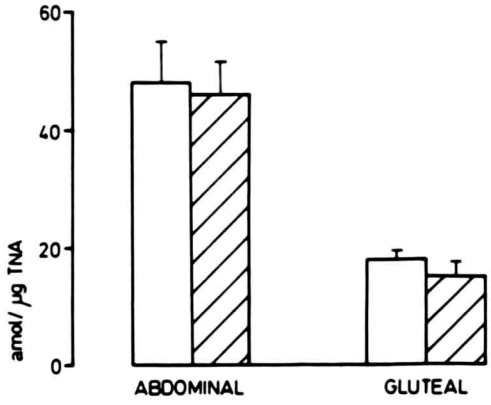

Fig. 3. Expression of lipoprotein lipase mRNA in isolated fat cells from the abdominal or gluteal subcutaneous regions. The amount of mRNA was determined by solution hybridization in the same individuals as in Fig. 1 (See legend to Fig. 1 for further information.)

The influence of the mode of tissue preparation on mRNA levels was also investigated. Mean data obtained with three women plus four men are shown in **Fig. 4**. Almost identical LPL mRNA values were found in fat segments as compared to isolated fat cells.

The solution hybridization assay was also applied to TNA extracts from liver tissue, which does not express the adipose tissue-muscle specific LPL (12). Subcutaneous adipose tissue from the same subjects contained significant amounts of LPL mRNA (about 40 amol/μg TNA). We did not, however detect LPL mRNA in either of the two liver samples investigated. This confirms previous findings (19) and suggests, together with the findings with Northern blot and DNA sequencing, that the probe detects the correct transcript.

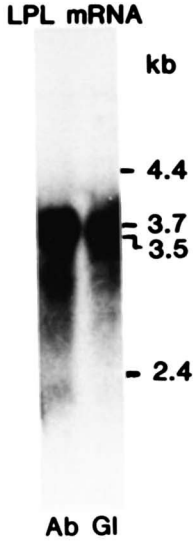

Fig. 2. Northern blot analysis of RNA from human adipose tissue. Total RNA from the abdominal (Ab) and gluteal (Gl) regions was subjected to RNA blot analysis and probed with a ^{32}P-labeled LPL cRNA probe. Positions of RNA markers are indicated. One experiment out of two is depicted.

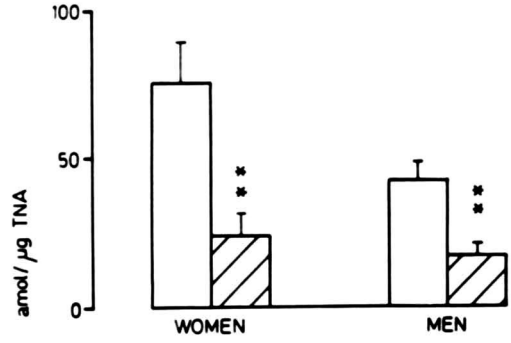

Fig. 4. Lipoprotein mRNA levels in adipose segments (hatched bars) or isolated fat cells (open bars). Fat segments and isolated fat cells were prepared from the same adipose tissue biopsy. Pooled data from four men and three women are presented. (See legend to Fig. 3 for more details.)

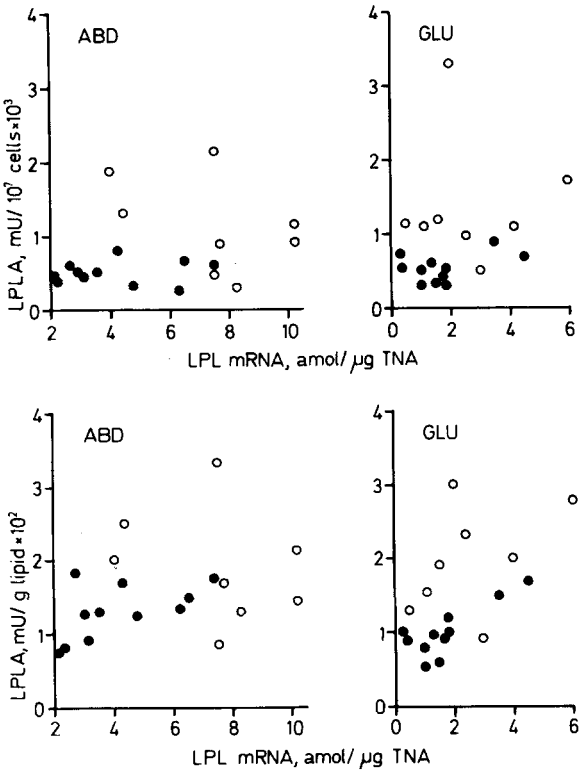

Fig. 5. Correlation between lipoprotein lipase activity (LPLA) and LPL mRNA in the abdominal (ABD) and gluteal (GLU) region. The individual values in the experiment described in Fig. 1 and Fig. 3 were correlated. Open circles represent results with women and filled circles represent results with men.

Correlation between mRNA and enzyme activity

A linear regression analysis was performed using LPL mRNA values in isolated fat cells and values for LPL activity (**Fig. 5** and **Fig. 6**). There was no significant relationship between mRNA and enzyme activity whether LPL activity was expressed per cell or per g lipid weight (r = 0.0–0.5). Similar results were obtained whether gluteal values, abdominal values, or values for men and women were investigated separately or together. However, there was a significant correlation between the abdominal and gluteal sites for LPL mRNA (r = 0.69) as well as LPL activity (r = 0.64). As regards LPL mRNA, a possible drop-out value shown in parentheses in Fig. 6 was omitted in the statistical calculations. Even when this value was included, the correlation between the gluteal and abdominal sites reached statistical significance in the whole material (r = 0.52, $P < 0.05$). Neither waist to hip ratio nor body fat correlated with LPL mRNA or LPL activity (data not shown).

DISCUSSION

This study demonstrates significant variations of steady-state LPL in fat cells between the sexes and between different subcutaneous regions of nonobese healthy subjects. These variations involve enzyme activity as well as the levels of mRNA for the LPL gene.

As regards LPL activity, we observed higher values in the gluteal as compared to the abdominal area in women when the activity was expressed per cell, but no regional differences when LPL activity was expressed per lipid weight. This is in accord with previous reports (8, 9) and stresses the importance of using alternative denominators for the calculation of LPL activity. It is yet unclear whether lipid weight or cell number is the most relevant

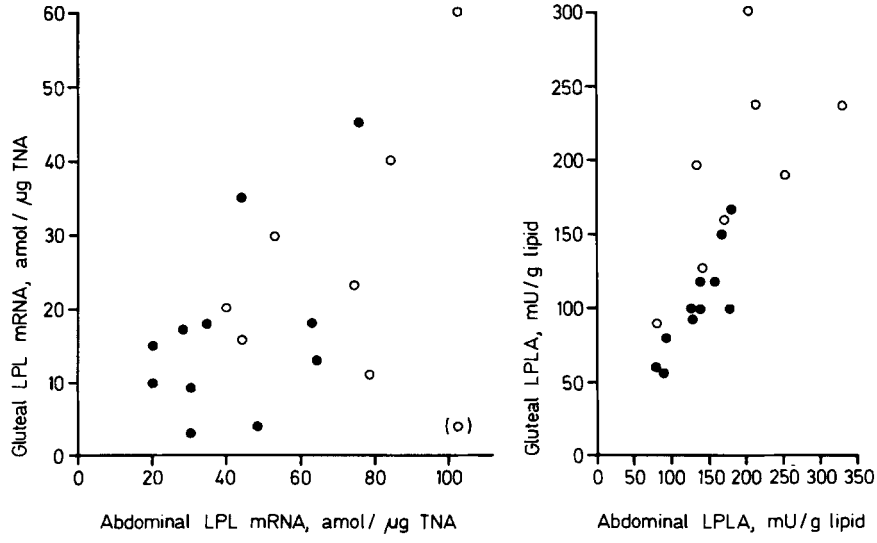

Fig. 6. Correlation between the abdominal and gluteal regions regarding LPL mRNA and LPLA. (See legend to Fig. 5 for further details.)

denominator for the enzyme activity. For men, the findings with LPL activity were opposite those for women. Thus, the values were higher in abdominal than in gluteal adipose tissue when they were expressed per lipid weight but not different when they were expressed per cell. In addition, LPL activity was much higher in both regions of women than men regardless of which denominator was used.

In a previous study, no regional difference in subcutaneous LPL activity was observed in nonobese men (25). However, femoral and gluteal tissues were compared in the latter study, whereas we compared the gluteal and abdominal regions. The physiological consequence of the observed variations in LPL activity between the regions and between the sexes may be that men have higher capacity to accumulate fat in the central than in the distal subcutaneous areas and vice versa in women. In addition, women have a higher capacity to accumulate fat in both regions as compared to men. These variations in LPL activity (presumably influencing lipid storage in fat cells) may be involved in the characteristic differences in total body fat and body fat distribution between men and women, which also were observed in this group of subjects. The significant correlation (r = 0.6–0.8) between the adipose tissue regions for LPL mRNA and LPL activity may favor this assumption. However, it should be kept in mind that changes in LPL activity might occur as a response to variation in fat cell size and body fat rather than be etiologic. Furthermore, LPL-deficient subjects have normal amounts of subcutaneous adipose tissue (12).

Region and sex influenced LPL mRNA differently than enzyme activity. The mRNA level was 40–80% higher female than in male adipocytes of both adipose regions. However, in adipocytes of either sex the mRNA levels were three times higher in the abdominal as compared to the gluteal region. At present it is not known whether these differences in LPL mRNA are due to variations in transcription, mRNA stability, or both. Unfortunately, the amount of adipose tissue that could be obtained was too small for the latter type of investigations. However, the values for LPL mRNA presently observed may reflect the actual in vivo values, since we found almost identical levels of LPL mRNA in fat segments and isolated fat cells. If there is an artificial degradation of the nucleic acids when cells are removed from the body, we would have found higher mRNA levels in fat segments, which were immediately processed for mRNA analysis, as compared to isolated adipocytes, which were processed after more than an hour of in vitro preparation. The results of the comparison between fat segments and isolated fat cells indicate also that stromal cells contribute insignificantly to the LPL mRNA concentration in adipose tissue. It is unlikely that the site difference in LPL mRNA is due to a general increase in mRNA in abdominal tissue as compared to gluteal tissue, since the level of beta-actin mRNA was almost identical in both regions. In addition, the site differences persisted when LPL mRNA was expressed as a function of beta-actin mRNA.

In this study we have used the solution hybridization assay for mRNA measurements. This method is very sensitive and allows actual quantification of mRNA in small samples. However, total mRNA encoding for a particular gene is measured. Therefore, it is not possible to study different species of mRNA. The present Northern blot analysis revealed two LPL mRNA species of 3.5 and 3.7 kilobases, respectively, which confirms previous results in humans (19).

By comparing the results with mRNA and enzyme activity it is possible to speculate to some extent about the mechanisms behind variations in steady-state LPL expression between different types of human adipocytes. Expression of LPL activity involves gene transcription, processing, transport, and translation of mRNA, as well as posttranslational modification, secretion, and extracellular activation of the enzyme. Some of the present findings may be explained by variations in the expression of the LPL gene, in particular the overall increase in LPL activity of female as compared to male adipose tissue. However, the regional variations in LPL activity are less likely due to differences in mRNA expression. Instead, they seem more likely to involve posttranscriptional steps in the processing and modification of the protein. The apparent lack of correlation between LPL mRNA and LPL activity argues for this hypothesis. It should also be noted that the regulation of adipocyte LPL activity may be different in basal and stimulated conditions. It has recently been demonstrated in 3T3-L1 adipocytes that insulin regulates LPL activity at posttranscriptional and posttranslational levels (26, 27). In addition we measured LPL enzyme activity and not the actual amount of LPL protein. It is possible that LPL mRNA correlates better with the amount of LPL protein than with the activity of LPL.

In summary, this study shows some regional and sex differences in LPL activity of human subcutaneous adipose tissue which may be of importance for the characteristic differences in body fat distribution and total body fat content between men and women. The mechanisms for variations in LPL expression between different adipocytes seem to be localized at the level of mRNA as well as at posttranslational levels.

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