CLINICAL STUDY

Hemostasis and fibrinolysis in non-diabetic overweight and obese men and women. Is there still a role for leptin?

Ilse Mertens¹, Robert V Considine³, Marc Van der Planken² and Luc F Van Gaal¹

¹Department of Diabetology, Metabolism and Clinical Nutrition and ²Laboratory of Hematology and Hemostasis, Faculty of Medicine, Antwerp University Hospital, University of Antwerp (UA), Antwerp, Belgium and ³Division of Endocrinology and Metabolism, Indiana University School of Medicine, Indianapolis, USA

(Correspondence should be addressed to L F Van Gaal; Email: luc.van.gaal@uza.be)

Abstract

Objective: Leptin has been associated with disturbances in hemostasis and fibrinolysis, with inconsistent results on the influence of fat mass. However, the influence of the amount of visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) has not yet been studied. In this study, we investigated the relationship between leptin and fibrinogen, von Willebrand factor antigen (vWF:Ag), and plasminogen-activator inhibitor-1 (PAI-1) activity and determined the influence of associated metabolic variables and VAT versus SAT.

Methods: Fibrinogen, vWF:Ag, PAI-1, VAT and SAT (CT-scan), and insulin resistance (homeostasis model assessment; HOMA-IR) were measured in 199 women and 81 men with overweight or obesity visiting the weight management clinic of a university hospital.

Results: Leptin did not relate to fibrinogen ($r=0.11$ and 0.13 in women and men respectively; $P>0.05$), a relationship with vWF:Ag was only found in men ($r=0.31$; $P=0.005$), while leptin related to PAI-1 activity in both men ($r=0.36$; $P<0.001$) and women ($r=0.23$; $P<0.001$). Further analysis showed leptin to have an effect on the variation of PAI-1 independent of VAT and HOMA-IR in women, but not in men. Multiple regression showed HOMA-IR to be the most important determinant of PAI-1, both in men and women, but leptin also showed an independent effect. As for vWF:Ag, leptin was an independent determinant in men only.

Conclusions: PAI-1 related to leptin levels independent of fat mass percentage, HOMA-IR, and the amount of VAT and SAT. For vWF:Ag this relationship was found only in men, and not in women, while a relationship with fibrinogen could not be demonstrated.

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Introduction

Obesity has been shown to be associated with an increased incidence of cardiovascular morbidity and mortality (1). However, the exact mechanisms linking an increased amount of adipose tissue mass to cardiovascular disease are not yet completely clear. Until about a decade ago, adipose tissue was considered as a passive tissue for the storage of excess energy. This changed with the discovery of leptin (2) showing adipose tissue to be an important endocrine organ, secreting a variety of bioactive substances, such as leptin and plasminogen activator inhibitor-1 (PAI-1) (2, 3). Although initially thought to be merely involved in the regulation of energy homeostasis, leptin has been found to influence different cardiovascular processes, such as platelet aggregation, angiogenesis, and oxidative stress (4). Consistent with these findings, reports from the MONICA (5) and WOSCOPS (6) cohorts have shown a relationship between leptin levels and cardiovascular disease. In contrast, the Quebec Cardiovascular study

could not show an association (7) and a recent preliminary study from our group (8) even showed a potential protective effect of leptin on coronary heart disease in patients with type-2 diabetes. The latter study was the only one correcting for the exact amount of abdominal subcutaneous adipose tissue (SAT), which has been shown to be important in the determination of leptin levels (9, 10). Obesity and an increased amount of adipose tissue have also been associated with an increase in prothrombotic factors, such as fibrinogen and von Willebrand factor antigen (vWF:Ag), and a decrease in fibrinolytic activity due to raised levels of its main inhibitor PAI-1 (11). Several studies investigated the relationship between leptin and factors of the hemostatic and fibrinolytic system, and found the relation to be independent (5, 12–15) or dependent (16–20) on the amount of fat mass and/or insulin resistance. Although potentially important in the relationship between leptin and hemostatic and fibrinolytic factors, none of these studies investigated the additional role of the amount of visceral adipose tissue

(VAT) versus SAT. In this study we wanted to investigate the relationship between leptin and fibrinogen, vWF:Ag, and PAI-1, as representatives of the hemostatic and fibrinolytic system, and to determine whether the observed relationships were independent of associated metabolic variables and the amount of VAT and/or SAT as measured with CT-scan.

Subjects and methods

Subjects

The subject characteristics are shown in Table 1. The study population consisted of 199 women (154 pre- and 45 postmenopausal) and 81 men: 44 subjects were overweight ($\text{BMI} \geq 25.0\text{--}29.9 \text{ kg/m}^2$) and 236 were obese ($\text{BMI} \geq 30 \text{ kg/m}^2$). The subjects were selected in chronological order from the outpatient clinic and did not participate in a structured weight-loss program at the time of enrolment. They were 18 years or older and medication influencing coagulation, such as oral contraceptives, hormonal replacement therapy, anticoagulants, platelet inhibitors, acetyl-salicyl-acid derivatives, non-steroidal antiinflammatory drugs, and recent antibiotic use were not allowed. Other exclusion criteria were Cushing's disease, thyroid illness ($\text{TSH} > 4$ or $< 0.1 \mu\text{U/ml}$), manifest hypertriglyceridemia ($> 4.52 \text{ mmol/l}$), treated or newly diagnosed diabetes, and acute illness. Patients were clinically examined by a physician and shown to be in generally good health. This study was approved by the ethical committee of the Antwerp University Hospital and all patients gave their informed consent. Comparing pre- and postmenopausal

women, after adjustment for age, no significant differences were found in the levels of fibrinogen ($P=0.810$), vWF:Ag ($P=0.064$) or PAI-1 ($P=0.326$) with a trend towards higher levels of leptin ($P=0.051$) in postmenopausal women. Data on smoking habits were available in 185 women and 74 men. In the female subgroup, 119 women were smokers, 21 were former smokers and 45 were non-smokers. In the male subgroup, 22 were smokers, 22 were former smokers and 30 were non-smokers. In men and women, smokers were compared with non-smokers separately. Although fibrinogen levels were higher in smokers compared with non-smokers both in women (356 ± 79 vs $338 \pm 61 \text{ mg/dl}$; $P=0.153$) and men (349 ± 78 vs $324 \pm 80 \text{ mg/dl}$; $P=0.261$), these differences were not statistically significant. In women and men, no significant differences were found between smokers and non-smokers in the levels of vWF:Ag (161 ± 54 vs $169 \pm 76\%$; $P=0.854$ and 164 ± 56 vs $145 \pm 52\%$; $P=0.223$ respectively), PAI-1 (15.6 ± 9.3 vs $16.7 \pm 9.4 \text{ AU/ml}$; $P=0.508$ and 22.0 ± 9.6 vs $20.1 \pm 9.9 \text{ AU/ml}$; $P=0.504$ respectively) or leptin (26.2 ± 14.3 vs $30.5 \pm 17.2 \text{ ng/ml}$; $P=0.112$ and 17.7 ± 9.1 vs $15.7 \pm 11.5 \text{ ng/ml}$; $P=0.506$ respectively).

Anthropometric measurements

All measurements were performed in the morning, with patients in fasting conditions and undressed. Height was measured to the nearest 0.5 cm and body weight with a digital scale to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms over height in meters squared. The waist circumference was

Table 1 Subject characteristics. Data are presented as mean $\pm$ s.d. (range).

	Women ($n=199$)	Men ($n=81$)
Age (years)	41 $\pm$ 12 (18–73)	41 $\pm$ 11 (18–66)
BMI (kg/m^2)	35.9 $\pm$ 6.9 (25.1–64.1)	37.7 $\pm$ 6.3 (26.5–52.1)*
Waist (cm)	107.0 $\pm$ 16.5 (78.0–150.0)	122.0 $\pm$ 14.5 (90.5–158.0) [†]
Hip (cm)	117.0 $\pm$ 11.0 (85.0–144.0)	114.0 $\pm$ 11.0 (91.0–144.0)*
WHR	0.91 $\pm$ 0.13 (0.68–1.31)	1.07 $\pm$ 0.11 (0.85–1.34) [†]
Fat mass (%)	49.3 $\pm$ 6.8 (26.1–64.8)	41.4 $\pm$ 7.2 (27.3–56.5) [†]
TAT (cm^2)	702 $\pm$ 178 (211–1058)	741 $\pm$ 193 (387–1136)
VAT (cm^2)	149 $\pm$ 73 (28–392)	226 $\pm$ 90 (44–469) [†]
SAT (cm^2)	553 $\pm$ 142 (105–878)	519 $\pm$ 155 (249–868)
Visceral/subcutaneous ratio	0.28 $\pm$ 0.13 (0.05–1.01)	0.46 $\pm$ 0.24 (0.11–1.59) [†]
Total cholesterol (mmol/l)	5.51 $\pm$ 0.96 (3.11–8.42)	5.56 $\pm$ 0.90 (3.52–7.85)
HDL cholesterol (mmol/l)	1.39 $\pm$ 0.39 (0.67–2.98)	1.08 $\pm$ 0.29 (0.44–2.31) [†]
Triglycerides (mmol/l)	1.42 $\pm$ 0.71 (0.42–3.89)	1.99 $\pm$ 0.96 (0.56–4.27) [†]
Fasting glucose (mmol/l)	4.7 $\pm$ 0.5 (3.6–6.9)	4.9 $\pm$ 0.6 (3.7–6.9)*
Fasting insulin (mU/l)	17.6 $\pm$ 9.4 (1.4–54.0)	22.5 $\pm$ 13.5 (2.4–83.0) [‡]
HOMA-IR	3.78 $\pm$ 2.31 (0.32–14.25)	4.95 $\pm$ 3.16 (0.47–18.02) [†]
Fibrinogen (mg/dl)	343 $\pm$ 67 (188–536)	333 $\pm$ 76 (203–597)
vWF:Ag (%)	167 $\pm$ 11 (59–541)	161 $\pm$ 58 (62–401)
PAI-1 (AU/ml)	16.9 $\pm$ 9.2 (0.8–38.6)	20.9 $\pm$ 9.7 (2.0–37.3) [†]
Leptin (ng/ml)	29.5 $\pm$ 16.9 (3.4–97.6)	16.4 $\pm$ 11.3 (1.4–66.5) [†]

BMI, body mass index; WHR, waist-to-hip ratio; TAT, total adipose tissue; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; vWF: Ag, von Willebrand factor antigen; PAI-1, plasminogen activator inhibitor-1. Differences between women and men: * $P \leq 0.05$; [†] $P \leq 0.001$; [‡] $P \leq 0.01$.

measured at the mid-level between the lower rib margin and the iliac crest. Hip circumference was measured at the level of the trochanter major and the waist-to-hip ratio (WHR) was calculated. Body composition was determined by bio-impedance analysis as described by Lukaski *et al.* (21), and fat mass percentage was calculated, using the formula of Deurenberg *et al.* (22). A CT-scan at L4–L5 level was performed to measure the amount of total adipose tissue (TAT), VAT, and abdominal SAT according to the methods described previously (23).

Laboratory analyses

A fasting blood sample was taken from an antecubital vein between 0800 and 1000 h to determine the fasting levels of leptin, total and high density lipoprotein (HDL) cholesterol, and triglycerides. For assay of fibrinogen, vWF:Ag and PAI-1 activity, blood was collected without stasis. A minimal oral glucose tolerance test (OGTT) was performed with 75 g glucose, with blood samples taken to determine glucose and insulin in the fasted state and 2 h after the glucose load. Diabetes was diagnosed based on the OGTT data according to the WHO criteria (24). Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) as described by Matthews *et al.* (25) and calculated as (insulin (mU/l) $\times$ glucose (mmol/l))/22.5, with a reference value for normal insulin sensitivity as 1. Plasma glucose, total cholesterol, and triglycerides were measured using Vitros 750 XRC (Ortho Clinical Diagnostics, Johnson & Johnson, USA). HDL cholesterol was measured using Hitachi 912 (Roche Diagnostics). Insulin levels were measured with the Medgenix two-site IRMA (Bio-Source, Nivelles, Belgium). Estradiol was measured with RIA (DiaSorin Saluggia, Italy). FSH and TSH were

measured using Vitros Immunodiagnostic Products (Ortho Clinical Diagnostics, Johnson & Johnson, USA). Prolactin levels were measured with the AutoDelfia Automatic Immunoassay System (AutoDelfia, Wallac, Finland). Leukocyte count was measured on ADVIA 120 (Bayer, US; normal range: $4.3\text{--}10.0 \times 10^9/\text{l}$), fibrinogen was assayed with a Clauss–Vermeylen-based thrombin-clotting assay with the STA-fibrinogen reagents on an STA-analyser (Diagnostic, Stago, France; normal range: 200–400 mg/dl). vWF:Ag was measured using an ELISA technique (Asserachrom, Stago, France; normal range: 60–160%). PAI-1 activity (expressed as AU/ml) was measured using a chromogen substrate method (Coatest, Chromogenix, Sweden; normal range: 5–15 AU/ml). Serum leptin was measured with an, as RIA described previously (26).

Statistical analyses

Statistical calculations were performed using the statistical package SPSS version 12.0 (SPSS, Chicago, IL, USA). Normality of distribution was verified with a Kolmogorov–Smirnov test, for women and men separately. Variables that were not normally distributed were log transformed. Partial correlation coefficients were calculated to control for influencing factors. Differences in continuous variables were tested with Student's *t*-test. A 2×2 ANOVA was used to determine the influence of leptin, VAT, SAT, and HOMA-IR on the variation of PAI-1 activity. Linear regression analyses with studentized residuals were used to test whether differences were independent of influencing factors. In order to determine the most important determinants of PAI-1 activity, a stepwise multiple regression analysis, with PAI-1 activity as the dependent variable, was performed. The results were considered significant when $P < 0.05$.

Table 2 Pearson correlation coefficients with anthropometric and metabolic variables.

	Women (<i>n</i> = 199)				Men (<i>n</i> = 81)			
	Fibrinogen (mg/dl)	vWF:Ag (%)	PAI-1 (AU/ml)	Leptin (ng/ml)	Fibrinogen (mg/dl)	vWF:Ag (%)	PAI-1 (AU/ml)	Leptin (ng/ml)
BMI (kg/m ²)	0.39*	0.14 [†]	0.25*	0.37*	0.24 [†]	0.21	0.32 [†]	0.60*
Waist (cm)	0.34*	0.09	0.35*	0.29*	0.22 [†]	0.24 [†]	0.35*	0.57*
Fat mass (%)	0.36*	0.11	0.21 [‡]	0.38*	0.16	0.20	0.32 [†]	0.55*
VAT (cm ²)	0.27*	0.22 [†]	0.32*	0.18 [†]	0.26 [†]	0.23 [†]	0.36*	0.32 [†]
SAT (cm ²)	0.26*	0.04	0.20 [‡]	0.37*	0.14	0.16	0.28 [†]	0.58*
Visceral/subcutaneous ratio	0.12	0.19 [†]	0.21 [‡]	−0.04	0.13	0.10	0.15	−0.20
HDL cholesterol (mmol/l)	−0.16 [†]	0.08	−0.22 [‡]	−0.02	0.04	0.03	−0.21	−0.07
Triglycerides (mmol/l)	0.08	0.11	0.24*	0.09	−0.18	0.15	0.11	−0.11
Fasting glucose (mmol/l)	0.03	0.09	0.31*	0.05	−0.01	0.10	0.23 [†]	0.11
Fasting insulin (mU/l)	0.18 [†]	0.13	0.38*	0.23 [‡]	0.05	0.18	0.34 [‡]	0.20
HOMA-IR	0.17 [†]	0.13	0.41*	0.22 [‡]	0.10	0.17	0.44*	0.24 [†]
Leptin (ng/ml)	0.11	0.11	0.23*	—	0.13	0.31 [†]	0.36*	—

* $P \leq 0.001$; [†] $P \leq 0.05$; [‡] $P \leq 0.01$.

vWF:Ag, von Willebrand factor antigen; PAI-1, plasminogen activator inhibitor-1; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance.

Results

Relationship between leptin and fibrinogen, vWF:Ag and PAI-1

Pearson correlation coefficient values for men and women are shown in Table 2. Leptin levels were not related to fibrinogen levels and a relationship between leptin and vWF:Ag was found only in men. However, leptin levels were found to be related to PAI-1 activity in both genders. In both women and men, the relationship between PAI-1 activity and leptin remained significant after correction for age and fat mass percentage ($r=0.18$; $P=0.016$ and $r=0.23$; $P=0.039$ respectively), age and VAT ($r=0.18$; $P=0.013$ and $r=0.25$; $P=0.025$ respectively), age and SAT ($r=0.15$; $P=0.032$ and $r=0.24$; $P=0.038$), and age and HOMA-IR ($r=0.15$; $P=0.044$ and $r=0.29$; $P=0.010$ respectively). After simultaneous adjustment for age, fat mass percentage, and HOMA-IR, the relationship was borderline significant in women ($r=0.15$; $P=0.049$), but non-significant in men ($r=0.20$; $P=0.09$), probably due to the lower number of subjects in the male subgroup. Again, after simultaneous adjustment for age, fat mass percentage, and VAT ($r=0.18$; $P=0.016$) or SAT ($r=0.16$; $P=0.033$), the relationship remained significant in women, and non-significant in men ($r=0.21$; $P=0.063$ and $r=0.22$; $P=0.062$ respectively). The relationship found between vWF:Ag and leptin in men remained significant after adjustment for age and fat mass percentage ($r=0.24$; $P=0.024$), age and VAT ($r=0.30$; $P=0.007$), age and SAT ($r=0.26$; $P=0.02$) and age and HOMA-IR ($r=0.32$; $P=0.004$). After simultaneous adjustment for age, fat mass percentage and HOMA-IR ($r=0.24$; $P=0.04$), VAT ($P=0.24$; $P=0.034$) or SAT ($r=0.24$; $P=0.034$), the relationship remained significant.

Contribution of leptin versus VAT, SAT, and HOMA-IR in the variation of PAI-1 activity

In order to elucidate the role of leptin versus VAT, SAT, and HOMA-IR in the variation of PAI-1 activity levels, we divided the female and male sample based on the median value of leptin and the median values of VAT, SAT, and HOMA-IR (Figs 1 and 2). In women, leptin and VAT, and leptin and HOMA-IR showed an independent effect on the variation of PAI-1 activity levels, while there was no effect of the interaction terms. In contrast, SAT or the interaction term leptin*SAT did not have an effect independent of leptin. In men, leptin did not contribute to the variation in PAI-1 activity levels, while the effect of VAT was borderline significant. While leptin did not show an effect, independent of SAT, the interaction term leptin*SAT did have a significant effect. The effect of leptin levels on the variation in PAI-1 was dependent on HOMA-IR, while there was a significant effect of the interaction term leptin*HOMA-IR.

Stepwise regression analyses

In order to investigate whether leptin was an independent determinant of PAI-1 activity, we performed a stepwise regression analysis with PAI-1 activity as a dependent variable (Table 3). We only included factors that correlated with PAI-1 ($r \geq 0.20$; $P < 0.05$) and did not include variables expressing the same parameter (BMI versus fat mass percentage, fasting insulin and glucose versus HOMA-IR). Since waist circumference reflects the amount of VAT and SAT, we decided to include VAT and SAT in the first model and waist circumference in the second. In men and women, and in both models, HOMA-IR was the most important determinant of PAI-1 activity, but leptin was also an independent determinant (Table 3).

Figure 1 Influence of high and low levels of leptin combined with high and low levels of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and insulin resistance (HOMA-IR) on the variation in plasminogen activator inhibitor-1 (PAI-1) activity in women (2×2 ANOVA). NS, Not significant.

Figure 2 Influence of high and low levels of leptin combined with high and low levels of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and insulin resistance (HOMA-IR) on the variation in plasminogen activator inhibitor-1 (PAI-1) activity in men (2×2 ANOVA). NS, Not significant.

In the male subgroup, we performed a multiple regression analysis to investigate whether leptin was an independent determinant of vWF:Ag and included fat mass percentage, leptin and VAT as independent variables, showing leptin as the only independent determinant of vWF:Ag, although explaining only 8% of the variance in vWF:Ag levels (data not shown).

Discussion

In this study of overweight and obese non-diabetic women and men, we found leptin to be a positive determinant of PAI-1 activity, independent of age, fat mass percentage and HOMA-IR. Leptin was also an independent determinant of vWF:Ag in men, while we did not find a relationship in women. We could not

demonstrate a relationship between leptin and fibrinogen. Among studies investigating the potential mediators of the relationship between PAI-1 and leptin, we are, to the best of their knowledge, the first to study the influence of the exact amount of VAT versus SAT, as measured by CT-scan. Only two previous studies, finding a relationship between PAI-1 and leptin, investigated the influence of body fat distribution and found the relationship to be independent of (12) or dependent (19) on BMI or fat mass percentage and WHR used as a crude estimate of VAT. However, the latter study was performed in obese children, which could have influenced the results. Among all adult patients, multiple regression analysis showed leptin to be a determinant of PAI-1 activity in both sexes, independent of fat distribution estimates, VAT or SAT. Results from ANOVA confirm these results in women, but not in

Table 3 Multiple regression analyses with plasminogen activator inhibitor-1 (PAI-1) activity as dependent variable.

	B	SEB	r ²	P value
Women				
<i>Model 1:</i> fat mass percentage, VAT, SAT, HDL cholesterol, triglycerides, HOMA-IR, leptin				
1. HOMA-IR	13.348	2.416	0.17	<0.001
2. Leptin	6.628	2.550	0.20	0.010
<i>Model 2:</i> fat mass percentage, waist, HDL cholesterol, triglycerides HOMA-IR, leptin				
1. HOMA-IR	11.065	2.613	0.18	<0.001
2. Leptin	6.061	2.608	0.20	0.021
3. Waist	0.087	0.043	0.21	0.045
Men				
<i>Model 1:</i> fat mass percentage, VAT, SAT, HOMA-IR, leptin				
1. HOMA-IR	11.941	3.788	0.17	0.002
2. VAT	0.025	0.012	0.24	0.034
3. Leptin	0.188	0.088	0.27	0.037
<i>Model 2:</i> fat mass percentage, waist, HOMA-IR, leptin				
1. HOMA-IR	13.712	3.819	0.17	0.001
2. Leptin	0.240	0.089	0.24	0.009

VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance.

men. This gender effect, however, could be due to the lower number of men in the study sample. *In vitro* data have shown that the expression of leptin is higher in subcutaneous compared with omental adipose tissue (9, 10), while for PAI-1, this seems to be the other way round (27). In the female group, the subcutaneous to visceral ratio was much higher, making the subcutaneous fat depot the major source of leptin. The question, however, remains whether the relationship observed between leptin levels and PAI-1 is causal or not, and if it is, in which direction? In obesity, leptin produced almost exclusively from adipocytes (2), could trigger PAI-1 production from various cell types, such as adipocytes, endothelial cells, smooth muscle cells, hepatocytes, and/or platelets (28). Indeed, leptin receptors have been found on hepatocytes (29), endothelial cells (30), and platelets (31). Surprisingly, a study in *ob/ob* mice, a genetic animal model lacking leptin, showed increased levels of plasma PAI-1 activity (32), which is in contrast with the positive association we found between leptin and PAI-1 activity. Recently, a study in predominantly normal weight women investigated the effects of physiological leptin administration and found no effect on serum PAI-1 levels (33). However, it has been shown previously that mechanisms postulated from the studies using the *ob/ob* mice (32) and lean individuals (33) cannot be extrapolated to obese humans showing increased leptin levels and (selective) leptin resistance (26). Next to a direct effect of leptin on PAI-1 expression, different indirect pathways linking leptin to PAI-1 activity in obesity could be put forward, such as the involvement of the sympathetic nervous system (34, 35), glucocorticoids (36, 37), inflammation (38), HOMA-IR, and/or body fat distribution (39, 11). In this study insulin resistance was found to be the major determinant of PAI-1 activity, although leptin levels seemed to relate to PAI-1 activity independent of insulin resistance. In the present study, we found an independent relationship between leptin and vWF:Ag in the male subgroup, while other studies in women or men did (14) or did not (13) find an association. In the study by Guagnano *et al.* (14), this relationship was also independent of body fat distribution, as measured by WHR. A specific leptin receptor (Ob-Rb) is expressed in human endothelial cells (30), which may link leptin expression to endothelial activation, leading to increased levels of the endothelial-derived vWF.

In this study, as previously found by others (19), we did not find an independent relationship between leptin levels and fibrinogen. Two other studies (5, 27), found an association between fibrinogen and leptin, which disappeared after adjustment for fat mass percentage or BMI. Only one study, in a small group of obese and non-obese men (13), found a relationship between fibrinogen and leptin independent of fat mass percentage.

It is clear that this study has some limitations. The authors do not have data on the regularity of the menstrual cycle of the pre-menopausal women and

blood samples were not taken in the same phase of the menstrual cycle for each patient. Since gonadotropins have been shown to influence leptin (40) and some hemostatic and fibrinolytic factors (41), this could have influenced our results. In contrast to most other studies investigating the influence of smoking on hemostatic and fibrinolytic factors (42–44), we did not find significantly higher levels of these factors in smokers compared with non-smokers, which could be due to the low absolute number of smokers in both men and women. Leptin (45) and the hemostatic and fibrinolytic system (46) have been related to inflammation, and prolactin has been suggested to influence platelet aggregation (47) and fibrinolysis (48). However, in this study, adjusting for prolactin levels or leukocyte count as a marker of inflammation, did not influence the relationships found between leptin and fibrinogen, and vWF:Ag or PAI-1 (data not shown).

In conclusion, PAI-1 activity related to leptin levels independent of fat mass percentage, insulin resistance, and the amount of VAT and SAT; for vWF:Ag, this relationship was found only in men, and not in women, while we could not demonstrate a relationship with fibrinogen.

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