# Plasma PAF-acetylhydrolase in patients with coronary artery disease: results of a cross-sectional analysis

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**Abstract Inflammation underlies both onset and perpetuation of atherosclerosis. Plasma lipoproteins transport the platelet-activating factor-acetylhydrolase (PAF-AH) with potentially anti-inflammatory activities. Our aim was to determine whether PAF-AH activity was associated with inflammatory markers and with coronary artery disease (CAD). PAF-AH activity and a panel of inflammatory mediators were measured in plasma of 496 patients with CAD and in 477 controls; 276 patients presented with stable angina pectoris and 220 with acute coronary syndrome (ACS). Individuals within the highest quartile of PAF-AH activity had an 1.8-fold increase in CAD risk [95% confidence interval (CI), 1.01 to 3.2; $P = 0.048$] compared with those in the first quartile (adjusted for clinical and metabolic factors). When excluding individuals receiving statin and angiotensin-converting enzyme-inhibitor medication, individuals within the highest quartile of PAF-AH activity revealed a 3.9-fold increase in CAD risk (95% CI, 2.0 to 7.7; $P < 0.0001$). In these subjects, the plasma PAF-AH activity increased gradually in stable angina and in ACS both in men ($P < 0.0001$) and in women ($P < 0.001$), as compared with controls. No correlation was found between PAF-AH levels and those of common markers of inflammation. This study and the previous ones raise the important issue of whether PAF-AH is simply a marker of risk or directly promotes atherosclerosis.**—Blankenberg, S., D. Stengel, H. J. Rupprecht, C. Bickel, J. Meyer, F. Cambien, L. Tiret, and E. Ninio. **Plasma PAF-acetylhydrolase in patients with coronary artery disease: results of a cross-sectional analysis. *J. Lipid Res.* 2003.** 44: **1381–1386.**

**Supplementary key words** inflammation • atherosclerosis • platelet-activating factor

Inflammation underlies the onset and the perpetuation of atherosclerosis. The inflammatory process is initiated by infiltration of both leukocytes and lipoproteins into the intimal space of the artery (1), where the lipoproteins become oxidized (2) and the monocyte-derived macrophages acquire the phenotype of foam cells. Plasma lipoproteins transport at least three enzymes with potentially antiinflammatory activities: platelet-activating factor-acetylhydrolase (PAF-AH) (3, 4), paraoxonase (5), and lecithin-cholesterol acyltransferase (6). PAF-AH, also known as LDL-$PLA_2$, associated mainly with LDL and, to a lower extent, with HDL (3, 4), degrades PAF by hydrolysing its acetate moiety in the *sn*-2 position of glycerol, and thus inhibits its proinflammatory activity. PAF-AH is a $Ca^{2+}$-independent $PLA_2$ belonging to group VII, which also degrades the short-chain *sn*-2-analogs of phosphatidylcholine generated upon oxidation of LDL (7) and, for this reason, might be important in atherosclerosis. The controversy exists whether PAF-AH is the sole enzyme with $PLA_2$ activity in HDL (8, 9). Mature macrophages and platelets synthesize and excrete this enzyme (10, 11); the myeloid origin of PAF-AH has subsequently been confirmed (12). We have recently shown that the macrophage PAF-AH is highly glycosylated, and this property determines its weak association with human HDL (13).

The cDNA encoding macrophage PAF-AH has been cloned, and the recombinant enzyme showed antiinflammatory properties in animal models (14). Additionally, we showed that the overexpression of PAF-AH by adenoviral gene transfer diminished by 2.5-fold the macrophage homing to aortic roots in atherosclerosis-prone C57Bl6 apolipoprotein E $(apoE)^{-/-}$ mice (15). Recently, Quarck et al. (16) showed, in the latter model, that the neointima formation (restenosis) induced by a wire-guided denudation of the endothelium of the common left carotid was diminished in males and females; however, the spontaneous atherosclerosis in aortic roots was diminished only in males.

A missense mutation (Val-Phe substitution) in exon 9 of PAF-AH leading to a complete loss of catalytic activity is

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Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin-6; OR, odds ratio; PAF-AH, platelet-activating factor-acetylhydrolase; SAP, stable angina pectoris.

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present in 4% of the Japanese population. Studies of this mutation suggest that the lack of PAF-AH is an independent risk factor for coronary artery disease (CAD) (17) and stroke (18). In Caucasian populations, the role of PAF-AH in cardiovascular disease is poorly documented. Packard et al. (19) demonstrated that circulating PAF-AH levels were an independent predictor of the risk of coronary heart disease in hyperlipidemic men, whereas this association was weaker in a cohort of initially healthy women (20).

The aim of our cross-sectional study was first to evaluate the correlation of plasma PAF-AH activity with different markers of inflammation in patients with documented CAD as well as in healthy control subjects. Furthermore, we aimed to investigate whether plasma PAF-AH activity was modified in patients with acute coronary syndrome (ACS) compared with stable angina and healthy control individuals.

## MATERIALS AND METHODS

### Study population

Between November 1996 and July 2000, 496 CAD patients of both sexes suffering from stable angina pectoris (SAP) (n = 276) or ACS (n = 220) were recruited at the Department of Medicine II of the Johannes Gutenberg-University Mainz and the Bundeswehrzentralkrankenhaus Koblenz on the occasion of a diagnostic coronary angiography. The inclusion criterion was the presence of a stenosis diameter >30% in at least one major coronary artery. The exclusion criterion was evidence of significant concomitant diseases, in particular hemodynamically significant valvular heart disease, known as cardiomyopathy, and malignant diseases, as well as febrile condition. At study entry, patients completed a questionnaire that provided information about smoking habits, any history of diabetes mellitus, hypertension, hyperlipoproteinemia, current drug use, and family history of premature CAD (documented CAD of one first-degree relative before the age of 65 years). Diabetes mellitus was diagnosed in patients who had previously undergone dietary treatment, had received additional oral antidiabetic or insulin medication, or had a current fasting blood sugar level >125 mg/dl; hypertension was diagnosed in patients who had received antihypertensive treatment or who had been diagnosed as hypertensive (blood pressure >160/90 mmHg); hyperlipoproteinemia was diagnosed in patients who had been given lipid-lowering medication or who had a history of cholesterol levels >240 mg/dl.

Healthy control subjects (n = 477) were recruited either from general practitioners' offices in the course of a routine check-up visit or by newspaper announcement. The newspaper announcement described briefly the study design and invited healthy German individuals aged ⩾40 years to participate in the AtheroGene study as control subjects. Of the individuals who presented, we selected those without any clinical or anamnestic evidence of a history of atherosclerosis and without evidence of any pathological electrocardiogram pattern. All individuals who presented received results of testing for classical and treatable risk factors for personal use later.

Subjects had German nationality and were inhabitants of the Rhein-Mainz area. The study was approved by the ethics committee of the University of Mainz. Participation was voluntary, and each study subject gave written informed consent.

### Laboratory methods

Blood was drawn from all subjects under standardized conditions after an overnight fasting period before coronary angiography was performed. Samples were placed on ice immediately, and within 30 min blood was centrifuged at 4,000 rpm for 10 min, divided into aliquots, and frozen at −80°C until analysis.

PAF-AH activity was measured by the trichloroacetic acid precipitation procedure as previously described (13), and the routine assays were performed in 96-well plates. Plasma was stored at −80°C and diluted 1:100 in 90 μl of PAF-AH assay buffer (pH 7.4), and 10 μl of 50 μM [$^3$H]acetyl PAF (NEN-Dupont de Nemour, Boston, MA; specific activity, 81,000 ± 2,000 dpm/nmol) was added. Samples, in duplicate, were incubated for 10 min at 37°C, and after precipitation, the radioactivity was assessed in the supernatant. The activity of PAF-AH is expressed in nmol PAF hydrolyzed/min per ml of plasma. The pool of control plasma (n = 10) served as an internal standard for all measurements.

Serum lipid levels (total cholesterol, Roche Diagnostics, Germany; HDL-cholesterol, Rolf Greiner Biochemica, Flacht, Germany; LDL-cholesterol, calculated according to the Friedewald formula; triglycerides, Roche Diagnostics) were determined immediately. ApoA-I and apoB-100 concentrations were determined by an immunoturbodimetric assay (Tina-quant, Roche Diagnostics). The lipoprotein [a] (Lp[a]) concentration was determined using an enzyme-linked immunosorbent assay-based method supplied by Immuno Ltd (Dunton Green, Kent, UK). C-reactive protein (CRP) was determined by a highly sensitive, latex particle-enhanced immunoassay (Roche Diagnostics), fibrinogen by derived method, and interleukin-6 (IL-6) by ELISA technique (EASI-ATM, Biosource Europe, Fleurus, Belgium) according to the manufacturers' instructions.

### Statistical analysis

Comparison of biochemical variables between CAD patients and controls was performed by ANOVA for variables with a normal distribution and by Mann-Whitney U test for variables with a skewed distribution. Sex-adjusted association of PAF-AH activity with cardiovascular risk factors was tested by ANOVA for categorical variables and by Pearson correlation coefficient for continuous variables. Association between PAF-AH activity and clinical status considered in three classes (control, SAP, and ACS) was tested separately in men and women by ANOVA adjusted for age, body mass index, ever smoking, and history of hypertension, and further adjusted for lipid parameters. Odds ratios (ORs) for CAD disease associated with increasing quartiles of PAF-AH were estimated by logistic regression analysis adjusted for the same variables. In all analyses, $P < 0.05$ was considered to be significant. All analyses were carried out using SPSS 10.07 software.

## RESULTS

Baseline data regarding case and control subjects are outlined in **Table 1**. Patients with CAD did not significantly differ from control subjects with respect to age and sex. As expected, the classical risk factors, diabetes, hypertension, and ever smoking were more frequent in cases than in controls. Although HDL-C and apoA-I levels were lower in cases than in controls, Lp[a] and triglyceride levels but not apoB levels were higher in cases. Paradoxically, total- and LDL-cholesterol levels were higher in controls than in patients. This might be explained in part by already existing lipid-lowering treatment in CAD patients, because 38.9% of patients as compared with 8% of con-

TABLE 1. Baseline characteristics in cases and controls

| Variable | Cases (n = 496) | Controls (n = 477) | *P* |
|---|---|---|---|
| Age, years | 59.9 ± 10.0 | 59.9 ± 7.2 | 0.9 |
| BMI, kg/m$^2$ | 27.8 ± 3.9 | 26.7 ± 4.3 | 0.3 |
| Sex, male, % | 75.6 | 73.0 | 0.3 |
| Ever smoking, % | 67.9 | 23.7 | <0.0001 |
| Diabetes mellitus, % | 17.9 | 1.7 | <0.0001 |
| Hypertension, % | 75.0 | 28.3 | <0.0001 |
| Family history of CAD, % | 35.7 | 22.0 | <0.0001 |
| Lipid status | | | |
| Total cholesterol, mg/dl | 220 ± 51 | 239 ± 40 | <0.0001 |
| LDL-cholesterol, mg/dl | 141 ± 43 | 156 ± 35 | <0.0001 |
| HDL-cholesterol, mg/dl | 50 ± 14 | 60 ± 16 | <0.0001 |
| LDL/HDL ratio | 2.9 ± 1.0 | 2.8 ± 0.9 | 0.008 |
| Triglycerides (mg/dl)[a] | 144 (107/200) | 123 (87/164) | <0.0001 |
| ApoA-I, g/l | 1.3 ± 0.2 | 1.6 ± 0.3 | <0.0001 |
| ApoB-100, g/l | 1.2 ± 0.3 | 1.2 ± 0.2 | 0.7 |
| Lipoprotein [a], mg/dl | 37.7 ± 43.5 | 24.9 ± 32.7 | <0.0001 |
| PAF-AH, nmol/min/ml | 42.2 ± 1.3 | 40.1 ± 1.4 | <0.06 |
| Medical treatment | | | |
| Statin, % | 38.9 | 8.0 | <0.0001 |
| β-blocker, % | 57.5 | 10.7 | <0.0001 |
| ACE inhibitor, % | 41.1 | 8.0 | <0.0001 |
| Calcium antagonist, % | 13.9 | 5.0 | <0.0001 |
| Antiplatelet therapy, % | 84.5 | 6.7 | <0.0001 |
| Inflammatory status | | | |
| hs-CRP (mg/l)[a] | 3.4 (1.7/9.3) | 1.4 (0.8/3.0) | <0.0001 |
| Fibrinogen (mg/dl)[a] | 328 (264/393) | 270 (241/306) | <0.0001 |
| IL-6 (pg/ml)[a] | 6.1 (3.9/13.4) | 3.9 (0.7/3.6.7) | <0.0001 |

ACE, angiotensin-converting enzyme; apo, apolipoprotein; BMI, body mass index; CAD, coronary artery disease; CRP, C-reactive protein; IL-6, interleukin 6; PAF-AH, platelet-activating factor-acetylhydrolase. Categorical variables are presented as percentage of patients; continuous variables are presented as mean values ± SD.

[a] Because of skewed distribution, variables are presented as median values (25th/75th interquartiles).

TABLE 2. Sex-adjusted mean of PAF-AH activity in cases and controls according to levels of cardiovascular risk factors

| | Cases (n = 496) | | | Controls (n = 477) | | |
|---|---|---|---|---|---|---|
| | n | PAF-AH Mean ± SEM | *P* | n | PAF-AH Mean ± SEM | *P* |
| | | *nmol/min/ml* | | | *nmol/min/ml* | |
| Sex | | | | | | |
| Male | 375 | 42.5 ± 0.7 | 0.3 | 348 | 43.0 ± 0.7 | <0.0001 |
| Female | 121 | 41.1 ± 1.2 | | 129 | 34.2 ± 1.1 | |
| Diabetes | | | | | | |
| − | 407 | 42.3 ± 0.7 | 0.7 | 469 | 40.6 ± 0.6 | 0.6 |
| + | 89 | 41.8 ± 1.1 | | 8 | 38.9 ± 3.0 | |
| Smoking | | | | | | |
| − | 159 | 42.5 ± 1.7 | 0.7 | 364 | 40.4 ± 0.7 | 0.7 |
| + | 337 | 42.0 ± 0.7 | | 113 | 41.0 ± 1.2 | |
| Hypertension | | | | | | |
| − | 124 | 46.6 ± 1.2 | <0.0001 | 342 | 40.5 ± 0.7 | 0.9 |
| + | 372 | 40.7 ± 0.7 | | 135 | 40.7 ± 1.1 | |
| BMI, kg/m$^2$ | | | | | | |
| <27 | 241 | 42.6 ± 0.9 | 0.5 | 275 | 39.4 ± 0.8 | 0.02 |
| ⩾27 | 255 | 41.8 ± 0.8 | | 201 | 42.2 ± 0.9 | |
| ACE inhibitor | | | | | | |
| − | 292 | 43.8 ± 0.8 | <0.001 | 438 | 40.4 ± 0.6 | 0.2 |
| + | 204 | 39.8 ± 0.9 | | 38 | 42.7 ± 2.1 | |
| Statins | | | | | | |
| − | 303 | 43.3 ± 0.8 | 0.013 | 438 | 40.2 ± 0.6 | 0.03 |
| + | 193 | 40.3 ± 0.9 | | 38 | 44.9 ± 2.1 | |
| ACS | | | | — | — | — |
| − | 276 | 40.0 ± 0.8 | <0.0001 | — | — | — |
| + | 220 | 44.9 ± 0.9 | | — | — | — |
| EF, % | | | | — | — | — |
| >40 | 360 | 42.1 ± 0.7 | 0.09 | — | — | — |
| ⩽40 | 37 | 38.2 ± 2.2 | | — | — | — |
| Troponin | | | | — | — | — |
| ⩽0.4 | 367 | 41.9 ± 0.7 | 0.4 | — | — | — |
| >0.4 | 129 | 43.1 ± 1.2 | | — | — | — |

ACS, acute coronary syndrome; EF, ejection fraction. Means are adjusted for sex.

trols took statin medication. Furthermore, the inflammatory markers hs-CRP, IL-6, and fibrinogen were significantly elevated in CAD patients. A slight, nonsignificant elevation of PAF-AH was observed in CAD patients compared with controls.

Women free of CAD had significantly lower PAF-AH activity than men, but this difference was abolished in CAD patients (**Table 2**). Diabetes or smoking did not affect PAF-AH in either group. By contrast, CAD patients with hypertension had significantly lower PAF-AH activity compared with nonhypertensive patients. This difference was not present in controls. Although angiotensin-converting enzyme (ACE)-inhibitor medication significantly decreased PAF-AH activity in CAD patients, this effect was not responsible for the lower levels of PAF-AH in hypertensive patients. Other antihypertensive drugs including β-blockers or calcium antagonists did not affect PAF-AH activity. Patients receiving statin medication also revealed lower PAF-AH activity, whereas this association was inverse in control subjects. Most interestingly, patients presenting with ACS had significantly higher PAF-AH activity levels than did stable patients (Table 2).

PAF-AH activity strongly correlated with LDL- and total cholesterol as well as apoB-100 in all subgroups (**Table 3**). Correlation coefficients between PAF-AH and lipid variables (total cholesterol, LDL-cholesterol, and apoB-100) were similar in both genders (data not shown). It was further negatively associated with HDL-cholesterol and apoA-I concentrations in controls and ACS patients. By contrast, no correlation could be detected with inflammatory markers, except in the group of patients with ACS.

In the entire population, PAF-AH activity was borderline-associated with the presence of CAD ($P = 0.06$; Table 1), but the case/control difference seemed mainly due to

TABLE 3. Sex-adjusted Pearson correlation coefficients between PAF-AH, lipid variables, and markers of inflammation

| | Controls (n = 477) | SAP (n = 276) | ACS (n = 220) |
|---|---|---|---|
| Age | ∼0 | ∼0 | −0.10 |
| Total cholesterol | 0.32[c] | 0.51[c] | 0.32[c] |
| LDL-cholesterol | 0.39[c] | 0.56[c] | 0.40[c] |
| HDL-cholesterol | −0.12[b] | 0.01 | −0.11 |
| Triglycerides[d] | 0.13[b] | 0.11 | 0.09 |
| ApoA-I | −0.19[b] | −0.10 | −0.22[b] |
| ApoB-100 | 0.39[c] | 0.54[c] | 0.43[c] |
| hs-CRP[d] | 0.07 | −0.08 | −0.10 |
| Fibrinogen[d] | −0.06 | −0.08 | −0.16[a] |
| IL-6[d] | 0.13 | −0.11 | −0.17[a] |
| Troponin I[d] | — | — | −0.06 |

SAP, stable angina pectoris.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] Log-transformed variable.

women (Table 2). However, when dividing patients according to stable or unstable angina, levels of PAF activity appeared elevated in patients suffering from ACS in both genders (**Table 4**). In women, PAF-AH activity in SAP patients was intermediate between levels of controls and ACS patients (Table 4). The difference remained significant after controlling for classical risk factors. The association was strengthened when excluding subjects receiving statin or ACE-inhibitor therapy, and the gradual increase among controls, SAP patients, and patients with ACS was present in both genders (Table 4, model 3). **Figure 1** shows the ORs for CAD associated with increasing quartiles of PAF-AH activity by reference to the first quartile. The highest quartile of PAF-AH activity was associated with a 1.8-fold increase in CAD risk [95% confidence interval (95% CI), 1.01 to 3.2; $P = 0.048$] after adjustment for clinical and metabolic factors. The association was stronger when excluding individuals receiving statin or ACE-inhibitor medication, patients within the highest quartile of PAF-AH activity revealing a 3.9-fold increase in CAD risk (95% CI, 2.0 to 7.7; $P < 0.0001$).

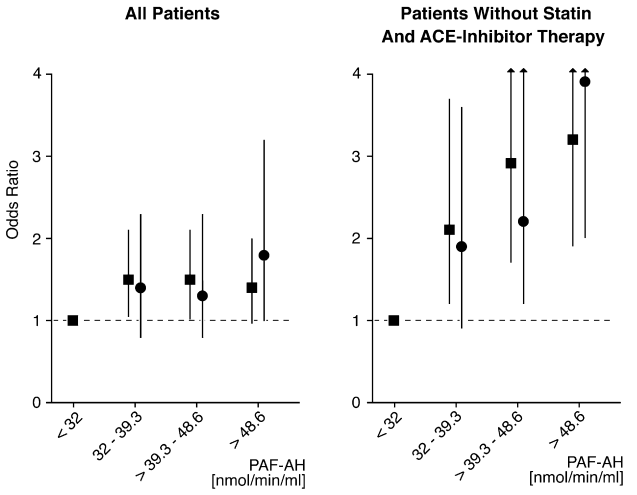


**Fig. 1.** Odds ratios (ORs) for coronary artery disease according to increasing quartiles of platelet-activating factor-acetylhydrolase in all patients and in the subgroup of patients not taking statin or angiotensin-converting enzyme-inhibitor therapy. Unadjusted ORs (squares) and ORs (circles) adjusted for age, sex, body mass index, ever smoking, history of hypertension, LDL-cholesterol, HDL-cholesterol, and triglycerides are shown.

## DISCUSSION

We showed for the first time in a cross-sectional study including 496 cases and 477 healthy controls (a subset of the AtheroGene study), that the plasma PAF-AH activity increases gradually in SAP and in ACS both in men and in women, as compared with healthy controls. Additionally, we showed that in cases with hypertension and/or with treatment with ACE inhibitors, the PAF-AH levels were diminished.

It has been shown earlier that PAF-AH activity in plasma LDL increases in ischemic stroke (18), leading the authors to postulate that the latter might be an adaptation to an increased amount of bioactive PAF-like phospholipids generated upon stroke (21). More recently, Packard et al. (19) found, in a prospective nested case-control study, that increased levels of PAF-AH showed a strong positive association with the risk of coronary heart disease in a population of middle-aged men with hypercholesterolemia.

TABLE 4. PAF-AH activity according to clinical status stratified by gender

| | n | Model 0 | Model 1 | Model 2 | Model 3 |
|---|---|---|---|---|---|
| Men | | | | | |
| Controls | 347 | 42.9 ± 0.7 | 42.1 ± 0.8 | 41.3 ± 0.7 | 41.6 ± 0.8 |
| SAP | 208 | 40.5 ± 0.9 | 41.3 ± 1.0 | 41.8 ± 0.9 | 44.3 ± 1.5 |
| ACS | 167 | 45.0 ± 1.0 | 45.6 ± 1.1 | 46.8 ± 1.0 | 49.3 ± 1.6 |
| *P* | | 0.005 | 0.004 | <0.0001 | <0.0001 |
| Women | | | | | |
| Controls | 129 | 34.2 ± 1.1 | 33.6 ± 1.4 | 34.7 ± 1.2 | 35.3 ± 1.1 |
| SAP | 68 | 38.3 ± 1.5 | 38.9 ± 1.7 | 37.7 ± 1.5 | 36.7 ± 2.5 |
| ACS | 53 | 44.8 ± 1.7 | 45.5 ± 1.9 | 44.4 ± 1.7 | 45.8 ± 2.3 |
| *P* | | <0.0001 | <0.0001 | <0.0001 | 0.0005 |

Model 0 is unadjusted; Model 1 is adjusted for age, BMI, ever smoking, and history of hypertension; Model 2 is further adjusted for LDL-cholesterol, HDL-cholesterol, and triglycerides (log-transformed); Model 3 excludes subjects with statin or ACE-inhibitor therapy.

Among healthy controls of the present study, women expressed much lower PAF-AH activity in comparison to men. Interestingly, in a recent study in a Japanese population, it was shown that only women under 50 years of age had lower PAF-AH as compared with men (22). The lower activity of PAF-AH in women may be due to their hormonal status, as hormone replacement therapy lowers PAF-AH levels (20). However, in this large prospective study of apparently healthy middle-aged women, PAF-AH did not appear to be a strong predictor of future cardiovascular risk among unselected women (20).

In the present study, we showed that the patients, but not the control subjects, with hypertension revealed decreased PAF-AH activity, a result that remains presently unexplained, insofar as it could not be clearly attributed to treatment with ACE inhibitors or other anti-hypertensive drugs. Similarly, patients with statin medication showed a significant reduction in PAF-AH levels, probably due to the decrease in LDL levels. Indeed, Tsimihodimos et al. (23) showed that atorvastatin (20 mg/day), when administered to patients with IIA and IIB dyslipidemia, lowered the activity of PAF-AH in plasma LDL. Furthermore, in IIB and IV dislipidemic patients, the administration of fenofibrate attenuated PAF-AH activity associated with apoB-containing lipoproteins but increased those of HDL-associated enzyme (24). Finally, there is a debate regarding whether PAF-AH associated with HDL could be more relevant to the protection against oxidative stress and atherosclerosis than that associated with LDL. From our earlier work (4) it is apparent that PAF-AH is associated with various subclasses of LDL; however, it is more abundant in small, dense LDLs, as these particles have prolonged life in circulation. This is probably the reason that in hypercholesterolemia, the PAF-AH levels are elevated in LDLs and especially in small, dense LDLs; however, they remain nor-

mal in HDLs (23). The association of PAF-AH with HDL is determined by its weak glycosylation, which does not affect its enzymatic activity, thus excluding the possibility that the altered activity of the enzyme is dependent on the transporting particle (13). Taken together, there is no strong evidence that PAF-AH associated with HDL would be more protective against atherosclerosis than that associated with LDL.

The lack of correlation between PAF-AH levels and other proinflammatory markers (CRP, fibrinogen, IL-6) was unexpected in our studies, as PAF-AH is believed to be induced in response to inflammation to protect against damage caused by PAF and PAF-like phospholipids. The promoter of PAF-AH contains the IL-1 response elements as well as the STAT consensus sites and is activated by inflammatory mediators, at least in transfection experiments with reporter gene constructs (25).

Our present study and the previous studies from other groups (19, 20, 26) therefore raise an important question: whether PAF-AH is simply a marker of risk or directly promotes atherosclerosis. It can be equally envisioned that under mild inflammatory and oxidative pressure, PAF-AH would serve as a protective enzyme against lipid oxidation and, under severe stress, convert into a factor that contributes to the proatherogenic status by, for example, releasing excessive levels of fatty acids or their oxidative products, augmenting the inflammatory reaction. Alternatively, PAF-AH could be always a protective factor, and if it was not upregulated in disease states, the conditions would be even worse. Indeed, in Japanese individuals, genetic deficiency of PAF-AH due to the missense mutation Val279Phe has been shown to be an independent risk factor for CAD (17) and stroke (18). More recently, the same mutation was shown to be associated with atherosclerotic disease (myocardial infarction or stroke), leading the authors to conclude that the PAF-AH gene may be one of the genetic determinants for atherosclerosis in the Japanese population (27). Interestingly, these same authors found that PAF-AH activity increased with age in both men and women, arguing for an adaptative mechanism that prevents the age-dependent vascular wall damage accelerated by PAF oxidized phospholipids.

The data from animal models are in favor of the protective role of PAF-AH in cardiovascular disease. Recombinant PAF-AH protects against myocardial ischemia reperfusion injury in rabbit (28), and we have shown that the overexpression of PAF-AH by adenoviral gene transfer diminished by 2.5-fold the macrophage homing to aortic roots in atherosclerosis-prone C57Bl6 apoE$^{-/-}$ mice (15). Moreover, we showed in this model that the neointima formation (restenosis) induced by a wire-guided denudation of the endothelium of the common left carotid was diminished in males and females; however, the spontaneous atherosclerosis in aortic roots was diminished only in males (16). In a recent paper, Noto et al. (29) showed that a massive adenoviral overexpression of PAF-AH (76- to 140-fold increase in circulation) in apoE$^{-/-}$ mice protected all lipoprotein classes from oxidation in vitro, diminished the oxLDL autoantibodies in plasma, and inhibited foam cell formation by facilitating cholesterol efflux from macrophages. Unfortunately, we are still lacking a suitable animal model with genetically manipulated PAF-AH (knockout or knockdown) that would definitively prove the exact function of this enzyme in atherogenesis.

In conclusion, plasma PAF-AH activity increases gradually in SAP and in ACS in both men and in women as compared with healthy controls. However, no correlation could be demonstrated between PAF-AH activity and inflammatory markers such as acute phase reactants or proinflammatory cytokines.

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