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# Is visceral adipose tissue a determinant of von Willebrand factor in overweight and obese premenopausal women?

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**Abstract**

Visceral obesity has been associated with an increased cardiovascular risk. However, the exact mechanisms are not completely clear. In this study we investigated the relationship between von Willebrand factor (vWF) and visceral adipose tissue (VAT) in a group of 181 overweight and obese premenopausal women visiting the weight management clinic of a university hospital. von Willebrand factor antigen (vWF:Ag), plasminogen activator inhibitor 1 (PAI-1) activity, VAT (computed tomography scan), insulin resistance (homeostasis model assessment of insulin resistance), and other anthropometric and metabolic parameters were measured. Subjects with VAT in the highest quintile had significantly higher levels of vWF:Ag (171 ± 60 vs 129 ± 40%; $P = .001$) and PAI-1 (24.7 ± 8.5 vs 15.2 ± 12.0 AU/mL; $P < .001$) compared with subjects in the lowest quintile. After correction for fat mass and homeostasis model assessment of insulin resistance the difference was still significant for vWF:Ag ($P = .046$), but not for PAI-1 ($P > .05$). Stepwise multiple regression analysis showed VAT and insulin resistance as independent determinants of vWF:Ag, whereas waist circumference, high-density lipoprotein cholesterol, and insulin resistance were independent determinants of PAI-1 activity. In a subgroup of 115 patients, we measured high-sensitivity C-reactive protein and found it to influence the relationship between VAT and vWF:Ag ($r = 0.16$; $P = .088$), whereas the relationship with PAI-1 was still significant ($r = 0.21$; $P = .025$). The results from this preliminary study suggest a plausible relation between visceral obesity and endothelial activation, possibly mediated by low-grade inflammation.


## 1. Introduction

Obesity, and visceral obesity in particular, has been accepted to be an important cardiovascular risk factor [1]. Although the exact mechanisms that link an increased amount of deep abdominal fat to vascular disease are not completely clear yet, chronic low-grade inflammation, endothelial dysfunction, and/or disturbances in the hemostatic and fibrinolytic system have been suggested to play a role [2,3]. von Willebrand factor (vWF) is a multimeric high-molecular-weight glycoprotein, synthesized by the endothelial cell and megakaryocytes and stored in the Weibel-Palade bodies of endothelial cells and in α-granules of platelets. In physiologic conditions, vWF is mainly synthesized by the endothelial cell [4], and elevated vWF levels have been suggested to be a reflection of endothelial cell activation [5]. However, vWF also acts as an acute-phase reactant [6] and is involved in the hemostatic process [4].

Endothelial dysfunction can be estimated by measuring endothelium-dependent vasodilatation or by measuring plasma levels of products of the activated endothelial cell such as cellular adhesion molecules, cytokines, vasoconstrictor molecules (endothelin-1), and hemostatic (vWF, thrombomodulin) and fibrinolytic factors (plasminogen activator inhibitor-1 [PAI-1]) [7]. Different studies have shown disturbances in endothelial-dependent vasodilatation in obese subjects [8,9], which seem to be closely related to an abdominal fat distribution as measured by the waist-to-hip ratio (WHR) [9,10], waist circumference [9], or the amount of visceral adipose tissue (VAT) [11]. Compared with endothelial-dependent vasodilation, endothelial cell products such as vWF and PAI-1 are easier to assay and can

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therefore be used in larger groups of subjects. Previous studies investigating the influence of body fat distribution on vWF mostly used indirect measures of visceral fat (waist circumference or WHR), and found conflicting results. A study by De Pergola et al [12] using waist circumference found an association with vWF, whereas most studies using WHR did not [13-16]. Because the waist circumference is a representation of the amount of subcutaneous adipose tissue (SAT) and VAT, the measurement of both abdominal fat compartments by computed tomography scan or magnetic resonance imaging seems a more appropriate way to study the impact of the amount of VAT. Only one recent study investigated the relationship between vWF and the amount of VAT and found a significant relationship between VAT and vWF, even after correction for other metabolic variables [17]. However, this study was performed in a group of predominantly normal-weight men and did not report on the influence of low-grade inflammation. The aim of the present study was to investigate the relationship between vWF antigen (vWF:Ag) and VAT, as a direct measure of abdominal fat accumulation, in a group of overweight and obese premenopausal women.

## 2. Subjects and methods

### 2.1. Subjects

Subjects were 45 overweight (body mass index [BMI] ≥25-30 kg/m$^2$) and 136 obese women (BMI ≥30 kg/m$^2$) selected from a larger cohort of patients visiting the obesity clinic of the university hospital. Subjects were 18 years or older, and only premenopausal women not taking oral contraceptives were included. Menopause was defined using clinical data (no menstruation during the previous year) combined with hormonal data (follicle-stimulating hormone >25 mU/mL and estradiol < 20 pg/mL). Other medication influencing coagulation such as anticoagulants, platelet inhibitors, acetylsalicylic acid derivatives, nonsteroidal anti-inflammatory drugs, or recent antibiotic use was not allowed. Other exclusion criteria were Cushing's disease, hypo- or hyperthyroidism (thyroid-stimulating hormone >4 or <0.1 μU/mL, respectively), and manifest hypertriglyceridemia (>4.52 mmol/L). Patients treated for diabetes or newly diagnosed type 2 diabetic patients according to the World Health Organization criteria [18] were also excluded. All patients were clinically examined by a physician and shown to be in general good health. The study was approved by the ethical committee of the University Hospital Antwerp, and all patients gave informed consent.

Table 1
Subject characteristics

| | Mean ± SD/median* | Range |
|---|---|---|
| Age (y) | 34 ± 9 | 18-53 |
| BMI (kg/m$^2$) | 35.5 ± 6.8 | 25.0-58.4 |
| Waist (cm) | 105.0 ± 16.0 | 75.0-145.0 |
| WHR | 0.89 ± 0.12 | 0.68-1.31 |
| Fat mass (%) | 49.3 ± 6.8 | 30.3-62.5 |
| TAT (cm$^2$) | 688 ± 188 | 288-1056 |
| SAT (cm$^2$) | 572 ± 159 | 198-880 |
| VAT (cm$^2$) | 105 | 30-292 |
| Total cholesterol (mmol/L) | 5.33 ± 1.01 | 3.11-9.01 |
| HDL-C (mmol/L) | 1.45 ± 0.42 | 0.65-2.98 |
| Triglycerides (mmol/L) | 1.23 | 0.42-4.07 |
| Fasting glucose (mmol/L) | 4.5 ± 0.4 | 3.7-6.0 |
| Fasting insulin (mU/L) | 117 ± 60 | 2-340 |
| Insulin resistance (HOMA-IR) | 3.43 ± 1.86 | 0.06-10.88 |
| vWF:Ag (%) | 144 | 52-336 |
| PAI-1 (AU/mL) | 19.8 ± 10.5 | 1.8-68.7 |

TAT indicates total abdominal adipose tissue.

* Data are represented as mean ± SD for normally distributed variables or as median when distribution of variable is skewed.

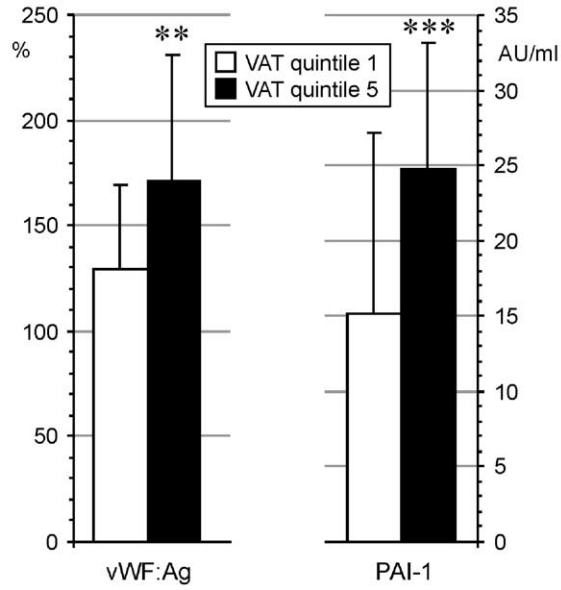


Fig. 1. Levels of vWF:Ag and PAI-1 in subjects in the lowest and highest quintiles of VAT. **$P$ < .01; ***$P$ < .001.

Characteristics of the 181 overweight and obese premenopausal women included in the study are listed in Table 1. Mean age was 34 ± 9 years, ranging from 18 to 53 years, and mean BMI was 35.5 ± 6.8 kg/m$^2$, ranging from 25.0 to 58.4 kg/m$^2$. The median value of vWF:Ag was 144% (range, 52-336%). Mean PAI-1 activity value was 19.8 ± 10.5 AU/mL, ranging from 1.8 to 68.7 AU/mL. Data on smoking habits were available in 174 patients: 107 were nonsmokers, 45 were smokers, and 22 were former smokers. Smokers were compared to nonsmokers, and no significant differences were found (vWF:Ag, 167 ± 59 vs 152 ± 58; $P$ = .120; PAI-1: 20.2 ± 10.2 vs 19.2 ± 9.5 AU/mL, $P$ = .582).

### 2.2. 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 was measured with a digital scale to the nearest 0.1 kg. Body mass index was calculated as the weight in kilograms divided by

Table 2
Pearson correlation coefficients with vWF:Ag and PAI-1 activity

| | vWF:Ag[a] (%) | PAI-1 (AU/mL) |
|---|---|---|
| Age (y) | 0.10 | −0.01 |
| BMI (kg/m$^2$) | 0.26*** | 0.40*** |
| Waist (cm) | 0.24*** | 0.44*** |
| WHR | 0.21** | 0.39*** |
| Fat mass (%) | 0.16* | 0.35*** |
| TAT (cm$^2$) | 0.24*** | 0.37*** |
| SAT (cm$^2$) | 0.20** | 0.32*** |
| VAT (cm$^2$)[a] | 0.25*** | 0.37*** |
| Total cholesterol (mmol/L) | 0.07 | 0.07 |
| HDL-C (mmol/L) | −0.08 | −0.41*** |
| Triglycerides | 0.11 | 0.37*** |
| Fasting glucose (mmol/L) | 0.12 | 0.31*** |
| Fasting insulin (mU/L) | 0.26*** | 0.36*** |
| Insulin resistance (HOMA-IR) | 0.26*** | 0.39*** |

[a] Log transformed because of skewed distribution.
* $P \leq .05$.
** $P \leq .01$.
*** $P \leq .001$.

the square of height in meters. Waist circumference was measured at the midlevel between the lower rib margin and the iliac crest. Hip circumference was measured at the level of the trochanter major and the WHR was calculated. A bioimpedance analysis (BIA) was performed to measure the total amount of body fat dividing the body into 2 compartments, fat-free mass and fat mass [19], using the formula of Deurenberg et al [20]. This technique measures the total fat percentage of the body without making a distinction between peripheral subcutaneous, abdominal subcutaneous, or deep abdominal fat. Because especially the amount of VAT has been suggested to be associated with an increased cardiovascular risk, we estimated the amounts of total abdominal adipose tissue, VAT, and SAT separately by a computed tomography scan at the L4 through L5 level according to previously described methods [21].

### 2.3. Laboratory analyses

A fasting blood sample was taken from an antecubital vein between 8:00 and 10:00 AM to determine fasting levels of triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol was calculated using the Friedewald formula [22]. For assay of vWF:Ag and PAI-1, blood was collected without stasis. An oral glucose tolerance test was performed with 75 g of glucose, with blood samples taken to determine glucose, insulin, and C-peptide in the fasted state and 2 hours after the glucose load.

Plasma glucose, total cholesterol, and triglycerides were measured on Vitros 750 XRC (Ortho Clinical Diagnostics, Johnson & Johnson, Rochester, NY). High-density lipoprotein cholesterol was measured on Hitachi 912 (Roche Diagnostics, Mannheim, Germany). Insulin levels were measured with the Medgenix 2-site IRMA assay (BioSource, Fleurus, Belgium), and C-peptide levels were measured using a specific radioimmunoassay with the Medgenix assay (BioSource). Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) as described by Matthews et al [23] and was calculated as (insulin [mU/L] × glucose [mmol/L])/22.5, with 1 as a reference value for normal insulin sensitivity. Estradiol was measured with a radioimmunoassay (DiaSorin, Saluggia, Italy). Follicle-stimulating hormone and thyroid-stimulating hormone were measured using Vitros Immunodiagnostic Products (Ortho-Clinical Diagnostics, Johnson & Johnson). High-sensitivity C-reactive protein (hs-CRP) was measured using latex-enhanced immunonephelometric assays on a BNII analyzer (Dade Behring, Marburg, Germany). von Willebrand factor antigen was measured with an enzyme-linked immunosorbent assay technique (Asserachrom, Stago, Asnieres, France; reference range, 60-160%). Plasminogen activator inhibitor-1 activity (expressed as AU/mL) was measured using a chromogen

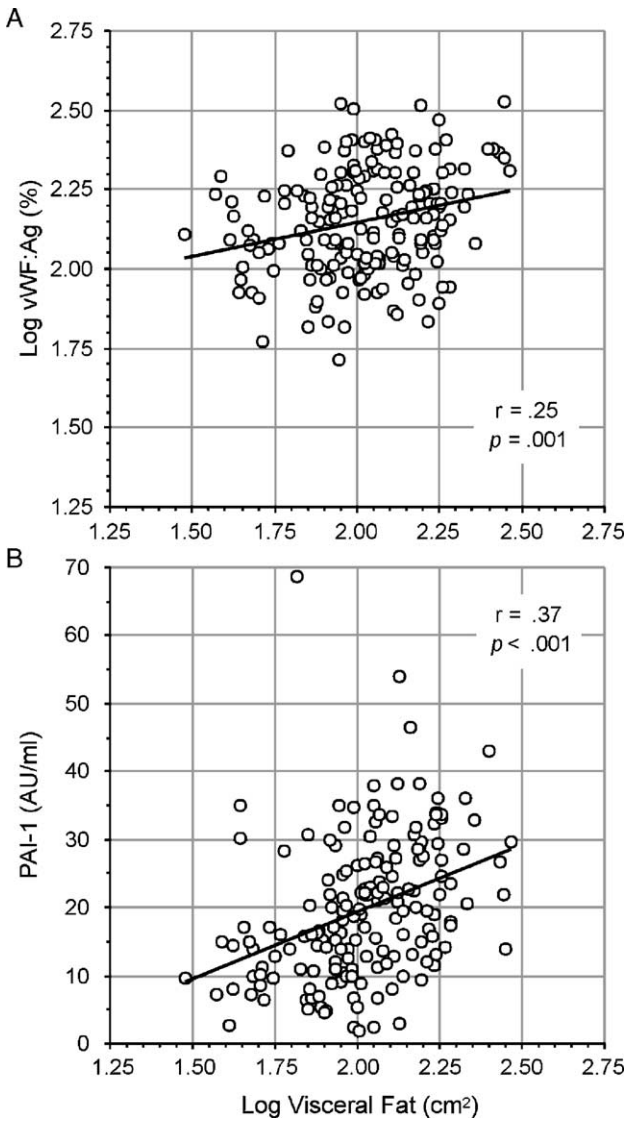


Fig. 2. Correlation between VAT and (A) vWF:Ag and (B) PAI-1 (after log transformation if needed).

Table 3
Stepwise multiple regression analyses with vWF:Ag[a] and PAI-1 activity as dependent variables, and age, fat mass percentage, waist, SAT, VAT[a], insulin resistance (HOMA-IR), HDL-C and triglycerides[a] as independent variables

| Dependent variable | *B* | SEB | $r^2$ of model | *P* |
|---|---|---|---|---|
| vWF:Ag[a] (%) | | | | |
| VAT[a] ($cm^2$) | 0.001 | <0.001 | 0.07 | .010 |
| Insulin resistance | 0.016 | 0.007 | 0.10 | .023 |
| PAI-1 (AU/mL) | | | | |
| Waist (cm) | 0.153 | 0.056 | 0.18 | .007 |
| HDL-C (mmol/L) | -6.276 | 1.903 | 0.25 | .001 |
| Insulin resistance | 1.064 | 0.453 | 0.27 | .020 |

[a] Log transformed because of skewed distribution.

substrate method (Coatest, Chromogenix, Mölndal, Sweden; reference range, 5-15 AU/mL).

### 2.4. Statistical analyses

Statistical analyses were performed using the statistical package SPSS version 12.0 (SPSS, Chicago, IL). Normality of distribution was verified with a Kolmogorov-Smirnov test. Not normally distributed variables were log transformed. Values are expressed as mean ± SD or as median for skewed variables. Pearson correlation coefficients were calculated, after log transformation if necessary. Partial correlations were calculated to control for influencing factors. Differences in continuous variables were tested with a Student *t* test with log-transformed values if necessary. Linear regression was used to test if these differences were independent of fat mass percentage and/or insulin resistance. A stepwise multiple regression was performed to evaluate the most important determinants of log vWF:Ag and PAI-1. Results were considered significant if $P < .05$.

## 3. Results

### 3.1. High and low levels of VAT

Subjects were divided in quintiles according to their levels of VAT, and subjects in the highest quintile of VAT had significantly higher levels of vWF:Ag (171 ± 60 vs 129 ± 40%; $P = .001$) and PAI-1 (24.7 ± 8.5 vs 15.2 ± 12.0 AU/mL; $P < .001$) compared with subjects in the lowest quintile of VAT (Fig. 1). After correction for fat mass (%) or insulin resistance these differences remained significant for vWF:Ag ($P = .013$ and $P = .039$), but were no longer significant for PAI-1 ($P = .095$ and $P = .056$). After correction for both fat mass (%) and insulin resistance the difference in vWF:Ag remained significant ($P = .046$).

### 3.2. Relation with anthropometric and metabolic variables

Pearson correlation coefficients (after log transformation if needed) with different anthropometric and metabolic parameters are given in Table 2. For vWF:Ag, strongest relations were found with BMI, VAT (Fig. 2), waist circumference, fasting insulin, and insulin resistance ($P < .001$). von Willebrand factor antigen did not show any association with lipid parameters. Plasminogen activator inhibitor-1 activity related most strongly to BMI, waist circumference, WHR, HDL-C, and insulin resistance ($P < .001$). Relationships with anthropometric and metabolic parameters were consistently stronger with PAI-1 as compared with vWF:Ag.

### 3.3. Influence of inflammation

In a subgroup of 115 patients, hs-CRP levels were determined as a marker of subclinical inflammation (mean, 0.51 ± 0.54 mg/dL). Levels of hs-CRP were significantly related to vWF:Ag ($r = 0.28$; $P = .002$), PAI-1 activity ($r = 0.29$; $P = .002$), VAT ($r = 0.42$; $P < .001$), SAT ($r = 0.45$; $P < .001$), insulin resistance ($r = 0.37$; $P < .001$), triglycerides ($r = 0.21$; $P = .023$), and HDL-C ($r = -0.33$; $P < .001$). The relationship between vWF:Ag and the amount of VAT was no longer significant after correction for hs-CRP ($r = 0.16$; $P = .088$), whereas the relationship between PAI-1 and VAT remained significant ($r = 0.21$; $P = .025$).

### 3.4. Multiple regression analysis

To determine the independent determinants of vWF:Ag and PAI-1, we performed a stepwise multiple regression analysis with vWF:Ag and PAI-1 as dependent variables and anthropometric and metabolic variables as independent variables (Table 3). Visceral adipose tissue was the most important determinant of vWF:Ag in this group of overweight and obese premenopausal women, with insulin resistance being an additional determinant. The most important determinant of PAI-1 activity was the waist circumference, explaining 18% of variation in PAI-1 activity. Other determinants of PAI-1 activity were HDL-C and insulin resistance.

Adding hs-CRP as an independent determinant showed, in the subgroup of patients with data on hs-CRP, insulin resistance as the only independent determinant of vWF:Ag, and waist circumference and HDL-C as independent determinants of PAI-1 activity (data not shown).

## 4. Discussion

Numerous studies, including the present, have shown an association between abdominal obesity, insulin resistance, and increased levels of PAI-1, an important cardiovascular risk factor. The fact that adipose tissue itself expresses PAI-1 could partly explain these observations [3].

In contrast to PAI-1, vWF has not been shown to be produced by adipose tissue, and the relation between abdominal obesity, insulin resistance, and vWF is not so clear. In this preliminary study, we wanted to investigate whether vWF, as a measure of endothelial activation, is increased in obese premenopausal women with high levels of VAT. We found higher levels of vWF in subjects in the highest quintile of VAT compared with subjects in the lowest quintile, independent of fat mass (%) and insulin resistance.

Abdominal obesity is an important component of the metabolic syndrome, and we found insulin resistance as measured with HOMA to be an additional significant determinant of vWF, although its contribution to the variation of plasma vWF was rather limited (3%). Previous studies investigating the relationship between insulin resistance and vWF:Ag, using indirect or direct measures of insulin resistance, found conflicting results with some studies [24,25], finding an association while others [26,27] did not. It should be noted that we did not find an association with other components of the metabolic syndrome such as fasting glucose, HDL-C, or triglycerides, making it unlikely, in contrast to PAI-1, that vWF should be considered as a real component of the metabolic syndrome.

Clearly, this preliminary study has some limitations. We do not have data on ABO blood group phenotype or genotype distribution, and because lower values of vWF were found in subjects with the O blood type [28], this could have influenced our findings. Inclusion of a normal-weight control group would give additional information on the mechanisms linking visceral obesity to endothelial activation.

However, the results from this preliminary study suggest a plausible relationship between VAT and vWF:Ag levels in overweight and obese premenopausal women. It could be hypothesized that there is a link between central obesity and/or insulin resistance and endothelial activation leading to increased vWF:Ag levels. Several mechanisms explaining the relationship between visceral obesity and endothelial activation have been put forward. Free fatty acids and/or high insulin levels, associated with visceral obesity and insulin resistance, could increase the production of endothelial factors [29]. In recent years, it has been shown that adipose tissue expresses various secretory proteins such as tumor necrosis factor (TNF)-α and interleukin-6, which stimulate the production of CRP, a marker of low-grade inflammation [30]. The pro-inflammatory cytokine TNF-α has been shown to be associated with endothelial dysfunction [31] and to be able to induce vWF release in healthy humans [32]. Yudkin et al [33] could demonstrate a strong relation between TNF-α and vWF:Ag. In addition, TNF-α has been shown to be associated with insulin resistance [34]. Therefore, circulating TNF-α, derived from adipose tissue, could be the link between (central) obesity, low-grade inflammation, insulin resistance, and endothelial activation. In this study we found that the relationship between vWF:Ag and VAT was not independent from hs-CRP, suggesting that the relationship between vWF:Ag and VAT indeed is a reflection of a state of low-grade inflammation associated with visceral obesity.

It is however not possible to conclude from the results of this cross-sectional study that there is a cause-consequence relationship between central obesity and endothelial cell activation. The higher levels of vWF:Ag could be the result of a change in the breakdown or clearance of vWF. Because VAT is a highly vascularized organ, it could be hypothesized that the increased levels of vWF simply represent the greater vascular mass associated with visceral adiposity. However, in contrast to PAI-1 [35], vWF:Ag has not been found to be secreted by the adipocyte or other cells of adipose tissue.

This study showed a relationship between vWF:Ag, a marker of endothelial activation, and the amount of VAT, possibly mediated by low-grade inflammation. The results from this study should be investigated in a broader population of men, pre- and postmenopausal women, and diabetic and nondiabetic subjects measuring vWF propeptide. Both parameters should be measured in patients with variable levels of vascular pathology to investigate whether vWF and its relationship with VAT can be seen as a marker of vessel wall pathology. Intervention studies looking at the effect of changes in VAT on changes in vWF are needed to speculate on the clinical significance of our findings.

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