Serum total 8-iso-prostaglandin $F_{2\alpha}$: A new and independent predictor of peripheral arterial disease

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Objective: **Circulating 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) has been proposed as new indicator of oxidative stress, which is involved in the pathophysiologic changes of atherosclerosis. We proposed to test the hypothesis that 8-iso-$PGF_{2\alpha}$ is an independent predictor of symptomatic peripheral arterial disease (PAD).**

Methods: **A case-control study in 100 patients with symptomatic PAD and 100 control subjects matched for age, sex, and diabetes mellitus was conducted. Smokers and subjects using lipid-lowering drugs were excluded. Serum total 8-iso-$PGF_{2\alpha}$ was quantified with an enzyme immunoassay.**

Results: **Median 8-iso-$PGF_{2\alpha}$ was higher in patients with PAD than in control subjects (63 vs 42 pg/mL; $P = .001$). Logistic regression with hypertension, body mass index, and creatinine, low-density lipoprotein (LDL) cholesterol, triglyceride, high-sensitivity C-reactive protein (hs-CRP), 8-iso-$PGF_{2\alpha}$, and total homocysteine concentrations as independent variables and case-control status as dependent variable revealed significant odds ratios (OR) for hypertension (OR, 3.74; 95% confidence interval [CI], 1.85-7.53), low-density lipoprotein cholesterol (OR, 1.16, for an increment of 10 mg/dL; 95% CI, 1.07-1.27), high-sensitivity C-reactive protein (OR, 1.02, for an increment of 1 mg/L; 95% CI, 1.00-1.03), and 8-iso-$PGF_{2\alpha}$ (OR, 1.11, for an increment of 10 pg/mL; 95% CI, 1.03-1.20).**

Conclusions: **Serum total 8-iso-$PGF_{2\alpha}$ was an independent predictor of PAD in the population studied. This finding supports the hypothesis that 8-iso-$PGF_{2\alpha}$ is a risk marker for PAD. Our results indicate increased systemic oxidative stress in patients with PAD. (J Vasc Surg 2004;40:768-73.)**

Peripheral arterial disease (PAD) is an important manifestation of systemic atherosclerosis, and is associated with elevated risk for cardiovascular and cerebrovascular events including death, myocardial infarction, and stroke.[1] Diabetes mellitus and smoking are the strongest risk factors for PAD.[1,2] Other well-known risk factors are advanced age, male sex, arterial hypertension, and hyperlipidemia.[1,2] Emerging risk factors for PAD include elevated levels of high-sensitivity C-reactive protein (hs-CRP) and total homocysteine (tHcy).[2,3] Although oxidative stress is involved in the pathophysiologic changes of atherosclerosis and cardiovascular disease,[4] only scarce data are available as to the extent that oxidative damage is also related to PAD.

In the atherosclerotic process lipids are the first line of radical attack, and the isoprostanes are stable end products of lipid peroxidation.[5] Isoprostanes, structural isomers of the prostaglandins, are a family of compounds produced from polyunsaturated fatty acids via a free radical–catalyzed mechanism. The isoprostane family includes F_2-isoprostane, D_2-isoprostane, E_2- isoprostane, and isothromboxane A_2, F_2-isoprostanes are the most abundant.[5] Isoprostanes are present in the human circulation mainly in their ester forms, whereas only hydrolized isoprostanes are excreted into urine.[6,7] F_2-isoprostanes, among them 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), have been proposed as new indicators of oxidative stress,[8-10] and it has been suggested that their measurement is the most accurate method for quantifying oxidant stress in human beings.[9]

Previous research focused on the role of circulating and urinary 8-iso-$PGF_{2\alpha}$ in coronary artery disease (CAD), and demonstrated that 8-iso-$PGF_{2\alpha}$ levels are elevated in patients with CAD and myocardial ischemia.[11-13] Inasmuch as there are currently no published data on PAD and 8-iso-$PGF_{2\alpha}$, we proposed to test the hypothesis that circulating 8-iso-$PGF_{2\alpha}$ is an independent predictor of symptomatic chronic limb ischemia, that is, PAD. The relation of 8-iso-$PGF_{2\alpha}$ with atherosclerotic disease may be affected by a number of confounding factors, such as age, diabetes mellitus, and cigarette smoking.[10] Because medication with 3-hydroxy-3-methylglutaryl coenzyme A inhibitors is also thought to affect 8-iso-$PGF_{2\alpha}$ levels,[14] statistical models testing the relation of underlying metabolic effects with PAD become increasingly complex in a study population including these conditions. Therefore we intended to address the association of 8-iso-$PGF_{2\alpha}$ in a population devoid of the mentioned confounding factors by conducting a case-control study, with the study subjects matched for age, sex, and diabetes mellitus, and excluding smokers as well as subjects using any lipid-lowering drugs.

METHODS

Patients and study protocol. The present study, performed at the Division of Vascular Surgery, Department of

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Table I. Clinical and biochemical data of patients with PAD and control subjects

	PAD group (n = 100)	*Control group (n = 100)*	P*
Male/female (no.)	47/53	47/53	1.000†
Age (y)	76 (70-80)	76 (69-80)	.826†
Body mass index (kg/m^2)	26 (24-29)	26 (24-28)	.795
Arterial hypertension (no.)	75	52	.001
Diabetes mellitus (no.)	27	27	1.000†
Coronary artery disease (no.)	33	0	—
Cerebrovascular disease (no.)	26	0	—
Carotid stenosis ≥50% (no.)	32	0	—
PAD-relevant data			
Previous PTA/stenting (no.)	21	0	—
Previous vascular surgery (no.)	20	0	—
Previous amputation (no.)	2	0	—
ABI (mm Hg/mm Hg)	0.82 (0.62-1.00)	1.23 (1.13-1.32)	—
Biochemical markers			
Creatinine (mg/dL)	1.0 (0.9-1.1)	0.9 (0.8-1.1)	.056
Total-cholesterol (mg/dL)	224 (192-273)	211 (186-246)	.037‡
LDL-cholesterol (mg/dL)	156 (118-190)	139 (107-155)	.001
HDL-cholesterol (mg/dL)	51 (41-60)	54 (43-69)	.155
Triglycerides (mg/dL)	127 (101-194)	114 (81-154)	.034‡
Fasting glucose (mg/dL)¶	97 (88-107)	95 (88-104)	.379
HbA_{1c} (%)¶	5.9 (5.7-6.3)	5.7 (5.4-6.1)	.010‡
hs-CRP (mg/L)	4.1 (2.0-9.1)	1.6 (0.8-4.2)	<.001
8-iso-$PGF_{2\alpha}$ (pg/mL)	63 (39-85)	42 (24-70)	.001
Total homocysteine (μmol/L)	17.0 (14.4-21.4)	16.2 (13.4-19.7)	.167
Folate (ng/mL)	7.4 (5.0-9.1)	7.0 (5.7-9.1)	.717
Vitamin B_{12} (pg/mL)	364 (241-479)	309 (234-441)	.218

PAD, Peripheral arterial disease; *PTA,* percutaneous transluminal angioplasty; *ABI,* resting ankle-brachial index; *LDL,* low-density lipoprotein; *HDL,* high-density lipoprotein; *HbA_{1c},* hemoglobin A_{1c}; *hs-CRP,* high-sensitivity CRP; *8-iso-$PGF_{2\alpha}$*; 8-iso-prostaglandin $F_{2\alpha}$.
Age, body mass index, ABI, and biochemical markers are presented as median (25^{th}-27^{th} percentiles).
*Nonparametric Mann-Whitney *U* test or Fisher exact test, as appropriate.
†Matched variables.
‡*P* not significant after Bonferroni correction for multiple comparisons.
¶Subjects with diabetes mellitus were excluded.

Surgery, St John of God Hospital, Linz, Austria, is a case-control study of patients with symptomatic chronic limb ischemia, that is, PAD, and control subjects without clinically relevant atherosclerotic disease. All study subjects underwent evaluation for the presence of risk factors for atherosclerosis and comorbid conditions, as recommended.[15] PAD was defined as atherosclerotic occlusive disease of the lower extremities associated with typical symptoms, such as claudication or leg pain upon exertion, rest pain, or minor or major tissue loss, and was verified by interview, physical examination, noninvasive techniques, and angiograpy. CAD was defined as remote myocardial infarction by history, occult myocardial infarction by electrocardiography, previous coronary bypass surgery or percutaneous transluminal coronary angioplasty, and stable or unstable angina (cardiac troponin positive) and acute coronary syndrome (cardiac troponin negative). Cerebrovascular disease (CVD) was defined as transient or temporary stroke, completed stroke with permanent neurologic deficit, or acute stroke. In patients and control subjects Doppler segmental blood pressure of the lower limbs, including continuous-wave spectral analysis and resting ankle-brachial index (ABI) measurements, as well as color duplex ultrasound scanning of the carotid bifurcation and the internal carotid artery were carried out as described.[16,17] Patients and control subjects had been nonsmokers for at least 10 years, and use of any lipid-lowering drugs or vitamin supplements were exclusion criteria for the PAD and control groups. The study protocol was approved by the local ethics committee in accordance to the Helsinki Declaration, and all study participants gave informed consent.

A total of 100 patients, admitted for inpatient treatment of symptomatic PAD (claudication or leg pain on exertion, rest pain, minor or major tissue loss), were recruited and examined according to standard procedures. In addition to Doppler segmental blood pressure of the lower limbs and resting ABI measurements, aortofemoral angiography was performed in all patients to confirm the presence of PAD and to determine the location and extent of wall changes. The control group consisted of 100 subjects matched to the patients with PAD in a 1:1 design by sex, age (±2 years), and diabetes mellitus. All control subjects were patients in our hospital, and fulfilled the following criteria: no clinical indication of PAD by history and physical examination; systolic brachial blood pressure equal to or less than the blood pressure in each of the right and left anterior tibial and posterior tibial arteries, respectively (ie, ABI ≥1.0); no pathologic pattern of pulse waves in lower limbs by continuous-wave spectral analysis; no CAD; no

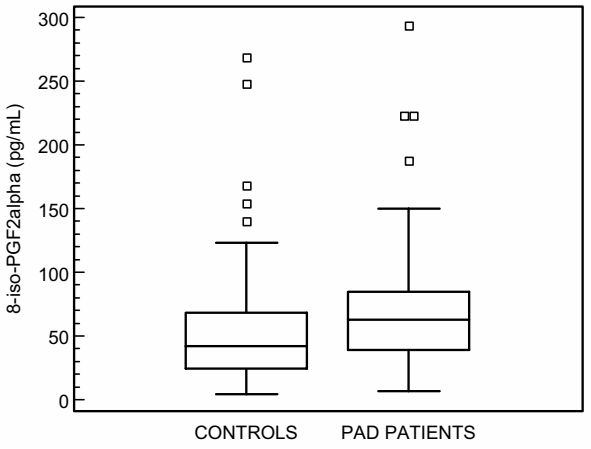

Fig 1. Box-and-whisker plots of 8-iso-prostaglandin $F_{2\alpha}$ (*$PGF_{2\alpha}$*) for patients with peripheral arterial disease (*PAD*; n = 100) and control subjects (n = 100). In box-and-whisker plots, central box represents values from lower to upper quartile, middle line represents median; whiskers extend from minimum to maximum value, excluding outside values (>1.5 box lengths from box) and far out values (>3 box lengths from box), which are displayed as separate points.

CVD; no previous vascular surgery or stenting of the internal carotid arteries; and no stenosis of the internal carotid artery 50% or greater on color duplex ultrasound scans. All control subjects were generally in good health, and were admitted for treatment of minor health problems, such as cataract surgery, vertebragene pain, or nonvascular surgery (eg, herniotomia, varicose vein extirpation).

Biochemical analysis. Blood was collected at venipuncture in Vacuette polyethylene terephthalate glycol clot activator tubes (Greiner Bio-One) after the patient had fasted overnight. Creatinine, fasting glucose, glycohemoglobin A_{1c} (HbA_{1c}), total cholesterol, and triglyceride concentrations were analyzed with standard assays on a COBAS Integra analyzer (Roche Diagnostics). For determination of high-density lipoprotein cholesterol and LDL cholesterol quantitative electrophoresis with enzymatic staining (Helena BioSciences) was used. tHcy, folate, and vitamin B12 assays were performed on an AxSYM analyzer (Abbott Diagnostics). Concentration of CRP was measured with a high-sensitivity assay (N High Sensitivity CRP) on a BN ProSpec analyzer (Dade Behring) with polystyrene particles coated with monoclonal mouse antibodies to CRP. Serum samples for total 8-iso-$PGF_{2\alpha}$ (ie, free and esterified 8-iso-$PGF_{2\alpha}$) determination were immediately frozen and stored at −80°C until assessment. After hydrolyzation of isoprostanes by incubation with potassium hydroxide at 40°C for 1 hour, the samples were brought to pH 7.0 to 7.4 with KH_2PO_4 and purified with a commercially available 8-isoprostane purification kit (immunoaffinity method; Cayman Chemicals). 8-iso-$PGF_{2\alpha}$ was then quantified with a competition enzyme immunoassay (Cayman Chemicals). Samples from corresponding patients with PAD and control subjects were assayed on the same microwell plates. The whole procedure of 8-iso-$PGF_{2\alpha}$ determination was performed according to the manufacturers' recommendations.

Statistical analysis. Statistical analysis was performed with SPSS version 10.0 software. Dichotomous variables are given as prevalence in number, continuous data are expressed as median (25th—75th percentiles). Univariate comparisons of risk factors and other dichotomous variables between study groups were calculated with the Fisher exact test, and continuous variables were evaluated with the nonparametric Mann-Whitney *U* test (respective *P* values were not adjusted for multiple comparisons, and are therefore only descriptive). To determine multivariate odds ratios (OR) for PAD, logistic regression without variable selection was performed. Dichotomous risk factors were coded with an indicator variable of 1 for having the condition and 0 for its absence. Spearman's coefficient of rank correlation (ρ) was used for assessment of the relationship of continuous data in the study population. Probabilities are 2-tailed, and $P < .05$ is regarded as statistically significant.

RESULTS

Patients with PAD were admitted because of mild to severe claudication or leg pain on exertion (n = 76), ischemic rest pain (n = 1), and minor or major tissue loss (n = 23). Of the 100 patients with PAD 33 had concomitant CAD and 26 had concomitant CVD. Furthermore, 25 patients with PAD exhibited internal carotid stenosis 50% or greater. Another 7 patients with PAD were classified as having stenosis 50% or greater as well, because they had undergone previous carotid surgery to treat stenosis. At enrolment, 21 patients with PAD had undergone remote percutaneous transluminal angioplasty with or without stenting, 20 patients with PAD had undergone vascular surgery, and 2 patients with PAD had undergone minor amputations. Per definition, none of the 100 control subjects matched to the patients with PAD for sex, age (±2 years), and diabetes mellitus had either CAD or CVD, or internal carotid stenosis 50% or greater. However, many control subjects (n = 91) exhibited carotid plaques as a sign of mild but not clinically relevant atherosclerosis. At enrolment, 47 patients with PAD and 16 control subjects received low-dose aspirin daily, and none of the 200 study participants received cyclooxygenase 1 or 2 inhibitors. After enrolment in the present study, 20 patients with PAD underwent vascular surgery, including open revascularization procedures or bypass graft placement; in 28 patients endovascular techniques, such as percutaneous transluminal angioplasty with or without stenting, were performed; 3 patients underwent lumbar sympathectomy; major amputation was performed in 1 patient, and minor amputation in 2 patients; and 46 patients with PAD received conservative treatment.

The clinical and biochemical characteristics of all study participants are described in Table I. Arterial hypertension was significantly more prevalent in the PAD group (n = 75) compared with the control group (n = 52; $P = .001$). In addition, median values of the following biochemical mark-

Table II. Results of logistic regression analyses of PAD risk factors (100 patients with PAD vs 100 control subjects): Statistical model with an incremental approach for continuous variables

Change in risk factor	*Multivariate odds ratio for PAD**	*95% CI*	P
Body mass index (+5 kg/m^2)	0.79	0.53-1.17	.244
Arterial hypertension (vs not)	3.74	1.85-7.53	<.001
Creatinine (+0.1 mg/dL)	0.99	0.93-1.06	.822
LDL-cholesterol (+10 mg/dL)	1.16	1.07-1.27	.001
Triglycerides (+10 mg/dL)	1.01	0.96-1.06	.779
hs-CRP (+1 mg/L)	1.02	1.00-1.03	.035
8-iso-$PGF_{2\alpha}$ (+10 pg/mL)	1.11	1.03-1.20	.006
Total homocysteine (+5 μmol/L)	1.14	0.88-1.47	.328

PAD, Peripheral arterial disease; *LDL,* low-density lipoprotein; *hs-CRP,* high-sensitivity C-reactive protein; *8-iso-PGF*$_{2\alpha}$; prostaglandin $F_{2\alpha}$; *CI,* confidence interval.
*Multivariate odds ratios were calculated with logistic regression analysis without variable selection technique (all variables were included simultaneously into the model).

Table III. Results of logistic regression analyses of PAD risk factors (100 patients with PAD vs 100 control subjects). Statistical model with continuous variables dichotomized according to median values of entire study population (n = 200)

Change in risk factor	*Multivariate odds ratio for PAD**	*95% CI*	P
Body mass index (≥ median value of 26 kg/m^2)	0.99	0.51-1.93	.977
Arterial hypertension (vs not)	2.02	1.42-2.88	<.001
Creatinine (≥ median value of 1.0 mg/dL)	1.04	0.53-2.06	.901
LDL cholesterol (≥ median value of 144 mg/dL)	2.77	1.42-5.41	.003
Triglycerides (≥ median value of 121 mg/dL)	1.06	0.55-2.07	.856
hs-CRP (≥ median value of 2.9 mg/L)	3.34	1.70-6.57	<.001
8-iso-$PGF_{2\alpha}$ (≥ median value of 51 pg/mL)	3.83	1.92-7.65	<.001
Total homocysteine (≥ median value of 16.6 μmol/L)	1.30	0.67-2.55	.438

PAD, Peripheral arterial disease; *LDL,* low-density lipoprotein; *hs-CRP,* high-senstitivity C-reactive protein; *8-iso-PGF*$_{2\alpha}$; 8-iso-prostaglandin $F_{2\alpha}$; *CI,* confidence interval.
*Multivariate odds ratios were calculated with logistic regression analysis without variable selection technique (all variables were included simultaneously into the model).

ers were significantly higher in patients with PAD than in control subjects: total cholesterol (224 vs 211 mg/dL; $P = .037$), LDL cholesterol (156 vs 139 mg/dL; $P = .001$), triglycerides (127 vs 114 mg/dL; $P = .034$), HbA_{1c} (5.9% vs 5.7%; $P = .010$), hs-CRP (4.1 vs 1.6 mg/L; $P < .001$), and total 8-iso-$PGF_{2\alpha}$ (63 vs 42 pg/mL; $P = .001$). The box-and-whisker plots in Fig 1 show the distribution of 8-iso-$PGF_{2\alpha}$ values in the 2 study groups. Investigating 8-iso-$PGF_{2\alpha}$ in the patients with PAD (n = 100), we found no difference of median 8-iso-$PGF_{2\alpha}$ values between those with claudication (n = 76) and those with critical limb ischemia (ischemic rest pain, minor or major tissue loss; n = 24; 66 vs 58 pg/mL; $P = .283$).

Multivariate ORs for symptomatic PAD in the population studied, calculated without variable selection technique, are shown in Tables II and III. Inasmuch as patient and control groups were matched for age, sex, and diabetes mellitus, these variables were not included in the regression analysis. In the multivariate model, total 8-iso-$PGF_{2\alpha}$ was an independent and significant predictor ($P = .006$) of case-control status, with an OR of 1.11 (95% confidence interval [CI], 1.03-1.20), for an increment of 10 pg/mL. In this analysis arterial hypertension (OR, 3.74; 95% CI, 1.85-7.53; $P < .001$), LDL cholesterol (OR, 1.16, for an increment of 10 mg/dL; 95% CI, 1.07-1.27; $P = .001$), and hs-CRP (OR, 1.02, for an increment of 1 mg/L; 95% CI, 1.00-1.03; $P = .035$) were also independently related to symptomatic PAD. All other variables failed to reach statistical significance; body mass index, and creatinine, triglyceride, and tHcy concentrations revealed no significant ORs. Stepwise logistic regression models revealed the same predictors, with similar ORs compared with the models without variable selection technique (data not shown).

To test the relation of the 3 main biochemical markers (LDL cholesterol, hs-CRP, 8-iso-$PGF_{2\alpha}$) with each other we used rank correlation analysis. As a result, there was no significant correlation for either 8-iso-$PGF_{2\alpha}$ and hs-CRP (rank correlation coefficient [r_s] −0.079; 95% CI, −0.216-0.060; $P = .264$), nor for 8-iso-$PGF_{2\alpha}$ and LDL cholesterol (r_s, 0.042; 95% CI, −0.097-0.180; $P = .555$). Even when using dichotomized variables according to the thresholds specified in Table III (median 8-iso-$PGF_{2\alpha}$, and hs-CRP and LDL cholesterol levels for the entire study population), there was no significant association between these analytes, indicating that there was no substantial degree of overlap between the elevated levels of the 3

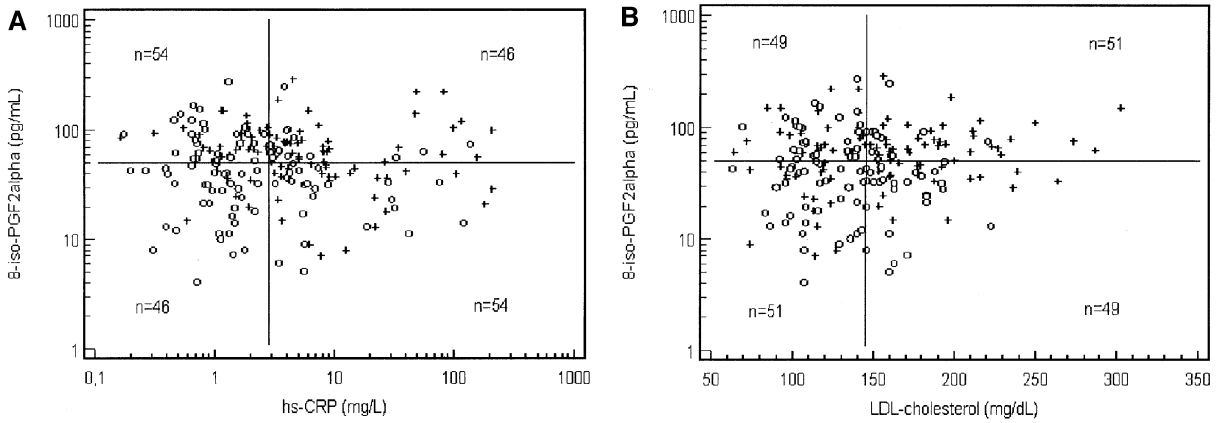

Fig 2. Scatterplots for 8-iso-prostaglandin $_{2\alpha}$ (*$PGF_{2\alpha}$*; *horizontal lines*, median) vs high-sensitivity C-reactive protein (*hs-CRP*) and low-density lipoprotein (LDL) cholesterol (*vertical lines*, median) for entire study population (n = 200). Distribution in entire study population (+, 100 patients with peripheral arterial disease; *open circles*, 100 control subjects) is demonstrated by number of subjects in each of the 4 squares. **A,** Spearman coefficient of rank correlation for 8-iso-$PGF_{2\alpha}$ vs hs-CRP: −0.079 (95% confidence interval, −0.216-0.060; P = .264). Fisher exact test for dichotomized variables according to median 8-iso-$PGF_{2\alpha}$ and hs-CRP levels, P = .322. **B,** Spearman coefficient of rank correlation for 8-iso-$PGF_{2\alpha}$ vs LDL cholesterol: 0.042 (95% confidence interval, −0.097-0.180; P = .555). Fisher exact test for dichotomized variables according to median 8-iso-$PGF_{2\alpha}$ and LDL cholesterol levels, P = .888.

markers for patients and control subjects. (More detailed information is provided in Fig 2, *A and B*).

DISCUSSION

To our knowledge, this is the first report on 8-iso-$PGF_{2\alpha}$ as a potential risk factor for PAD. The main finding of the present study was an approximately 1.5-fold higher median circulating total 8-iso-$PGF_{2\alpha}$ level in patients with symptomatic PAD compared with control subjects. Given that 8-iso-$PGF_{2\alpha}$ has been proposed as a reliable marker of oxidative damage,[8-10] our results indicate increased systemic oxidative stress in patients with PAD. Furthermore, total 8-iso-$PGF_{2\alpha}$ was a predictor of PAD in the multivariate logistic regression model, independent of other covariates associated with 8-iso-$PGF_{2\alpha}$,[10] such as age, sex, and diabetes mellitus (matched variables), as well as arterial hypertension, body mass index, hypercholesterolemia (controlled variables). In addition, the previously reported effect of cigarette smoking[18] and intake of 3-hydroxy-3-methylglutaryl coenzyme A inhibitors[14] on 8-iso-$PGF_{2\alpha}$ was not evident in our population, because both conditions were exclusion criteria of the present study. As demonstrated with logistic regression analysis (Table III), the association of 8-iso-$PGF_{2\alpha}$ with PAD was similar to the effect of other risk factors for PAD, that is, arterial hypertension, elevated LDL cholesterol, and hs-CRP. Thus our results are in line with recent findings that demonstrate that circulating and urinary 8-iso-$PGF_{2\alpha}$ may be independent risk markers for CAD.[11,13]

As shown in Fig 2, *A*, no statistically significant correlation between 8-iso-$PGF_{2\alpha}$ and hs-CRP was found in our study population. A previous study[13] showed a weak correlation between these 2 analytes, possibly resulting in part from involvement of inflammatory and oxidative processes on atherosclerosis. However, although inflammation works hand in hand with oxidative modifications, oxidative stress may also occur independent of inflammation. In support of this, we found 8-iso-$PGF_{2\alpha}$ and hs-CRP to be independent predictors of PAD (Tables II and III). Thus, evaluation of both 8-iso-$PGF_{2\alpha}$ and hs-CRP should be superior to measurement of either parameter alone.

Mass spectrometric methods and immunologic methods (radio immunoassay, enzyme immunoassay) have been developed and validated for 8-iso-$PGF_{2\alpha}$ determination.[8,10] Enzyme immunoassays and gas chromatography–mass spectrometry revealed rather incomparable results[10]; the circulating 8-iso-$PGF_{2\alpha}$ levels obtained in our study therefore must be compared with the results of other studies using the same assay (Cayman Chemicals).[11,12] One of these studies[12] focusing on circulating total 8-iso-$PGF_{2\alpha}$ and myocardial ischemia found 8-iso-$PGF_{2\alpha}$ values similar to our results. In another study[11] investigating oxidative stress in patients with CAD and control subjects mean 8-iso-$PGF_{2\alpha}$ plasma concentrations were higher than in our report. However, in contrast to our work, both studies included smokers, who exhibit higher 8-iso-$PGF_{2\alpha}$ concentrations,[18] and the latter study had a great proportion of smokers, especially in the CAD group. In addition, the prevalence of other confounding factors such as advanced age, sex, hypertension, obesity, and dyslipidemia in the populations studied might have also influenced circulating total 8-iso-$PGF_{2\alpha}$ values to a different extent. Furthermore, it must be mentioned that many of the control subjects of our study, who had no PAD, no CAD, and no CVD, per definition, exhibited carotid plaques as a sign of mild but not clinically relevant atherosclerosis. Thus, it is

theoretically conceivable that our investigation rather underestimated the real difference in circulating total 8-iso-$PGF_{2\alpha}$ levels in patients and control subjects.

In conclusion, serum total 8-iso-$PGF_{2\alpha}$ was an independent predictor of PAD in the present study. This finding supports the hypothesis that 8-iso-$PGF_{2\alpha}$ as an indicator for oxidative stress is a risk marker for PAD, but awaits confirmation in other studies.

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