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Elevated plasminogen activator inhibitor 1 in sleep apnea and its relation to the metabolic syndrome: an investigation in 2 different study samples

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Abstract

Increased circulating levels of plasminogen activator inhibitor 1 (PAI-1) have been associated with atherothrombosis. Plasminogen activator inhibitor 1 levels are elevated in obstructive sleep apnea (OSA) and in the metabolic syndrome, both of which confer excess coronary risk. We investigated whether apnea-hypopnea index (AHI) and the metabolic syndrome would interact in determining plasma concentration of PAI-1. Full-night polysomnography was performed in 2 different groups consisting of a total of 180 unmedicated apneic and nonapneic subjects of whom 20% met the diagnostic criteria for the metabolic syndrome. Distinct AHI cutoffs were selected to define 3 OSA groups with different apnea severity: (*a*) AHI of at least 5 (n = 115), (*b*) AHI of at least 10 (n = 84), and (*c*) AHI of at least 15 (n = 72). Plasminogen activator inhibitor 1 concentration was determined in plasma and statistical analyses controlled for age, sex, ethnicity, and smoking status. In both study groups, PAI-1 was positively correlated with AHI (P's < .002) and was also higher in subjects with the metabolic syndrome than in those without (P' < .013). The interaction between AHI and the metabolic syndrome independently predicted PAI-1 across all subjects and in all 3 OSA groups (P < .05). The AHI was not a significant predictor of PAI-1 in the presence of the metabolic syndrome. If the metabolic syndrome was absent, AHI accounted for between 10% and 13% of the variance in PAI-1 across all subjects and in all 3 OSA severity groups (P < .05). In conclusion, more severe apnea was independently associated with higher PAI-1 concentration in subjects without the metabolic syndrome. Once the metabolic syndrome is clinically manifest, it may be more important than apnea in determining PAI-1 levels.

1. Introduction

Obstructive sleep apnea (OSA) is a frequent sleep disorder that is characterized by repeated disruptions of breathing in the form of apneas or hypopneas during sleep. The sleep fragmentation and accompanying hypoxemia lead to many negative consequences for the cardiovascular system, thereby promoting atherosclerosis [1]. Obstructive sleep apnea is prevalent in subjects who had a myocardial infarction [2,3] and is an independent risk factor of coronary artery disease (CAD) [4]. Moreover, OSA prospectively predicted cardiovascular morbidity and mortality in patients with established CAD [5,6]. A prothrombotic state is pertinent to atherosclerosis progression [7] and atherothrombotic events [8]. Increasing evidence suggests that apnea-induced hypoxia and sympathetic nerve overactivity may elicit prothrombotic changes possibly contributing to the link between OSA and atherothrombosis [9-11].

We previously found that the antifibrinolytic enzyme plasminogen activator inhibitor 1 (PAI-1) was positively associated with apnea-hypopnea index (AHI) in patients with OSA [12] and in a sample of subjects, most of which do not have apnea [13]. Another study found significantly higher PAI-1 in apneic subjects than in nonapneic controls [14]. Plasminogen activator inhibitor 1 is the major physiologic inhibitor of tissue-type plasminogen activator, which, in turn, initiates fibrinolysis resulting in dissolution of blood clots [15]. Excess concentration of circulating PAI-1 gives rise to intravascular accumulation of fibrin, thereby promoting the development of atherosclerosis and intravascular thrombosis [15,16]. In accordance with this notion,

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elevated plasma PAI-1 levels have been associated with an increased risk of CAD [17,18].

The metabolic syndrome is prevalent in OSA and could contribute to the high cardiovascular risk in apneic subjects [19]. Factors clustering in the metabolic syndrome such as obesity, dyslipidemia, hypertension, and insulin resistance have consistently been related to PAI-1 [16]. In fact, increased PAI-1 is viewed as a true component of the metabolic syndrome [20] that is characterized by a prothrombotic state [21], of which circulating PAI-1 reflects fat distribution during obesity [22]. In a complex interplay, several mechanisms including inflammatory processes, glucidolipidic disturbances, and oxidative stress may induce PAI-1 production, mainly by macrophages in ectopic fat depots [20].

Given that so many apneic individuals have a metabolic syndrome and that elevated PAI-1 belongs to the metabolic syndrome, the question is whether the metabolic syndrome contributes independent risk over and beyond apnea in predicting circulating PAI-1 concentration. We therefore examined whether AHI and the metabolic syndrome would show an independent association with PAI-1 levels and whether AHI and metabolic syndrome would interact in predicting circulating PAI-1 concentration. The finding of a distinct contribution of AHI and metabolic syndrome to PAI-1 levels might help tailor clinical interventions aimed at reducing cardiovascular risk in OSA.

We performed our analyses in 2 different samples of unmedicated apneic and nonapneic subjects to increase the overall sample size and, therefore, statistical power. However, subjects of the 2 study groups differed in several characteristics and blood samples were not handled identically between studies (for instance, samples were drawn at somewhat different times of the day and PAI-1 level shows some diurnal fluctuation [15]). We therefore performed primary analyses in the 2 groups of subjects separately, as well as together while controlling for differences in designs between studies.

Several AHI cutoffs ranging between 5 and 20 events per hour have previously been used to define OSA in studies investigating hemostatic function [9]. We applied 3 commonly used cutoffs for AHI to explore whether findings would be robust across different degrees of OSA severity across all of our subjects.

2. Methods

2.1. Study participants

All participants were recruited from the community by advertisement, by word of mouth, or by referral from local medical practitioners. All subjects gave informed written consent on the study protocol approved by the University of California San Diego Institutional Review Board. This study combines 180 men and women from 2 separate studies on sleep apnea, blood pressure (BP), and cardiovascular physiology described in more detail elsewhere [12,13]. In previous work we reported that AHI was independently associated with PAI-1 antigen level, but we did not investigate the extent to which this relationship would be affected by the presence of the metabolic syndrome. From these previous 2 studies [12,13], we considered for the present study all subjects who had data available for PAI-1, polysomnographically obtained AHI, and the metabolic syndrome.

The sample of study A consisted of 125 subjects with resting BP of less than 180/110 mm Hg at screening. Those patients taking antihypertensive drugs underwent drug tapering under close monitoring of BP. Administration of antihypertensive drugs was stopped after tapering, which was followed by a 3-week washout period before the start of the study. No subjects took any other medication on a regular basis. Some subjects had uncomplicated essential hypertension and some were later found to have OSA, but, otherwise, physical examination and electrocardiograms were normal. All subjects underwent full-night polysomnography (PSG) at the University of California San Diego General Clinical Research Center (GCRC) to determine AHI. The sample of study B included 55 unmedicated subjects diagnosed with OSA as defined by an AHI of 15 events or more per hour after full-night PSG on the GCRC. Antihypertensive drugs were tapered and stopped for 3 weeks before the study, as in study A. Eligible subjects had a body weight 1.0 to 2.0 times the ideal body weight as determined from Metropolitan Life Insurance tables [23].

Specified exclusion criteria applying to both study protocols were congestive heart failure, symptomatic obstructive pulmonary, coronary and cerebrovascular diseases, history of life-threatening arrhythmias, cardiomyopathy, history of psychosis, narcolepsy, current alcohol or drug abuse, previous surgery for treatment of OSA, and periodic limb movement index of 15 events or more per hour.

2.2. Demographic and metabolic characteristics

Ethnicity was defined by subjects' self-identification. Subjects who currently smoked 1 cigarette or more per day were termed *smokers*. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). The average BP was computed based on 3 seated resting measurements. Blood, to determine serum high-density lipoprotein (HDL) cholesterol, serum triglycerides, and plasma glucose levels, was obtained after an overnight fast.

Subjects were diagnosed with the metabolic syndrome if they demonstrated at least 3 of the 5 clinical identification criteria listed in Table 1. There are different ways to define metabolic syndrome [21]. We adopted a modified version of the definitions of the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) and the World Health Organization (WHO). The ATP III definition previously predicted CAD [21,24]. Because the waist circumference recommended by the ATP III to define

Table 1
Diagnostic criteria of metabolic syndrome in the present study*

Risk factor	Defining level
1. Obesity	BMI >30 kg/m^2
2. Triglycerides	≥150 mg/dL
3. HDL cholesterol (mg/dL)	
Men	<40
Women	<50
4. BP	≥140 mm Hg (systolic) and/or 90 mm Hg (diastolic)
5. Fasting glucose	≥100 mg/dL

* The presence of 3 or more of these diagnostic criteria defined the metabolic syndrome.

obesity was not available in all of our subjects, we used BMI of greater than 30 kg/m^2 for this purpose (as recommended by the WHO). For the BP criterion, we defined mild hypertension as per WHO criteria instead of the ≥130/≥85 mm Hg level set by the ATP III. As suggested by the ATP III but not yet applied in its definition [21], we set the cut point for fasting plasma glucose at 100 mg/dL or higher.

2.3. Sleep recordings

Sleep was recorded using the Grass Heritage (model PSG36-2, West Warwick, RI) sleep recording system. Rechtshaffen and Kales' [25] criteria were used to score sleep recordings. An *apnea* was defined as a decrement in airflow of 90% or higher from baseline for 10 seconds or more. A *hypopnea* was defined as a decrement in airflow of 50% or higher but less than 90% from baseline for 10 seconds or more. *Apnea-hypopnea index* was defined as the number of apnea plus hypopnea events per hour of sleep. *Degree of OSA severity* was defined as per the following 3 cutoffs: (*a*) AHI of at least 5, (*b*) AHI of at least 10, and (*c*) AHI of at least 15. All sleep scorers had interrater reliability indexes (κ) of greater than 0.85 for AHI.

2.4. Biochemical analysis

An indwelling 22-gauge venous catheter was placed on top of the wrist at 5:00 PM before the PSG night. The morning after the PSG night, blood samples were drawn from resting subjects into 10-mL plastic tubes containing 3.8% sodium citrate (9:1 ratio). The first 2 mL of blood was discarded and blood flow was obtained with minimal trauma. In study A, blood for PAI-1 was obtained after an overnight fast at 6:00 AM and spun in a refrigerated centrifuge between 4°C and 8°C for 10 minutes at 3000*g*. In study B, blood for PAI-1 was obtained after a light standardized breakfast at 8:00 AM and spun twice for 15 minutes at 2000*g* at room temperature. Plasma from both studies was frozen in polypropylene tubes at −80°C.

Plasma levels of PAI-1 antigen were determined by an enzyme-linked immunosorbent assay following the manufacturer's instructions (Asserachrom, Diagnostica Stago, Asnières, France). Inter- and intra-assay coefficients of variation were 10% or less.

2.5. Statistical analyses

Data were analyzed using the SPSS Graduate Pack 13.0 for Windows (SPSS, Chicago, IL) and are given as mean ± SE. The level of significance was set at $P \leq .05$ (2-tailed). We used the Blom transformation to obtain a normal distribution of values for AHI, PAI-1, BMI, glucose, and triglycerides [26]. Independent-samples *t* test and Pearson χ^2 test or Fisher exact test, where appropriate, were used for group comparisons of continuous and categorical variables, respectively. Pearson correlation analysis was used to estimate the univariate relationship between 2 variables. Hierarchical linear regression analysis using the "enter" method identified whether AHI (continuous variable), metabolic syndrome (dummy variable: 0 = no, 1 = yes), or their interaction term was a predictor of PAI-1, independent of study design and demographic characteristics. In case of a significant interaction, post hoc analyses

Table 2
Characteristics of the 180 subjects studied

	Study group A (n = 125)	Study group B (n = 55)	*P*
Men/women	52%/48%	80%/20%	<.001
White/Black/other	50%/50%/0%	62%/14% / 24%	<.001
Age (y)	36.8 ± 0.7	47.5 ± 1.4	<.001
Current smoker (y/n)	17%/83%	9%/91%	.175
BMI (kg/m^2)	27.1 ± 0.6	31.9 ± 0.8	<.001
Systolic BP (mm Hg)	126.4 ± 1.3	132.7 ± 2.1	.011
Diastolic BP (mm Hg)	75.3 ± 0.9	79.4 ± 1.4	.013
HDL cholesterol (mg/dL)			
Women	50.9 ± 1.8	41.5 ± 3.2	.048
Men	41.8 ± 1.7	39.7 ± 1.7	.409
Triglycerides (mg/dL)	90.7 ± 4.8	161.6 ± 14.4	<.001
Fasting glucose (mg/dL)	87.1 ± 2.2	93.8 ± 1.9	.001
Metabolic syndrome (y/n)	10%/90%	42%/58%	<.001
AHI (events per hour)	7.6 ± 0.8	64.8 ± 4.1	<.001
PAI-1 antigen (ng/mL)	43.0 ± 7.2	68.7 ± 16.1	<.001

Values are expressed as mean ± SE.

identified the strength (β coefficient) of the relationship between AHI and PAI-1 in subjects with the metabolic syndrome and in those without. A dummy control variable designating the respective study was used in all multivariate models investigating the data of the 2 study groups combined to account for subtle differences between study protocols (eg, blood processing methods, fasting state).

3. Results

3.1. Subjects' characteristics

Table 2 shows that except for smoking status and HDL cholesterol in men, subject characteristics were different between study group A and study group B. Thirty-six (20%) of all subjects fulfilled the identification criteria for the metabolic syndrome. In group A, 48% of subjects had AHI of at least 5, 23% had AHI of at least 10, and 14% had AHI of at least 15. In group B, all subjects had AHI of at least 15 as per study design.

3.2. Bivariate associations with PAI-1

Plasminogen activator inhibitor 1 levels were significantly higher in subjects with the metabolic syndrome than in those without in study group A (121.3 ± 33.7 vs 33.9 ± 6.6 ng/mL, $P < .001$) and in study group B (69.0 ± 26.8 vs 68.4 ± 10.6 ng/mL, $P = .013$). In both study groups, PAI-1 showed correlations with AHI (group A: $r = 0.37$, $P < .001$; group B: $r = 0.41$, $P = .002$), BMI (group A: $r = 0.58$, $P < .001$; group B: $r = 0.49$, $P < .001$), and triglyceride levels (group A: $r = 0.32$, $P < .001$; group B: $r = 0.39$, $P = .004$).

In study group A, PAI-1 correlated with systolic BP ($r = 0.30$, $P = .001$), diastolic BP ($r = 0.20$, $P = .030$), HDL cholesterol ($r = -0.24$, $P = .009$), and fasting glucose ($r = 0.20$, $P = .030$). In study group B, PAI-1 correlated with age ($r = -0.29$, $P = .035$). There were no significant differences in PAI-1 levels between men and women, ethnic groups, and current smokers and nonsmokers in each study group.

3.3. Independent predictors of PAI-1

Hierarchical linear regression analysis was used to determine the extent to which AHI, the metabolic syndrome, and their interaction independently predicted PAI-1 concentration in each study group controlling for age, sex, ethnicity, and smoking status. The building of the models

Table 3A
Regression model for plasma PAI-1 concentration for study group A (n = 125)

	β Coefficient	P	ΔR^2
Step 1: forced entry of covariates[a,b]			
Step 2: forced entry of covariates[a,c] plus			
Apnea hypopnea index	0.41	<.001	0.132
Step 3: forced entry of covariates[a,d] plus			
Apnea hypopnea index	0.33	.001	0.132
Metabolic syndrome status	0.28	.002	0.068
Step 4: forced entry of covariates[a,e] plus			
Apnea hypopnea index	0.34	.001	0.132
Metabolic syndrome status	0.28	.002	0.068
AHI × metabolic syndrome status	−0.03	.716	0.001

The presence of the metabolic syndrome was coded as "1."
[a] Age, sex, ethnicity, and smoking status.
[b] Model accounted for 4.1% of variance in PAI-1 ($F_{4,120} = 1.28$, $P = .281$).
[c] Model accounted for 17.3% of variance in PAI-1 ($F_{5,119} = 4.97$, $P < .001$).
[d] Model accounted for 24.0% of variance in PAI-1 ($F_{6,118} = 6.22$, $P < .001$).
[e] Model accounted for 24.1% of variance in PAI-1 ($F_{7,117} = 5.31$, $P < .001$).

Table 3B
Regression model for plasma PAI-1 concentration for study group B (n = 55)

	β Coefficient	P	ΔR^2
Step 1: forced entry of covariates[a,b]			
Step 2: forced entry of covariates[a,c] plus			
Apnea hypopnea index	0.30	.026	0.081
Step 3: forced entry of covariates[a,d] plus			
Apnea hypopnea index	0.24	.077	0.081
Metabolic syndrome status	0.23	.103	0.041
Step 4: forced entry of covariates[a,e] plus			
Apnea hypopnea index	0.49	.004	0.081
Metabolic syndrome status	0.93	.004	0.041
AHI × metabolic syndrome status	−0.85	.016	0.082

The presence of the metabolic syndrome was coded as "1."
[a] Age, sex, ethnicity, and smoking status.
[b] Model accounted for 17.0% of variance in PAI-1 ($F_{4,50} = 2.56$, $P = .050$).
[c] Model accounted for 25.1% of variance in PAI-1 ($F_{5,49} = 3.29$, $P = .012$).
[d] Model accounted for 29.2% of variance in PAI-1 ($F_{6,48} = 3.30$, $P = .008$).
[e] Model accounted for 37.4% of variance in PAI-1 ($F_{7,47} = 4.02$, $P = .002$).

Table 3C
Regression model for plasma PAI-1 concentration for all subjects (n = 180)

	β Coefficient	P	ΔR^2
Step 1: forced entry of covariates[a,b]			
Step 2: forced entry of covariates[a,c] plus			
Apnea hypopnea index	0.54	<.001	0.112
Step 3: forced entry of covariates[a,d] plus			
Apnea hypopnea index	0.46	<.001	0.112
Metabolic syndrome status	0.25	.001	0.049
Step 4: forced entry of covariates[a,e] plus			
Apnea hypopnea index	0.51	<.001	0.112
Metabolic syndrome status	0.40	<.001	0.049
AHI × metabolic syndrome status	−0.24	.018	0.023

The presence of the metabolic syndrome was coded as "1."
[a] Study design, age, sex, ethnicity, and smoking status.
[b] Model accounted for 11.3% of variance in PAI-1 ($F_{5,174} = 4.45$, $P < .001$).
[c] Model accounted for 22.6% of variance in PAI-1 ($F_{6,173} = 8.41$, $P < .001$).
[d] Model accounted for 27.4% of variance in PAI-1 ($F_{7,172} = 9.29$, $P < .001$).
[e] Model accounted for 29.8% of variance in PAI-1 ($F_{8,171} = 9.07$, $P < .001$).

was done in 4 steps. We first forced age, sex, ethnicity, and smoking status as independent control variables into the equation. In a second step, we entered AHI. Metabolic syndrome status was then entered in a third step. In a last step, we also entered the interaction term between AHI and metabolic syndrome status. After the primary analysis for each group, we rerun the regression model with subjects from both studies combined additionally controlling for study design.

3.4. Analysis on subjects of study group A

Table 3A shows that after age, sex, ethnicity, and smoking status had been entered, AHI accounted for 13% of the variance in PAI-1. The presence of the metabolic syndrome accounted for an additional 7% of the variance in PAI-1 concentration. The interaction between AHI and the metabolic syndrome was not significant, suggesting AHI and metabolic syndrome contributed to PAI-1 level independent of each other.

3.5. Analysis on subjects of study group B

Table 3B shows that after age, sex, ethnicity, and smoking status had been entered, AHI and the metabolic syndrome did not explain a significant amount of variance in PAI-1 concentration in steps 2 and 3 of the model. However, when the interaction between AHI and metabolic syndrome was entered in the last step, AHI, metabolic syndrome, and their interaction all were independent predictors of PAI-1, accounting for 8%, 4%, and 8% of the variance, respectively. In post hoc analysis, AHI was a significant predictor of PAI-1 in subjects without the metabolic syndrome (β = .38, P = .031, ΔR^2 = 0.120) but not in those with the metabolic syndrome (β = −.15, P = .55, ΔR^2 = 0.020).

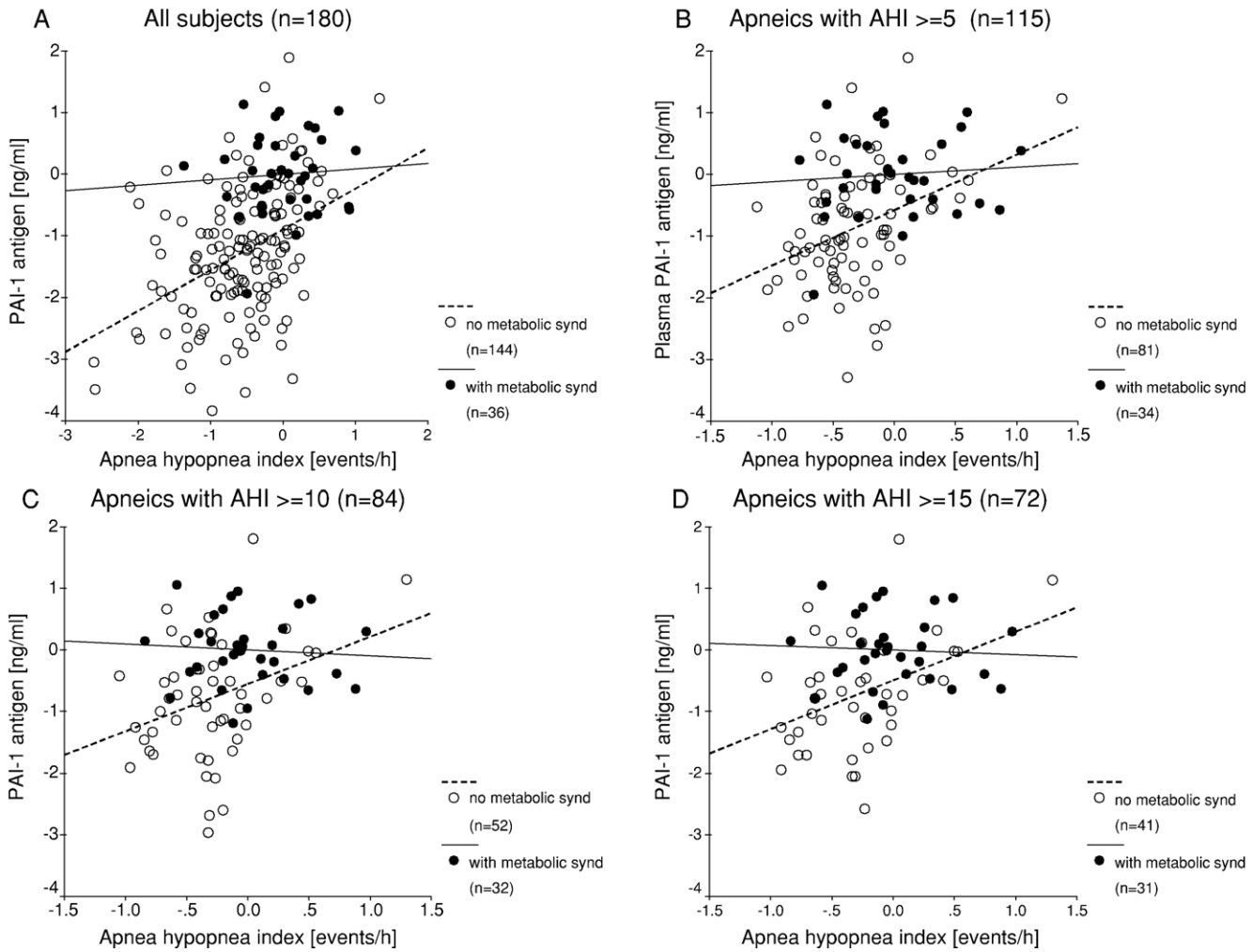

Fig. 1. Interaction between AHI and the metabolic syndrome for PAI-1 concentration. Independent associations between AHI and PAI-1 concentration across all subjects, and in patients with OSA categorized as per 3 different AHI cutoffs. Control variables were study design, age, sex, ethnicity, and smoking status. In the absence of the metabolic syndrome (synd), the relationship between more severe apnea and higher PAI-1 levels was significant in all subjects (A) and in the 3 groups of patients affected by OSA with AHI of at least 5 (B), AHI of at least 10 (C), or AHI of at least 15 (D). When metabolic syndrome was present, AHI was not a significant predictor of PAI-1 concentration in all equations. Values are given as normalized scores.

3.6. Analysis on subjects of study groups A and B combined

Table 3C shows that after study design, age, sex, ethnicity, and smoking status had been entered as independent control variables into the equation, AHI accounted for 11% of the variance in PAI-1. The presence of the metabolic syndrome accounted for an additional 5% of that variance over and beyond AHI. The interaction term between AHI and metabolic syndrome status was also significant, implying that the relationship between AHI and PAI-1 differed between subjects with and those without the metabolic syndrome. Post hoc analysis revealed that AHI was a significant predictor of PAI-1 levels in subjects without the metabolic syndrome (β = .48, P < .001, ΔR^2 = 0.101) but not in those with the metabolic syndrome (β = .10, P = .70, ΔR^2 < 0.01) (Fig. 1A). Study design (ie, study A vs study B), age, sex, ethnicity, and smoking status did not emerge as significant predictors of PAI-1 concentration in the final model and in post hoc analyses.

3.7. Analysis considering patients with sleep apnea

We computed separate hierarchical linear regression equations for the 3 groups of patients with different OSA severity, namely, those with (*a*) AHI of at least 5, (*b*) AHI of at least 10, and (*c*) AHI of at least 15. The regression models with forced entry of study design, age, sex, ethnicity, and smoking status as control variables were built exactly the same as described above for all subjects. The final models for the 3 apnea categories are shown in Table 4. With all 3 definitions of OSA, AHI, metabolic syndrome status, and the interaction between AHI and metabolic syndrome status consistently emerged as significant predictors of PAI-1 levels.

Table 4
Final regression models for plasma PAI-1 concentration in patients with apnea

	β Coefficient	P	ΔR^2
Patients with AHI ≥5 events per hour			
Forced entry of covariates[a,b] plus			
AHI	0.58	.001	0.104
Metabolic syndrome status	0.51	.001	0.053
AHI × metabolic syndrome status	−0.37	.035	0.032
Patients with AHI ≥10 events per hour			
Forced entry of covariates[a,c] plus			
AHI	0.48	.004	0.088
Metabolic syndrome status	0.72	.001	0.089
AHI × metabolic syndrome status	−0.54	.021	0.054
Patients with AHI ≥15 events per hour			
Forced entry of covariates[a,d] plus			
AHI	0.50	.003	0.088
Metabolic syndrome status	0.81	.001	0.079
AHI × metabolic syndrome status	−0.65	.015	0.066

The presence of the metabolic syndrome was coded as "1."

[a] Study design, age, sex, ethnicity, and smoking status.

[b] Model accounted for 24.9% of variance in PAI-1 ($F_{8,106}$ = 4.39, P < .001).

[c] Model accounted for 27.6% of variance in PAI-1 ($F_{8,75}$ = 3.58, P = .001).

[d] Model accounted for 32.6% of variance in PAI-1 ($F_{8,63}$ = 3.80, P = .001).

Post hoc analyses were performed to investigate the independent association between AHI and PAI-1 levels in apneic patients with and in those without the metabolic syndrome. All equations controlled for study design, age, sex, ethnicity, and smoking status. In the presence of the metabolic syndrome, AHI predicted PAI-1 in apneic patients with AHI of at least 5 (β = .43, P = .003, ΔR^2 = 0.109; Fig. 1B), in apneic patients with AHI of at least 10 (β = .43, P = .021, ΔR^2 = 0.101; Fig. 1C), and in apneic patients with AHI of at least 15 (β = .43, P = .018, ΔR^2 = 0.125; Fig. 1D). In contrast, AHI was not a significant predictor of PAI-1 levels in all 3 groups of patients affected by OSA with the metabolic syndrome (all β values <.11, all P values >.68, all ΔR^2 values <0.01; Fig. 1B-D).

4. Discussion

We found that AHI and metabolic syndrome were independent predictors of PAI-1 in the final regression models. These findings corroborate previous studies [12-14,27-29] and are in agreement with the notion that elevated PAI-1 could contribute to increased atherothrombotic risk in OSA [9] and the metabolic syndrome [20]. Importantly, the finding of a linear increase of PAI-1 levels with more severe apnea was robust because it was observed across all subjects and the 3 patient groups with different OSA severity.

The main and novel finding of the present study was that in a sample of patients with OSA only (study group B) and in all apneic and nonapneic subjects combined (study groups A and B), AHI interacted with metabolic syndrome in predicting plasma PAI-1 concentration. We found that only in absence of the metabolic syndrome was PAI-1 significantly predicted by AHI. When subjects fulfilled the clinical criteria for the metabolic syndrome, AHI on its own was not a significant predictor of PAI-1 levels in study group B and in all subjects combined. This observation was consistently observed when splitting the sample into those with apnea and those without as per 3 different AHI cutoffs roughly corresponding to very mild, mild, and moderately severe OSA. We did not find a significant interaction between AHI and metabolic syndrome in study group A perhaps because only 10% of this sample had a metabolic syndrome, and thus statistical power was limited. Altogether, these analyses suggest that the many effects of the metabolic syndrome [20] could override those of apnea [9] in determining PAI-1 levels across a range of apnea severity. In contrast, if patients with different degrees of OSA severity, even those with subclinical stages of sleep apnea, do not present with metabolic syndrome, AHI consistently accounted for a substantial amount of variance in PAI-1 levels.

Our findings may have important implications for hemostasis research and treatment algorithms aimed at alleviating cardiovascular risk in OSA. The metabolic syndrome is prevalent in patients with OSA [19,30]. We

found that above and beyond the main effects of apnea and the metabolic syndrome on PAI-1, metabolic syndrome status and apnea-related sleep disruption interacted in predicting PAI-1 levels. It follows from our study that if an independent relationship between OSA and PAI-1 is to be postulated, one needs to account for the metabolic syndrome. Fibrinogen and PAI-1 are the 2 mostly acknowledged prothrombotic factors clustering in the metabolic syndrome [21]. Like PAI-1, fibrinogen is a risk factor for atherothrombotic diseases [31] and elevated fibrinogen levels have been shown in OSA [32-34], but these studies did not control for metabolic syndrome. To possibly distinguish between distinct contributions of apnea and the metabolic syndrome to hemostatic factors other than PAI-1, we propose that future studies account for the metabolic syndrome.

Therapeutic reduction of PAI-1 levels by different means has recently been advocated to better control atherothrombotic diseases [20]. Plasminogen activator inhibitor 1 could be lowered by restoration of insulin resistance, weight reduction, insulin-sensitizing drugs, and angiotensin-converting enzyme inhibitors all targeting specific biological disturbances thought to be involved in PAI-1 overexpression with the metabolic syndrome [20]. Plasminogen activator inhibitor 1 levels could also be lowered by treatment with continuous positive airway pressure in patients with OSA [12]. Our data suggest that for their atherothrombotic risk, patients with OSA who also have metabolic syndrome may primarily profit from treatments targeting metabolic perturbation. In contrast, the patient with OSA but without overt metabolic syndrome may primarily profit from therapies decreasing AHI. However, OSA physiology exerts detrimental metabolic effects on its own right such as weight gain and arterial hypertension [30], and some of these improve with continuous positive airway pressure treatment [35]. Therefore, even in the presence of a metabolic syndrome, apnea treatment could indirectly lower PAI-1 levels via a beneficial effect on individual components of the metabolic syndrome.

Our study had several strengths. It applied a highly standardized protocol in a research environment of a GCRC. We pooled nonapneic subjects and subjects with a broad range of apnea severity who also met diagnostic criteria for the metabolic syndrome in one fifth of cases. Importantly, a possible influence of drugs on PAI-1 levels, for example, by angiotensin-converting enzyme inhibitor use in hypertensives, was ruled out because all subjects were unmedicated. In addition, our sample size allowed us to control for possible confounders of apnea and PAI-1 levels. A methodological weakness of our study could be the difference in PAI-1 assessment across the 2 studies (ie, blood sampling at 6:00 vs 8:00 AM and, in fasting vs nonfasting state, centrifugation of blood samples at room temperature vs at 4°C). However, we statistically controlled for study design by introducing a dummy variable. This variable did not predict PAI-1, suggesting that differences in PAI-1 assessment across the 2 studies did not introduce a significant bias into our PAI-1 measures. Moreover, PAI-1 levels may be high in diabetes [36]. Although none of our subjects were known to have diabetes, 6 of them had plasma fasting glucose values of at least 126 mg/dL corresponding to the diagnosis of diabetes as per the American Diabetes Association standards [37]. To assure that data from these subjects were not altering the findings, we reran the analyses eliminating these 6 subjects. The significance levels of findings were unchanged.

In conclusion, elevated PAI-1 in patients with OSA was independently predicted by apnea severity and by the metabolic syndrome. However, the relationship between apnea and PAI-1 was particularly effective in apneic patients without a metabolic syndrome, whereas metabolic syndrome effects seemed to outweigh those of apnea in apneic patients who met the clinical criteria for metabolic syndrome.

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References

[1] Parish JM, Somers VK. Obstructive sleep apnea and cardiovascular disease. Mayo Clin Proc 2004;79:1036-46.
[2] Hung J, Whitford EG, Parsons RW, et al. Association of sleep apnoea with myocardial infarction in men. Lancet 1990;336:261-4.
[3] Andreas S, Schulz R, Werner GS, et al. Prevalence of obstructive sleep apnoea in patients with coronary artery disease. Coron Artery Dis 1996;7:541-5.
[4] Shahar E, Whitney CW, Redline S, et al. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. Am J Respir Crit Care Med 2001;163:19-25.
[5] Mooe T, Franklin KA, Holmstrom K, et al. Sleep-disordered breathing and coronary artery disease: long-term prognosis. Am J Respir Crit Care Med 2001;164:1910-3.
[6] Peker Y, Hedner J, Kraiczi H, et al. Respiratory disturbance index: an independent predictor of mortality in coronary artery disease. Am J Respir Crit Care Med 2000;162:81-6.
[7] Kannel WB. Overview of hemostatic factors involved in atherosclerotic cardiovascular disease. Lipids 2005;40:1215-20.
[8] Shah PK. Thrombogenic risk factors for atherothrombosis. Rev Cardiovasc Med 2006;7:10-6.
[9] von Känel R, Dimsdale JE. Hemostatic alterations in patients with obstructive sleep apnea and the implications for cardiovascular disease. Chest 2003;124:1956-67.
[10] Quan SF, Gersh BJ. Cardiovascular consequences of sleep-disordered breathing: past, present and future: report of a workshop from the National Center on Sleep Disorders Research and the National Heart, Lung, and Blood Institute. Circulation 2004;109:951-7.
[11] von Känel R, Loredo JS, Powell FL, et al. Short-term isocapnic hypoxia and coagulation activation in patients with sleep apnea. Clin Hemorheol Microcirc 2005;33:369-77.
[12] von Känel R, Loredo JS, Ancoli-Israel S, et al. Association between sleep apnea severity and blood coagulability: treatment effects of nasal continuous positive airway pressure. Sleep Breath 2006;10:139-46.

[13] von Känel R, Loredo JS, Ancoli-Israel S, et al. Association between polysomnographic measures of disrupted sleep and prothrombotic factors. Chest 2007;131:733-9.
[14] Rangemark C, Hedner JA, Carlson JT, et al. Platelet function and fibrinolytic activity in hypertensive and normotensive sleep apnea patients. Sleep 1995;18:188-94.
[15] Vaughan DE. PAI-1 and atherothrombosis. J Thromb Haemost 2005;3:1879-83.
[16] Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. N Engl J Med 2000;342:1792-801.
[17] Hamsten A, de Faire U, Walldius G, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet 1987;2:3-9.
[18] Thogersen AM, Jansson JH, Boman K, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998;98:2241-7.
[19] Coughlin SR, Mawdsley L, Mugarza JA, et al. Obstructive sleep apnoea is independently associated with an increased prevalence of metabolic syndrome. Eur Heart J 2004;25:735-41.
[20] Alessi MC, Juhan-Vague I. PAI-1 and the metabolic syndrome: links, causes, and consequences. Arterioscler Thromb Vasc Biol 2006; 26:2200-7.
[21] Grundy SM, Brewer Jr HB, Cleeman JI, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/ American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
[22] Bastelica D, Morange P, Berthet B, et al. Stromal cells are the main plasminogen activator inhibitor-1–producing cells in human fat: evidence of differences between visceral and subcutaneous deposits. Arterioscler Thromb Vasc Biol 2002;22:173-8.
[23] Metropolitan Life Foundation. Metropolitan height and weight tables. Stat Bull Metrop Insur Co 1983;64:1.
[24] National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.
[25] Rechtshaffen A, Kales A. A manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects. NIH, Washington, DC: US Government Printing Office: Publication No. 204; 1968.
[26] Blom G. Statistical estimates and transformed beta-variables. Wiley: New York; 1958.
[27] Vague P, Juhan-Vague I, Aillaud MF, et al. Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism 1986;35:250-3.
[28] Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia 1991;34:457-62.
[29] Juhan-Vague I, Alessi MC, Mavri A, et al. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost 2003;1:1575-9.
[30] Wolk R, Somers VK. Sleep and the metabolic syndrome. Exp Physiol 2007;92:67-78.
[31] Fibrinogen Studies Collaboration. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 2005;294: 1799-809.
[32] Wessendorf TE, Thilmann AF, Wang YM, et al. Fibrinogen levels and obstructive sleep apnea in ischemic stroke. Am J Respir Crit Care Med 2000;162:2039-42.
[33] Nobili L, Schiavi G, Bozano E, et al. Morning increase of whole blood viscosity in obstructive sleep apnea syndrome. Clin Hemorheol Microcirc 2000;22:21-7.
[34] Reinhart WH, Oswald J, Walter R, et al. Blood viscosity and platelet function in patients with obstructive sleep apnea syndrome treated with nasal continuous positive airway pressure. Clin Hemorheol Microcirc 2002;27:201-7.
[35] Norman D, Loredo JS, Nelesen RA, et al. Effects of continuous positive airway pressure versus supplemental oxygen on 24-hour ambulatory blood pressure. Hypertension 2006;47:840-5.
[36] Meigs JB, Mittleman MA, Nathan DM, et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: the Framingham Offspring Study. JAMA 2000;283:221-8.
[37] Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.