Insulin resistance is a common metabolic disorder that includes a cluster of abnormalities such as hyperinsulinemia, hypertriglyceridemia, a decrease in HDL cholesterol, and obesity with a predominant fat distribution in the upper part of the body. The insulin resistance syndrome is associated with an increased risk of ischemic heart disease. Among the mechanisms explaining this relation, an increase in type 1 plasminogen activator inhibitor (PAI-1) concentration in plasma has been invoked. PAI-1 is a specific inhibitor of plasminogen activators. Its modulation in vivo affects fibrin deposition and smooth muscle cell migration, 2 mechanisms involved in atherosclerosis development. Clinical and epidemiological studies conducted in healthy populations or in patients with coronary heart disease suggest that an increased plasma PAI-1 level is a biological risk factor for the development of atherosclerosis complications, mainly in patients with insulin resistance. Indeed, the ability of PAI-1 to predict such sequelae disappears after adjustment for parameters belonging to the insulin resistance syndrome. The mechanisms responsible for this original association between a metabolic situation and a fibrinolytic inhibitor remain unclear. Several studies conducted in humans and animals have underlined the importance of fat mass in explaining such a relation. We have recently contributed to this discussion by demonstrating the synthesis of PAI-1 by human adipose tissue, this production being higher in omental than in subcutaneous tissue. Furthermore, Eriksson et al have shown that this production is higher in the fat of obese individuals and is related to the size of the adipocytes. To evaluate the relevance of this phenomenon in patients, we have investigated the relation between the production of PAI-1 by adipose tissue, the plasma PAI-1 level, and variables related to the insulin resistance state. To observe this relation, blood and adipose tissue samples were taken nearly simultaneously. Tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) are potent inducers of PAI-1 synthesis. Moreover, TNF-α expressed in adipose tissue is an important component of the link between obesity and insulin resistance. We thus evaluated in vitro the relationship between PAI-1, TNF-α, and TGF-β produced by adipose tissue.

Methods

Correlation analysis between plasma and adipose tissue parameters was performed on subcutaneous tissue obtained during elective abdominoplasty from 30 patients (group 1), 3 men and 27 women, whose age ranged from 16 to 70 years (mean, 47) and with a body mass index (BMI: weight in kilograms divided by the square of height in meters) ranging from 21 to 42 kg/m² (mean, 27).
Comparison between omental and subcutaneous adipose tissue was made in a group of 16 patients (group 2), 7 men and 9 women, whose age ranged from 35 to 79 years and whose BMI ranged from 18 to 39 kg/m² (mean, 27). Tissues were obtained during elective abdominal surgery. Informed consent was obtained from each patient, and the study protocol was approved by the ethics committee of Marseille. The investigation was conducted according to the principles expressed in the Declaration of Helsinki.

Tissue explant incubations for measurements of PAI-1 protein secretion by adipose tissue were made as previously described. In brief, freshly obtained fat specimens were cut into small pieces (1 mm³) under sterile conditions, rinsed once in PBS, weighed, and incubated (1 mL medium per 300 mg tissue) in a medium consisting of minimal essential medium/HAMF12, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/l L-glutamine, 1% FCS, and 1% BSA at 37°C under a 5% CO₂, 95% O₂ atmosphere. Media were collected after a 19-hour incubation, centrifuged, and frozen at −80°C. We have previously shown that the secretion of PAI-1 is linear during an incubation time of at least 19 hours.15

Venous blood samples were obtained just before anesthesia in the 30 patients from group 1. For PAI-1 antigen, samples were drawn into chilled trisodium citrate tubes and were centrifuged as previously described to obtain platelet-free plasma. Parameters reflecting the insulin resistance state, such as fasting insulin triglycerides (TGs) and HDL cholesterol, were evaluated from serum samples by using routine clinical assays. PAI-1 antigen from conditioned media (expressed as ng per mL or ng per g of adipose tissue) and plasma (ng per mL) was assayed using a specific ELISA as previously described.21 PAI-1 activity was quantified using a commercially available kit (Chromolize, Biopool).

Total TGF-β1 protein and TNF-α from conditioned media (expressed as pg per mL) were assayed using ELISA assays from R&D Systems in 27 patients from group 1. All measurements were performed in triplicate. All supplies and reagents were obtained as described in detail previously.15 Results were expressed as mean±SD. The value n represents the number of independent tissue preparations. In the study aimed to compare visceral and subcutaneous production of PAI-1, the between-group comparison was tested by 2-tailed, paired Student’s t test. Significance was defined at P≤0.05. The nonparametric correlation coefficient (Spearman’s) was utilized to examine the relations among study variables.

Results
Relation Between PAI-1 Production by Adipose Tissue and Plasma PAI-1 Level
A significant correlation was observed between plasma PAI-1 antigen or activity and the PAI-1 antigen level measured in conditioned media from subcutaneous adipose tissue explants (r=0.54, P=0.004; r=0.52, P=0.005, respectively; Figure 1).

Relation Between PAI-1 Production by Adipose Tissue, Plasma PAI-1 Level, BMI, and Insulin Resistance Parameters
Plasma PAI-1 level and PAI-1 antigen measured in conditioned media were significantly correlated with plasma variables belonging to the insulin resistance syndrome, such as TGs (r=0.51, P=0.006; r=0.46, P=0.01, respectively) and HDL cholesterol (r=−0.59, P=0.003; r=−0.50, P=0.01, respectively), whereas no relation was observed between PAI-1 levels and insulinemia, PAI-1, or BMI.

Within the cluster of variables related to insulin resistance, associations were found between TGs and HDL cholesterol (r=−0.65, P=0.001), insulin, and TGs (r=0.41, P=0.038).

Discussion
An increased plasma PAI-1 level belongs to the insulin resistance syndrome. Among the variables grouped with insulin resistance, obesity appears to be particularly relevant for explaining the relationships observed between plasma PAI-1 levels and insulin resistance. Indeed, PAI-1 may be an adipose tissue–derived circulating peptide. Sawdey and Loskutoff9 showed that murine adipose tissue expressed PAI-1 mRNA. Mice with genetically induced obesity have an increased PAI-1 expression in adipose tissue, this local increase in PAI-1 concentration being a potential circulating PAI-1.80

In the case of human obesity, PAI-1 expression in adipose tissue was investigated in humans. Recently, we have demonstrated production of PAI-1 by human adipose tissue.15 In the case of human obesity, PAI-1 expression in adipose tissue was investigated in humans. Recently, we have demonstrated production of PAI-1 by human adipose tissue.15

Figure 1. Scatterplot showing the correlation between PAI-1 plasma levels and those of PAI-1 secreted by subcutaneous adipose tissue expressed per g tissue (n=30). Values were compared using a nonparametric correlation coefficient (Spearman’s).

Relation Between PAI-1, TNF-α, and TGF-β Productions by Adipose Tissue
TNF-α production by adipose tissue correlated well with that of PAI-1 (r=0.50, P=0.01; Figure 2). TNF-α production by adipose tissue was correlated with fasting insulinemia (r=0.49, P=0.03). A significant correlation was observed between the production of PAI-1 and TGF-β by adipose tissue (r=0.53, P=0.007; Figure 2).

Relation Between Visceral and Subcutaneous Adipose Tissue Productions of PAI-1
We compared the production of PAI-1 antigen by subcutaneous and visceral fat in patients from group 1. The relation between these productions after a 19-hour incubation period is represented in Figure 3. A high correlation was observed between the production of PAI-1 by visceral and subcutaneous adipose tissue (r=0.91, P<0.001). As we have previously shown, PAI-1 antigen level (expressed in ng per g of tissue) produced in the conditioned medium from omental tissue was higher than that secreted from subcutaneous tissue. The mean±SD and (range) values for omental and subcutaneous tissues were 712.2±464.8 (92 to 1442) and 335.8±183 (46 to 620) ng/g, respectively (P<0.001).
present work, we describe a positive association between the PAI-1 production rate by human subcutaneous adipose tissue and plasma PAI-1 levels. This observation is in favor of a role for adipose tissue in the increased plasma PAI-1 levels found in obesity.

Elevated plasma PAI-1 levels are associated with excessive visceral rather than subcutaneous adiposity. The relation between PAI-1 produced by visceral territories and PAI-1 plasma levels was not analyzed in this study, as blood samples could not be obtained for all of the patients from group 2. However, the strong correlation observed between the PAI-1 quantities produced by the 2 fat territories suggests a similar regulatory pathway of PAI-1 in these 2 tissues despite their different anatomic and metabolic characteristics and validates the use of subcutaneous tissue as a suitable model for studying the production of PAI-1 by adipose tissue. The confirmation in a larger population that omental tissue explants produced more PAI-1 than did explants from subcutaneous tissue during an incubation that lasted 4 times longer underlines the role of omental tissue as a major source of PAI-1.

On examining the relation between the insulin resistance parameters and PAI-1, we found that plasma PAI-1 concentration as well as PAI-1 antigen level measured in conditioned medium was correlated with TGs and HDL cholesterol, whereas no relation was observed with insulinenia and BMI. The lack of correlation between PAI-1 and each of these last 2 parameters could in part be due to the time of blood sampling. Indeed, in most studies showing a correlation between plasma PAI-1 concentration, BMI, and insulinenia, blood samples were obtained early in the morning to avoid the influence of circadian variations in plasma PAI-1 levels. As our main interest was to compare PAI-1 in plasma and PAI-1 production by adipose tissue, plasma samples were not always obtained in the early morning (at the peak of PAI-1) but as soon as possible to the time of induction of anesthesia (which ranged from 8 AM to 4 PM). Moreover, because our study population was mostly female, we could evoke the inclusion of patients presenting a gynoid fat distribution that is known to be not associated with insulin resistance and increased PAI-1 levels. Then, the fact that circadian variations in plasma PAI-1 were not considered for blood sampling and the particular composition of the population studied could both contribute to the lack of correlation observed between PAI-1, insulinenia, and BMI. Furthermore, despite demonstration of an in vitro effect of insulin on PAI-1 production by some cultured cells, our results do not favor the hypothesis of a direct contribution of insulin to PAI-1 level regulation. They also underline the link between PAI-1 expression and a qualitative rather than a quantitative aspect of adipose tissue.

Several studies inferred a role for TNF-α in the relation between obesity and insulin resistance. Recent experiments conducted in TNF-α-deficient obese mice have shown that the absence of TNF-α resulted in a significantly improved insulin sensitivity in obesity. Moreover, an infusion of TNF-α into humans has been reported to result in reduced insulin sensitivity. TNF-α is a potent inducer of expression of inflammatory cytokines, including PAI-1.

**Figure 2.** Relationship between secretion of PAI-1 and those of TNF-α (upper graph) and TGF-β (lower graph) by subcutaneous fat tissue (n=27). Values were compared using a nonparametric correlation coefficient (Spearman’s).

**Figure 3.** Scatterplot showing the correlation between subcutaneous and visceral PAI-1 secretion (n=16). Values are expressed per g of tissue. Subcutaneous and visceral PAI-1 secretion was compared using a nonparametric correlation coefficient (Spearman’s).
of PAI-1 synthesis; when administered to mice, it increased PAI-1 mRNA expression in adipose tissue. The correlation between the secretion rate of PAI-1 and TNF-α secreted by human adipose tissue suggests that TNF-α is involved in the regulation of PAI-1 production in insulin-resistant patients with obesity. It has been proposed that TNF-α could function in an autocrine fashion to regulate insulin sensitivity in adipocytes. We confirmed the link between insulinemia and TNF-α production by adipose tissue: a strong correlation between insulinemia and TNF-α measured in conditioned media was found, as was previously reported in mice. Since Hotamisligil et al. have demonstrated a strong, positive correlation between TNF-α mRNA expression levels in fat tissue and the level of insulinemia, our results emphasize the fact that the measurement of TNF-α protein in conditioned media after a 19-hour incubation could be a surrogate for the quantification of TNF-α mRNA.

TGF-β is a multifunctional agent present in many cells such as platelets, monocytes, and tissue macrophages. It has been implicated in a number of biological processes, including cell adhesion and migration, extracellular matrix production, tissue remodeling, and wound repair. TGF-β stimulates PAI-1 synthesis in human endothelial cells, vascular smooth muscle cells, and HepG2 cells. Injected in vivo into mice, TGF-β is one of the major inducers of PAI-1 expression in adipose tissue. Moreover, Samad et al. have shown that TGF-β mRNA and protein levels were increased in the adipose tissues of obese mice compared with their lean counterparts. The correlation that we have observed between the production of PAI-1 and of TGF-β by human fat tissue suggests, as for TNF-α, an involvement of TGF-β in the regulation of PAI-1 production by human adipose tissue. However, further larger studies are needed to evaluate the respective contribution of each effector. By increasing the production of PAI-1 by adipose tissue, TNF-α and TGF-β could be important determinants in the link between circulating PAI-1 concentrations and the insulin resistance syndrome. Evaluation of the importance of TGF-β and TNF-α in adipose tissue metabolism and insulin resistance is a question that should be addressed.

Acknowledgments

This work was supported by INSERM and Ministère de l’Éducation Nationale, de la Recherche Médicale et de la Technologie (Contrat Quadrannuel) and by Fondation de France. The authors wish to thank Pr H.R. Lijnen (Center for Molecular and Vascular Biology, Leuven, Belgium) for the gift of PAI-1 antibodies; Drs Farnarier and Marin (Laboratory of Immunology, CHU Sainte-Marguerite, Marseilles, France) for the TNF-α and TGF-β1 assays; and D. Nivière, O. Geel, B. Bonardo, and A. Olivi for their skillful assistance.

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PAI-1 Produced Ex Vivo by Human Adipose Tissue Is Relevant to PAI-1 Blood Level
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_Arterioscler Thromb Vasc Biol_. 1999;19:1361-1365
doi: 10.1161/01.ATV.19.5.1361

_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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