Plasma Plasminogen Activator Inhibitor-I Is Associated With Plasma Leptin Irrespective of Body Mass Index, Body Fat Mass, and Plasma Insulin and Metabolic Parameters in Premenopausal Women

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Leptin, the satiety hormone expressed almost exclusively in adipose tissue, is a marker of body fat accumulation in humans. Recent studies have shown that plasminogen activator inhibitor-1 (PAI-1), a prothrombotic factor associated with atherosclerosis complications, is also produced in adipose tissue. The objective of the present study was to determine whether PAI-1 antigen plasma concentrations are associated with leptin plasma levels or the body fat mass (FM) independently of the variables known to influence PAI-1 production. Sixty-one nondiabetic women aged 18 to 45 years with a wide range of values for the body mass index ([BMI] 18.1 to 37.7 kg/m^2) were evaluated for (1) body FM and fasting plasma levels of (2) PAI-1 antigen, (3) PAI-1 activity, (4) leptin, (5) insulin, (6) blood glucose, and (7) lipids (cholesterol, high-density lipoprotein [HDL]-cholesterol, and triglycerides [TG]). Body FM and fat-free mass (FFM) were estimated during fasting conditions by the bioimpedance analysis (BIA) method using a tetrapolar device. Body fat distribution was evaluated by the waist circumference and the waist to hip ratio (WHR). FM was directly associated with both PAI-1 antigen ($r = .585$, $P < .001$) and PAI-1 activity ($r = .339$, $P < .001$). Seemingly, leptin was positively related to both PAI-1 antigen ($r = .630$, $P < .001$) and PAI-1 activity ($r = .497$, $P < .001$). Moreover, both PAI-I antigen and PAI-1 activity were directly correlated with FFM ($r = .285$, $P < .05$, and $r = .336$, $P < .01$, respectively), BMI ($r = .594$, $P < .001$, and $r = .458$, $P < .001$, respectively), and WHR ($r = .510$, $P < .001$, and $r = .391$, $P < .005$, respectively). Insulin was directly related to PAI-1 antigen ($r = .540$, $P < .001$), PAI-1 activity ($r = .259$, $P < .05$), leptin ($r = .447$, $P < .001$), and FM ($r = .435$, $P < .001$). The association between PAI-1 antigen (dependent variable) and leptin or FM was tested by a stepwise regression model simultaneously including leptin, FM, BMI, WHR, age, FFM, and fasting insulin, blood glucose, TG, cholesterol, and HDL-cholesterol as independent variables. PAI-1 antigen maintained a significant positive independent relationship only with leptin ($t = 2.923$, $P < .01$), insulin ($t = 3.489$, $P < .001$), and fasting blood glucose ($t = 2.092$, $P < .05$), and a negative independent relationship with HDL-cholesterol ($t = -2.634$, $P < .05$). In conclusion, the strong relationship between PAI-1 antigen and leptin irrespective of other variables known to influence these factors seems to indicate that leptin per se may potentially increase PAI-1 plasma concentrations in obese subjects.

OBESITY AND VISCERAL FAT accumulation, in particular, are well-known risk factors for the development of coronary heart disease.[1-7] The increased prevalence of thrombotic events in subjects with excess body fat has been related to the various metabolic and nonmetabolic abnormalities associated with obesity, including insulin resistance, hyperinsulinemia, impaired glucose metabolism, hypertriglyceridemia, low high-density lipoprotein (HDL)-cholesterol levels, hypertension, and increased prothrombotic factors.[2-20] Each of these abnormalities may favor the atherosclerotic process and increase the occurrence of thrombotic events through multiple and distinct mechanisms. Concerning prothrombotic factors, over the last few years, experimental cross-sectional and prospective cohort studies have underlined the association between plasma plasminogen activator inhibitor-1 (PAI-1) levels and atherosclerosis complications.[21] Plasma PAI-1 levels are high in patients with coronary artery disease[22] and ischemic stroke[23] and have prognostic value in predicting the recurrence of myocardial infarction.[24] Interestingly, PAI-1 concentrations are in strong correlation with a cluster of variables included in the insulin resistance syndrome, such as the body mass index (BMI), central fat accumulation, and plasma insulin, triglyceride (TG), and HDL-cholesterol levels.[13-21,25] The recent demonstration of synthesis of PAI-1 by adipose tissue, especially visceral fat,[26-28] which accumulates in insulin resistance, has attracted much attention. Higher PAI-1 production by adipose tissue deserves special attention since it may represent a fundamental determinant of the overall risk of atherosclerosis in obese individuals.

Leptin, a recently discovered 167–amino acid protein encoded by the *ob* gene,[29] is expressed exclusively in adipose tissue,[30,31] and its plasma concentration is strongly related to the body fat mass (FM) in lean and obese subjects.[32-35] Therefore, although a wide variability in the plasma leptin concentration has been reported in obese patients and several factors may influence the synthesis of this protein for a given level of total body FM, the plasma leptin level may well be considered a biological marker of body fat accumulation. It is noteworthy that both leptin and PAI-1 are produced by adipocytes, but an association between leptin and PAI-1 plasma levels or between FM and PAI-1 has never been investigated.

This study was performed to examine the possible relationship between leptin and PAI-1 plasma concentrations independently of the variables known to influence these two factors. To this end, 61 nondiabetic women aged 18 to 45 years with a wide range of BMI values were investigated. Body fat distribution, blood pressure, and the fasting plasma insulin, blood glucose,

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and lipid profile (cholesterol, HDL-cholesterol, and TG) were also measured. In addition, to examine whether leptin has a role in influencing PAI-1 antigen plasma levels independently of body composition, FM and fat-free mass (FFM) were also quantified by bioimpedance analysis (BIA). Finally, since PAI-1 antigen and PAI-1 activity may have a different biological significance, with the antigen reflecting more the production and the activity reflecting more the antifibrinolytic potential of PAI-1, PAI-1 activity was also measured in the same subjects.

SUBJECTS AND METHODS

Subjects

This study enrolled 61 women, including 39 overweight and obese (BMI > 25) and 22 non-obese (BMI ≤ 25) subjects. The cutoff for the BMI to identify obesity was greater than 25.0 kg/m^2 according to Ferrannini et al.[36] Overweight and obese patients were recruited consecutively at the Outpatient Clinic for the Study of Obesity, Institute of Internal Medicine, Endocrinology and Metabolic Diseases, University of Bari School of Medicine (Bari, Italy). Normal-weight subjects were represented by healthy individuals, recruited consecutively among physicians and medical students. Participants were aged between 18 and 45 years, and all provided informed consent for enrollment in the study, which was performed according to the Helsinki Declaration.

The women had no history or physical or electrocardiographic signs of coronary heart disease; moreover, patients affected by stable hypertension and familial hyperlipidemia were excluded from the study. Biochemical markers of thyroid, liver, and kidney function were within normal range in all subjects, and none of the individuals were receiving any medication when they entered the study. They all had normal glucose tolerance in accordance with the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[37]; moreover, they were spontaneously menstrually active (nine to 13 menses per year), and did not take estrogens or oral contraceptives during the prior 6 months. All of the women were not regular smokers or alcohol drinkers, and during the testing period, they were asked to keep their normal mixed diet and not to perform any sports activity. Blood pressure was recorded on at least three separate occasions using a mercury manometer with appropriate cuff size.

Anthropometric Parameters

Body fat distribution and central fat accumulation were evaluated by the waist to hip ratio (WHR). Waist circumference was measured as the minimum measurement between the xyphoid process and the umbilicus; hip circumference was measured at the most protruding points of the greater trochanters. A direct measurement of the visceral FM by computed tomographic scan was not performed in this study.

Body Composition

Body FM and FFM were estimated in the fasting condition by the BIA method using a tetrapolar device (BIA 101/S; RJL Systems, Detroit, MI). FFM was calculated by Heitmann's equation,[38] already used in another study performed in a group of Italian obese women of similar age and BMI,[39] and FM was calculated as the difference between body weight and FFM.

Hormone and Metabolic Parameters

Blood for hormonal and metabolic determinations was drawn between 8 and 9 AM after overnight fasting; in particular, blood samples were taken on days 5 to 7 of the menstrual cycle in women. The oral glucose tolerance test was performed by collecting venous blood samples during fasting and every 30 minutes following an oral load with 75 g glucose for 2 hours.

Plasma concentrations of insulin were measured by radioimmunoassay using a commercially available kit (Behring, Scoppitto, Italy). Blood glucose was determined by the glucose-oxidase method (Sclavo, Siena, Italy). Total cholesterol, HDL-cholesterol, and TG levels were measured using enzymatic assays (Boehringer, Mannheim, Germany). Both the intraassay and interassay coefficients of variation were less than 7.5% for all of the methods.

The plasma leptin level was measured by a specific radioimmunoassay previously described in detail.[40] The sensitivity was 0.03 ng/L and the intraassay and interassay coefficients of variation were 0.8% and 8.5%, respectively.

PAI-1

For measurements of PAI-1, subjects were placed at rest in the supine position for 20 minutes. Then, venous blood was drawn using a no. 19 butterfly needle without venous stasis and discarding the first 3 mL. Blood samples were drawn in siliconized vacutainer tubes containing sodium citrate (0.38% final concentration). PAI-1 antigen and activity were determined as previously described.[20] Both the intraassay and interassay coefficients of variation for the method were less than 7.5%.

Statistics

Statistical analysis was performed using STATISTICA 6.0 for Windows (1995; StatSoft, Tulsa, OK). Results are presented as the average, median, standard deviation, and range for all parameters. Pearson correlation coefficients were used to quantify univariate associations among variables, and logarithmic transformation ($\log_{10}$) was performed for non–normally distributed variables (PAI-I antigen, insulin, and TG) in these statistical analyses. A multiple regression analysis was performed to test the joint effect of different variables on PAI-1 (antigen or activity).

RESULTS

General characteristics, body composition, and fasting plasma PAI-I (antigen and activity), leptin, insulin, and metabolic parameters are listed in Table 1.

Table 2 shows correlation coefficients between plasma PAI-I antigen or PAI-1 activity and all other parameters. Both PAI-I antigen and activity were directly associated with leptin, FM, FFM, BMI, WHR, and insulin levels and negatively associated with HDL-cholesterol. Only PAI-1 antigen was positively correlated with blood glucose and TG, whereas only PAI-1

Table 1. General Characteristics, Plasma PAI-1 Antigen and Activity, Plasma Leptin, and Metabolic Parameters in the Women Under Study (N = 61)

Variable	Average	Median	Standard Deviation	Range
Age (yr)	28.2	26.0	7.48	18.0-45.0
BMI (kg/m^2)	27.5	27.0	5.62	18.1-37.7
FM (kg)	26.9	27.7	11.1	7.20-46.7
FFM (kg)	44.2	44.4	7.78	24.3-65.4
WHR	0.82	0.83	0.07	0.68-0.98
MBP (mm Hg)	88.5	86.6	9.04	71.6-115.0
PAI-1 antigen (ng/mL)	13.3	8.40	12.6	0.50-58.5
PAI-1 activity (AU/mL)	12.7	11.7	9.46	0.15-38.1
Leptin (ng/mL)	24.4	24.4	16.2	1.56-65.8
Insulin (μU/mL)	12.9	11.9	8.96	2.0-45.9
Fasting blood glucose (mg/dL)	83.8	83.0	9.49	68.0-107.0
Cholesterol (mg/dL)	168.5	168.0	30.1	97.0-241.0
HDL-cholesterol (mg/dL)	42.2	38.0	14.2	29-72
TG (mg/dL)	89.3	81.0	36.8	52-205

Table 2. Correlation Coefficients Between Plasma PAI-1 Antigen or PAI-1 Activity and All Other Parameters (N = 61)

Variable	PAI-1 Antigen (ng/mL)		PAI-1 Activity (AU/mL)	
	r	P	r	P
PAI-1 activity (IU/mL)	.671	<.001	/	/
Leptin (ng/mL)	.630	<.001	.497	<.001
FM (kg)	.585	<.001	.339	<.01
FFM (kg)	.285	<.05	.336	<.01
Age (yr)	.156	NS	.135	NS
BMI (kg/m²)	.594	<.001	.458	<.001
WHR	.510	<.001	.391	<.005
MBP (mm Hg)	.193	NS	.268	<.05
Insulin (μU/mL)	.540	<.001	.259	<.05
Fasting blood glucose (mg/dL)	.388	<.005	.240	NS
Cholesterol (mg/dL)	.008	NS	.117	NS
HDL-cholesterol (mg/dL)	−.495	<.001	−.342	<.01
TG (mg/dL)	.384	<.005	.246	NS

Abbreviation: NS, nonsignificant.

activity was directly associated with mean blood pressure (MBP) levels. PAI-1 antigen was strongly correlated with PAI-1 activity.

Table 3 shows correlation coefficients between leptin or FM and all other parameters. Both leptin and FM were directly associated with FFM, BMI, WHR, MBP, and insulin, blood glucose, and TG levels and negatively associated with HDL-cholesterol. Leptin was strongly correlated with FM.

To verify whether leptin was a significant independent determinant of PAI-1 antigen, the association between PAI-1 antigen (dependent variable) and leptin was tested by a stepwise regression model simultaneously including leptin, FM, BMI, WHR, age, FFM, and fasting insulin, blood glucose, TG, cholesterol, and HDL-cholesterol as independent variables; it is worth noting that BMI, TG, and cholesterol did not enter into the regression model. PAI-1 antigen maintained a significant positive independent relationship with leptin, insulin, and fasting blood glucose and a significant negative independent association with HDL-cholesterol (Table 4).

The association between PAI-1 activity and leptin was also tested by a stepwise regression model examining PAI-1 activity as a dependent variable and leptin, FM, BMI, WHR, age, FFM, and fasting insulin, blood glucose, TG, cholesterol, and HDL-cholesterol as independent variables; insulin, FFM, blood glucose, TG, and cholesterol did not enter into the model. PAI-1 activity maintained a significant positive independent relationship with leptin, FM, and BMI (Table 5).

Table 3. Correlation Coefficients Between Leptin Level or FM and All Other Parameters (N = 61)

Variable	Leptin (ng/mL)		FM (kg)	
	r	P	r	P
FM (kg)	.887	<.001	—	—
FFM (kg)	.392	<.005	.411	<.001
Age (yr)	−.094	NS	113	NS
BMI (kg/m²)	.829	<.001	.920	<.001
WHR	.480	<.001	.526	<.001
MBP (mm Hg)	.309	<.05	.314	<.05
Insulin (μU/mL)	.447	<.001	.435	<.001
Fasting blood glucose (mg/dL)	.269	<.05	.389	<.005
Cholesterol (mg/dL)	.169	NS	.162	NS
HDL-cholesterol (mg/dL)	−.381	<.005	−.429	<.001
TG (mg/dL)	.258	<.05	.282	<.05

Table 4. Regression Summary for Dependent Variable: PAI-1 Antigen (R^2 = .641, adjusted R^2 = .586, F = 11.614, P < .001)

Variable	β	r	t	P
Leptin (ng/mL)	0.550	.376	2.923	<.01
Insulin (μU/mL)	0.351	.435	3.489	<.001
HDL-cholesterol (mg/dL)	−0.254	−.343	−2.634	<.05
Fasting blood glucose (mg/dL)	0.208	.279	2.092	<.05
WHR	0.184	.240	1.782	NS
FM (kg)	−0.309	−.208	−1.537	NS
FFM (kg)	−0.110	−.155	−1.134	NS
Age (yr)	0.110	.152	1.109	NS

DISCUSSION

We found a significant relationship between leptin and PAI-1 (both antigen and activity) plasma levels in obese and nonobese women irrespective of factors known to influence PAI-1 plasma levels.

Although leptin synthesis also has been demonstrated in the placenta[41] and the stomach,[42] leptin is produced almost exclusively in adipose tissue and is therefore considered a marker of body fat accumulation in humans.[29-31] Thus, even though correlations do not always imply biological causation and some groups have shown that the absolute leptin levels for a given body weight are notably variable,[30,33] the independent association between leptin and PAI-1 plasma levels may indicate that a progressive increase of leptin production is paralleled by a simultaneous increase of PAI-1 synthesis.

It is well known that hepatocytes and endothelial cells may produce PAI-1[21]; however, interestingly, it has been recently shown that this antifibrinolytic factor may also be produced in adipose tissue.[26-28] Therefore, it is also possible that the parallel increase of PAI-1 and leptin plasma levels may be an expression of body fat accumulation.

Our findings are in line with those of Folsom et al,[15] who showed that the reduction in PAI-1 antigen after weight loss was related more to the degree of weight loss than to changes in TG or insulin plasma levels. It was also emphasized that plasma levels of adipsin, a serine protease mainly produced by adipocytes, were independently and positively associated with those of PAI-1.[43]

On the basis of our results, we cannot exclude that leptin and/or other metabolites (FFA, etc.) produced by adipocytes

Table 5. Regression Summary for Dependent Variable: PAI-1 Activity (R^2 = .475, adjusted R^2 = .416, F = 8.139, P < .001)

Variable	β	r	t	P
Leptin (ng/mL)	0.937	.498	4.223	<.001
FM (kg)	−1.301	−.499	−4.232	<.001
BMI (kg/m²)	0.716	.347	2.720	<.01
HDL-cholesterol (mg/dL)	−0.159	− 189	−1.416	NS
Age (yr)	0.125	.159	1.182	NS
WHR	0.128	.138	1.028	NS

may have a direct stimulating effect on the expression of PAI-1 in adipose tissue. For example, since insulin, as well as FFA, and TG have been shown to increase PAI-1 plasma levels,[21,44] it may well be that these factors act locally in adipose tissue. Since insulin maintained an independent association with PAI-1 antigen irrespective of leptin (or FM, BMI, or WHR), it seems possible that body fat and insulin are the most important factors influencing PAI-1 antigen plasma levels. However, it must be taken into account that genetic factors also have an important role in controlling PAI-1 levels.[45]

In the stepwise regression analysis, PAI-1 antigen maintained a significant independent relationship with leptin, but not with FM. Therefore, whether leptin is produced almost exclusively in adipose tissue, and these results may suggest that the adipose tissue excess participates in the increase of PAI-1 levels in obese subjects, it cannot be excluded that leptin per se may have an additive role to that of adipose tissue in influencing PAI-1 production. From this point of view, high leptin levels could be considered a cardiovascular risk factor, and it is worth noting that this possibility has been raised recently by Haynes et al.[46] However, since correlations do not always imply biological causation, further in vitro studies are needed to test this hypothesis. Moreover, a recent prospective study has shown that hyperleptinemia is not an independent cardiovascular risk factor, at least in middle-aged men.[47] In addition, since we did not directly measure visceral adipose tissue by computed tomography or magnetic resonance imaging, it may be premature to conclude that there is an association between leptin and PAI-1 independently of body fat distribution.

Obesity, central fat accumulation, hyperinsulinemia, hypertension, and hypertriglyceridemia have been shown to increase PAI-1 antigen and activity[13-21,25]; in this study, statistical analyses have shown that insulin (stepwise regression analysis) maintained an independent association with PAI-1 antigen. These findings are in line with previous studies in women showing a significant association between PAI-1 and fasting insulin.[14,25,48-50] By contrast, PAI-1 activity has not shown an independent association with insulin. Therefore, according to previous studies,[14,19,20] PAI-1 antigen and activity seem to be influenced differently by similar factors.

In conclusion, the present study shows that PAI-1 plasma levels are significantly associated with body FM and leptin, a marker of body fat accumulation, independently of other factors known to affect PAI-1 plasma concentrations. These results indicate that body fat accumulation per se may have a pivotal role in PAI-1 production and thereby in the development of atherosclerosis in obese patients. Moreover, since only leptin, not FM, maintained an independent association with PAI-1 antigen in a stepwise regression analysis, it cannot be excluded that leptin has a direct role independent from that of FM in influencing PAI-1 production.

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