Lower Androgenicity Is Associated With Higher Plasma Levels of Prothrombotic Factors Irrespective of Age, Obesity, Body Fat Distribution, and Related Metabolic Parameters in Men

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The purpose of this study was to examine the relationships between androgenic status and plasma levels of both prothrombotic and antithrombotic factors in men, irrespective of obesity, body fat distribution, and metabolic parameters. Sixty-four apparently healthy men, 40 with a body mass index (BMI) greater than 25 kg/m^2 (overweight and obese [OO]) and 24 non-obese controls with a BMI less than 25, were selected and evaluated for (1) plasma concentrations of plasminogen activator inhibitor-1 (PAI-1) antigen, PAI-1 activity, fibrinogen, von Willebrand factor (vWF) antigen, vWF activity, and factor VII (FVII) as the prothrombotic factors; (2) plasma levels of tissue plasminogen activator (TPA) antigen, protein C, and antithrombin III as the antithrombotic factors; (3) fasting plasma concentrations of insulin and glucose and the lipid pattern (triglycerides [TG] and total and high-density lipoprotein [HDL] cholesterol) as the metabolic parameters; and (4) free testosterone (FT), dehydroepiandrosterone sulfate (DHEAS), and sex hormone–binding globulin (SHBG) serum levels as the parameters of androgenicity. Body fat distribution was evaluated by the waist to hip ratio (WHR). In OO and non-obese subjects taken together, plasma levels of PAI-1 antigen, fibrinogen, and FVII were inversely associated with FT ($r = .255$, $P < .05$, $r = -3.14$, $P < .05$, and $r = -.278$, $P < .05$, respectively), and the negative relationships of both fibrinogen and FVII with FT were maintained after stepwise multiple regression analysis. Plasma concentrations of PAI-1 antigen and PAI-1 activity were also negatively correlated with SHBG ($r = -.315$, $P < .05$ and $r = -.362$, $P < .01$, respectively), and these associations held irrespective of the other parameters investigated. None of the antithrombotic and fibrinolytic factors were independently related to serum androgen levels. Subjects with a BMI higher than 25 kg/m^2 had higher plasma concentrations of PAI-1 antigen, PAI-1 activity, and fibrinogen as compared with non-obese controls ($P < .001$, $P < .001$, and $P < .01$, respectively). In addition, PAI-1 activity, fibrinogen, vWF antigen, and vWF activity were independent of any other metabolic and hormonal parameters. Plasma concentrations of PAI-1 antigen, PAI-1 activity, and fibrinogen were also directly correlated with WHR in all subjects taken together, irrespective of the other parameters investigated. Evaluation of antithrombotic factors showed that OO subjects had higher TPA plasma concentrations than non-obese controls ($P < .001$), whereas protein C and antithrombin III did not differ in the two groups. TPA was also directly correlated with BMI ($r = .415$, $P < .001$) and WHR ($r = .393$, $P < .001$) in all subjects. The results of this study indicate that (1) men with lower FT serum levels have higher fibrinogen and FVII plasma concentrations, and those with lower SHBG serum levels also have higher levels of PAI-1 antigen and activity; (2) irrespective of other factors, obesity per se may account for higher concentrations of PAI-1, fibrinogen, and vWF; (3) plasma levels of PAI-1 (antigen and activity) and fibrinogen correlate independently with WHR; and (4) among the investigated antithrombotic factors (TPA antigen, protein C, antithrombin III), only TPA antigen plasma concentrations are higher in men with abdominal obesity. Thus, because of the increase in several prothrombotic factors, men with central obesity, particularly those with lower androgenicity, seem to be at greater risk for coronary heart disease (CHD). Apparently, this risk is not counteracted by a parallel increase in plasma concentrations of antithrombotic factors.

OBESITY AND VISCERAL FAT accumulation, in particular, are known risk factors for the development of coronary heart disease (CHD).[1-5] The increased prevalence of thrombotic events in individuals with central obesity has been related to various metabolic and nonmetabolic abnormalities, including insulin resistance, hyperinsulinemia, impaired glucose metabolism, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol levels, hypertension, and sex hormone changes.[1-7] By acting with multiple and distinct mechanisms, each of these abnormalities, when associated with abdominal obesity, may contribute to enhance the risk of atherosclerosis and thrombotic events.[1-7] Basically, the interaction of metabolic and nonmetabolic abnormalities with the endothelium and with the coagulation system may play a key role in development of the disease.

Recent epidemiological studies have shown that CHD is associated with high plasma levels of prothrombotic factors such as fibrinogen,[8-14] factor VII (FVII),[8,10,11] von Willebrand factor (vWF),[13,15] and plasminogen activator inhibitor-1 (PAI-1)[16]; in particular, elevated fibrinogen,[8] FVII,[8] and PAI-1[16] plasma levels have been shown to be independent risk factors for CHD. Increased fibrinogen,[9,10,12,13] FVII,[10] vWF,[13,15] and PAI-1[17-19] have recently been reported in obese subjects, particularly in patients with a higher waist to hip ratio (WHR),[10,13,15,17-22] thus contributing to the well-known higher cardiovascular risk in patients with central obesity.[1-5] However, several issues presented in those studies make the potential role of the coagulation system in central obesity difficult to evaluate. First, most of the studies were conducted on subjects of both sexes or on individuals with a broad age range. Second, investigation of factors involved in regulation of the coagula-

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tion process has often been incomplete, as only some of the prothrombotic factors have been considered. Third, little is known about the effect of excess body fat and fat distribution on important factors mediating the fibrinolytic process and/or inhibiting coagulation, such as antithrombin III and protein C, which counteract the thrombotic process under physiological conditions and thus protect against the overall risk of atherosclerosis and thrombosis. Fourth, central obesity in men is associated with lower testosterone plasma levels, and in very obese patients, lower free testosterone (FT) concentrations.[23] Since lower testosterone and dehydroepiandrosterone sulfate (DHEAS) plasma levels have been associated with a higher prevalence of CHD,[24,25] it may well be that the higher cardiovascular risk in men with central obesity is partly due to lower androgenicity. In fact, a significant inverse correlation between testosterone and PAI-1 activity has been found in healthy non-obese men[26] and in those with myocardial infarction[27] or hypogonadism.[28] Furthermore, a significant negative association between sex hormone–binding globulin (SHBG) and PAI-1 has been shown in normal-weight men[26] and in men with myocardial infarction.[27] Conflicting findings have been reported on the existence of an association between androgens and FVII activity: in fact, while no correlation has been found between androgenic markers (testosterone, SHBG, and DHEAS) and FVII in non-obese subjects by some investigators,[26] FVII correlates negatively with testosterone in healthy middle-aged men according to others.[29] As for fibrinogen, a positive correlation between fibrinogen and the FT to total testosterone ratio has been shown in healthy men,[26] and testosterone treatment has proved to increase fibrinogen concentrations.[30] Finally, testosterone proprionate administration has been reported to increase fibrinolytic activity.[31]

To the best of our knowledge, no simultaneous measurements of all of the main anthropometric, hemostatic, metabolic, and androgenic parameters in men have been reported.

The purpose of this study was to examine the independent relationship between androgenic activity (evaluated by FT, DHEAS, and SHBG serum levels) and prothrombotic and antithrombotic factors in apparently healthy men, irrespective of obesity, body fat distribution (measured by WHR), and associated metabolic changes (hyperinsulinemia, higher fasting blood glucose [FBG] levels, hypertriglyceridemia, low HDL cholesterol levels). Plasma concentrations of PAI-1 antigen, PAI-1 activity, fibrinogen, vWF antigen, vWF activity, and FVII were determined as prothrombotic factors, because most of them have been shown to predict the risk of atherothrombosis in large-scale population studies.[9-15] In addition to prothrombotic factors, plasma levels of antithrombin III, protein C, and antigen were also evaluated because these blood factors have been shown to exert a protective effect against the risk of thrombosis.[32]

Since fasting insulin, FBG, and the lipid pattern (triglycerides [TGs], total cholesterol, and HDL cholesterol) are known to be regulated by body weight and by the pattern of body fat distribution,[1-5] and since they may also influence plasma concentrations of hemostatic factors,[8-19] we measured these metabolic parameters in all subjects enrolled into this study to assess the relationship of hemostatic factors with androgenicity and central obesity independently of metabolic parameters.

SUBJECTS AND METHODS

Subjects

Sixty-four healthy men, 40 overweight and obese (body mass index [BMI] > 25, OO) and 24 non-obese (BMI < 25, control) were enrolled into the study. They were aged 18 to 45 years. OO 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. Normal-weight men were represented by apparently healthy volunteers recruited consecutively among physicians, medical students, and their relatives. All participants gave informed consent for enrollment, and the study was performed according to the Helsinki Declaration.

The men had no history or physical or ECG signs of CHD. Biochemical markers of thyroid, liver, and kidney function were within normal range in all subjects. They all had normal glucose tolerance as determined by World Health Organization criteria.[33] The subjects were not regular smokers or alcohol drinkers, and all were asked not to take any drugs for at least 15 days before the tests. During this period, they were also asked to maintain their normal mixed diet and not to engage in any intense sporting activity. Blood pressure was recorded on at least three separate occasions using a mercury manometer with appropriate cuff size (Baumanometer; Baum, New York, NY). Blood for hormonal, metabolic and hemostatic determinations was drawn between 8 and 9 AM after overnight fasting. An 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.

Metabolic Parameters

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

Hemostatic Parameters

Before measuring hemostatic parameters, the subjects were allowed to rest in the supine position for 20 minutes. Then, venous blood (25 mL) was drawn using a no. 19 butterfly needle without venous stasis and discarding the first 3 mL. Blood samples were drawn into siliconized vacutainer tubes containing sodium citrate (0.38% final concentration). Plasma for FVII and fibrinogen determinations was always handled at room temperature to avoid cold activation, and measurements were made on the day of blood collection. Blood samples for other hemostatic determinations were kept on crushed ice until centrifugation. Samples were centrifuged at 2,500 × *g* for 15 minutes at 4°C within 15 minutes after collection. The resulting plasma was stored in small aliquots in a −70°C freezer until assay.

Plasma FVII was determined by a one-stage clotting assay using thromboplastin (PT-Fibrinogen HS; Instrumentation Laboratory, Milan, Italy), acFVII-deficient plasma (Instrumentation Laboratory), a control plasma (Instrumentation Laboratory), and a plasma pool from our own laboratory.

Fibrinogen concentrations were determined according to the method of Clauss[34] using a commercially available kit (Ortho Diagnostic Systems, Raritan, NJ).

vWF antigen was quantified by an enzyme-linked immunoadsorbent assay (ELISA) (Boehringer Mannheim, Asnieres, France). For vWF antigen measurements, a plastic tube coated with specific rabbit anti-human vWF antibodies was used. These antibodies bind vWF in the test plasma. According to this method, rabbit anti-vWF antibodies coupled with peroxidase are then added and bind the remaining free

antigenic determinants of vWF; bound peroxidase can then be revealed by its activity on *o*-phenylenediamine in the presence of hydrogen peroxide. A standard curve for this method was prepared with a universal reference plasma, and the results are reported as a percentage of the standard.

vWF activity was evaluated by a modification of the Weiss test.[35]

Antithrombin III was determined by the chromogenic substrate method using a commercially available colorimetric assay (Instrumentation Laboratory) and calibrating against an Antithrombin III calibrator (Instrumentation Laboratory).

Measurements of plasma protein C concentrations were performed by an automated functional assay based on prolongation of the activated partial thromboplastin time (Instrumentation Laboratory), using the ACL 2000 device (Instrumentation Laboratory).

PAI-1 antigen concentrations were determined by an ELISA (Boehringer Mannheim, Asnieres, France), and PAI-1 activity was measured by a photometric determination method (Kabivitrum, Molndal, Sweden) as previously described.[19]

Plasma TPA antigen concentrations were quantified by an ELISA method (Boehringer Mannheim), similar to the determination of vWF antigen. Results are reported as a percentage of the standard.

Both intraassay and interassay coefficients of variation in all of these determinations were less than 7.5%.

WHR

Waist circumference was measured as the minimum measurement between the xyphoid process and the umbilicus. Hip circumference was measured at the most protrusive points of the greater trochanters.

Statistical Analyses

Results are presented as the mean ± SEM. A logarithmic transformation ($\log_{10}$) of the values was performed for nonnormally distributed variables (eg, PAI-1 antigen, fasting insulin, and TG); however, natural levels of these parameters are presented in the descriptive Table 1. The unpaired *t* test was used to compare mean values in obese and non-obese men. Pearson correlation coefficients (*r*) were used to quantify univariate associations between variables. Since the selection process does not allow a full assessment of the distribution of relative ponderosity, obese and normal weight subjects were pooled. Thus, we had only one group with a wide range of BMI and androgenicity. Moreover, a stepwise multiple regression analysis was performed to test the joint effect of different variables on hemostatic parameters. Age, mean blood pressure (MBP), cholesterol, HDL cholesterol, TGs, fasting glucose, fasting insulin, FT, DHEAS, and SHBG were the independent variables included in the multiple regression models; BMI or WHR were also alternatively entered into the multiple regression analyses as anthropometric parameters.

RESULTS

General Characteristics

General characteristics and metabolic, hormonal, and hemostatic parameters of OO subjects versus non-obese controls are listed in Table 1. As expected, BMI and WHR were significantly higher in OO subjects. Moreover, OO subjects had significantly higher systolic, diastolic, and MBP, higher plasma concentrations of insulin, FBG, and TG, and lower plasma levels of HDL cholesterol, FT, and SHBG.

Prothrombotic Factors

Plasma concentrations of prothrombotic factors determined in OO and non-obese subjects are shown in Table 1. The correlation coefficients for prothrombotic factors versus anthropometric parameters, MBP, plasma concentrations of fasting insulin, glucose, and the lipid profile, and androgen levels in all of the subjects are shown in Table 2.

Table 1. General Characteristics and Anthropometric, Metabolic, and Hemostatic Parameters in the Controls and OO Men

Variable	Controls (n = 24)	OO Subjects (n = 40)
Age (yr)	28.5 ± 1.03	29.1 ± 1.24
BMI (kg/m²)	22.6 ± 0.31	33.0 ± 1.23‡
Systolic blood pressure (mm Hg)	115.0 ± 1.70	123.1 ± 1.92†
Diastolic blood pressure (mm Hg)	71.2 ± 1.09	77.0 ± 1.53†
MBP (mm Hg)	85.8 ± 1.07	92.3 ± 1.49‡
WHR	0.86 ± 0.01	0.94 ± 0.01‡
Cholesterol (mg/dL)	159.0 ± 8.09	173.8 ± 6.60
HDL cholesterol (mg/dL)	43.8 ± 2.06	36.5 ± 1.57†
TG (mg/dL)	86.2 ± 7.37	144.7 ± 6.69‡
FBG (mg/dL)	79.7 ± 1.38	86.5 ± 1.48†
Fasting insulin (μU/mL)	9.87 ± 1.08	16.8 ± 1.36‡
FT (pg/mL)	21.9 ± 1.20	18.8 ± 0.85*
DHEAS (μg/mL)	2,237 ± 255	2,486 ± 182
SHBG (ng/mL)	2.05 ± 0.21	1.52 ± 0.08‡
PAI-1 antigen (ng/mL)	6.20 ± 1.33	22.1 ± 1.91‡
PAI-1 activity (AU/mL)	10.6 ± 1.41	19.1 ± 1.21‡
TPA antigen (ng/mL)	0.93 ± 0.10	1.41 ± 0.08‡
Fibrinogen (mg/dL)	209.2 ± 12.1	266.7 ± 12.8†
vWF antigen (%)	85.9 ± 4.14	90.7 ± 4.22
vWF activity (%)	80.8 ± 9.15	81.8 ± 7.28
FVII activity (%)	81.2 ± 4.16	91.6 ± 3.61
Protein C (%)	111.7 ± 4.89	110.5 ± 3.85
Antithrombin III (%)	103.4 ± 2.59	105.8 ± 2.45

NOTE. Results are the mean ± SEM.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

Concentrations of PAI-1 antigen, PAI-1 activity, and fibrinogen were found to be significantly higher in OO patients than in controls. In addition, when all subjects in the study were considered together for statistical analysis, plasma concentrations of PAI-1 antigen, PAI-1 activity, and vWF antigen were found to correlate directly with BMI (Table 2). vWF antigen, vWF activity, and FVII did not differ statistically between OO men and the controls (Table 1), although they did show a significant correlation with BMI when all subjects were considered together (Table 2).

Next, we investigated the effects of body fat distribution on plasma concentrations of prothrombotic factors in all of the subjects. Plasma concentrations of PAI-1 antigen, PAI-1 activity, fibrinogen, and FVII were positively associated with WHR (Table 2).

Additional correlations were found between certain prothrombotic factors and other metabolic and nonmetabolic parameters. Plasma concentrations of both PAI-1 antigen and activity were associated positively with fasting insulin, TG, and MBP and negatively with HDL cholesterol, whereas fibrinogen, PAI-1 antigen, and TPA antigen were directly correlated with FBG (Table 2). As for androgens, plasma concentrations of both PAI-1 antigen and activity were negatively associated with SHBG, and PAI-1 antigen was also inversely correlated with FT (Table 2).

When multiple stepwise regression analysis was performed

Table 2. Correlation Coefficients Between Hemostatic Factors and BMI, WHR, MBP, Fasting Insulin, Glucose, and Lipid Profile, and Androgens in All Subjects Entered Onto the Study (N = 64)

Parameter	BMI	WHR	MBP	Age	Insulin	FBG	TG§	Cholesterol	HDL Cholesterol	FT	DHEAS	SHBG
PAI-1 antigen§	.604‡	.540‡	.484‡	.063	.585‡	.287‡	.539‡	.216	−.361†	−.255*	.160	−.315*
PAI-1 activity	.500‡	.431‡	.364†	.024	.382†	.167	.472‡	.172	−.290*	−.230	.236	−.362†
Fibrinogen	.591‡	.572‡	.317*	.083	.299*	.262*	.364†	.319†	−.245	−.314*	−.007	−.074
vWF antigen	.299*	.205	.157	.138	.158	.053	.001	.052	−.153	−.193	−.086	−.031
vWF activity	.281*	.229	.014	.095	.021	.122	.039	.054	−.101	−.214	.055	−.232
FVII	.256*	.358†	.257*	.257*	.266*	.196	.197	.413‡	.069	−.278*	−.139	−.030
TPA antigen	.415‡	.393‡	.310*	.310‡	.291*	.315*	.266*	.371†	−.261*	−.251*	.050	−.210
Protein C	−.003	−.055	.069	.069	−.226	−.066	−.012	.014	.197	.035	−.286*	.090
Antithrombin III	−.053	.061	.026	−.142	.126	−.082	.080	−.025	.103	.050	.067	−.204

*$P < .05$.
†$P < .01$.
‡$P < .001$.
§Values were $\log_{10}$-transformed.

and PAI-1 antigen was examined as the dependent variable (fitted model: adjusted $R^2 = .520$, F ratio = 10.77, $P < .001$), PAI-1 antigen maintained an independent association with WHR ($t = 2.064$, $P < .05$), MBP, and SHBG ($t = -2.040$, $P < .05$). When BMI was entered into the regression analysis, PAI-1 antigen did not show an independent association with BMI ($t = 1.239$, NS) and was independently associated only with SHBG ($t = -2.047$, $P < .05$).

When PAI-1 activity was examined as the dependent variable (fitted model: adjusted $R^2 = .339$, F ratio = 7.468, $P < .001$), it maintained an independent association with WHR ($t = 2.094$, $P < .05$) and TG ($t = 2.602$, $P < .05$). When BMI was entered into the regression analysis (fitted model: adjusted $R^2 = .388$, F ratio = 9.386, $P < .001$), PAI-1 activity maintained an independent association with both BMI ($t = 2.931$, $P < .01$) and TG ($t = 2.039$, $P < .05$).

Fibrinogen showed a positive correlation with fasting insulin, FBG, cholesterol, TG, and MBP, and a negative association with HDL cholesterol when all subjects were considered together (Table 2). With androgens, plasma concentrations of fibrinogen were inversely associated with FT (Table 2). However, after multiple stepwise regression analysis (fitted model: adjusted $R^2 = .408$, F ratio = 6.449, $P < .001$), fibrinogen maintained a significant association with WHR ($t = 4.535$, $P < .001$), FT ($t = -2.238$, $P < .05$), and age ($t = -2.506$, $P < .05$). When BMI was entered into the regression analysis (fitted model: adjusted $R^2 = .366$, F ratio = 8.267, $P < .001$), fibrinogen maintained an independent association only with BMI ($t = 4.250$, $P < .001$) and cholesterol ($t = 2.247$, $P < .05$).

FVII plasma concentrations were directly correlated with MBP levels and fasting insulin and cholesterol concentrations, and were inversely associated with FT levels (Table 2). However, after multiple stepwise regression analysis (fitted model: adjusted $R^2 = .252$, F ratio = 8.076, $P < .001$), FVII maintained a significant association with cholesterol ($t = 3.272$, $P < .05$) and FT ($t = -2.176$, $P < .05$), whereas WHR did not enter into the statistical model. When BMI was entered into the regression analysis (fitted model: adjusted $R^2 = 0.239$, F ratio = 10.914, $P < .001$), FVII maintained an independent association with cholesterol ($t = 3.272$, $P < .05$) and FT ($t = -2.176$, $P < .05$), whereas BMI did not enter into the statistical model.

vWF antigen and activity were not significantly associated with MBP or with metabolic and hormonal parameters in the whole population.

After multiple stepwise regression analysis (fitted model: adjusted $R^2 = .030$, F ratio = 1.988, P = NS), vWF antigen did not maintain significant associations with any of the investigated parameters when WHR entered into the model. When BMI entered into the regression analysis (fitted model: adjusted $R^2 = .806$, F ratio = 3.761, $P < .05$), vWF antigen maintained an independent association only with BMI ($t = 2.742$, $P < .01$).

vWF activity did not maintain significant associations with any of the investigated parameters when WHR was entered into the model (fitted model: adjusted $R^2 = .071$, F ratio = 2.209, NS). When BMI was entered into the regression analysis (fitted model: adjusted $R^2 = .101$, F ratio = 3.376, $P < .05$), vWF activity maintained an independent association only with BMI ($t = 2.413$, $P < .05$).

Antithrombotic Factors

Plasma concentrations of antithrombotic factors determined in OO and non-obese subjects are shown in Table 1. Correlation coefficients between antithrombotic factors and anthropometric parameters, MBP, plasma concentrations of fasting insulin, glucose, and the lipid profile, and androgen levels in all investigated subjects are shown in Table 2.

When antithrombotic factors were measured in the OO and non-obese men under investigation, OO men were found to have significantly higher plasma concentrations of TPA antigen but not of protein C and antithrombin III compared with non-obese controls (Table 1). Moreover, plasma concentrations of TPA antigen but not protein-C and antithrombin III showed positive associations with BMI, WHR, and MBP (Table 2). TPA was also correlated with some of the investigated metabolic and nonmetabolic parameters. Indeed, plasma concentrations of TPA antigen showed a positive association with fasting insulin, glucose, TG, and cholesterol and a negative association with HDL cholesterol (Table 2). As for androgens, plasma concentrations of TPA were negatively associated with FT, but not with DHEAS and SHBG. Interestingly, after multiple stepwise regression analysis, TPA antigen maintained a significant correlation with cholesterol ($t = 2.623$, $P < .05$) and HDL cholesterol ($T = -2.128$, $P < .05$) when WHR was entered into the

model (fitted model: adjusted $R^2 = .279$, F ratio = 4.478, $P < .01$). On the other hand, TPA antigen maintained a significant correlation only with cholesterol ($t = 2.708$, $P < .01$) when BMI was entered into the model (fitted model: adjusted $R^2 = .260$, F ratio = 4.174, $P < .001$).

Plasma concentrations of protein C were not correlated with metabolic and nonmetabolic parameters (Table 2). Moreover, while protein C plasma concentrations were negatively associated with DHEAS, this correlation was not maintained after stepwise multiple regression analysis, irrespective of whether WHR or BMI were entered into the regression model.

Plasma concentrations of antithrombin III were not correlated with metabolic and nonmetabolic parameters (Table 2).

DISCUSSION

The purpose of this study was to examine the relationships between androgenic status and plasma levels of both prothrombotic and antithrombotic factors in men, irrespective of obesity, body fat distribution, and metabolic parameters.

Concerning SHBG and androgens, both PAI-1 antigen and PAI-1 activity were negatively correlated with SHBG, and only PAI-1 antigen was inversely related to FT. Our data are in line with previous studies showing a negative correlation between PAI-1 activity and both total testosterone and SHBG in healthy non-obese men[26] and in those with myocardial infarction[27] or hypogonadism.[28] In this study, SHBG was negatively and independently correlated with PAI-1 antigen and FT was negatively and independently correlated with fibrinogen and FVII. These results suggest that lower SHBG levels may be predictive of lower fibrinolysis, and that lower testosterone levels may be predictive of higher fibrinogen and FVII levels in men. Therefore, it may well be that lower androgenicity is associated with a higher risk of atherothrombosis in men. In particular, our data seem to confirm that low testosterone levels may precede myocardial infarction in men,[36] and may partly explain the results of an intervention study showing that testosterone undecanoate given orally significantly improved angina pectoris in 62 patients with CHD[37] and of 18 cross-sectional studies reporting reduced testosterone concentrations in CHD patients compared with normals.[38] Animal studies (six in male rabbits and one in male chicks) have also suggested an antiatherogenic effect of testosterone.[38]

DHEA has also been suggested to have a beneficial impact on CHD, and this effect seems to be mediated by preventing platelet aggregation, inhibiting accumulation of macrophages in the intima, interfering with arterial uptake of cholesterol, suppressing local generation of superoxide radicals produced by macrophages, and inhibiting proliferation of smooth muscle cells from the media into the intima.[38] However, DHEAS did not show an independent association with any of the hemostatic parameters evaluated in this study, thus possibly excluding that the antiatherogenic effect of this proandrogen may also be due to an influence on the circulating levels of prothrombotic and antithrombotic factors. On the other hand, not all cohort and cross-sectional studies have demonstrated a favorable effect of DHEAS on CHD in males.[38]

The lack of any significant relationship between androgenic markers and protein C and antithrombin III plasma levels seems to suggest that the increased cardiovascular risk in men with lower testosterone levels, due to higher PAI-1, fibrinogen, and FVII plasma concentrations, is not compensated for by a parallel increase in antithrombotic and profibrinolytic factors.

OO men showed increased plasma concentrations of prothrombotic factors such as PAI-1 antigen, PAI-1 activity, and fibrinogen. In addition, when all subjects were considered together, each of these factors and vWF antigen were found to correlate directly with BMI values. The association of PAI-1 activity, fibrinogen, and vWF (antigen and activity) with BMI was independent of MBP, metabolic parameters, and hormone levels. These results strongly suggest that obesity may be responsible for higher production of PAI-1, fibrinogen, and wWF, leading to their higher plasma concentrations and activity and thus to an increased risk of thrombosis.

In the OO and non-obese men as a whole, PAI-1 antigen, PAI-1 activity, fibrinogen, and FVII were also directly correlated with WHR. In addition, multiple stepwise regression analysis showed that PAI-1 antigen, PAI-1 activity, and fibrinogen were associated with WHR independently of other investigated parameters. Taken together, these findings show that accumulation of central fat is associated with higher plasma concentrations of PAI-1 (antigen and activity) and fibrinogen in men. Similar results were found by Cigolini et al.[21,22] Since PAI-1, fibrinogen, and FVII have been recognized as factors predictive of the risk of CHD,[9-15] it may well be that an increase in these prothrombotic factors contributes to the well-known higher risk of CHD in patients with central obesity. Hypertension has been shown to be associated with an increase of PAI-1 activity,[39,40] fibrinogen,[12,39] and FVII.[11,40] Although hypertensive patients were not enrolled in the study, a linear relationship between MBP levels and plasma concentrations of PAI-1 (both antigen and activity), fibrinogen, and FVII was found in our population, but only PAI-1 antigen concentrations were independently associated with MBP levels. These results suggest that a moderate increase in blood pressure levels may account, at least in part, for higher levels of PAI-1 (antigen and activity).

Both PAI-1 antigen and activity were not only correlated with the BMI, the amount of central fat, and MBP levels, but were also positively associated with fasting serum TG and insulin concentrations and negatively associated with HDL cholesterol, SHBG, and FT. In particular, PAI-1 activity maintained an independent association with TG levels, suggesting that TG might influence PAI-1 production directly; this hypothesis is supported by population studies[41] and by in vitro experiments showing that TG-rich purified VLDL lipoproteins directly increase the synthesis of PAI-1 by endothelial and liver cells.[42] Thus, TG plasma concentrations may represent an important determinant of PAI-1 activity in men.

Although several previous studies found a significant association between fasting insulin levels and PAI-1,[17-19,43] insulin did not maintain an independent relationship with PAI-1 antigen or PAI-1 activity after adjustment for other investigated variables (WHR, TG, and MBP levels in particular). However, the significance of the relationship between plasma concentrations of PAI-1 and insulinemia is still poorly understood. In fact, while studies on human hepatoma cells (HepG2) proved an insulin-stimulating effect on PAI-1 synthesis and release,[44] this effect was not confirmed in cultured human umbilical vein

endothelial cells.[45] Moreover, a population study performed in 1,564 men found no independent relationship between PAI-1 activity and insulin levels or insulin sensitivity[41]; in clamp studies, Mykkanen et al[46] excluded the possibility that insulin sensitivity could be an independent determinant of PAI-1 activity, and Landin et al[47] even reported a decrease in PAI-1 activity during acute euglycemic hyperinsulinemia. Thus, central fat accumulation and TG and blood pressure levels can possibly account for higher PAI-1 concentrations irrespective of insulin levels.

FVII was also directly correlated with fasting insulin levels, but again this association was not maintained after multiple regression analysis. Plasma concentrations of fibrinogen were also associated with fasting insulin, FBG, cholesterol, and TG (positively) and HDL cholesterol (negatively); however, only the association between fibrinogen and cholesterol was maintained after multiple regression analysis, and of note, plasma cholesterol levels have previously been reported to be directly associated with fibrinogen levels.[11,13]

As regards TPA antigen, OO men showed higher plasma concentrations than non-obese men; in addition, when all subjects were pooled together, TPA antigen was found to be directly correlated with WHR. The interest of these findings seems to be in the fact that TPA antigen levels may be inversely correlated with TPA activity, and it has recently been shown that TPA mass concentrations may be predictive of long-term mortality in patients with CHD[48]; therefore, higher plasma levels of this factor are further proof of the increased risk of thrombotic events in subjects with central obesity. It should be noted that plasma concentrations of TPA antigen were also independently associated with cholesterol levels (positively) and with HDL cholesterol (negatively). Unlike TPA antigen, protein C and antithrombin III did not show significant independent relationships with BMI, WHR, metabolic parameters, and androgens. The lack of any significant independent relationship between plasma concentrations of these antithrombotic factors and all of the other investigated parameters suggests that protein C and antithrombin III are not remarkably influenced by changes in metabolic and nonmetabolic parameters during development of central obesity.

In conclusion, while it is understood that statistical correlations should be interpreted with caution, this study indicates that lower androgenicity (lower FT levels in particular) is associated with higher plasma concentrations of several prothrombotic factors irrespective of obesity, body fat distribution, and related metabolic factors, thus providing an additional explanation for the higher cardiovascular risk in individuals with central obesity. Moreover, our study seems to confirm that men with central obesity usually have higher plasma concentrations of several prothrombotic factors (especially PAI-1 and fibrinogen). The lack of a parallel increase in protein C and antithrombin III plasma levels may well be a further mechanism responsible for the increased risk of atherothrombosis in patients with central fat accumulation.

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