

Impact of Adipose Tissue on Plasma Plasminogen Activator Inhibitor-1 in Dieting Obese Women

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Abstract—The increased incidence of cardiovascular diseases in obese subjects could be partially attributed to impaired fibrinolysis due to elevated plasma levels of tissue plasminogen activator inhibitor 1 (PAI-1). The associations between changes in plasma PAI-1, metabolic variables, and adipose tissue during weight loss and regain were studied in 52 healthy, premenopausal, obese women participating in a weight reduction program with a hypocaloric diet. PAI-1, insulin, triglyceride, leptin, and adipsin levels were determined at entry, after the first week, after completion of the program, and after 5 months of follow-up. In the 33 obese women who completed the program, decreases in PAI-1 antigen (−54%), PAI activity (−74%), and leptin (−51%), but not of adipsin, were observed. Changes in PAI-1 were associated with changes in body mass index (BMI), body fat, leptin, and insulin. The decreased level of PAI-1 remained low after follow-up in the 14 women who maintained their reduced weight but increased in the 16 women who regained weight. This increase in PAI-1 was correlated with an increase in body fat and leptin. On multivariate analysis, BMI was the major determinant of PAI-1 level. In conclusion, during weight reduction with a hypocaloric diet, the decrease in PAI-1 is more closely related to changes in adipose tissue than to changes in metabolic variables, suggesting a significant role for adipose tissue in regulating plasma levels of PAI-1. (*Arterioscler Thromb Vasc Biol.* 1999;19:1582-1587.)

Key Words: adipose tissue ■ adipsin ■ diet ■ leptin ■ plasminogen activator inhibitor 1

An increased level of plasma tissue plasminogen activator inhibitor 1 (PAI-1), the main regulator of blood fibrinolytic activity,¹ has repeatedly been shown to be associated with obesity.^{2,3} PAI-1 is positively correlated with body mass index (BMI) in men⁴ as well as in women.^{5,6} PAI-1 is further correlated with other measures of obesity, such as waist-to-hip circumference ratio (WHR), reflecting abdominal fat, and with several metabolic factors, such as plasma triglycerides and insulin.⁷⁻⁹ It is, however, unclear which of these parameters is the major determinant of plasma PAI-1. Elevated PAI-1 levels were found in young survivors of myocardial infarction and were also predictive for future cardiovascular events.^{10,11} In obese women, mortality due to cardiovascular events is increased 4-fold compared with lean women.¹² Therefore, the abnormal expression of PAI-1 in obesity might represent 1 of the mechanisms through which the risk for the development of cardiovascular diseases is increased in obese individuals.

There is ample evidence that weight loss due to a low-calorie diet or fasting affects fibrinolysis by reducing plasma PAI-1 levels.¹³⁻¹⁹ The decrease in PAI-1 could be attributed to either a reduction in body weight and body fat or alterations in blood lipids and/or insulin levels. The former presumption is supported by data showing high concentra-

tions of PAI-1 mRNA in mouse fat tissue²⁰ and by the demonstration of PAI-1 mRNA in mouse adipocytes.²¹ PAI-1 is also synthesized by cultured 3T3-L1 cells, an adipocyte line.²¹ Therefore, adipose tissue might be an important contributor to the elevated plasma PAI-1 levels in obese individuals.²²

Adipocytes are the site where triglycerides are stored or free fatty acids are released, depending on the body's energy demands. In addition, adipocytes produce several mediators. One of them is adipsin, a serine protease identical to complement factor D,²³ involved in the generation of acylation-stimulating factor C3adesArg and proposed to be a marker of some genetic and metabolic obesities.^{24,25} Another mediator is leptin, the product of the *obese* gene that might regulate body weight by signaling the amount of body fat.²⁶ Furthermore, adipocytes release certain cytokines such as tumor necrosis factor- α (TNF- α).²⁷

To elucidate whether changes in PAI-1 plasma levels reflect changes in the amount and synthetic activity of adipocytes, or rather, of other metabolic variables associated with such changes, plasma adipsin and leptin levels as well as metabolic variables (triglycerides and insulin levels) were followed during a body weight reduction program and follow-up in obese, premenopausal women.

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TABLE 1. Anthropometric, Metabolic, and Fibrinolytic Variables in Lean and Obese Premenopausal Women at Entry, After the First Week, and After Completion of the Body Weight Reduction Program

	Obese Women			
	Lean Women (n=19)	At Entry (n=52)	After First Week (n=51)	After Completion of Program (n=33)
BMI, kg/m ²	22.97±2.64	30.77±4.59*	29.60±4.53†	25.52±2.67†
WHR, relative	0.77±0.05	0.79±0.07	0.78±0.07	0.77±0.06‡
Body fat, %	32.5±3.9	38.8±4.0*	37.6±4.0†	33.5±3.8†
Triglycerides, mmol/L	0.9 (0.6–1.1)	1.3 (1.0–1.7)*	0.8 (0.7–1.0)†	1.1 (0.8–1.3)
Glucose, mmol/L	5.2 (4.8–5.4)	5.2 (4.9–5.5)	5.0 (4.6–5.5)‡	4.9 (4.6–5.2)‡
Insulin, mIU/L	6.3 (4.7–10.8)	9.4 (6.9–14.0)§	7.5 (5.8–11.6)‡	7.5 (5.9–9.7)
Adipsin, µg/mL	1.4±0.2	2.1±0.4*	2.1±0.5	2.0±0.4
Leptin, ng/mL	6.7 (5.6–8.6)	19.7 (12.3–34.6)*	11.8 (7.7–22.19)†	8.4 (5.2–13.4)†
PAI activity, IU/mL	6.2 (2.9–15.8)	8.5 (2.4–18.4)	3.3 (0.0–15.8)†	2.9 (0.0–4.3)†
PAI-1 antigen, ng/mL	7.6 (3.1–17.0)	15.9 (6.1–33.2)§	9.5 (4.4–20.7)†	7.6 (3.5–10.3)†

Values are mean±SD or medians and the 25th to 75th percentiles.

**P*<0.01, §*P*<0.05 compared with lean women.

†*P*<0.01, ‡*P*<0.05 compared with the corresponding values at entry.

Methods

Subjects and Study Protocol

Fifty-two obese (BMI ≥25 kg/m²), premenopausal women (age range, 21 to 53 years; mean±SD age, 40±8 years) were recruited at the outpatient Medical-Aesthetic Center in Ljubljana, Slovenia. They volunteered to participate in a body weight reduction program under medical supervision. Nineteen lean (BMI <25 kg/m²), premenopausal women of comparable age (age range, 29 to 50 years; mean±SD age, 41±7 years) served as controls. No woman had a history of thromboembolic disease or diabetes mellitus, and no woman was taking oral contraceptives. Twelve obese (23%) and 5 lean (26%) women were smokers. All women had given their fully informed consent to participate in the study, which was approved by the Slovene Ethics Committee.

The weight reduction program lasted 10 to 12 weeks and included a low-calorie diet (3.4 to 5.0 MJ/d; consisting of 20% fat, 40% carbohydrates, and 40% protein) combined with moderate physical activity (10 minutes of aerobics twice a day and 1 hour of supervised physical activity at a constant heart rate of 65 to 75 bpm twice a week).

In the obese women, blood samples were obtained at the time of entry into the weight reduction program, after 1 week of the program, at completion of the program, and 5 months thereafter. In control lean women, blood was sampled only once.

Anthropometric and Laboratory Measurements

At each blood sampling, the following anthropometric parameters were measured: body weight and height and waist and hip circumferences. From these data, BMI (body weight in kilograms divided by the square of body height in meters squared),²⁸ WHR (waist circumference in centimeters divided by hip circumference in centimeters), and the percentage of body fat ($[0.439 \times \text{waist circumference in cm}] + [0.221 \times \text{age in years}] - 9.4$) adjusted for sex²⁹ were calculated.

Blood samples were obtained from fasting women between 7:30 and 9 AM after a 20-minute rest in a sitting position. Blood was sampled from an antecubital vein with the aid of a tourniquet, which was applied for 2 minutes or less. For measurement of hemostatic factors, 9 volumes of blood flowed directly into precooled, siliconized glass vacuum tubes (Becton Dickinson Vacutainer System) with 1 volume of 0.13 mol/L trisodium citrate. Tubes were placed in ice water and then centrifuged within 1 hour for 30 minutes at 2000g and 4°C. Platelet-poor plasma was transferred to small plastic vials, frozen in LN₂, and

stored at -70°C until analyzed. For biochemical analysis, blood was collected into vacuum tubes without an anticoagulant. After 1 hour, serum was harvested and analyzed on the same day.

In plasma samples, PAI-1 antigen was determined by an ELISA (Imulysse PAI-1, Biopool) and PAI activity by an amidolytic assay (Spectrolyse/fibrin, Biopool). Fasting glucose³⁰ and triglyceride³¹ levels were determined by routine biochemical methods. Insulin and leptin were determined in serum by commercially available radioimmunoassays (Sorin, Biomedica, and Wak-Chemie, Medical GmbH, respectively) and adipsin by an ELISA according to Oppermann and coworkers,³² with the following modifications: for coating the plates, monoclonal anti-human factor D IgG (4 µg/mL D10/4, Connex GmbH) in 5.5 mmol/L Na₂CO₃, 35 mmol/L NaHCO₃, and 0.01% thimerosal (pH 9.6) was used. Instead of gelatin, 1% BSA was used in the blocking and sample dilution buffers. The sample incubation time was 2 hours. Biotinylated monoclonal anti-human factor D IgG (1 µg/mL D8/1, Connex GmbH) was used as a second antibody. In serum samples of 1/3 of the women completing the program, TNF-α was determined by an ELISA (Biotrak, Amersham) and was found to be below the detection limit in all samples.

Statistical Methods and Calculations

Normal distribution of all variables was tested by the Kolmogorov-Smirnov test. Variables with a normal distribution were described by means and SDs and variables with nonnormal distribution by medians and the 25th and 75th percentiles. Differences between obese and lean women were tested by either the *t* test or the

TABLE 2. Spearman's Correlations Coefficients for Associations Between Variables in All Women at Entry

	PAI Activity	PAI-1 Antigen	Leptin	Adipsin
BMI	0.35*	0.53*	0.77*	0.62*
WHR	0.30†	0.26†	0.06	0.03
%Body fat	0.43*	0.54*	0.52*	0.46*
Triglycerides	0.32*	0.35*	0.43*	0.37*
Glucose	0.35*	0.29†	0.12	-0.05
Insulin	0.30*	0.40*	0.44*	0.31*
Leptin	0.27†	0.49*	...	0.61*
Adipsin	0.14	0.32*

**P*<0.01, †*P*<0.05.

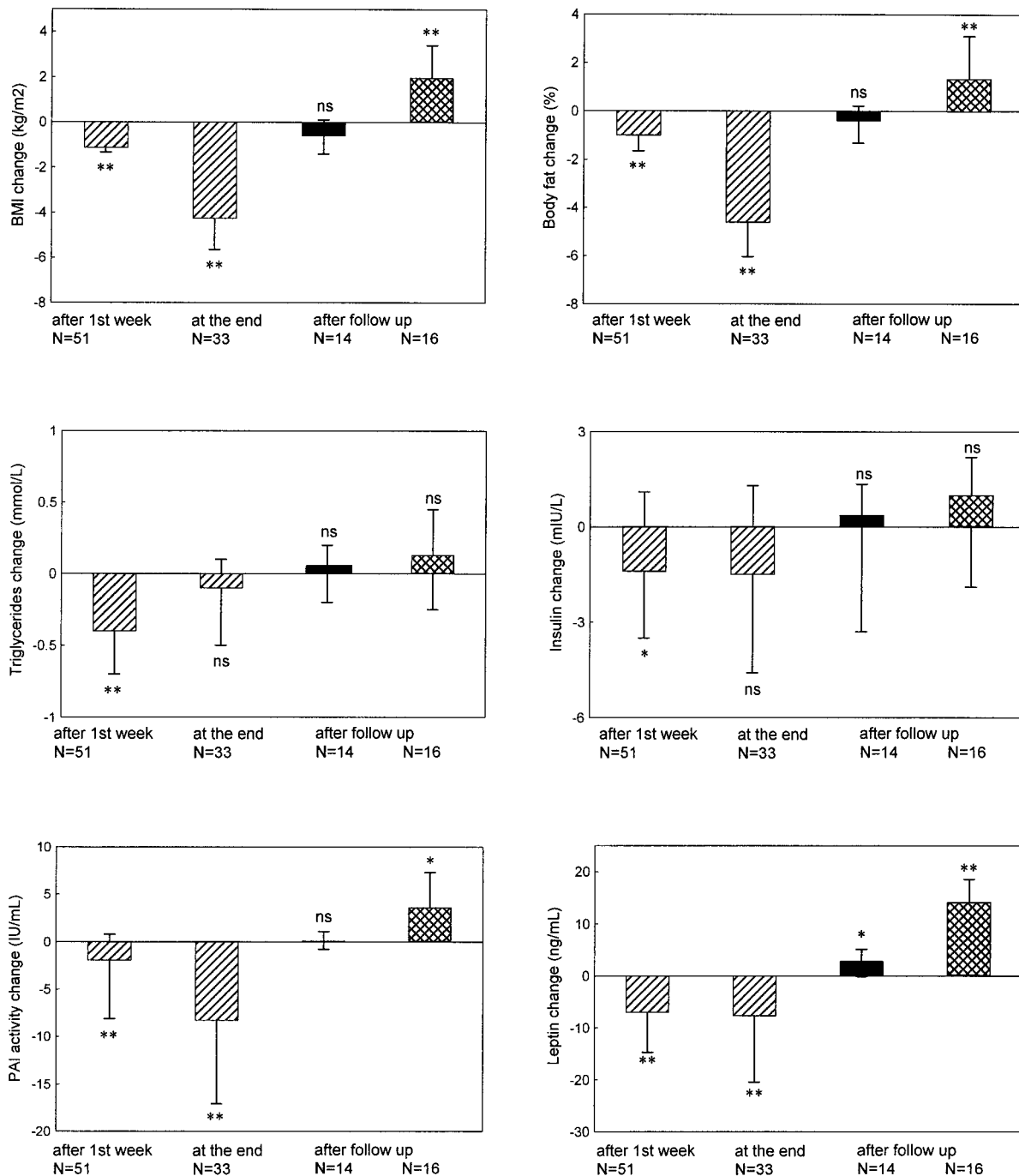


Figure 1. Changes in BMI, body fat, triglycerides, insulin, PAI-1 activity, and leptin from entry to the first week (▨), from entry to the end of the body weight reduction program (▩), and from the end of the body weight reduction program to the end of follow-up in women who maintained reduced weight (■) and in women who regained weight (▤). Medians and 25th to 75th percentiles are shown. ** $P < 0.01$, * $P < 0.05$; ns, not significant.

Mann-Whitney U test. Differences within the group of obese women were tested with the t test or the Wilcoxon test with Bonferroni's correction of P values. Associations between 2 variables were tested with Spearman's correlation coefficient. Multiple linear regression analysis was performed to evaluate the independence of associations.

The changes in variables during the observation period were calculated as follows: (1) values at entry minus values after the first week of the program; (2) values at entry minus values at completion of the program; and (3) values at the end of the follow-up period minus values at completion of the program. All calculations were performed using the Statistica for Windows computer program (StatSoft, Inc).

Results

Before participating in the body weight reduction program, the obese women had significantly higher BMI, percentage of body fat, and levels of triglycerides, insulin, adiponectin, leptin, and PAI-1 antigen than did the lean women (Table 1). In all women at entry, a positive association of PAI activity, PAI-1 antigen, adiponectin, and leptin was found with almost all anthropometric and metabolic variables measured. Significant associations between PAI-1, adiponectin, and leptin were also observed (Table 2).

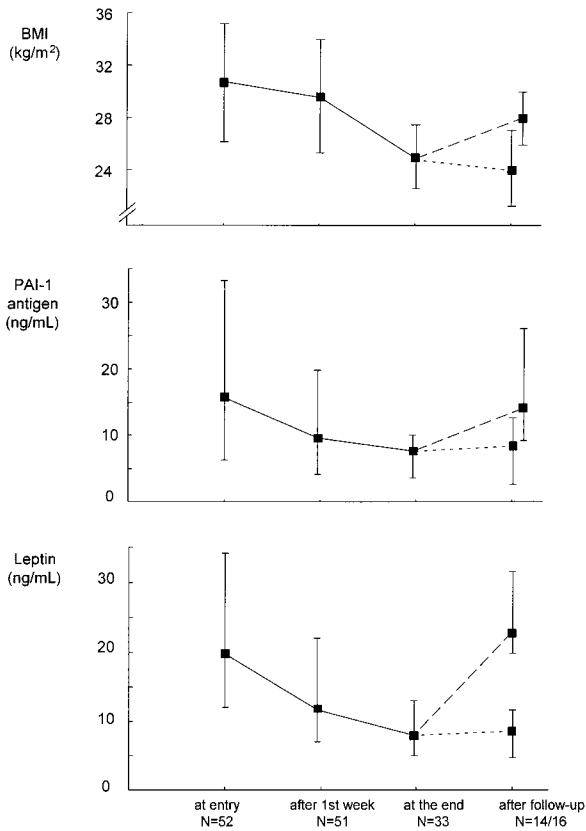


Figure 2. BMI, PAI-1 antigen, and leptin at entry and during the body weight reduction program. After follow-up, 14 women maintained their reduced weight (· · · · ·) and 16 women regained weight (- - - - -). Mean and SD for BMI and medians and 25th to 75th percentiles for PAI-1 antigen and leptin are shown.

After the first week of the program, the obese women had lost, on average, 4% (3.0±1.0 kg) of body weight and 3% of body fat. Significant reductions of triglycerides, glucose, and insulin levels by 28%, 2%, and 16%, respectively, were also observed. The levels of leptin declined by 37%, but no significant change was observed for adipsin. During the same period, a significant decrease in PAI activity (by 31%) and PAI-1 antigen (by 26%) was recorded (Table 1 and Figure 1).

Thirty-three women completed the program and 19 women dropped out. Women who completed the program reduced their initial body weight by 17% (14.0±8.0 kg) and their initial body fat by 13%. Compared with the values after 1 week of the program, there was no further decrease in insulin

and glucose levels. Triglyceride levels even increased almost to the values observed at entry (1.1 and 1.3 mmol/L, respectively). In contrast, PAI activity, PAI-1 antigen, and leptin levels further declined and had decreased at completion of the program by 74%, 54%, and 51%, respectively. Adipsin, however, remained almost unchanged during the whole program (Table 1 and Figure 1).

During the 5-month follow-up after completion of the program, 16 women regained >25% (on average, 8.0±4.0 kg) of their body weight originally lost during the program. Fourteen women managed to maintain their reduced weight and 3 women dropped out. There were no significant changes in triglycerides, insulin, or adipsin levels at the end of the follow-up period compared with the values at the end of the program either in women who regained weight or in woman who maintained their reduced weight. However, in women who regained body weight, a significant increase in PAI-1 antigen, PAI activity, and leptin levels paralleled the increase in BMI (Figure 2).

Changes in PAI-1, changes in anthropometric variables, as well as alterations in adipocyte synthetic products during the program and during follow-up and correlations between these changes are presented in Figure 1 and Table 3. After the first week of the program, changes in PAI-1 were correlated significantly and positively with changes in BMI, insulin, and leptin levels. At completion of the program, these associations were even stronger. Additionally, a positive correlation between changes in PAI-1 and changes in body fat became significant. After the 5-month follow-up period, changes in PAI-1 were correlated with changes in BMI and body fat. The decline in leptin levels was correlated with declines in BMI, triglycerides, and insulin levels after the first week of the program; at completion of the program, correlations with changes in BMI and body fat were still highly significant (Table 3).

To test the independence of associations between changes in PAI-1 antigen and changes in BMI, leptin, and insulin during the program, a multivariate linear regression analysis was performed. Before entering the model, the dependent variable (changes in PAI-1 antigen) was logarithmically transformed. Only changes in BMI remained significantly and independently associated with changes in PAI-1 (*P*<0.0008) and accounted for 34% of the variance of changes in PAI-1.

In some samples obtained during the body weight reduction program, TNF-α was determined. TNF-α was below the detection limit in all samples.

TABLE 3. Spearman's Correlations Coefficients for Associations Between Changes in Observed Variables After the First Week, After Completion of the Program, and After the Follow-Up Period

	After First Week (n=52)			After Completion of the Program (n=33)			After Follow-Up (n=30)		
	Δ PAI ac	Δ PAI-1 ag	Δ Leptin	Δ PAI ac	Δ PAI-1 ag	Δ Leptin	Δ PAI ac	Δ PAI-1 ag	Δ Leptin
Δ BMI	0.22	0.37*	0.42*	0.69*	0.70*	0.62*	0.33†	0.28	0.76*
Δ Body fat	0.20	0.26†	0.28	0.62*	0.68*	0.56*	0.42‡	0.40‡	0.51*
Δ Triglycerides	0.20	0.20	0.42*	0.23	0.23	0.39‡	0.23	0.22	0.13
Δ Insulin	0.32‡	0.42*	0.31‡	0.49*	0.44*	0.28	0.17	-0.17	-0.04
Δ Leptin	0.23	0.58*	...	0.52*	0.55*	...	0.16	0.11	...

ac indicates activity; ag, antigen.
**P*<0.01, †*P*=0.07, ‡*P*<0.05.

Discussion

This study shows that in obese, premenopausal women with elevated levels of PAI-1, adipsin, and leptin, a decrease in body weight and/or body fat during a body weight reduction program affected fibrinolysis by reducing plasma PAI-1 levels. This effect persisted in those women who maintained their reduced weight during the follow-up period but was lost in those women who regained body weight. Throughout the observation period, leptin changes followed tightly those of body weight and body fat while adipsin levels remained unchanged and elevated compared with corresponding values in lean controls.

The source of plasma PAI-1 and its regulation are poorly understood. Data obtained from in vitro experiments suggest that PAI-1 is synthesized by hepatocytes and stimulated endothelial cells and that its synthesis can be affected by high insulin and lipoprotein levels.^{33,34} Insulin might affect cell synthesis of PAI-1 either directly or indirectly through alterations of plasma lipoproteins.³⁵ In a rodent model, it was demonstrated recently that PAI-1 is also produced by adipocytes²¹ and human adipose tissue; in particular, visceral adipose tissue is capable of expressing PAI-1.³⁶ This could suggest that elevated plasma PAI-1 levels in obese subjects might result from constitutive synthesis of PAI-1 by the increased amount of adipose tissue in obesity.

A strong, positive correlation between PAI-1 and anthropometric measures of obesity as well as between PAI-1 and 2 products of adipocytes, leptin and adipsin, were observed initially in the present study. These results support the presumption that adipose tissue is a potential source of plasma PAI-1 in obese subjects. A correlation between PAI-1 and adipsin was also observed previously by Alessi and coworkers,³⁷ but this is the first report of a correlation between plasma PAI-1 and leptin levels.

During the body weight reduction program, a dramatic decrease in plasma PAI-1 (>50% decrease in PAI-1 antigen and >70% decrease in PAI activity) was observed. This decrease in PAI-1 was already significant after the first week of the program. At this time, BMI, body fat, insulin, triglycerides, and glucose levels were also significantly decreased, indicating that important metabolic and fibrinolytic changes occur early during body weight reduction. While PAI-1 further decreased until the end of the program, as did BMI and body fat, insulin and triglycerides remained at the same level or even increased compared with the first week. Consistently during follow up, PAI-1 levels increased concomitantly with increases in body fat in women who regained body weight and stayed low in women who maintained their reduced body weight. In this period, plasma PAI-1 was not related to insulin or triglyceride levels, similar to that during the late period of weight loss (Table 3). From these data, it can be concluded that PAI-1 plasma levels are more closely related to the amount of adipose tissue than to insulin or triglycerides levels, supporting the results of other studies.^{13,14}

To obtain better insight whether changes in PAI-1 plasma levels reflect changes in the amount or synthetic activity of adipocytes, plasma adipsin and leptin levels were followed during weight loss and weight regain. Leptin levels were associated with the amount of adipose tissue, as reflected by

BMI and body fat, and as reported before.³⁸ During body weight (and fat) loss, leptin concentrations declined, probably owing to decreased production by adipocytes. This decline was closely related to the plasma PAI-1 reduction, but in multivariate regression analysis, it was found that changes in PAI-1 levels were independently influenced by changes in BMI only, whereas changes in the leptin level did not affect PAI-1 directly. These results also provide indirect evidence of adipose tissue as a source of PAI-1. In contrast to leptin, adipsin levels stayed unchanged during the whole observation period, despite a strong correlation observed between adipsin and body fat at entry. Therefore, it seems likely that adipsin is not as useful a marker of adipose tissue mass and/or adipocyte secretion in humans as suggested previously.³⁹

In conclusion, this study shows that in premenopausal women, changes in plasma PAI-1 levels during body weight reduction and body weight regain are correlated with changes in the amount of body fat, as are plasma leptin levels. These data strongly suggest that the elevated PAI-1 levels seen in obesity are linked with the amount of fat stored in adipose tissue rather than with other metabolic parameters. The fact that the elevated plasma levels of adipsin, another synthetic product of adipocytes, remained unchanged during weight reduction suggests that adipsin might rather be related to "obesity" itself and not to the actual mass of body fat.

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