Depot-Specific Differences in Adipose Tissue Gene Expression in Lean and Obese Subjects

Anne-Marie Lefebvre, Martine Laville, Nathalie Vega, Jean Paul Riou, Luc van Gaal, Johan Auwerx, and Hubert Vidal

Intra-abdominal and subcutaneous adipose tissue display important metabolic differences that underlie the association of visceral, but not subcutaneous, fat with obesity-related cardiovascular and metabolic problems. Because the molecular mechanisms contributing to these differences are not yet defined, we compared by reverse transcription–polymerase chain reaction the expression of 15 mRNAs that encode proteins of known importance in adipocyte function in paired omental and subcutaneous abdominal biopsies. No difference in mRNA expression between omental and subcutaneous adipose tissue was observed for hormone sensitive lipase, lipoprotein lipase, 6-phosphofructo-1-kinase, insulin receptor substrate 1, p85α regulatory subunit of phosphatidylinositol-3-kinase, and Rad. Total amount of insulin receptor expression was significantly higher in omental adipose tissue. Most of this increase was accounted for by expression of the differentially spliced insulin receptor lacking exon 11, which is considered to transmit the insulin signal less efficiently than the insulin receptor with exon 11. Perhaps consistent with a less efficient insulin signaling, a twofold reduction in GLUT4, glycogen synthase, and leptin mRNA expression was observed in omental adipose tissue. Finally peroxisome proliferator activated receptor-γ (PPAR-γ) mRNA levels were significantly lower in visceral adipose tissue in subjects with a BMI <30 kg/m^2, but not in obese subjects, indicating that relative PPAR-γ expression is increased in omental fat in obesity. This suggests that altered expression of PPAR-γ might play a role in adipose tissue distribution and expansion. *Diabetes* 47:98–103, 1998

Intra-abdominal body fat accumulation is an independent risk factor of obesity-related health problems, including NIDDM, cardiovascular disease, hypertension, and hyperlipidemia, and is associated with a significant increase in overall morbidity and mortality (1,2).

From INSERM U.325, Département d'Athérosclérose (A.-M.L., J.A.), Institut Pasteur, Lille; INSERM U.449 and Centre de Recherche en Nutrition Humaine de Lyon (M.L., N.V., J.P.R., H.V.), Faculté de Médecine R. Laënnec, Lyon, France; and University Hospital UIA (L.V.G.), Edegen, Belgium.

Address correspondence and reprint requests to Dr. Hubert Vidal, INSERM U 449, Faculté de Médecine R. Laënnec, Rue G. paradin, F-69373 Lyon Cedex 08, France. E-mail: vidal@cimac-res.univ-lyon1.fr.

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GS, glycogen synthase; HSL, hormone sensitive lipase; IRS-1, insulin receptor substrate 1; LPL, lipoprotein lipase; PCR, polymerase chain reaction; PFK-1, 6-phosphofructo-1-kinase; PI, phosphatidylinositol; PPAR, peroxisome proliferator activated receptor; Rad, protein Ras associated with diabetes; RT, reverse transcription.

The mechanisms responsible for the association between intra-abdominal fat accumulation and health outcomes are not well known, but several possible factors might be implicated. Visceral fat (mesenteric and omental adipose tissues) is drained into the portal venous system, leading to a direct supply of free fatty acids and other secreted products of adipocytes to the liver (3). In addition to the localization of the tissue, the metabolic consequences of visceral obesity might be related to specific differences in the properties of the adipocytes. Several lines of evidence indicate that adipocytes can function as endocrine cells, producing not only fatty acids but also several bioactive peptides (4). Production of such secreted signaling molecules might be profoundly different between adipocytes in different adipose tissue depots. For example, omental adipocytes were recently shown to express more plasminogen activator inhibitor 1 than subcutaneous adipocytes, providing a possible link between visceral fat and vascular disease (5,6). Moreover, leptin, the adipocyte-specific product of *ob* gene that plays a major role in the control of body weight in rodents (7), is expressed more in adipocytes from the subcutaneous depots (8,9). Finally, metabolism is also somewhat different in adipocytes that originate from superficial or visceral depots. For example, omental adipocytes have been shown to possess higher catecholamine-induced lipolytic activity than subcutaneous cells (10,11). In addition, resistance to the antilipolytic action of insulin is more pronounced in omental adipocytes (12), a difference that has been attributed, at least in part, to a decrease in insulin receptor affinity (12).

Although depot-specific differences in the intrinsic properties of adipocytes are well established (5,6,8–12), little is known regarding the mechanisms leading to these differences. Distinct regulations of specific key genes could be involved. In the present study, we compared the levels of 15 mRNAs that encode proteins of known importance in adipocyte metabolism and function in paired omental and abdominal subcutaneous adipose tissue biopsies from lean and obese humans. We specifically investigated the expression of the genes that code for *1*) the lipoprotein lipase (LPL) and the hormone sensitive lipase (HSL), two key enzymes controlling important aspects of fatty acid metabolism in adipocytes; *2*) the insulin-sensitive GLUT 4, glycogen synthase (GS), and 6-phosphofructo-1-kinase (PFK-1) that catalyze important steps of intracellular glucose metabolism; *3*) the two insulin receptor variants (with and without exon 11), insulin receptor substrate 1 (IRS-1), the p85α regulatory subunit of phosphatidylinositol (PI) 3-kinase (PI 3-kinase), and the protein Ras associated with diabetes (Rad), that represent some of the main components of the insulin signaling cascade; and *4*) leptin, which is already known to be less expressed in

omental than in subcutaneous fat (8,9). In addition, we also studied the expression of the four subtypes (α, β, γ1, and γ2) of the peroxisome proliferator activated receptors (PPARs), since nuclear receptors of the PPAR family have been implicated in the control of adipocyte differentiation and metabolic functions (13). We recently demonstrated that the expression of the PPARs is not different in subcutaneous adipose tissue in lean and obese subjects (14). However, the possibility that depot-specific alterations in PPAR expression participate in the different metabolic properties of the adipocytes has never been explored. Due to the small tissue sample available and the very low yields in total RNA from adipose tissue, we used sensitive reverse transcription–competitive polymerase chain reaction (RT-competitive PCR) assays (15) to quantify the mRNA levels of these 15 target genes.

RESEARCH DESIGN AND METHODS

Subjects. Abdominal subcutaneous and visceral (omental) adipose tissue biopsies were obtained from 12 patients (7 women, 5 men) during elective open abdominal surgery. None of the patients had familial or personal history of diabetes or suffered from severe systemic illness. Their age ranged from 31 to 68 years (mean ± SD, 44 ± 10 years), and all had a stable body weight for at least 3 months (range BMI, 20–53 kg/m^2). Tissue samples were immediately frozen in liquid nitrogen and stored at –80°C until total RNA preparation. All patients gave their informed consent, and the project was approved by local ethics committees of Hospice civil de Lyon and the University Hospital of Antwerp.

Total RNA preparation and specific mRNA quantification. Adipose tissue total RNA was prepared using the RNeasy total RNA kit (Qiagen, Courtaboeuf, France), as indicated previously (14,16). RNA samples were quantified by spectrophotometry, and integrity was verified on ethidium bromide stained agarose gel. Average yields were similar in samples from subcutaneous (24 ± 9 μg of total RNA/g of tissue) and omental (18 ± 9 μg/g of tissue) adipose tissue.

The concentration of 15 different mRNAs was measured by specific RT reaction followed by competitive PCR (RT–competitive PCR) (15). We have developed a multispecific competitor DNA molecule that allowed us to quantify precisely the levels of the mRNAs encoding GLUT4, GS, PFK-1, LPL, HSL, insulin receptor (total mRNA and mRNA variant with exon 11), IRS-1, PI-3K, and Rad (17). In addition to these mRNAs, we also set up RT–competitive PCR assays for the four PPAR subtypes (α, β, γ1, and γ2) (14) and for leptin (16) mRNAs. The construction of the different competitor DNA molecules, the sequences of the primers, and the validation of the assays were presented elsewhere (14–18). The sense primers used in the PCR were 5′-end labeled with the fluorescent dye Cy-5 (Eurogentec, Seraing, Belgium), and the PCR products were analyzed with an automated laser fluorescence DNA sequencer (ALFexpress; Pharmacia, Upsala, Sweden) using the Fragment Manager software (Pharmacia), as already presented (14,18). The concentration of the target mRNAs was determined at the competition equivalence point as described in detail previously (15).

To ensure that the variations in mRNA levels were not due to subtle methodological differences, total RNA were always prepared simultaneously from two biopsies from the same subject, and the target mRNAs were measured in the same run of PCR, with the same dilutions of competitor.

Presentation of data. Specific mRNA levels were determined as attomoles (10^{-18} mol) per microgram of total RNA. The main results are presented as the mRNA level ratio between omental and subcutaneous adipose tissue. Data are expressed as means ± SE. The relative mRNA levels in omental versus subcutaneous fat were compared using a nonparametric Wilcoxon's test for paired values. The correlations between BMI and mRNA levels (or mRNA level ratio) were examined using the nonparametric Spearman's rank correlation coefficient. The threshold for significance was set at $P < 0.05$.

RESULTS

To investigate whether the expression of important genes of insulin action and lipid and glucose metabolism differs in visceral and in subcutaneous fat depots, the levels of 15 mRNAs were quantified by RT–competitive PCR in paired biopsies from 12 individuals with various degrees of obesity. Figure 1 shows the omental/subcutaneous level ratio of 11 of these mRNAs. Significant differences were found for the mRNAs encoding GLUT4, GS, insulin receptor, leptin, and PPARγ.

The mRNA levels of LPL and HSL, two essential enzymes of fatty acid metabolism in adipose tissue, were not different in omental and in subcutaneous depots. Among all the mRNA analyzed, these two RNAs were the most abundantly expressed in adipose tissues (LPL: 373 ± 61 vs. 368 ± 491 HSL: 468 ± 100 vs. 465 ± 83 amol/μg total RNA, in omental vs. subcutaneous fat). Furthermore, PI-3K (228 ± 39 vs. 267 ± 37), IRS-1 (12.8 ± 2.5 vs. 11.5 ± 1.3), PFK-1 (6.1 ± 1.7 vs. 6.8 ± 1.6), and Rad (10.5 ± 2.5 vs. 12.3 ± 2.7 amol/μg total RNA in omental vs. subcutaneous fat) mRNA levels were not different between the two depots (Fig. 1).

GLUT4 (14.2 ± 2.0 vs. 27.4 ± 4.1) and GS (29.5 ± 5.4 vs. 47.2 ± 7.4 amol/μg total RNA in omental vs. subcutaneous fat) mRNA levels were about two times lower in visceral fat than in the superficial depot. As shown in Fig. 2, this difference was consistently found in all subjects, regardless of their BMI. Leptin mRNA levels were also markedly lower in omental adipose tissue (Fig. 1). This twofold difference in leptin expression was similarly found in lean and obese subjects (Fig. 2). However, absolute leptin mRNA levels (ranging from 0.1 to 172 amol/μg total RNA) were dramatically higher in obese subjects and showed positive correlations with BMI both in subcutaneous ($r = 0.85$, $P = 0.035$) and in visceral ($r = 0.76$, $P = 0.027$) adipose tissue depots.

The mRNA levels of the nuclear receptor PPAR-γ was slightly, but significantly ($P = 0.037$, Fig. 1), lower in omental (42 ± 8 amol/μg total RNA) than in subcutaneous (53 ± 7 amol/μg total RNA) fat depot. Figure 2 shows that the omental/subcutaneous PPAR-γ mRNA ratio strongly correlated with the BMI of the subjects ($r = 0.83$; $P = 0.017$). Therefore, a marked difference in PPAR-γ mRNA expression between the two fat depots exists in lean subjects (mRNA level ratio = 0.54 ± 0.06 in the six subjects with a BMI < 30 kg/m^2), whereas in the obese subjects, PPAR-γ mRNA levels were similar in the two tissues (mRNA level ratio = 0.99 ± 0.08 in the six subjects with BMI > 30 kg/m^2). When the absolute PPAR-γ mRNA values were analyzed, there was no relation with BMI in subcutaneous adipose tissue ($P = 0.562$). In the omental fat, however, a trend to a positive correlation was observed ($r = 0.55$; $n = 12$), but it did not reach significance ($P = 0.066$) in the population tested. PPAR-γ exists as two spliced isoforms, PPAR-γ1 and PPAR-γ2. At the mRNA level, PPAR-γ2 was less abundant than PPAR-γ1 in human adipose tissue, representing 13.2 ± 4.8% of total PPAR-γ mRNA in subcutaneous and 14.6 ± 1.6% in omental depot (not significantly different). These percentages were similar in lean and obese subjects, and did not correlate with BMI in either subcutaneous ($P = 0.693$) or in visceral ($P = 0.693$) adipose tissue. Finally, the mRNA levels of PPAR-α and PPAR-β, two other members of the PPAR family, were not different in visceral and subcutaneous fat depots ($n = 6$; data not shown). These PPAR subtypes were expressed at very low levels in human adipose tissue (PPAR-α = 2.1 ± 0.4 and PPAR-β = 1.8 ± 0.2 amol/μg total RNA; means ± SE of the determinations in subcutaneous and omental fat).

Insulin receptor was expressed at higher mRNA levels in omental fat (Fig. 1). This difference tended to be more pronounced in obese (ratio omental/subcutaneous insulin receptor mRNA = 2.5 ± 0.8 in the six subjects with BMI > 30 kg/m^2) than in lean subjects (ratio = 1.5 ± 0.5 in the six subjects with BMI < 30 kg/m^2). However, the correlation between this ratio and BMI did not reach significance ($P = 0.057$). Figure

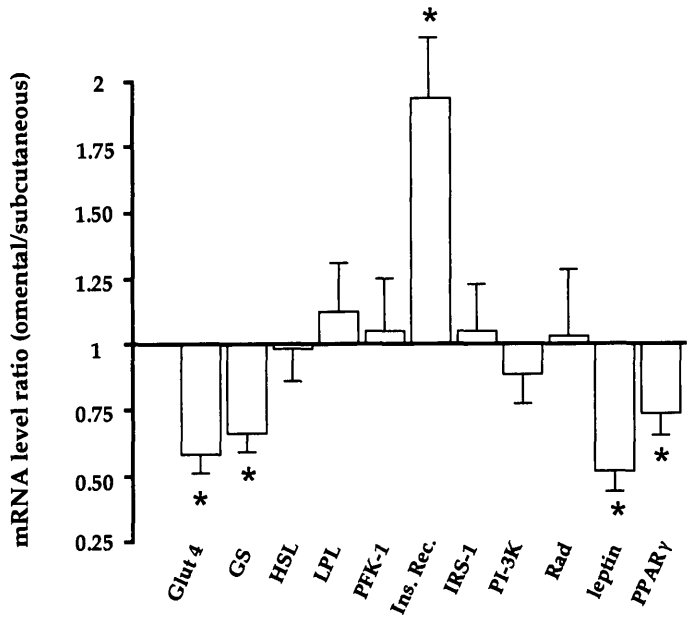

FIG. 1. Relative expression of 11 target mRNAs in omental versus subcutaneous adipose tissue biopsies. Specific mRNAs were quantified by RT-competitive PCR in paired adipose biopsies (n = 8–12 subjects). Data are means ± SE. *P < 0.05.

3 demonstrates that the increase in the mRNA levels of insulin receptor in omental adipose tissue resulted almost exclusively from an increased expression of the insulin receptor isoform without exon 11. The mRNA levels of the variant with exon 11 were not different in the two fat depots. Therefore, the insulin receptor isoform with exon 11 represented 27 ± 4% of total insulin receptor in omental and 51 ± 3% in subcutaneous adipose tissue (P = 0.003). There was no difference in the relative expression of the two insulin receptor mRNA isoforms associated with obesity in both depots.

DISCUSSION

Visceral and subcutaneous adipose tissue are known to be distinct (19). The main reported metabolic differences between human omental and subcutaneous adipocytes are the higher lipolytic capacities of the cells from the visceral depot (10,11,20). The results of this study suggest that this property is not related to differences in the expression levels of the main enzymes of triacylglycerol hydrolysis. Indeed, the mRNA levels of LPL, and more importantly, of HSL, the key enzyme in intracellular lipolysis, were similar in omental and subcutaneous adipose tissue, both in lean and in obese subjects. Therefore, the increased lipolytic rates of omental adipocytes are due to differences in the hormonal control of this metabolic pathway rather than to absolute differences in the expression levels of the enzymes involved. Lower α_2-adrenergic–mediated antilipolytic effect of catecholamines (10,11,20) and higher β_3-adrenoreceptor sensitivity (21) have been observed in omental adipocytes. Moreover, cells from these intra-abdominal depots are less responsive to the antilipolytic action of insulin (10,11). Because the mRNA levels of IRS-1 and PI-3K were not different in the omental and subcutaneous adipose tissues, it seemed unlikely that variation in the level of expression of these major post-receptor actors of the insulin-signaling cascade contribute to the reduced insulin responsiveness of the omental adipocytes. Furthermore, the decreased lipolytic activity of insulin in visceral adipose tissue has been attributed, at least in part, to a lower insulin binding affinity to its receptor (12,22). The marked difference found in the relative expression of the

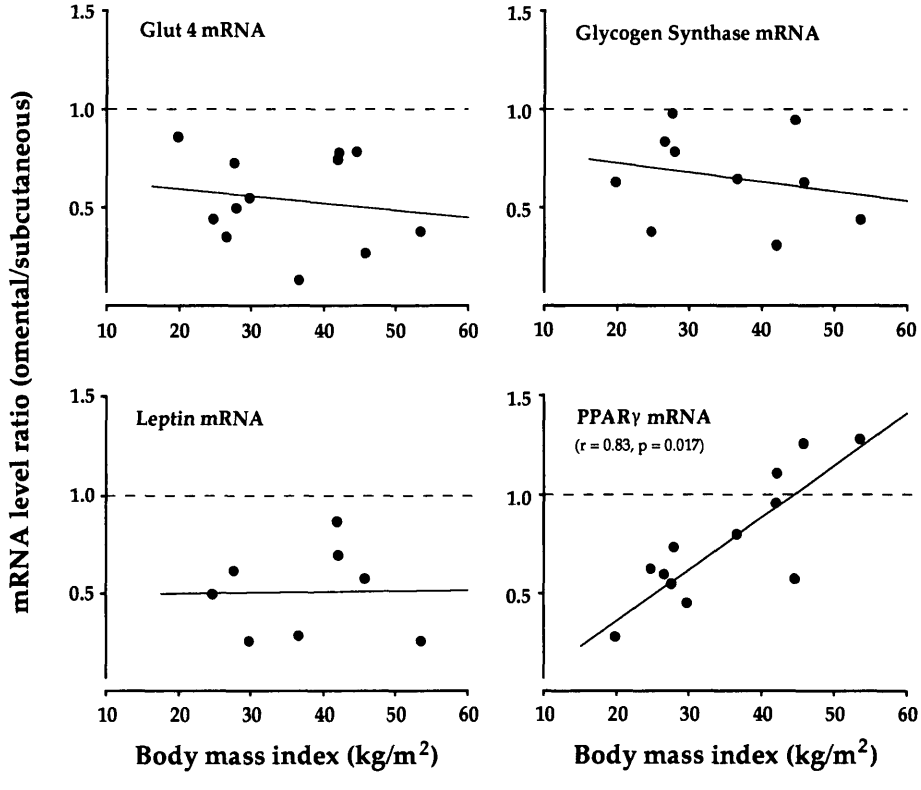

FIG. 2. Relationship between BMI and the relative mRNA levels of GLUT4, glycogen synthase, leptin, and PPAR-γ in omental versus subcutaneous adipose tissue biopsies.

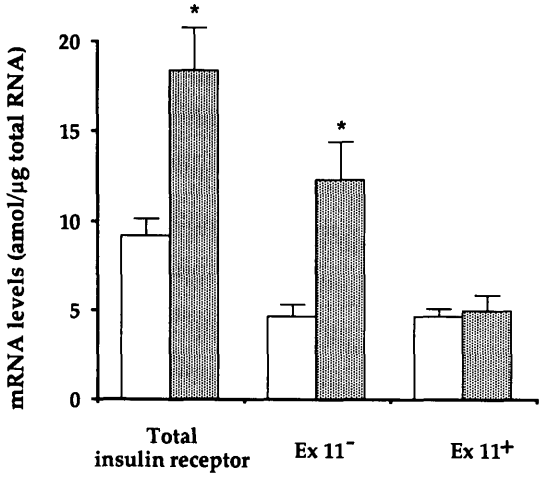

FIG. 3. Absolute insulin receptor mRNA levels in subcutaneous (□) and omental (▨) in paired adipose tissue biopsies (n = 12). Ex11⁻, insulin receptor spliced variant without exon 11; Ex11⁺, insulin receptor variant with exon 11. Data are means ± SE. *P < 0.05.

two spliced variants of the insulin receptor between the two fat depots might contribute to the molecular explanation of this phenomenon. The insulin receptor with the 12 amino acids encoded by exon 11 is indeed considered to transmit the insulin signal more efficiently into the cell than the receptor without exon 11 (23). It is interesting that the form with exon 11 is proportionally less expressed in omental than in subcutaneous fat tissue.

Our results also clearly indicate that total insulin receptor mRNA expression was increased in omental adipose tissue. This result contradicts results from previous reports showing that the number of receptor binding sites, calculated on a per cell basis, was not significantly different between adipocytes isolated from omental and subcutaneous regions (12,22). This would suggest that the observed increase in insulin receptor mRNA levels observed in the present study was not directly translated into mature receptor molecules that are able to bind insulin in collagenase-isolated adipocytes. Regarding the causes of the difference in mRNA levels between the two depots, one might speculate that the reduced responsiveness of the omental adipocytes to insulin could contribute to the increased expression of insulin receptor mRNA. Indeed, insulin is well known to downregulate the expression of its own receptor (24). Furthermore, we have recently demonstrated that insulin resistance was associated with upregulation of the mRNA levels of the insulin receptor variant without exon 11, with no change in the form with exon 11, in adipose tissue in a model of mildly insulin-deficient rats obtained by neonatal streptozotocin treatment (25).

In addition to inhibiting lipolysis in adipocytes, insulin stimulates glucose uptake. The incorporated glucose is mainly used for the re-esterification of fatty acids and the production of lactate, with a small proportion being converted into glycogen (26,27). Using a semi-quantitative RT-PCR method, it was very recently reported that GLUT4 mRNA levels tended to be reduced in omental adipocytes from severely obese women, but the difference did not reach significance (28). Furthermore, the same authors indicated that the stimulatory effect of insulin on glucose transport was similar in omental and subcutaneous fat cells (28), suggesting a lack of difference in the GLUT4-mediated glucose uptake between the two depots. Here, however, using quantitative RT-PCR assay, we clearly found a twofold decrease in GLUT4 mRNA levels, associated with a significant reduction in the levels of GS mRNA, in omental adipose tissue. Moreover, these differences were consistently observed in lean and in obese subjects. If these differences at the mRNA level are translated at the protein level, these results might suggest a lower GLUT4-mediated glucose uptake, and perhaps glucose storage, in omental adipocytes. This apparent discrepancy with the results of Marette et al. (28) requires more careful investigation to verify whether differences in glucose uptake and metabolism exist between omental and subcutaneous adipose tissue in humans.

Depot-specific differences in leptin expression have been already reported (8,9), and our data confirm these observations. Leptin mRNA levels were about twice as high in subcutaneous compared with omental adipose tissue, and this difference was consistently found in lean and obese subjects. The potential mechanisms that could be responsible for this difference in leptin expression between the two adipose tissue depots have been discussed in detail recently by Montague et al. (9). As suggested by those authors (9), if the reduced responsiveness to insulin of omental adipocytes also affects the regulation of gene expression, it is likely to play a role in the decreased leptin expression in the visceral depot. However, the positive effect of insulin on leptin gene expression is relatively weak in humans (16) when compared with the situation in rodents (29), and, more importantly, adipocytes from obese subjects produce more leptin (16,30) while they are markedly resistant to the action of insulin (31). These data suggest that other regulators of leptin gene expression (e.g., catecholamines [9]) more likely participate in the depot-specific difference.

Over the last years, the nuclear receptors of the PPAR family have emerged as one of the central regulators of the expression of the main genes controlling adipocyte differentiation and metabolic functions (13,32,33). PPARs are transcription factors that are activated by fatty acid metabolites. The adipose specific isoform PPAR-γ is activated by prostaglandin J derivatives (34,35) as well as by insulin-sensitizing agents of the thiazolidinedione family (36). The key role of PPAR-γ in adipose tissue development and function supports the speculation that PPARs could be involved in obesity and pathologies with altered lipid metabolism (33,37). Recently, we demonstrated that there was no major alteration in the expression of the four PPAR isoforms in abdominal subcutaneous adipose tissue of obese patients (14). In the present study, we found that PPAR-γ mRNA levels were about twofold higher in subcutaneous than in omental adipose tissue in lean and moderately overweight subjects. In contrast, in obese patients, PPAR-γ mRNA levels were similar in the two depots. This latter situation appeared to be mainly due to an obesity-linked increased expression of PPAR-γ in the visceral depot, without a significant change in the subcutaneous adipose tissue. This difference was specific to the PPAR-γ isoform since neither PPAR-α nor PPAR-β mRNA levels were different between the two adipose depots. In addition, both the γ1 and γ2 isoforms of PPAR-γ were affected. Therefore, the expression ratio between the two spliced isoforms (18) of PPAR-γ remained the same in all the adipose depots, independently of the degree of obesity of the subjects. The impli-

cations of the relative increase in PPAR-γ mRNA levels in omental adipose tissue in severely obese subjects are currently unknown. Because these changes are proportional to the increase in BMI, it is tempting to speculate that the alteration in PPAR-γ expression could be related to the expansion of visceral adipose tissue mass observed in these patients. Although there is no difference in the expression levels of PPAR-γ in subcutaneous adipose tissue (14, and this study), these data suggest that this nuclear receptor could play a possible role in the pathogenesis of altered fat depot regionalization, and perhaps also in the function of the omental adipocytes, in human obesity.

The comparison of the steady-state mRNA expression levels of 15 genes in paired omental and subcutaneous adipose tissue biopsies was made possible by the use of a very sensitive RT–competitive PCR assay (15). However, this study focused only on mRNA levels, and some of the differences in gene expression that were observed might not occur at the protein levels. Conversely, important differences could occur in the regulation of the protein without alteration in the mRNA levels. In addition, it should be pointed out that, since whole adipose tissues were studied, the differences among depots in the numbers and characteristics of other cells than mature adipocytes could contribute to the variations in mRNA observed. However, this was probably not the case for leptin and PPAR-γ, which are adipocyte-specific genes (7,15).

In summary, we have quantified for the first time the mRNA levels of 15 important proteins of adipocyte metabolism and function in subcutaneous and omental adipose tissues in humans. Although most of the measured mRNA displayed similar levels of expression in the two fat depots, we found consistent depot-related differences in the levels of GLUT4, GS, insulin receptor, and leptin mRNAs, reinforcing the concept that subcutaneous and visceral fat possess distinct and specific metabolic properties. In addition, a difference in the relative expression of the adipose tissue specific nuclear receptor PPAR-γ was associated with severe obesity and the expansion of the visceral fat depot. The implications of these alterations in the physiopathology of obesity remain to be defined.

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