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Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues

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Abstract

We have investigated the difference in gene expression of six proteins secreted by adipocytes in paired biopsies from visceral and abdominal subcutaneous adipose tissue in nine individuals with various degrees of obesity. The mRNAs levels of leptin, TNFα, angiotensinogen, acylation stimulating protein (ASP), cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) were quantified by RT-competitive PCR. ASP and angiotensinogen mRNA levels were higher in the visceral fat, whereas the mRNA levels of leptin and CETP were higher in the subcutaneous depot. TNFα mRNA expression was similar in the two sites. For angiotensinogen, the difference was more pronounced in the subjects with body mass index (BMI) lower than 30 kg/m^2 whereas for ASP, CETP and leptin, the difference was observed regardless the BMI of the subjects. PLTP mRNA levels in subcutaneous, but not in the visceral, adipose tissue were positively related to the BMI of the subjects. These results strongly suggest that visceral and subcutaneous adipocytes may have different properties in the production of bioactive molecules.

Keywords: Angiotensinogen; Acylation stimulating protein; Cholesterol ester transfer protein; Phospholipid transfer protein; TNFα; Leptin

1. Introduction

Subjects with accumulation of visceral fat are at a higher risk for the development of obesity-related metabolic derangements, such as type-2 diabetes, cardiovascular disease, hypertension and hyperlipidemia [1–3]. The molecular basis of this association between intra-abdominal obesity and health problems is still largely unknown. Different properties between adipocytes from the intra-abdominal or the subcutaneous depots have been reported, especially regarding the control of lipolysis [4,5]. In addition, because adipocytes are now regarded as endocrine-like cells, capable of secreting several molecules and hormones [6,7], depot-related differences in the production of some of them could participate in the disorders linked to visceral obesity. This concept was recently reenforced with the demonstration that visceral adipocytes express and produce more plasminogen-activator inhibitor 1 (PAI-1) than subcutaneous cells

Abbreviations: TNFα, tumor necrosis factor α; LPL, lipoprotein lipase; ASP, acylation stimulating protein; CETP, cholesterol ester transfer protein; PLTP, phospholipid transfer protein; PAI-1, plasminogen-activator inhibitor 1; RT-cPCR, reverse transcription-competitive polymerase chain reaction; BMI, body mass index

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[8,9]. Among the numerous adipocyte-derived products identified so far, some play a role in the regulation of the fat mass and the control of adipocyte differentiation [6,7,10]. Others are involved in the control of lipid and cholesterol metabolisms [11,12]. Some of them also participate in the immune system [13,14] and in the regulation of the blood flow and vascular functions [15–17]. With regard to their multiple biological functions, it is likely that they participate in the metabolic consequences of obesity, such as altered lipid metabolism, insulin resistance and cardiovascular complications. It is therefore important to verify whether adipose tissue depot-specific differences exist in the expression and production of these factors in humans.

In the present study, we have evaluated the gene expression of several of these proteins in paired omental and abdominal subcutaneous adipose tissue biopsies from subjects spanning a wide range of body mass index (BMI). Due to the small size of the tissue samples and to the very low yields in total RNA from adipose tissue, we have elaborated a multispecific competitor cDNA construct for the quantification by reverse transcription-competitive polymerase chain reaction (RT-cPCR) assay [18] of mRNAs encoding adipocyte-secreted proteins. We have investigated the fat depot-related difference in mRNA expression of acylation stimulating protein (ASP), cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP), three major determinants of lipid and lipoprotein metabolism, leptin and tumor necrosis factor α (TNFα), two cytokines that participate in the control of the fat mass and potentially in the development of insulin-resistance, and angiotensinogen, a possible contributor to the obesity-related cardiovascular risk.

2. Materials and methods

2.1. Subjects and tissue sampling

Abdominal subcutaneous and visceral (omental) adipose tissue biopsies were obtained from nine patients (five women, four men) during elective open abdominal surgery. Patients with a previous history of coronary heart disease (angina pectoris or myocardial infarction) were excluded from the study. None of the subjects suffered from severe systemic illness, diabetes mellitus or other diseases known to interfere with lipid metabolism. Their age ranged from 30 to 66 years (mean ± S.E.M. = 42 ± 5 years) and all had a stable body weight for at least 3 months (BMI ranging from 18 to 58 kg/m^2, mean = 34 ± 4 kg/m^2). Tissue samples were immediately frozen in liquid nitrogen and stored at −80°C until total RNA preparation. All patients gave their written informed consent, and the project was approved by the ethics committee of Hospice Civil de Lyon.

2.2. Total RNA preparation and target mRNA quantification

Adipose tissue total RNA was prepared using the RNeasy total RNA kit (Qiagen, Courtaboeuf, France). RNA samples were quantified by spectrophotometry and the absorption ratios at 260 to at 280 nm were between 1.8 and 2.0. Average yields were similar in samples from subcutaneous (1.2 ± 0.2 μg of total RNA/100 mg of tissue) and omental adipose tissue (1.4 ± 0.2 μg/100 mg of tissue). Total RNA samples were diluted in water and stored at −80°C until use.

The concentration of the different mRNAs was determined by an RT-cPCR assay that consists, after a specific reverse transcription reaction, of the co-amplification of the target single-strand cDNA with known amounts of a DNA competitor molecule added in the same PCR tube [18]. We have developed a multispecific competitor DNA to quantify the levels of the mRNAs encoding ASP, CETP, PLTP, LPL, angiotensinogen, PAI-1, leptin, TNFα and β-actin (see after for details of the construction). For the assay of the target mRNAs, specific reverse transcription reactions were performed from 0.1 or 0.2 μg of tissue total RNA with 2.5 U of thermostable reverse transcriptase (Tth, Promega, Charbonières, France) and in the presence of 15 pmol of one of the specific antisense primer. The conditions required to obtain the transformation of all the target mRNA molecules into single strand cDNA during the reverse transcription reaction have been described in detail previously [18]. For cPCR amplification, the reverse transcription medium was added to a PCR master mix (10 mM Tris-HCl pH 8.3, 100 mM KCl, 0.75 mM EGTA, 5% glycerol) con-

taining 0.2 mM deoxynucleoside triphosphates, 5 U of *Taq* polymerase (Life Technologies, Cergy Pontoise, France), 30 pmol of the specific antisense primer and 45 pmol of the specific sense primer. The sense primers were 5′-labelled with the CY-5 fluorescent dye (Eurogentec, Seraing, Belgium). The final volume was 100 μl and four aliquots of 20 μl were transferred to 0.5-ml microtubes containing 5 μl of a defined working solution of the DNA competitor. After 120 s at 94°C, the PCR mixtures were subjected to 40 cycles of PCR amplification with a cycle profile including denaturation for 60 s at 94°C, hybridization for 60 s at 58°C and elongation for 60 s at 72°C. The PCR products were separated and analyzed in 4% polyacrylamide gel electrophoresis (6% for CETP mRNA assay) on a ALFexpress DNA sequencer (Pharmacia, Upsala, Sweden) using the Fragment Manager software (Pharmacia) [19]. The concentration of the target mRNAs was determined at the competition equivalence point as described in detail previously [18]. The absence of contamination with genomic DNA was checked by omitting reverse transcriptase in the reaction. To ensure that the difference in mRNA levels between paired subcutaneous and visceral adipose tissue samples were not due to subtle methodological differences, total RNA were always prepared simultaneously from the two biopsies of the same subject and target mRNAs were measured in the same run of PCR, with the same dilutions of competitor.

2.3. Construction of the competitor

Fig. 1 depicted the organization of the homologous multispecific competitor DNA (as defined in [18]) that was used to quantify the target mRNAs. First, we have constructed the nine specific competitors individually. A cDNA fragment of each target mRNA was obtained by RT-PCR from human adipose tissue using the specific sense and antisense primers (Table 1) and high-fidelity *pfu* DNA polymerase (Stratagen, La Jolla, CA). Each fragment was subcloned (pGEM-T Vector System, Promega) and used to generate a specific competitor either by restriction enzyme digestion and ligation (for ASP, PLTP, LPL, leptin, TNFα and β-actin) or by a PCR-based deletion/mutagenesis method [20] (for CETP, angiotensinogen and PAI-1). Each competitor gave rise to a PCR product that was slightly shorter in size than the normal cDNA (Table 1). To generate the complete molecule, individual competitor cDNAs were associated together (leptin+LPL, CETP+ASP+angiotensinogen, PLTP+β-actin+TNFα, PAI-1) using a PCR overlap extension method [20] and high-fidelity *pfu* DNA polymerase. The four fragments were then subcloned successively in the same plasmid (pGEM) to obtain the full length (2969 bp) multispecific homologous competitor (Fig. 1). Its sequence, verified by automatic DNA sequencing, matched perfectly the target mRNA sequences reported in GenBank. The plasmid was further purified, carefully quantified and stored at −20°C. Working solutions of the competitor plasmid (20 amol/μl to 10^{-3} amol/μl) were prepared by serial dilutions in 10 mM Tris-HCl (pH 8.3), 1 mM EDTA buffer.

To validate the assays, nine RNAs corresponding to part of the target mRNAs were synthetised by in vitro transcription (Riboprobe system, Promega) using the plasmids containing the cDNA fragments initially obtained by RT-PCR and subcloned in pGEM (see before). Known amounts of these RNAs (0.1–50 amol added into the RT medium) were measured by RT-cPCR to generate dose–response curves that were all linear demonstrating that the assays were really quantitative. The coefficient of variation of the different assays was between 9 and 18% when a given mRNA was measured several times in the same tissue sample. The validations of the RT-cPCR-based assays have been reported in detail in a methodological paper [18].

2.4. Presentation of the results

Absolute mRNA levels were determined and presented as amol/μg of total RNA. Data are shown as means ± S.E.M. Because we have previously examined the expression of LPL in the two fat depots [21], we did not measured LPL mRNA in the present study. Moreover, due to the low amounts of total RNA obtained with some biopsies and because depot-related difference in its expression has been already reported [8,9], we also did not investigate the expression of PAI-1 mRNA in this study. The differences between omental and subcutaneous mRNA levels were compared using a non-parametric Wil-

coxon test for paired values. The correlations between BMI and mRNA levels (or mRNA level ratio) were examined using the non-parametric Spearman's rank correlation coefficient. The threshold for significance was set at $P < 0.05$.

3. Results

The sequences of the different primers used for the RT-cPCR and the sizes of the target and competitor PCR products that are generated during the reactions are presented in Table 1. All the target mRNAs can be properly amplified from 0.1 or 0.2 μg of total RNA from human adipose tissue (data not shown). Fig. 2 shows the absolute mRNA levels (expressed as amol/μg of total RNA) determined in abdominal subcutaneous and visceral fat depots. Large differences in the expression levels of the target mRNAs were observed in human adipose tissue. ASP mRNA was abundantly expressed, PLTP and leptin mRNAs were at intermediary levels whereas CETP, angiotensinogen and TNFα transcripts were expressed at extremely low levels (Fig. 2). The mRNA encoding β-actin was measured to verify the integrity of the total RNA preparations. As compared with the other mRNAs of interest, β-actin was expressed at high levels both in subcutaneous and visceral fat.

The mRNA levels of ASP and angiotensinogen were significantly higher in the visceral adipose tissue ($P = 0.03$ for ASP and $P = 0.01$ for angiotensinogen mRNA, respectively). On the opposite, leptin ($P = 0.02$) and CETP ($P = 0.01$) mRNAs were more expressed in the subcutaneous adipose tissue (Fig. 2). The average values of PLTP and TNFα mRNA levels were similar in subcutaneous and in visceral adipose tissue (Fig. 2). Because of the low number of subjects, and to ensure that the differences were real and not due to chance findings, we determined the statistical power of the analyses. We found values higher than 80% for leptin, CETP and angiotensinogen and of 65% for ASP, indicating thus that the results likely correspond to real differences. For ASP, leptin and CETP mRNAs, the depot-related difference was found in almost all subjects, regardless of their BMI (Fig. 3). For angiotensinogen mRNA, a

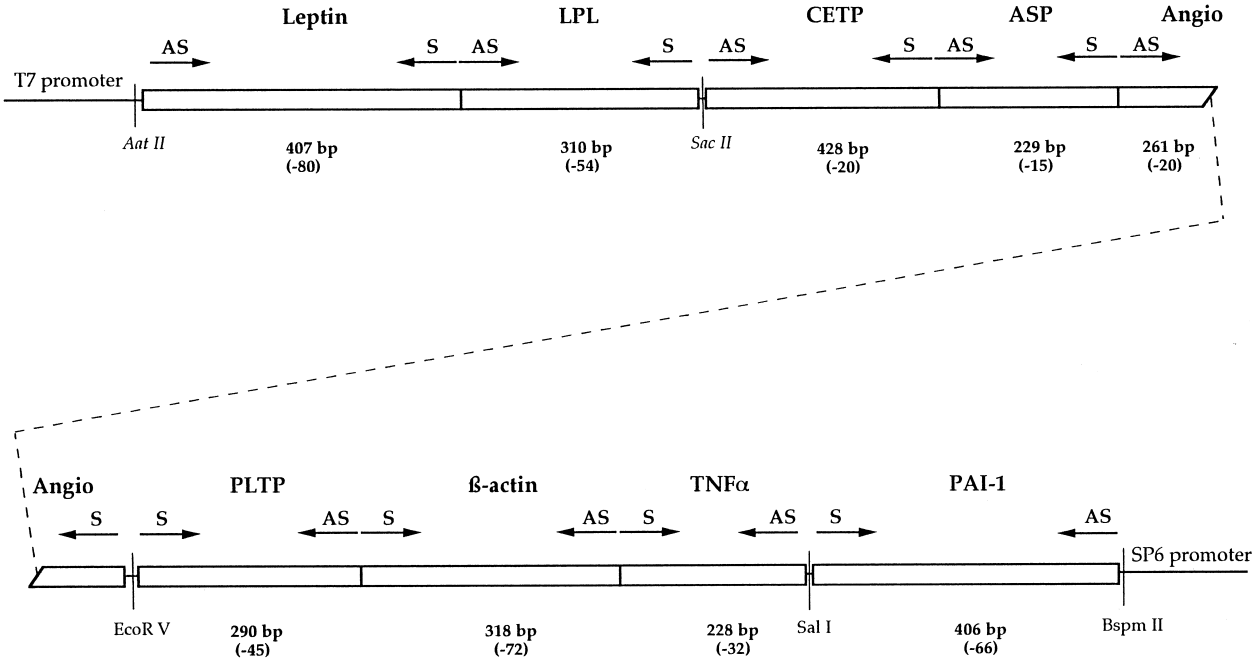

Fig. 1. Organization of the multispecific competitor. The nine homologous competitors were constructed individually and cloned in the pGEM plasmid vector to generate the full length (2969 bp) multispecific competitor, as described in Section 2. The arrows indicate the position and orientation of the sense (S) and antisense (AS) primers for each target (see Table 1 for their sequences). The number of nucleotides that was deleted from the normal cDNA sequences is indicated in brackets.

Table 1
Sequences of the primers used for the RT-cPCR assays

mRNA species	Sense primers	Antisense primers	Size of the PCR products	
			mRNA	Competitor
Angiotensinogen (K02215)	$_{1211}$5′-TTCTGCACACCGAGCTGAAC-3′$_{1230}$	$_{1489}$5′-TTGCCAGGCACTGTGTTCTG-3′$_{1470}$	281	261
ASP (K02765)	$_{176}$5′-TCCTGGACTGCTGCAACTAC-3′$_{195}$	$_{417}$5′-AATCTCCCACGTGGTGATGG-3′$_{398}$	244	229
CETP (M30185)	$_{1030}$5′-AAGATGCCCAAGATCTCCTG-3′$_{1049}$	$_{1475}$5′-AAGCTCTGGAGGAAATCCAC-3′$_{1456}$	448	428
Leptin (U43653)	$_{1}$5′-ATGCATTGGGGAACCCTGTGCGG-3′$_{23}$	$_{485}$5′-AGGTCCAGCTGCCACAGCATGTC-3′$_{463}$	487	407
LPL (M15856)	$_{652}$5′-GGTCGAAGCATTGGAATCCAG-3′$_{672}$	$_{1013}$5′-TAGGGCATCTGAGAACGAGTC-3′$_{993}$	364	310
PAI-1 (M16006)	$_{1}$5′-ATGCAGATGTCTCCAGCCCTCAC-3′$_{23}$	$_{472}$5′-TGAATCTGGCTCTCTCCACCTC-3′$_{451}$	472	406
PLTP (L26232)	$_{315}$5′-GCTCTACTGGTTCTTCTATG-3′$_{334}$	$_{647}$5′-TCAATGCCAACAAGCTCGTC-3′$_{628}$	335	290
TNFα (X01394)	$_{261}$5′-GCCTGTAGCCCATGTTGTAG-3′$_{280}$	$_{518}$5′-ATGGCAGAGAGGAGGTTGAC-3′$_{499}$	260	228
β-Actin (X00351)	$_{338}$5′-AGGCCAACCGCGAGAAGATGAC-3′$_{359}$	$_{725}$5′-AGCTCGTAGCTCTTCTCCAGGG-3′$_{704}$	390	318

The location of the primers on the coding sequences of the target mRNAs is indicated by indexed numbers. The GenBank accession numbers of the nine targets are indicated in brackets. The target mRNAs and the competitors generated PCR products of different sizes allowing their separation on polyacrylamide gel electrophoresis. The sense primers were 5′-end labeled with the fluorescent dye CY-5 for analysis on an automated fluorescence DNA sequencer.

marked depot-related difference was found in the four subjects with BMI lower than 30 kg/m^2. These latter had an about 4-fold higher level of angiotensinogen mRNA in the visceral as compared to the subcutaneous fat depot (Fig. 3). Regarding PLTP mRNA expression, there was no depot-related difference when the mean level values were considered (Fig. 2). However, there was a negative correlation between the visceral to subcutaneous ratio of PLTP mRNA and the BMI of the subjects (Fig. 3). Finally, there was no depot-related difference in the mRNA levels of TNFα, either in lean or in obese subjects (Figs. 2 and 3).

We further analyzed the possible relationships between the target mRNA expression levels in the two fat depots and the degree of obesity of the subjects (Table 2). Leptin mRNA levels were positively correlated with the BMI of the subjects both in the subcutaneous and the visceral depots. Similar results were found with TNFα mRNA levels. Angiotensinogen mRNA levels correlated positively with the BMI in the subcutaneous but not in the visceral adipose tissue. Similarly, a significant positive correlation was found between PLTP mRNA levels and the BMI in the subcutaneous but not in the visceral depot. Interestingly, PLTP mRNA levels in the subcutaneous adipose tissue was also associated with the waist to hip circumference ratio of the subjects ($r = 0.956$,

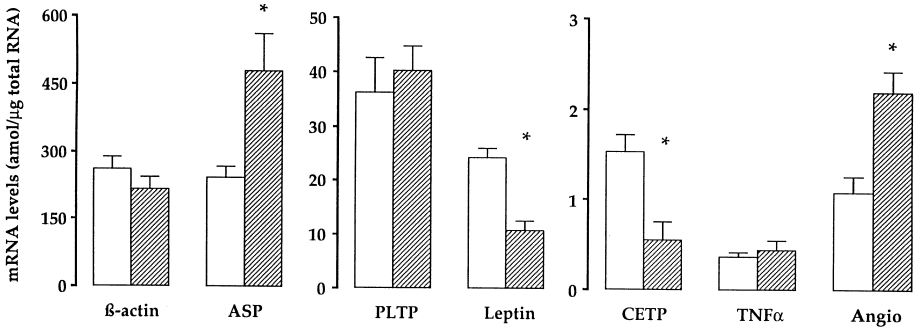

Fig. 2. Expression levels of the target mRNA in the subcutaneous and in the visceral adipose tissue. The absolute levels of the target mRNAs were determined by RT-cPCR as described in Section 2 in paired adipose tissue biopsies from abdominal subcutaneous (open boxes) and visceral (hatched boxes) fat depots. *$P < 0.05$, $n = 9$ subjects.

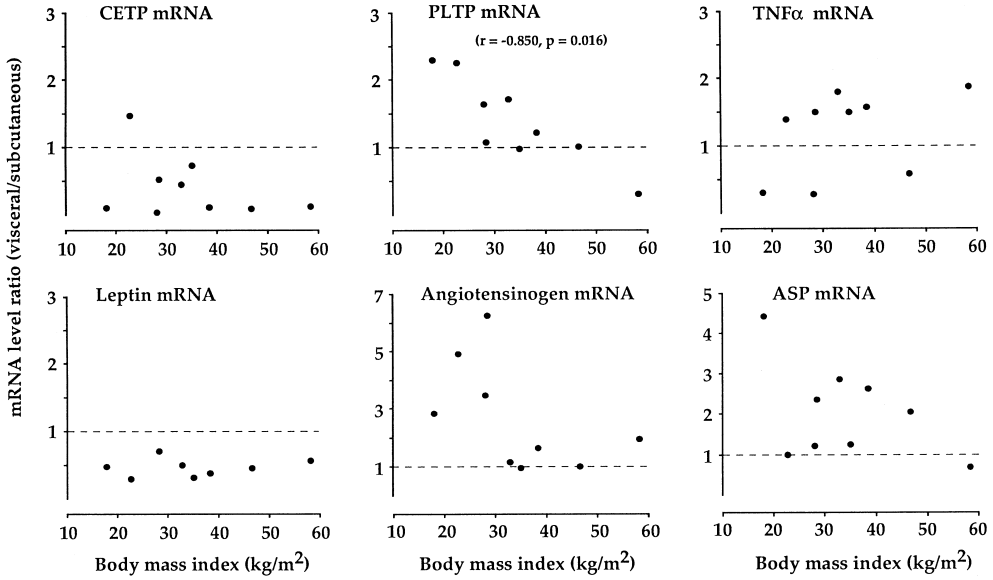

Fig. 3. Relationship between BMI and the relative mRNA levels of the target genes in visceral versus subcutaneous adipose tissue biopsies.

$P = 0.011$). None of the other target mRNA levels was correlated with this parameter.

Despite a tendency for leptin ($r = 0.666$, $P = 0.08$) and for TNFα ($r = 0.633$, $P = 0.07$) mRNAs, there was no significant correlation between the mRNA levels in the subcutaneous fat and the mRNA levels in the visceral adipose tissue for any of the target genes.

4. Discussion

To investigate whether the gene expression of important proteins secreted by adipocytes differs between visceral and subcutaneous human adipose tissue depots, the mRNAs levels of ASP, PLTP, leptin, CETP, TNFα and angiotensinogen were quantified by RT-cPCR in paired biopsies from individuals with various degrees of obesity. In agreement with previous reports, leptin mRNA was found to be more expressed in subcutaneous than in omental fat [21–23]. We also confirmed the lack of depot-related difference in the mRNA expression of TNFα in human adipose tissue [24]. On the other hand, our study demonstrated, for the first time, that mRNA expressions of ASP, angiotensinogen, CETP and PLTP are different in intra-abdominal compared to subcutaneous adipose tissues in humans.

Depot-related differences in the metabolic functions of adipocytes have already been reported and suggested to participate in the link between visceral fat accumulation and a higher risk for cardiovascular disease and other obesity-related outcomes [1]. Visceral adipocytes are less responsive to the anti-lipolytic action of insulin and more responsive to the lipolytic action of catecholamines [4,5]. These metabolic differences are not related to differences in the expression levels of the genes coding either key enzymes of triacylglycerol hydrolysis [21,24] or the main steps of insulin action [21]. Therefore, the increased lipolytic rates of omental adipocytes are most

Table 2
Spearman correlation coefficients for the relationships between target mRNA levels and the BMI of the subjects in the subcutaneous and in the visceral adipose tissues

	Subcutaneous adipose tissue		Visceral adipose tissue	
	ρ	P	ρ	P
β-actin	0.300	0.396	0.567	0.109
ASP	0.550	0.120	−0.017	0.962
PLTP	0.733	0.038	−0.177	0.637
Leptin	0.673	0.048	0.644	0.049
CETP	0.159	0.652	−0.200	0.572
TNFα	0.903	0.011	0.753	0.034
Angiotensinogen	0.837	0.018	−0.132	0.706

probably due to differences in the hormonal control of the metabolic pathway, as previously discussed [21]. In addition to this metabolic aspect, adipocytes are now known to secrete several bioactive molecules that may be involved in lipid metabolism, cardiovascular functions and insulin resistance [7–17,25]. Therefore, the finding that some of these molecules are expressed differently in visceral and in subcutaneous adipose tissue sheds new additional light on the possible molecular link between intra-abdominal fat accumulation and obesity-related metabolic diseases. However, because we reported data at the mRNA levels only, further studies are now needed to verify whether the protein levels and their secretion rates are also different between fat depots.

TNFα produced by adipose tissue is regarded as a possible mediator of insulin resistance [25] and its expression is increased in subcutaneous adipose tissue of obese subjects [26,27]. We found that the correlation between TNFα mRNA levels and the BMI of the subjects is also present in the visceral adipose tissue. However, the expression levels of TNFα in human fat tissues are extremely low ([24], this study), and therefore the contribution of adipose tissue to the circulating levels of TNFα is questionable. It was recently demonstrated using arteriovenous balance technique that the subcutaneous adipose tissue do not release measurable amount of TNFα protein into the circulation in lean and in obese subjects [13]. Our data indicated that a greater contribution of the visceral fat is unlikely because there is no difference in the expression of TNFα mRNA between visceral and subcutaneous depots.

We found that angiotensinogen mRNA levels are higher in visceral fat, particularly in individuals with BMI lower than 30 kg/m^2 in whom a more than 4-fold difference was observed between omental and subcutaneous mRNA abundances. In the more obese subjects, this difference was less apparent, mainly due to significantly increased expression of angiotensinogen in subcutaneous adipose tissue without a change in its expression in the visceral fat. Angiotensinogen is the precursor of angiotensin II, an important regulator of blood pressure and of salt and water balance. Interestingly, angiotensin II can also participate in the regulation of adipocyte differentiation [28]. The mRNA expression of angiotensinogen, as well as of the enzymes required for its conversion into angiotensin II, has been already reported in human adipose tissue [15,16]. Our study demonstrates that there is an increased expression of angiotensinogen in abdominal subcutaneous adipose tissue in obese subjects. The observed depot-related difference in angiotensinogen expression might therefore be of importance in the regulation of adipogenesis and in the development of diseases secondary to fat accumulation, such as hypertension.

ASP has been proposed to be a major determinant of the rate of triglyceride synthesis in adipocytes (see [29] for review). ASP is a 77 amino-acid long peptide derived from the third component of the complement (C3a) by interactions of the proteins of the alternative complement pathway factor B, factor D and C3 [29]. These three proteins are all produced by adipocytes [11,30]. Since ASP is generated by post-translational modification of the protein C3a (removal of the carboxy-terminal arginine), the RT-cPCR assay developed in the present work is specific for the mRNA encoding the fragment C3a of the C3 gene [31]. Therefore, if the post-translational modifications are limiting steps in the production of ASP, the measured ASP/C3a mRNA levels might not estimate correctly the expression of the ASP protein. Nevertheless, our results showed that ASP/C3a mRNA is expressed at very high levels in human adipose tissue. The abundance of ASP/C3a mRNA is about two-fold higher in visceral adipose tissue as compared with abdominal subcutaneous fat. If this difference is reflected at the protein level, a higher local production of ASP in omental fat should result in increased rates of fatty acid uptake and reesterification. However, this seems not to be the case since omental adipocytes have been shown to be characterized by a greater lipolytic rate and reduced responsiveness to insulin [4]. It may be possible that ASP/C3a gene expression is increased in omental fat as a compensatory phenomenon to counteract the greater lipolytic capacity of the visceral cells.

Obesity, and predominantly intra-abdominal fat accumulation, is usually associated with major abnormalities in lipoprotein profile [1,3]. Movements of neutral lipids and phospholipids between the different plasma lipoproteins involve the action of the lipid transfer proteins CETP and PLTP (see [32] for review). CETP mediates the net transfer of cholesterol ester and its exchange with triglycerides from

HDL to VLDL, and PLTP possesses a phospholipid transfer activity from VLDL to HDL as well as an HDL conversion activity [32]. CETP mRNA expression has already been demonstrated in human adipocytes and is related to the membrane cholesterol content of the cells [12]. In addition, a recent report suggested that adipose tissue production may contribute to the plasma concentration of CETP protein [33]. Plasma CETP protein mass and activity are increased in obese subjects [34]; however, we did not find correlation between CETP mRNA expression in adipose tissue and the BMI of the subjects. Subcutaneous adipose tissue level of CETP mRNA is about two-fold higher than the expression in visceral fat, regardless of the degree of obesity of the subjects. Interestingly, subcutaneous adipocytes have been shown to possess higher cholesterol content and HDL binding capacities than omental fat cells [35]. The greater expression of CETP in subcutaneous adipose tissue may therefore participate in this depot-specific difference in cholesterol metabolism. Alternatively, however, the difference in cholesterol metabolism in the two fat depots may influence the expression of CETP gene.

Contrary to CETP, PLTP expression has never been reported in human adipose tissue. In this study, we demonstrated that PLTP mRNA is actually expressed at relatively high level both in subcutaneous and in visceral adipose tissue. The average PLTP mRNA levels were similar to those found for leptin mRNA in obese subjects. These results suggest thus that adipose tissue may contribute to the plasma level of PLTP. In addition, a significant depot-related difference in PLTP mRNA expression was observed, particularly in lean subjects. The greater expression of PLTP mRNA observed in the visceral fat in lean subjects was compensated, in obese subjects, by an increased levels of PLTP mRNA in subcutaneous adipose tissue. Recently, plasma PLTP activity has been related to insulin resistance and to alterations in HDL metabolism in obese type-2 diabetic patients [36]. The same group has also demonstrated that plasma PLTP activity was positively related to the BMI, the waist to hip circumference ratio and fasting blood glucose in obese non-diabetic men [37]. In view of the high level of PLTP mRNA expression in human adipose tissue and of its correlations with the BMI and the waist to hip ratio of the subjects, it will be important to verify to which extent adipose tissue-derived PLTP is involved in the regulation of HDL metabolism and whether or not it plays a role in the insulin resistance syndrome.

In summary, human adipose tissue presents significant depot-specific differences in the mRNA expression levels of several important proteins with bioactive functions. Abdominal subcutaneous adipose tissue displays a greater expression of leptin and CETP mRNAs, whereas omental adipose tissue is characterized by higher levels of ASP, angiotensinogen and PLTP mRNAs, particularly in subjects with BMI lower than 30 kg/m^2. It is now important to verify whether the secretion rates of these factors are also different between the fat depots in order to define new molecular links between adipose tissue distribution and the metabolic derangements associated with obesity.

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