Diabetologia (2001) 44: 2025–2031

Diabetologia

Subcutaneous abdominal, but not femoral fat expression of plasminogen activator inhibitor-1 (PAI-1) is related to plasma PAI-1 levels and insulin resistance and decreases after weight loss

A. Mavri[1], M. C. Alessi[2], D. Bastelica[2], O. Geel-Georgelin[2], F. Fina[3], J. T. Sentocnik[4], M. Stegnar[1], I. Juhan-Vague[2]

[1] University Medical Centre, Department of Angiology, Ljubljana, Slovenia
[2] Faculty of Medicine, Laboratory of Haematology, Marseille, France
[3] Faculty of Medicine, Laboratory of Experimental Cancerology – AP-HM, Marseille, France
[4] Medical-Aestetic Centre, Ljubljana, Slovenia

Abstract

Aims/hypothesis. Abdominal fat produces plasminogen activator inhibitor-1 (PAI-1) and could contribute to increased plasma PAI-1 values in human obesity associated with insulin resistance. Femoral fat, which is not associated with insulin resistance, is thought to be metabolically different from the abdominal fat. This study aimed to assess PAI-1 expression in these two fat territories in obese and lean subjects and to determine if concomitant changes of plasma and adipose tissue PAI-1 values occur after weight reduction.
Methods. In 24 obese and 16 lean subjects, PAI-1 expression in abdominal and femoral subcutaneous fat, plasma PAI-1, insulin, triglyceride concentrations and insulin resistance were determined at the start of the study and in obese subjects after a 3-month weight reduction programme as well.
Results. PAI-1 mRNA content in the abdominal subcutaneous fat was higher in obese than in lean subjects and positively correlated with plasma PAI-1 values ($p < 0.01$) and markers of insulin resistance ($p < 0.05$). In 18 obese subjects, re-examined after successful dieting, PAI-1 mRNA content decreased in the abdominal subcutaneous fat along with plasma PAI-1. However, the absolute changes of these two variables were not associated. In contrast, PAI-1 mRNA content in the femoral subcutaneous fat did not differ between lean and obese subjects, was not associated with plasma PAI-1 values or with markers of insulin resistance, and did not change after weight loss.
Conclusion/interpretation. Only the abdominal, but not the femoral subcutaneous fat PAI-1 expression is a potential contributor to increases in plasma PAI-1 in obesity. Both plasma and abdominal subcutaneous fat PAI-1 values decreased significantly after weight reduction, although their absolute changes were not associated. [Diabetologia (2001) 44: 2025–2031]

Keywords PAI-1, abdominal, femoral, adipose tissue, obesity, weight reduction.

Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of tissue-type plasminogen activator and has an important role in regulating the fibrinolytic system and thrombus formation. Higher plasma PAI-1 concentrations impair fibrinolysis, which could account for its predictability for cardiovascular events [1, 2]. Obesity, an independent risk factor for cardiovascular diseases is associated with increased plasma PAI-1 values [3, 4].

The mechanisms involved in increased PAI-1 production in obesity have not been fully explained. The insulin-resistance syndrome, a cluster of metabolic abnormalities, accompanying visceral types of obesity could be an important regulator of PAI-1 expression. Indeed, all the variables related to the insulin resistance syndrome (insulinaemia, BMI, waist-to-hip ratio (WHR), serum triglyceride and HDL cholesterol concentration) are strongly related to

Received: 19 March 2001and in revised form: 3 August 2001

Corresponding author: A. Mavri, University Medical Centre, Department of Angiology, Riharjeva 24, 1000 Ljubljana, Slovenia, e-mail: alenka.mavri@trnovo.kclj.si
Abbreviations: PAI-1, Plasminogen activator inhibitor-1; BMI, body mass index; WHR, waist-to-hip ratio

plasma PAI-1 [5]. Furthermore, treatment with troglitazone which improves insulin resistance, also affects plasma PAI-1 [6].

Plasma PAI-1 concentrations have been observed to correlate independently with the visceral fat depot [7–10]. The fact that human adipose tissue expresses and, when maintained in culture, produces PAI-1 suggests a direct contribution of this tissue to higher plasma PAI-1 values [11, 12]. Ex vivo experiments show that PAI-1 expression from both abdominal subcutaneous and visceral adipose tissues was positively associated with plasma PAI-1 and BMI [12–14], suggesting that both fat territories could control plasma PAI-1 values. Whereas increases in abdominal fat (visceral and subcutaneous) characterise insulin resistance with higher plasma PAI-1 values, femoral fat is the feature of gynoid obesity, which is not associated with insulin resistance and marked increases in plasma PAI-1 values [8]. There are no data on PAI-1 expression in the femoral fat. In order to evaluate whether femoral fat PAI-1 expression is also enhanced in obesity, we assessed PAI-1 expression in the abdominal and femoral subcutaneous adipose tissues of lean and obese subjects.

Weight reduction by hypocaloric diet or surgical treatment leads to dramatic reduction in plasma PAI-1 concentrations [15–21]. Data from these studies indicate that weight reduction-induced reductions in plasma PAI-1 values is more closely related to the changes in fat depot than to markers of insulin resistance. Therefore, in the second part of our study we investigated if the changes in PAI-1 mRNA content in two subcutaneous fat territories (abdominal and femoral) are associated with changes in plasma PAI-1 values after weight reduction.

Methods

Subjects, blood and adipose tissue sampling, and biochemical methods. A total of 40 subjects, 26 women and 14 men, 40 ± 10 years of age (means ± SEM) were included in the study. Of these, 24 were obese (17 women and 7 men; BMI > 25 kg/m^2), and 16 were lean subjects (9 women and 7 men; BMI < 25 kg/m^2). The obese and the lean study groups were matched for age. None of the subjects had a history of thromboembolic disease or diabetes mellitus. Three obese subjects were treated for arterial hypertension. There were eight smokers in the obese group and one in the lean group. Altogether four obese and two lean women were post-menopausal. Obese subjects volunteered to participate in a 3-month body weight reduction programme with a hypocaloric diet under medical supervision. The energy-deficient diet of 5.0 MJ a day consisted of selected conventional foods and provided 40 % energy in the form of protein, 40 % as carbohydrates, and 20 % as fats. All subjects gave their informed consent to participation in the study, which was approved by the medical ethical committee of the Slovenian Ministry of Health.

Anthropometric measurements, blood sampling, and adipose tissue sampling were performed between 0730 h and 0900 h in all subjects at the beginning of the study and were repeated in obese subjects at completion of the weight reduction programme. BMI was calculated as weight in kilograms divided by the square of the height in meters and WHR as waist circumference divided by hip circumference. Blood taken from an antecubital vein from fasting subjects was sampled after a 15-min rest. To measure haemostatic factors, nine volumes of blood were allowed to flow directly into precooled siliconized glass vacuum tubes containing one volume of 0.13 mol/l trisodium citrate (Becton Dickinson Vacutainer System, New York, USA). The tubes were placed in ice water and then centrifuged within 1 h for 30 min at 2000 g and 4 °C. Platelet poor plasma was transferred to small plastic vials, frozen in liquid nitrogen and stored at –70 °C until analysed. For biochemical analysis, blood was collected in vacuum tubes without an anticoagulant. Serum was obtained and stored at –70 °C until analysed. In plasma samples, PAI-1 antigen was determined by an enzyme-linked immunosorbent assay (Imulyse PAI-1, Biopool, Umeå, Sweden), and PAI activity by an amidolytic assay (Spectrolyse/fibrin, Biopool). Triglyceride and glucose were determined in serum by routine biochemical method and insulin by a commercially available radioimmunoassay (Sorin, Biomedica, Saluggia, Italy). Homeostasis model assessment (HOMA) was used to assess insulin resistance (insulin resistance = insulin(mIU/l) · glucose(mmol/l) / 22.5) [22].

Subcutaneous adipose tissue samples were obtained from subumbilical abdominal region and lateral femoral region under local anaesthesia with 2 % lidocaine. A sterile needle (3 cm · 2 mm) attached to a 20 ml sterile plastic syringe was inserted through the skin and adipose tissue was drawn into the syringe by suction. Adipose tissue was immediately frozen in liquid nitrogen and stored at –70 °C until analysed. Subcutaneous adipose tissue from abdominal region was obtained in all subjects, and from femoral region in all lean and 13 obese subjects.

RNA extraction and quantitative reverse transcription polymerase chain reaction (RT PCR TaqMan). Total RNA from adipose tissue were extracted using a method previously described [23]. The integrity of the RNA was verified by electrophoresis in ethidium bromide containing agarose gels and the RNA concentration was determined spectrophotometrically. Reverse transcription was performed as previously described [14]. Amplification was carried out in 25 μl samples (Abiprism 7700, Perkin Elmer, Boston, Mass., USA). Each sample contained 2 μl cDNA in 1X universal Master Mix, 1 μl (400 nmol/l) of each primer and of the TaqMan probe. The TaqMan system is based on Taq polymerase 5'-3' nuclease activity which cleaves a dual labelled non-extendible TaqMan probe designed to hybridize to a sequence between the forward and the reverse primer for every particular amplicon. Fluorescence emission from the reporter (3' end, 6-carboxyfluorescein-FAM) is quenched by the quencher dye (5' end, 6-carboxytetramethylrhodamine-TAMRA) until nuclease degradation during the extension phase of the PCR separates the two dyes and enables detection of the reporter dye fluorescence. The TaqMan PCR conditions were: 2 min at 50 °C, 10 min at 95 °C, then 40 cycles each of 15 s at 95 °C and 1 min at 60 °C, on MicroAmp Optical tubes, covered with MicroAmp Optical caps. Primers and probe for PAI-1 and 18S were designed using the Primer Express (Perkin-Elmer) software.

During the assay the linear increase in fluorescence signals was quantified every 7 s by an Applied Biosystem (Prism 7700). The fluorescence threshold was set within the linear phase across all the samples in a particular run. The point at which each individual sample crossed the threshold was recorded as its Ct value. This is linearly related to the log of the initial amount of template in each reaction, allowing estima-

Table 1. Characteristics of the lean and obese subjects at entry (medians with the 25th and 75th percentile are shown)

	Lean (n = 16)	Obese (n = 24)	p
Weight (kg)	64.8 (53.6–72.5)	100.9 (86.5–118.6)	< 0.001
BMI (kg/m^2)	21.4 (20.3–23.0)	35.6 (31.5–38.6)	< 0.001
WHR	0.80 (0.75–0.84)	0.86 (0.81–0.90)	< 0.01
Insulin (mIU/l)	4.9 (3.1–5.6)	10.3 (4.7–19.0)	< 0.005
Triglycerides (mmol/l)	0.7 (0.6–1.0)	1.2 (1.0–1.9)	< 0.001
HOMA insulin resistance	1.0 (0.7–1.3)	2.3 (0.9–4.8)	= 0.01
Plasma PAI-1:			
Antigen (ng/ml)	3.3 (2.3–4.7)	24.6 (9.2–39.0)	< 0.001
Activity (IU/ml)	2.9 (0.2–4.7)	16.2 (8.9–33.1)	< 0.001
PAI-1 mRNA (fg/10 ng 18S):			
Abdominal sc tissue	6.9 (0.7–9.8)	14.3 (7.8–24.5)	0.0025
Femoral sc tissue	11.3 (4.2–16.8)	12.7 (4.9–28.6)[a]	*ns*

ns, not significant; sc, subcutaneous
[a] Results available from 13 obese subjects

Table 2. Spearman's correlation coefficients for associations between PAI-1 mRNA, from the abdominal or femoral subcutaneous adipose tissue, and observed variables in all subjects investigated

	PAI-1 mRNA Abdominal (n = 40)		Femoral (n = 27)	
	r	p	r	p
BMI	0.49	< 0.01	0.13	*ns*
WHR	0.58	< 0.01	–0.10	*ns*
Insulin	0.39	0.02	0.11	*ns*
Triglycerides	0.33	0.04	0.18	*ns*
HOMA insulin resistance	0.41	< 0.01	0.28	*ns*
PAI-1 antigen	0.55	< 0.01	0.36	*ns*
PAI activity	0.50	< 0.01	0.19	*ns*

tion of initial number of copies in the unknown samples by comparison with standards. Each experiment included a standard curve for the individual amplicon and no template control for each reaction mix. Each sample was tested in triplicate and results were accepted if coefficient of variation was less than 0.5 Ct. Results were expressed as femtograms (fg) of PAI-1 per 10 ng 18S.

Statistical analyses. Results were expressed as medians with range between the 25th and 75th percentiles. Differences between obese and lean subjects were tested by the Mann-Whitney U test. Differences within the obese group (before and after weight reduction) were tested with the Wilcoxon test. Spearman's correlation coefficient was used to examine relations among the variables. Backward stepwise multiple linear regression analysis was performed to evaluate the independence of associations. The independent variable was log transformed before entering the model. A p value of less than 0.05 was considered to be statistically significant.

Results

Table 1 shows the medians with ranges for studied variables. As expected, obese subjects have higher insulin resistance, insulin, triglycerides, as well as plasma PAI-1 concentrations than lean subjects. PAI-1 mRNA content in abdominal subcutaneous adipose tissue of obese subjects was significantly higher than PAI-mRNA content in abdominal subcutaneous adipose tissue of lean subjects. However, PAI-1 mRNA content in femoral subcutaneous adipose tissue did not differ significantly between the two groups. An identical pattern was observed when the analysis was restricted to the 13 subjects in whom both femoral and abdominal tissue biopsies were done simultaneously (data not shown).

In all subjects, PAI-1 mRNA expression in abdominal subcutaneous adipose tissue, but not in femoral, was significantly associated with plasma PAI-1 antigen and activity. Furthermore, only PAI-1 expression in abdominal subcutaneous adipose tissue was positively associated with BMI, WHR, insulin resistance, insulin, and triglyceride concentrations (Table 2). In analysis, restricted to the 27 subjects in whom two adipose tissue biopsies were done simultaneously, PAI-1 expression in abdominal subcutaneous tissue remained in association with BMI, WHR and plasma PAI-1, but not with insulin resistance, insulin or triglyceride concentrations. To assess the importance of these correlations, a multiple linear regression analysis was done, with abdominal subcutaneous adipose tissue PAI-1 content as a dependent variable and BMI, WHR, insulin resistance, and triglycerides as an independent variables. Only WHR was associated with the abdominal subcutaneous adipose tissue

Table 3. Characteristics of the obese subjects ($n = 18$), who successfully completed a 3-month weight reduction programme (medians with the 25th and 75 percentile are shown)

	Before weight reduction	After weight reduction	p
Weight (kg)	94.2 (84.8–115.6)	73.4 (67.0–87.5)	< 0.001
BMI (kg/m^2)	33.7 (29.3–37.7)	26.5 (24.1–29.7)	< 0.001
WHR	0.85 (0.77–0.87)	0.82 (0.78–0.85)	= 0.01
Insulin (mIU/l)	9.4 (3.2–14.7)	5.4 (3.5–7.8)	< 0.01
Triglycerides (mmol/l)	1.1 (0.9–1.6)	0.8 (0.6–1.2)	< 0.05
HOMA insulin resistance	2.3 (0.6–3.3)	1.1 (0.7–1.7)	= 0.01
Plasma PAI-1:			
Antigen (ng/ml)	26.8 (8.7–34.3)	4.4 (2.3–6.5)	< 0.001
Activity (IU/ml)	18.4 (8.4–30.7)	3.8 (1.0–7.0)	< 0.001
PAI-1 mRNA (fg/10 ng 18S):			
Abdominal sc tissue	18.1 (7.7–24.5)	6.1 (3.5–15.6)	= 0.001
Femoral sc tissue	12.0 (5.1–21.1)[a]	11.8 (5.3–18.9)[a]	ns

ns, not significant; sc, subcutaneous
[a] Results available from eight obese subjects

PAI-1 expression ($\beta = 0.38$, $p < 0.02$) and contributed to 12 % of its variability.

The contribution of the abdominal subcutaneous adipose tissue to circulating PAI-1 levels was assessed by a multiple linear regression analysis in which PAI-1 plasma concentrations were dependent and BMI, insulin resistance, insulin, triglycerides, and adipose tissue PAI-1 mRNA were an independent variables. BMI ($\beta = 0.30$, $p < 0.02$), triglycerides ($\beta = 0.34$, $p < 0.01$), and PAI-1 mRNA from abdominal subcutaneous adipose tissue ($\beta = 0.26$, $p < 0.01$) were associated with plasma PAI-1 antigen, whereas insulin resistance and insulin were excluded from the model. Those three variables accounted for 72 % of the variance in plasma PAI-1 antigen.

Altogether 18 subjects successfully completed the weight reduction programme. The body weight was reduced on average by 20.7 kg. There was a significant decrease in insulin resistance, insulin, and triglyceride concentrations. Plasma PAI-1 antigen and activity declined by 67 and 68 %, respectively. PAI-1 mRNA content significantly decreased in the abdominal subcutaneous tissue (-57 %). There was no significant change in PAI-1 mRNA content in the femoral subcutaneous adipose tissue (Table 3). The reduction in PAI-1 mRNA content in abdominal subcutaneous adipose tissue remains significant even if the analysis was restricted to the eight subjects in whom both femoral and abdominal tissue biopsies were performed simultaneously ($p = 0.02$). Of note, after weight reduction, insulin resistance, as well as plasma concentrations of insulin, triglycerides, PAI-1 antigen and activity, as well as abdominal subcutaneous adipose tissue PAI-1 mRNA content in obese subjects were similar to the values in lean subjects. In contrast, BMI was still higher (26.5 vs 21.4 kg/m^2, $p < 0.01$).

Changes of variables were calculated as differences between the values before and after weight loss. The absolute change in plasma PAI-1 antigen during the weight reduction programme was associated with a change in BMI ($r = 0.48$, $p = 0.05$), WHR, and insulin but not with changes in triglycerides or PAI-1 mRNA content in abdominal subcutaneous tissue (Fig. 1). Similar results were obtained for plasma PAI activity (data not shown).

Discussion

The expression of PAI-1 in the adipose tissue has lately attracted considerable attention because this tissue could be an important source of plasma PAI-1 in human obesity. In this study, PAI-1 expression from two subcutaneous territories (abdominal and femoral) was assessed in obese and lean subjects. Additionally, we investigated whether plasma and tissue PAI-1 levels showed concomitant variation during weight reduction. Only the abdominal (but not the femoral) subcutaneous adipose tissue PAI-1 expression was found to be associated with the features of insulin resistance syndrome and could be a potential contributor to increased plasma PAI-1 in obesity. Concomitant reductions in plasma PAI-1 concentrations and abdominal (but not femoral) subcutaneous fat PAI-1 expression were observed after weight reduction, but these were however not associated.

In comparison to lean subjects, higher plasma PAI-1 values, but also PAI-1 mRNA content in the abdominal subcutaneous adipose tissue were found in obese subjects. Both variables were strongly related with the level of obesity as previously reported [12, 14]. Furthermore, we showed a strong positive association between the abdominal subcutaneous adipose tissue PAI-1 expression and markers of insulin resistance syndrome, suggesting that insulin resistance might be involved in the regulation of PAI-1 gene expression in abdominal adipose tissue. However, the finding from multivariate regression analysis of an independent association between abdominal adipose tissue PAI-1 expression and WHR only, does not favour a direct influ-

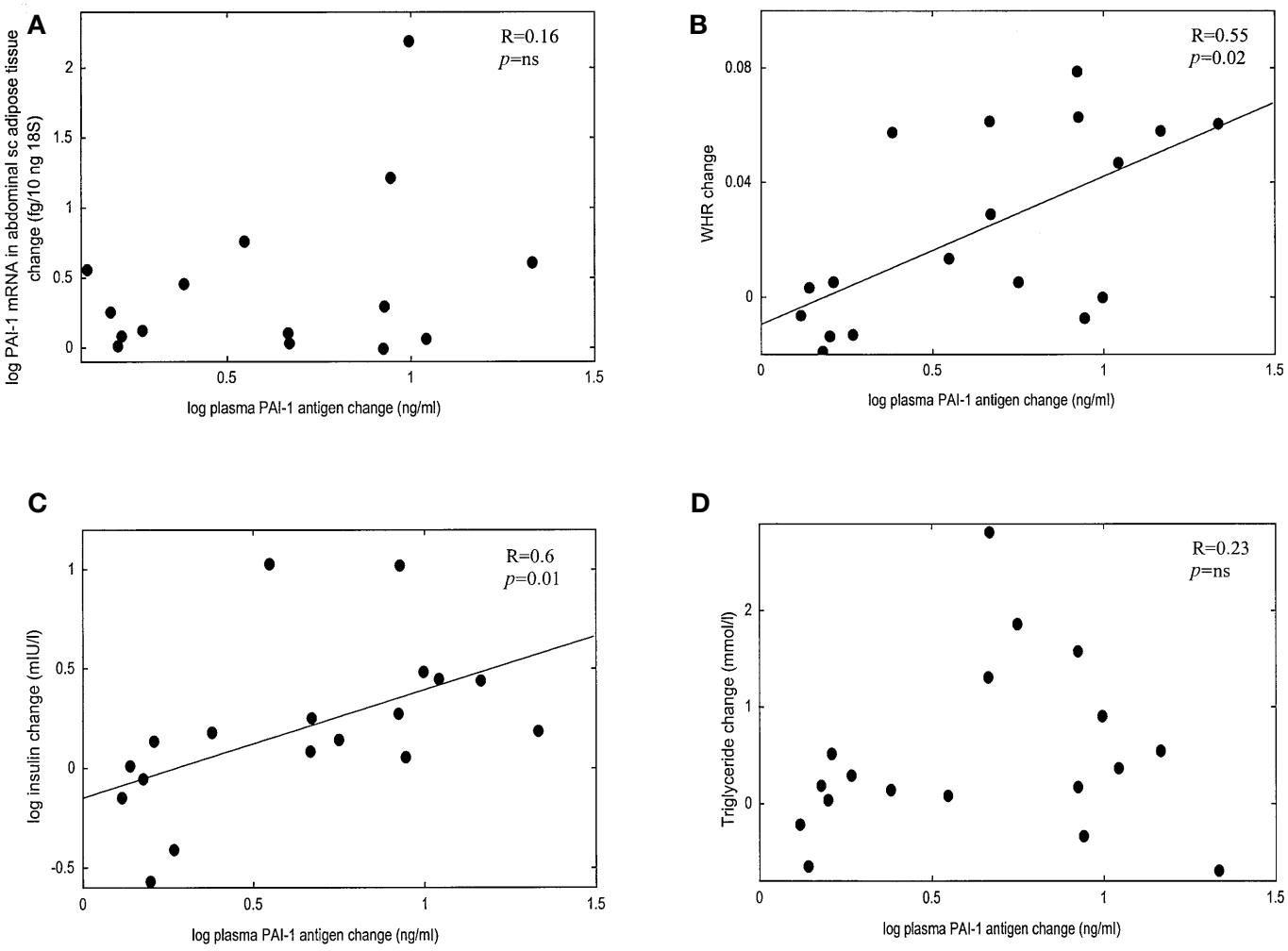

Fig. 1. Relation between absolute changes after weight loss in plasma PAI-1 antigen and abdominal subcutaneous adipose tissue PAI-1 expression (**A**), WHR (**B**), insulin (**C**) and triglycerides (**D**). Log transformed values of plasma PAI-1, abdominal subcutaneous (sc) tissue PAI-1 mRNA content and insulin are shown

ence of insulin or triglycerides on PAI-1 regulation in adipose tissue. This is in agreement with the recent finding that subcutaneous PAI-1 expression is not acutely regulated by insulin [24]. Thus, other mechanisms must be involved. From recent data, the potential relevance of TNF-α and transforming growth factor-β (TGF-β) in the control of PAI-1 expression in adipose tissue have been underlined [14, 25–29] and would deserve further investigation.

Interestingly, plasma PAI-1 concentrations were independently associated with PAI-1 expression in the abdominal subcutaneous adipose tissue. This suggests that PAI-1 over-expression in abdominal adipose tissue could contribute to the increased plasma PAI-1 values in obesity or indicates that plasma and abdominal adipose tissue PAI-1 are influenced by the same regulatory pathways.

In contrast to the abdominal, femoral subcutaneous adipose tissue shows similar amounts of PAI-1 mRNA in obese and lean subjects. Furthermore, femoral tissue PAI-1 expression correlated neither with circulating PAI-1 nor with markers of insulin resistance. This is in agreement with the fact that plasma PAI-1 values are not increased in gynoid obesity where excess of adipose tissue is distributed only in the femoral region. Our results clearly showed that PAI-1 expression is not uniformly distributed within human subcutaneous adipose tissue, suggesting that regulation of PAI-1 expression must be different in various body fat regions. It has been previously shown that visceral tissue explants produce more PAI-1 than explants from abdominal subcutaneous tissue [11, 13, 28]. However, the strong correlations observed between PAI-1 production from both abdominal fat depots (visceral and subcutaneous) and markers of insulin resistance, have led investigators to conclude that, in both tissues, PAI-1 production is controlled by a similar pathway [13]. In this study, the lack of correlation between PAI-1 expression in the femoral fat depot and markers of insulin resistance indicates that regulation of PAI-1 synthesis in this territory is not under the control of insulin resistance. Discrepancies be-

tween the abdominal and femoral subcutaneous adipose tissue compartments have been previously demonstrated. The stromal-to-adipocyte ratio as well as the lipolytic and lipoprotein lipase activities were higher in the abdominal compared to the femoral subcutaneous adipose tissue [30, 31]. Different structure and metabolic activity of these two fat territories might partly explain discrepant PAI-1 mRNA expression, however local (abdominal) distribution of a specific PAI-1 synthesis inducer, like TNF-α or TGF-β might also play an important role. Our findings indicate that the abdominal subcutaneous adipose tissue is a model well suited to study the regulation of PAI-1 expression during insulin resistance while femoral subcutaneous adipose tissue is not relevant.

Several clinical studies have shown a sustained reduction in plasma PAI-1 concentrations after weight loss in very obese, moderately obese [15–21] as well as in lean subjects [32]. The reduction in plasma PAI-1 was more strongly related to changes in fat mass than to markers of insulin resistance [17, 18, 21]. These results are consistent with the hypothesis that a reduction in total fat mass and perhaps in adipose PAI-1 overproduction is more important in regulating plasma PAI-1 than improvements in markers of insulin resistance. In this study, obese patients achieved substantial weight loss. Although the average BMI of obese patients decreased almost to the normal level, it was still higher than the BMI of lean subjects. In contrast, markers of insulin resistance as well as plasma and adipose tissue PAI-1 contents reached the levels of lean subjects. This emphasised that plasma PAI-1 values are not directly determined by the fat quantity in itself but rather by the environment that accompanies increased BMI. We found that absolute changes in plasma PAI-1 were related to those of BMI, WHR, and insulin concentrations. In contrast, changes in plasma PAI-1 did not parallel those of PAI-1 mRNA content in the abdominal subcutaneous adipose tissue. Thus, although PAI-1 expression in the abdominal subcutaneous adipose tissue decreases after weight reduction, we failed to demonstrate its involvement in lowering plasma PAI-1. Of note, baseline plasma PAI-1 values were almost tenfold higher in obese than in lean subjects, whereas PAI-1 mRNA content in abdominal subcutaneous fat in obese patients were only twice that of lean subjects. Therefore, other potential sources of PAI-1 as well as factors regulating PAI-1 synthesis should be investigated in the future. Similarly, the influence of the abdominal subcutaneous adipose tissue on circulating PAI-1 was not observed in another study, even though an increase in adipose PAI-1 mRNA (determined by a different quantitative RT PCR procedure as used in the present study) was observed after moderate weight reduction [33]. It has been shown previously that only an absolute change in visceral fat mass, but not a change in subcutaneous fat depot (assessed by computer-tomography scan) correlates with a change in plasma PAI-1 concentrations after weight reduction [34]. Therefore, changes in visceral fat mass could be more important determinants of changes in circulating PAI-1 after weight reduction. Improvement in hyperinsulinaemia and liver function during weight reduction could also affect changes in plasma PAI-1 concentrations. Finally, because there is no data on correlation between adipose tissue PAI-1 mRNA and antigen, we could not assure that mRNA correctly reflects the PAI-1 protein synthesis.

In conclusion, our results show that the over-expression of PAI-1 in abdominal, but not in femoral subcutaneous adipose tissue is associated with increases in circulating PAI-1 in obese patients. Weight loss leads to a marked decrease in circulating PAI-1 and PAI-1 expression in the abdominal subcutaneous adipose tissue. However, mechanisms other than normalisation of PAI-1 expression in abdominal subcutaneous adipose tissue must contribute to the marked reduction in circulating PAI-1 seen after weight loss.

Acknowledgements. Support by the Slovenian Ministry of Science and Technology, grant no. J3–7882 (Slovenia), and by the INSERM (EPI 99/36) and MENESR (France) is gratefully acknowledged. D. Bastelica is the recipient of French Atherosclerosis Society (France) fellowship.

References

1. Hamsten A, Walldius G, Szamozi A et al. (1987) Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet ii: 3–9
2. Juhan-Vague I, Pyke SDM, Alessi MC, Jespersen J, Haverkate F, Thompson SG (1996) Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. Circulation 94: 2057–2063
3. Vague P, Juhan-Vague I, Aillaud MF et al. (1986) Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism 35: 250 – 253
4. McGill JB, Schneider DJ, Arfken CL, Lucore CL, Sobel BE (1994) Factors responsible for impaired fibrinolysis in obese subjects and NIDDM patients. Diabetes 43: 104–109
5. Juhan-Vague I, Alessi MC, Vague P (1991) Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia 34: 457–462
6. Kruszynska YT, Yu JG, Olefsky JM, Sobel BE (2000) Effects of troglitazone on blood concentrations of plasminogen activator inhibitor 1 in patients with type 2 diabetes and in lean and obese normal subjects. Diabetes 49: 633–639
7. Vague P, Juhan-Vague I, Chabert V, Alessi MC, Atlan C (1989) Fat distribution and plasminogen activator inhibitor activity in nondiabetic obese women. Metabolism 38: 913–915
8. Landin K, Stigendal L, Eriksson E et al. (1990) Abdominal obesity is associated with an impaired fibrinolytic activity

and elevated plasminogen activator inhibitor-1. Metabolism 39: 1044–1048
9. Cigolini M, Targher G, Bergamo Andreis IA, Tonoli M, Agostino G, De Sandre G (1996) Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. Arterioscler Thromb Vasc Biol 16: 368–374
10. Shimomura I, Funahashi T, Takahashi M et al. (1996) Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 2: 800–803
11. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I (1997) Production of plasminogen activator inhibitor 1 by human adipose tissue. Possible link between visceral fat accumulation and vascular disease. Diabetes 46: 860–867
12. Eriksson P, Reynisdottir S, Lönnqvist F, Stemme V, Hamsten A, Arner P (1998) Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. Diabetologia 41: 65–71
13. Morange PE, Alessi MC, Verdier M, Casanova D, Magalon G, Juhan-Vague I (1999) PAI-1 produced ex vivo by human adipose tissue is relevant to PAI-1 blood level. Arterioscler Thromb Vasc Biol 19: 1361–1365
14. Alessi MC, Bastelica D, Morange P et al. (2000) Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. Diabetes 49: 1374–1380
15. Huisveld IA, Leenen R, vd Kooy K et al. (1990) Body composition and weight reduction in relation to antigen and activity of plasminogen activator inhibitor (PAI-1) in overweight individuals. Fibrinolysis 4 [suppl 2]:84–85
16. Primrose JN, Davies JA, Prentice CR, Hughes R, Johnston D (1992) Reduction in factor VII, fibrinogen and plasminogen activator inhibitor-1 activity after surgical treatment of morbid obesity. Thromb Haemost 68: 396–399
17. Folsom AR, Qamhieh HT, Wing RR et al. (1993) Impact of weight loss on plasminogen activator inhibitor (PAI-1), factor VII, and other hemostatic factors in moderately overweight adults. Arterioscler Thromb 13: 162–169
18. Palaretti G, Legnani C, Poggi M et al. (1994) Prolonged very low calorie diet in obese subjects reduces factor VII and PAI but not fibrinogen levels. Fibrinolysis 8: 16–21
19. Svendsen OL, Hassager C, Christiansen C, Nielsen JD, Winther K (1996) Plasminogen activator inhibitor-1, tissue-type plasminogen activator, and fibrinogen: effect of dieting with or without exercise in overweight postmenopausal women. Arterioscler Thromb Vasc Biol 16: 381–385
20. Calles-Escandon J, Ballor D, Harvey-Berino J, Ades P, Tracy R, Sobel B (1996) Amelioration of the inhibition of fibrinolysis in elderly, obese subjects by moderate energy intake restriction. Am J Clin Nutr 64: 17–11
21. Mavri A, Stegnar M, Krebs M, Sentocnik JT, Geiger M, Binder BR (1999) Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. Arterioscler Thromb Vasc Biol 19: 1582–1587
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419
23. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol chloroform extraction. Anal Biochem 161: 156–159
24. Koistinen HA, Dusserre E, Ebeling P, Vallier P, Koivisto VA, Vidal H (2000) Subcutaneous adipose tissue expression of plasminogen activator inhibitor (PAI-1) in nondiabetic and type 2 diabetic subjects. Diabetes Metab Res Rev 16: 364–369
25. Samad F, Yamamoto K, Pandey M, Loskutoff DJ (1997) Elevated expression of transforming growth factor- in adipose tissue from obese mice. Mol Med 3: 37–48
26. Samad F, Uysal KT, Wiesbrock SM, Pandey M, Hotamisligil GS, Loskutoff DJ (1999) Tumor necrosis factor alpha is a key component in the obesity-linked elevation of plasminogen activator inhibitor 1. Proc Natl Acad Sci USA 96: 6902–6907
27. Cigolini M, Tonoli M, Borgato L et al. (1999) Expression of plasminogen activator inhibitor-1 in human adipose tissue: a role for TNF? Atherosclerosis 143: 81–90
28. Gottschling-Zeller H, Birgel M, Rohrig K, Hauner H (2000) Effect of tumor necrosis factor alpha and transforming growth factor beta 1 on plasminogen activator inhibitor-1 secretion from subcutaneous and omental human fat cells in suspension culture. Metabolism 49: 666–671
29. Sakamoto T, Woodcock-Mitchell J, Marutsuka K, Mitchell JJ, Sobel BE, Fujii S (1999) TNF-alpha and insulin, alone and synergistically, induce plasminogen activator inhibitor-1 expression in adipocytes. Am J Physiol 276: C1391-C1397
30. Rink JD, Simpson ER, Barnard JJ, Bulun SE (1996) Cellular characterization of adipose tissue from various body sites of women. J Clin Endocrinol Metab 81: 2443–2447
31. Mauriege P, Marette A, Atgie C et al. (1995) Regional variation in adipose tissue metabolism of severely obese premenopausal women. J Lipid Res 36: 672–684
32. Peternel P, Stegnar M, Mavri A, Salobir-Pajnic B (1994) The effect of fasting and/or body weight reduction on tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1). Fibrinolysis 8 [Suppl 2]:41–43
33. Bastard JP, Vidal H, Jardel C et al. (2000) Subcutaneous adipose tissue expression of plasminogen activator inhibitor-1 gene during very low calorie diet in obese subjects. Int J Obes Relat Metab Disord 24: 70–74
34. Janand-Delenne B, Chagnaud C, Raccah D, Alessi MC, Juhan-Vague I, Vague P (1998) Visceral fat as a main determinant of plasminogen activator inhibitor 1 level in women. Int J Obes Relat Metab Disord 22: 312–317