

## LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A<sub>2</sub> AS AN INDEPENDENT PREDICTOR OF CORONARY HEART DISEASE

CHRIS J. PACKARD, D.Sc., DENIS S.J. O'REILLY, M.D., MURIEL J. CASLAKE, Ph.D., ALEX D. McMAHON, Ph.D., IAN FORD, Ph.D., JOSEPHINE COONEY, COLIN H. MACPHEE, Ph.D., KEITH E. SUCKLING, D.Sc., MALA KRISHNA, Ph.D., FRANCIS E. WILKINSON, Ph.D., ANN RUMLEY, Ph.D., AND GORDON D.O. LOWE, M.D.,  
FOR THE WEST OF SCOTLAND CORONARY PREVENTION STUDY GROUP

### ABSTRACT

**Background** Chronic inflammation is believed to increase the risk of coronary events by making atherosclerotic plaques in coronary vessels prone to rupture. We examined blood constituents potentially affected by inflammation as predictors of risk in men with hypercholesterolemia who were enrolled in the West of Scotland Coronary Prevention Study, a trial that evaluated the value of pravastatin in the prevention of coronary events.

**Methods** A total of 580 men who had had a coronary event (nonfatal myocardial infarction, death from coronary heart disease, or a revascularization procedure) were each matched for age and smoking status with 2 control subjects (total, 1160) from the same cohort who had not had a coronary event. Lipoprotein-associated phospholipase A<sub>2</sub>, C-reactive protein, and fibrinogen levels and the white-cell count were measured at base line, along with other traditional risk factors. The association of these variables with the risk of coronary events was tested in regression models and by dividing the range of values according to quintiles.

**Results** Levels of C-reactive protein, the white-cell count, and fibrinogen levels were strong predictors of the risk of coronary events; the risk in the highest quintile of the study cohort for each variable was approximately twice that in the lowest quintile. However, the association of these variables with risk was markedly attenuated when age, systolic blood pressure, and lipoprotein levels were included in multivariate models. Levels of lipoprotein-associated phospholipase A<sub>2</sub> (platelet-activating factor acetylhydrolase), the expression of which is regulated by mediators of inflammation, had a strong, positive association with risk that was not confounded by other factors. It was associated with almost a doubling of the risk in the highest quintile as compared with the lowest quintile.

**Conclusions** Inflammatory markers are predictors of the risk of coronary events, but their predictive ability is attenuated by associations with other coronary risk factors. Elevated levels of lipoprotein-associated phospholipase A<sub>2</sub> appear to be a strong risk factor for coronary heart disease, a finding that has implications for atherogenesis and the assessment of risk. (N Engl J Med 2000;343:1148-55.)

©2000, Massachusetts Medical Society.

THE discovery of inflammatory cells in the cap of atherosclerotic plaques led to the postulate that inflammation has a key role in the cascade of events leading to plaque rupture.<sup>1,2</sup> Supporting this idea are recent reports that levels of plasma markers of inflammation such as C-reactive protein are elevated in those at risk for coronary heart disease.<sup>3-5</sup> A<sub>2</sub> phospholipases are a family of enzymes that can hydrolyze phospholipids at the *sn*2 position to generate lysophospholipids and fatty acids. Several recent reports link type II secretory phospholipase A<sub>2</sub> to atherogenesis and the risk of coronary heart disease.<sup>6-8</sup> This enzyme, found in the media of normal and diseased arteries,<sup>9</sup> may be involved in modifying low-density lipoprotein (LDL) that is present in the artery wall.<sup>6,10</sup>

We examined the role of a distinct phospholipase, lipoprotein-associated phospholipase A<sub>2</sub>, also known as platelet-activating factor acetylhydrolase, in a small case-control study and found it to be a potential predictor of the risk of coronary heart disease.<sup>11</sup> The expression of this enzyme is regulated by mediators of inflammation.<sup>12</sup> It circulates bound mainly to LDL and has a structure and properties distinct from those of the 14-kd calcium-dependent type II secretory phospholipase A<sub>2</sub>.<sup>13</sup> We evaluated its association with the risk of coronary events in a prospective study with a nested case-control design, drawing samples from the biologic bank of the West of Scotland Coronary Prevention Study.<sup>14</sup> The enzyme, in theory, could promote atherogenesis if the products it releases from LDL phospholipids have a deleterious effect on the artery wall,<sup>15</sup> or it could be protective if, in hydrolyzing platelet-activating factor, it reduces inflammation and the thrombotic tendency of blood.<sup>16</sup>

The West of Scotland Coronary Prevention Study demonstrated that pravastatin therapy reduced the incidence of coronary events and death from cardiac

From the Departments of Pathological Biochemistry (C.J.P., D.S.J.O., M.J.C., J.C.) and Medicine (A.R., G.D.O.L.), Glasgow Royal Infirmary, Glasgow, Scotland; the Robertson Centre for Biostatistics, Glasgow University, Glasgow, Scotland (A.D.M., I.E.); SmithKline Beecham Pharmaceuticals, Harlow, United Kingdom (C.H.M., K.E.S.); and diaDexus, Santa Clara, Calif. (M.K., F.E.W.). Address reprint requests to Dr. Packard at the Department of Pathological Biochemistry, Glasgow Royal Infirmary University NHS Trust, 4th Fl. Queen Elizabeth Bldg., 10 Alexandra Parade, Glasgow G31 2ER, Scotland, or at chris.packard@clinmed.gla.ac.uk.

Other authors were Gillian Docherty, B.Sc., Robertson Centre, Glasgow University, Glasgow, Scotland; and John D. Burczak, Ph.D., diaDexus, Santa Clara, Calif.

causes by about one third in men with hypercholesterolemia.<sup>14</sup> We evaluated the extent to which levels of lipoprotein-associated phospholipase A<sub>2</sub> and C-reactive protein, as measured by a sensitive assay in stored base-line samples, and other markers of inflammation (such as the white-cell count and fibrinogen levels) predicted the risk of a coronary event in this primary prevention study. Findings regarding white-cell counts and fibrinogen levels have been reported previously.<sup>17</sup>

## METHODS

### Study Design and Subjects

In the West of Scotland Coronary Prevention Study, 6595 men who had LDL cholesterol levels between 174 and 232 mg per deciliter (4.5 and 6.0 mmol per liter) but who had no history of a myocardial infarction were randomly assigned to receive 40 mg of pravastatin or placebo daily.<sup>14</sup> All subjects provided written informed consent. The study was approved by the ethics committees of the University of Glasgow and all participating health boards. The first patient was enrolled on February 1, 1989, and the study ended on May 15, 1995. The incidence of the primary end point, a composite of nonfatal myocardial infarction and death from coronary heart disease, was 31 percent lower with pravastatin treatment. Risk reductions of the same magnitude were seen for revascularization procedures (coronary-artery bypass and percutaneous transluminal coronary angioplasty). Since the same underlying atherothrombotic process is believed to give rise to myocardial infarction and the need for revascularization, in the present case-control study we used an expanded end point comprising the primary end point plus revascularization as a first event to increase the statistical power of the study. A total of 580 men had such an event and were included in the current analysis: 503 had a myocardial infarction or death from cardiac causes as a first event, and 77 underwent revascularization as a first event. We matched each patient with 2 controls (also drawn from the original cohort of 6595 men), for a total of 1160 controls, on the basis of age (using two-year age categories) and smoking status, with subjects categorized as either nonsmokers (those who had never smoked or who had quit smoking) or current smokers. At randomization, 6.2 percent of patients with an event and 2.9 percent of controls were taking aspirin.

### Measurements

All major risk factors were assessed during recruitment.<sup>18</sup> Plasma total cholesterol, triglycerides, and very-low-density lipoprotein, LDL, and high-density lipoprotein (HDL) cholesterol were measured twice during screening, and the average was used as the base-line level.<sup>18</sup> At the third screening visit,<sup>18</sup> hematologic variables, including the white-cell count, were determined (model STKR or S+1, Beckman Coulter, Luton, United Kingdom) and fibrinogen was assayed by heat-precipitation nephelometry.<sup>19</sup>

C-reactive protein and lipoprotein-associated phospholipase A<sub>2</sub> were measured in aliquots of plasma collected at the third screening visit and stored at -70°C. A high-sensitivity, two-site enzyme-linked immunoassay was developed with use of a peroxidase-conjugated rabbit antihuman C-reactive protein antibody (DK2600, Dako, Glostrup, Denmark) and a polyclonal anti-C-reactive protein capture antibody. The assay was calibrated with a standard (CRM470-CAP/IFCC; lot 91/0619, Behringwerke, Marburg, Germany). The lower limit of the working range of the assay was 0.1 mg per liter. Values obtained in this study ranged from 0.1 to 45.2 mg per liter. The intraassay and interassay coefficients of variation were 1.9 percent and 6.2 percent, respectively (the assay yielded results similar to those of Ridker et al.<sup>3</sup>).

Lipoprotein-associated phospholipase A<sub>2</sub> mass was measured with an enzyme-linked immunoassay according to previously described methods.<sup>11</sup> Samples were captured with a monoclonal antibody

against lipoprotein-associated phospholipase A<sub>2</sub>. The enzyme was identified by a second monoclonal antibody labeled with biotin and a streptavidin-alkaline phosphatase conjugate. The standard was purified lipoprotein-associated phospholipase A<sub>2</sub>. The range of detection was 0.5 to 6.0 mg per liter, and the intraassay and interassay coefficients of variation were 4.5 percent and 8.3 percent, respectively. There was no cross-reactivity with other A<sub>2</sub> phospholipases. The results of the mass assay correlated well with levels of enzyme activity ( $r=0.86$ ) when both were measured in fresh samples.<sup>11</sup> All analyses were conducted by personnel who did not know whether the samples were from the patients with coronary events or the controls.

### Statistical Analysis

The distributions of C-reactive protein and plasma triglyceride levels were markedly skewed and were therefore log-transformed. We established quintile ranges according to the values in the control subjects, and we obtained risk ratios by comparing the frequency of the end point in patients in quintiles 2 through 5 with that in the reference quintile 1. We used multivariate conditional logistic-regression models to assess the independent prognostic value of variables. Each was included as a continuous variable and in a separate analysis as a categorical variable (in which quintiles were used). We calculated relative risks and 95 percent confidence intervals. We assessed associations among variables in the 1160 control subjects with use of Spearman's rank-correlation coefficient.

We assessed differences between plasma levels of lipoprotein-associated phospholipase A<sub>2</sub> and inflammatory markers in smokers and nonsmokers with use of a two-sample t-test. We examined the effect of smoking on the relation of C-reactive protein and lipoprotein-associated phospholipase A<sub>2</sub> levels to risk with separate conditional logistic-regression models for smokers and nonsmokers and then in a model that included all 1740 subjects and in which these variables were introduced first as main effects; all interactions between smoking status and C-reactive protein levels and lipoprotein-associated phospholipase A<sub>2</sub> levels were then investigated. We used a similar approach to evaluate the association of these variables with risk among 833 men in the pravastatin group and 907 men in the placebo group.

## RESULTS

The base-line characteristics of the patients and controls in the current analysis as well as of the patients in the original study group are shown in Table 1. As compared with the entire original study cohort, the patients who had a coronary event were older and more likely to be smokers and had higher blood pressure and LDL cholesterol levels and lower HDL cholesterol levels. A history of diabetes, hypertension, angina, and nitrate use were all predictors of coronary events in the trial itself,<sup>20</sup> and the proportions of subjects with these characteristics differed between patients and controls in the current study (Table 1). Base-line levels of C-reactive protein, lipoprotein-associated phospholipase A<sub>2</sub>, and fibrinogen and the white-cell count, all of which are potentially perturbed in a state of chronic inflammation, were evaluated as predictors of the risk of coronary events (Table 2). In univariate analyses, increasing levels of all four variables were associated with a significantly greater risk of the composite end point of nonfatal myocardial infarction, death from cardiac causes, or revascularization as a first event; a change of 1 SD (the standard deviations are given in Table 1) generated an increase in risk of 19 to 27 percent.

TABLE 1. BASE-LINE CHARACTERISTICS OF THE SUBJECTS.\*

CHARACTERISTIC	PATIENTS (N=580)	CONTROLS (N=1160)	ORIGINAL STUDY COHORT (N=6595)†
Age (yr)	56.8±5.2	56.8±5.2	55.2±5.5
Body-mass index	26.1±3.3‡	25.7±3.2	26.0±3.2
Systolic blood pressure (mm Hg)	140±17§	135±17	136±17
Diastolic blood pressure (mm Hg)	86±10§	84±10	84±10
Plasma cholesterol (mg/dl)	274±23	272±22	272±23
Plasma triglycerides (mg/dl)	173±74‡	163±68	163±69
LDL cholesterol (mg/dl)	194±17‡	192±17	192±17
HDL cholesterol (mg/dl)	41±9§	44±10	44±10
Fibrinogen (g/liter)	4.51±0.90§	4.36±0.86	—
White-cell count (×10 <sup>-3</sup> /mm <sup>3</sup> )	7.07±1.96§	6.75±1.86	—
C-reactive protein (mg/liter)	2.36±2.81§	1.88±2.92	—
Lipoprotein-associated phospholipase A <sub>2</sub> (mg/liter)	2.37±0.52§	2.27±0.57	—
Smoker (%)	54.3	54.5	44.1
Diabetes (%)	2.3	1.2	1.2
Hypertension (%)	23.6§	15.9	15.7
Angina (%)¶	13.8§	5.1	5.1
Nitrate use (%)	7.6§	2.6	2.1

\*Plus-minus values are means ±SD; for C-reactive protein, the values are geometric means ±SD calculated from the log-transformed distribution. Patients and controls were matched for age (in two-year age groups) and smoking status. Body-mass index was calculated as the weight in kilograms divided by the square of the height in meters. To convert values for cholesterol to millimoles per liter, multiply by 0.02586; to convert values for triglycerides to millimoles per liter, multiply by 0.01129. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein. The two-sample t-test was used for continuous variables, and the chi-square test was used for categorical variables.

†The characteristics of the entire cohort of the West of Scotland Coronary Prevention Study are given for comparison.<sup>14,18</sup> A formal analysis of the ability of these variables (not including the fibrinogen level, white-cell count, C-reactive protein level, or lipoprotein-associated phospholipase A<sub>2</sub> level) to predict the risk of coronary heart disease has been published elsewhere.<sup>20</sup>

‡P<0.01 for the comparison with controls.

§P<0.001 for the comparison with controls.

¶The Rose questionnaire was used to determine whether a subject had angina.

A similar association was present when each component of the composite end point was analyzed separately (Table 2).

C-reactive protein levels correlated significantly with both the white-cell count and fibrinogen levels, and the latter two variables also showed a strong positive interdependence (Table 3). Other risk factors showed significant associations with C-reactive protein, including age, systolic blood pressure, plasma triglycerides, LDL cholesterol, and HDL cholesterol. Likewise, the white-cell count was associated positively with plasma triglycerides and negatively with HDL cholesterol. C-reactive protein levels, white-cell counts,

and fibrinogen levels were significantly higher in smokers than in nonsmokers in the control group (Table 4). Lipoprotein-associated phospholipase A<sub>2</sub> exhibited a weak positive association with fibrinogen but little relation to C-reactive protein or the white-cell count. It also exhibited a positive relation with LDL cholesterol but little association with other risk factors (Table 3). Furthermore, it was not affected by smoking status (Table 4).

The independence of these variables as predictors of coronary events was assessed, as shown in Table 5 and Figure 1. When the white-cell counts and the C-reactive protein and lipoprotein-associated phospholipase A<sub>2</sub> levels in the patients were divided into quintiles, the risk for the highest quintile of each variable was approximately twice the risk for the lowest quintile (Fig. 1). Adjustment for the presence of other inflammatory markers markedly attenuated the risk associated with the fibrinogen level (Table 5) but had a less dramatic effect on risk associated with the C-reactive protein level and the white-cell count (Table 5 and Fig. 1); these two variables remained significant predictors of risk in this model. The lipoprotein-associated phospholipase A<sub>2</sub> level remained significant in a model that included inflammatory markers, whether it was entered as a continuous variable (Table 5) or a categorical variable (Fig. 1).

After adjustment for age, systolic blood pressure, and lipoprotein levels, the white-cell count was no longer associated with a significant risk except in the highest quintile (more than 8100 per cubic millimeter) (Fig. 1). A similar effect was seen with C-reactive protein: there was a trend toward increased risk with increasing levels of the protein when these other factors were included in the model, but again, the risk ratio was significantly increased only in the highest quintile (more than 4.59 mg per liter). Furthermore, when both traditional risk factors and other inflammatory markers were adjusted for, the risk ratio for the highest quintile of C-reactive protein was 1.49 (95 percent confidence interval, 0.95 to 2.33). C-reactive protein levels, the white-cell count, and fibrinogen levels were not significantly associated with risk when they were included as continuous variables in a model that also included age, systolic blood pressure, lipoprotein levels, and inflammatory markers (Table 5). For C-reactive protein the main confounding factor was the white-cell count (Table 5). In contrast, the association of lipoprotein-associated phospholipase A<sub>2</sub> with risk remained significant when other factors were included (Fig. 1 and Table 5).

In separate univariate models, the relative risks and 95 percent confidence intervals were estimated for smokers as compared with nonsmokers and for patients in the pravastatin group as compared with patients in the placebo group. An increase of 1 SD in the lipoprotein-associated phospholipase A<sub>2</sub> level was associated with a relative risk of 1.32 (95 percent

**TABLE 2.** UNIVARIATE ANALYSIS OF THE ASSOCIATION BETWEEN INFLAMMATORY MARKERS AND THE RISK OF A CORONARY EVENT.\*

VARIABLE	MYOCARDIAL INFARCTION OR DEATH FROM CARDIAC CAUSES (N=503)	REVASCULARIZATION AS A FIRST EVENT (N=77)	MYOCARDIAL INFARCTION OR DEATH FROM CARDIAC CAUSES OR REVASCULARIZATION AS A FIRST EVENT (N=580)	P VALUE†
	relative risk (95 percent confidence interval)			
Age	2.88 (1.03–6.47)	6.41 (0.77–53.5)	2.91 (1.21–6.98)	0.02
Systolic blood pressure	1.32 (1.19–1.47)	1.22 (0.97–1.54)	1.30 (1.18–1.44)	<0.001
Plasma triglycerides	1.13 (1.02–1.25)	1.34 (1.09–1.66)	1.16 (1.05–1.27)	0.003
LDL cholesterol	1.14 (1.03–1.26)	1.43 (1.16–1.78)	1.18 (1.07–1.30)	<0.001
HDL cholesterol	0.77 (0.68–0.86)	0.50 (0.37–0.68)	0.73 (0.66–0.82)	<0.001
Fibrinogen	1.17 (1.06–1.36)	1.28 (1.01–1.61)	1.19 (1.07–1.31)	0.001
White-cell count	1.22 (1.09–1.37)	1.23 (0.96–1.57)	1.22 (1.10–1.36)	<0.001
C-reactive protein	1.28 (1.14–1.43)	1.21 (0.94–1.55)	1.27 (1.14–1.42)	<0.001
Lipoprotein-associated phospholipase A <sub>2</sub>	1.20 (1.08–1.35)	1.17 (0.92–1.50)	1.20 (1.08–1.34)	<0.001

\*The relative risk is the risk associated with an increase of 1 SD in the variable (the standard deviations are given in Table 1). Revascularization consisted of coronary-artery bypass or percutaneous transluminal coronary angioplasty as a first event. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.

†The P value refers to the relative risk of the composite end point of myocardial infarction or death from cardiac causes or revascularization as a first event.

**TABLE 3.** ASSOCIATIONS BETWEEN INFLAMMATORY MARKERS AND CORONARY RISK FACTORS IN CONTROL SUBJECTS.

VARIABLE*	C-REACTIVE PROTEIN	LIPOPROTEIN- ASSOCIATED PHOSPHOLIPASE A <sub>2</sub>	WHITE-CELL COUNT	FIBRINOGEN
Spearman rank-correlation coefficient				
Age	0.085†	0.013	0.042	0.12‡
Body-mass index	0.13‡	–0.034	–0.039	0.010
Systolic blood pressure	0.12‡	–0.008	0.020	0.022
Plasma cholesterol	0.088†	0.17†	0.033	0.015
Plasma triglycerides	0.19‡	–0.048	0.17‡	0.039
LDL cholesterol	0.11‡	0.21‡	0.063	0.096†
HDL cholesterol	–0.20‡	0.044	–0.21‡	–0.18‡
Fibrinogen	0.49‡	0.086†	0.31‡	—
C-reactive protein	—	0.019	0.41‡	0.49‡
Lipoprotein-associated phospholipase A <sub>2</sub>	0.019	—	0.023	0.086†
White-cell count	0.41‡	0.023	—	0.31‡

\*LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.

†P<0.01.

‡P<0.001.

confidence interval, 1.00 to 1.76) among nonsmokers and a relative risk of 1.44 (95 percent confidence interval, 1.11 to 1.87) among smokers. For nonsmokers the relative risk associated with an increase of 1 SD in the C-reactive protein level was 1.19 (95 percent confidence interval, 1.03 to 1.38), and for smok-

ers it was 1.30 (95 percent confidence interval, 1.13 to 1.50). Interaction terms included in a regression model that included all 1740 subjects in the study were not significant.

Patients who were receiving pravastatin were, of course, at lower overall risk than those receiving pla-

**TABLE 4.** EFFECT OF SMOKING STATUS ON INFLAMMATORY MARKERS IN CONTROL SUBJECTS.\*

SMOKING STATUS	NO. OF SUBJECTS	C-REACTIVE PROTEIN	LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A <sub>2</sub>	WHITE-CELL COUNT	FIBRINOGEN
			mg/liter	×10 <sup>-3</sup> /mm <sup>3</sup>	g/liter
Former smoker or nonsmoker	513	1.49±2.86	2.23±0.53	5.87±1.33	4.20±0.83
Current smoker	614	2.28±2.85†	2.29±0.59	7.48±1.91†	4.49±0.86†

\*Plus–minus values are means ±SD; for C-reactive protein, the values are geometric means ±SD calculated from the log-transformed distribution.

†P<0.001 by the two-sample t-test.

**TABLE 5.** MULTIVARIATE ASSESSMENT OF THE EFFECT OF INFLAMMATORY MARKERS ON THE RISK OF A CORONARY EVENT.\*

VARIABLE	RELATIVE RISK (95% CI)†	P VALUE
Model 1		
Fibrinogen	1.04 (0.92–1.17)	0.53
White-cell count	1.15 (1.02–1.31)	0.03
C-reactive protein	1.21 (1.06–1.39)	0.004
Lipoprotein-associated phospholipase A <sub>2</sub>	1.19 (1.07–1.33)	0.002
Model 2		
Age	4.92 (1.88–12.9)	0.001
Systolic blood pressure‡	1.31 (1.17–1.47)	<0.001
Plasma triglycerides	1.04 (0.93–1.16)	0.54
LDL cholesterol	1.09 (0.98–1.22)	0.11
HDL cholesterol	0.75 (0.65–0.86)	<0.001
Fibrinogen	1.02 (0.90–1.15)	0.79
White-cell count	1.10 (0.97–1.25)	0.14
C-reactive protein	1.13 (0.98–1.29)	0.09
Lipoprotein-associated phospholipase A <sub>2</sub>	1.18 (1.05–1.33)	0.005
Variants of model 2		
C-reactive protein (white-cell count omitted)	1.16 (1.01–1.32)	0.04
C-reactive protein (fibrinogen omitted)	1.12 (0.99–1.27)	0.07
C-reactive protein (fibrinogen and white-cell count omitted)	1.15 (1.03–1.29)	0.02

\*Model 1 included the factors shown and tested the independence of the factors relative to each of the other variables in predicting the risk of coronary heart disease. Model 2 included the variables in model 1 as well as the traditional risk factors shown. In the three variants of model 2, the association of C-reactive protein with the risk of a coronary event was assessed after the removal of fibrinogen, the white-cell count, or both. CI denotes confidence interval, LDL low-density lipoprotein, and HDL high-density lipoprotein.

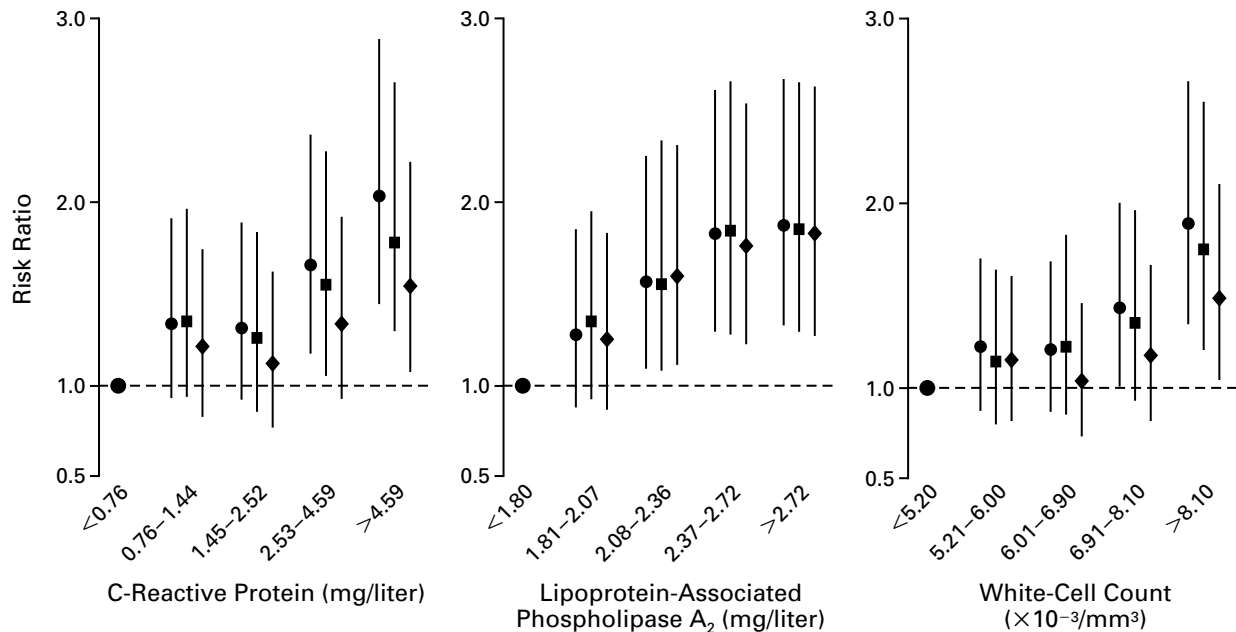
†The relative risk is the risk of the composite end point of nonfatal myocardial infarction, death from cardiac causes, or revascularization as a first event associated with an increase of 1 SD in the variable (the standard deviations are given in Table 1).

cebo.<sup>14</sup> Within the pravastatin and placebo groups, an increase of 1 SD in the C-reactive protein level was associated with a relative risk of 1.39 (95 percent confidence interval, 1.17 to 1.65) and 1.17 (95 percent confidence interval, 1.01 to 1.35), respectively. Similarly, an increase of 1 SD in the lipoprotein-associated phospholipase A<sub>2</sub> level was associated with a relative risk of 1.67 (95 percent confidence interval, 1.24 to 2.25) in the pravastatin group and of 1.17 (95 percent confidence interval, 0.90 to 1.51) in the placebo group. Again, interaction terms in a regression model that included all 1740 subjects were not significant.

## DISCUSSION

We confirmed that recognized indicators of a pro-inflammatory state — the C-reactive protein level, the white-cell count, and the fibrinogen level — were predictors of the risk of a coronary event in a population of middle-aged men with hypercholesterolemia. However, our principal finding was that the level of lipoprotein-associated phospholipase A<sub>2</sub> (platelet-activating factor acetylhydrolase), an enzyme that is also regulated by mediators of inflammation,<sup>12</sup> had a strong positive association with the risk of coronary heart disease.

When we compared the subjects in the highest quintile for C-reactive protein, white-cell count, and fibrinogen with those in the lowest quintile, the risk was approximately doubled — a finding that is in line with the results of a recent meta-analysis.<sup>21</sup> As others have found in recent studies,<sup>22-24</sup> we found that these markers of inflammation correlated with one another and were also related to the body-mass index, systolic blood pressure, plasma triglyceride levels, and HDL cholesterol levels. In our large, prospective study, the association of C-reactive protein levels, possibly the most sensitive of the inflammatory markers, was substantially attenuated in multivariate analyses that included these covariates and was no longer significant when the white-cell count was



**Figure 1.** Associations of the C-Reactive Protein Level, Lipoprotein-Associated Phospholipase A<sub>2</sub> Level, and the White-Cell Count with the Risk of a Coronary Event.

Levels of C-reactive protein and lipoprotein-associated phospholipase A<sub>2</sub> and the white-cell count at base line in the patients were divided according to the quintile values in the control subjects. In each case, the group of patients with the lowest value serves as the reference group (relative risk, 1.0). The circles indicate unadjusted relative risks. The squares indicate relative risks adjusted for lipoprotein-associated phospholipase A<sub>2</sub> levels, the white-cell count, and fibrinogen levels in the case of C-reactive protein; for C-reactive protein levels, the white-cell count, and fibrinogen levels in the case of lipoprotein-associated phospholipase A<sub>2</sub>; and for C-reactive protein levels, lipoprotein-associated phospholipase A<sub>2</sub> levels, and fibrinogen levels in the case of the white-cell count. The diamonds indicate risk ratios adjusted for age, systolic blood pressure, plasma triglyceride levels, low-density lipoprotein cholesterol levels, and high-density lipoprotein cholesterol levels. Vertical bars denote 95 percent confidence intervals.

included in the model. Our findings indicate that an assessment of inflammatory status is important in risk stratification and that measurement of C-reactive protein is helpful in this regard, as exemplified by the findings of Ridker et al.<sup>3</sup> The statistical independence of the association of the C-reactive protein level appears to depend on which of the other markers of inflammation are included in the models. Our cohort was not a random sample of the population, and C-reactive protein may retain its independent association with risk in other populations.

Why a marker of inflammation such as C-reactive protein should show such strong associations with other risk factors, as noted earlier<sup>22,24</sup> and confirmed in our study, has until recently been unclear. This liver-derived protein is regulated by interleukin-6, which is produced by inflammatory cells.<sup>24</sup> Thus, elevated C-reactive protein levels may simply reflect the presence of atherosclerotic disease and potentially unstable plaque. However, as pointed out by Ridker et al.,<sup>3</sup> high levels of C-reactive protein can predate a coronary event by many years.

Clues as to the mechanism underlying the association of C-reactive protein with classic risk factors,

such as hypertension, elevated plasma lipid levels, and insulin resistance,<sup>23,24</sup> come from the work of Yudkin et al.<sup>25</sup> and Mohamed-Ali et al.,<sup>26</sup> who found that subcutaneous adipose tissue was a further source of interleukin-6 and that plasma levels of C-reactive protein, interleukin-6, and tumor necrosis factor  $\alpha$  were all related to measures of obesity.<sup>25</sup> Therefore, in addition to marking the presence of a proinflammatory condition, high levels of C-reactive protein (and of fibrinogen, since it too has an interleukin-6-responsive promoter<sup>24</sup>) may to some extent be yet another facet of the insulin-resistant, obese state. The same may also be true of type II secretory phospholipase A<sub>2</sub>, which also responds to cytokine stimulation.<sup>7</sup> The plasma level of this enzyme, unlike that of lipoprotein-associated phospholipase A<sub>2</sub>, exhibits a strong association with C-reactive protein levels ( $r=0.53$ ,  $P<0.001$ ).<sup>7</sup> It has recently been suggested that C-reactive protein may not be an entirely passive marker of the atherogenic process. Reports have appeared indicating that the protein can bind to damaged LDL and trigger the activation of complement.<sup>24,27</sup>

The importance of our finding regarding lipoprotein-associated phospholipase A<sub>2</sub> is threefold. First, it

clarifies the clinical significance of this enzyme in atherosclerosis. Platelet-activating factor has potent biologic effects, including the activation of platelets and monocytes and macrophages<sup>28</sup>; thus, the hydrolysis of this phospholipid by lipoprotein-associated phospholipase A<sub>2</sub> may be expected to lead to a decreased risk of disease. Our observation that the level of lipoprotein-associated phospholipase A<sub>2</sub> was strongly related to the risk of a coronary event indicates that other actions of the enzyme are more pertinent to the atherosclerotic process. In this regard, it has been shown that lipoprotein-associated phospholipase A<sub>2</sub> on LDL is solely responsible for the hydrolysis of oxidized phospholipids in the particle. Blocking the enzyme does not alter the rate of LDL oxidation, but it does inhibit the release of products with biologic activity, such as factors that promote the chemotaxis of monocytes.<sup>15</sup> On the basis of our findings and previous findings,<sup>15,29</sup> we propose that, owing to its properties and location on the LDL particle, lipoprotein-associated phospholipase A<sub>2</sub> is placed to act as a key agent in the release of products of LDL oxidation into the artery wall.

Second, lipoprotein-associated phospholipase A<sub>2</sub> appears to be a novel risk factor that is statistically independent of markers of inflammation or classic risk factors. If our findings are confirmed in other populations, then measurement of lipoprotein-associated phospholipase A<sub>2</sub> mass will be a valuable addition to risk assessment in the future.

Third, inhibition of the activity of the enzyme has demonstrable biologic effects,<sup>15</sup> at least in vitro. Now that its association with the risk of coronary events is clear, it can become a new therapeutic target that is separate from current lipid-lowering or antiinflammatory approaches. In this context, it is noteworthy that the increase in risk with increasing lipoprotein-associated phospholipase A<sub>2</sub> levels was as strong in the men who received pravastatin in this study as in those who received placebo.

In conclusion, indicators of chronic inflammation were strongly associated with the risk of coronary heart disease in this nested case-control study and may be useful in risk stratification. C-reactive protein, fibrinogen, and the white-cell count are interrelated markers whose levels are influenced not only by chronic inflammation but also potentially by the presence of obesity and the insulin-resistance syndrome (as evidenced by increased blood pressure and plasma triglyceride levels and decreased HDL cholesterol levels in our patients). Lipoprotein-associated phospholipase A<sub>2</sub> is a potential risk factor that may have a direct role in atherogenesis.

Supported by a project grant (PG 97/160) from the British Heart Foundation and by a grant from Bristol-Myers Squibb (to the study data center).

*We are indebted to Ms. Shelley Wilkie for her excellent help in preparing the manuscript.*

## APPENDIX

In addition to the authors, the following persons are members of the West of Scotland Coronary Prevention Study Group: J. Shepherd, S.M. Cobbe, A.R. Lorimer, P.W. Macfarlane, and J.H. McKillop, Glasgow Royal Infirmary, Glasgow, Scotland; and C.G. Isles, Dumfries and Galloway Royal Infirmary, Dumfries, Scotland.

## REFERENCES

- Davies MJ, Richardson PD, Woolf N, Katy DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J* 1993;69:377-81.
- Ross R. Atherosclerosis — an inflammatory disease. *N Engl J Med* 1999;340:115-26.
- Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973-9. [Erratum, *N Engl J Med* 1997;337:356.]
- Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relationship of C-reactive protein and coronary heart disease in the MRFIT nested case-control study: Multiple Risk Factor Intervention Trial. *Am J Epidemiol* 1996;144:537-47.
- Koenig W, Sund M, Frohlich M, et al. C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 1999;99:237-42.
- Leitinger N, Watson AD, Hama SY, et al. Role of group II secretory phospholipase A<sub>2</sub> in atherosclerosis. 2. Potential involvement of biologically active oxidized phospholipids. *Arterioscler Thromb Vasc Biol* 1999;19:1291-8.
- Kugiyama K, Ota Y, Takazoe K, et al. Circulating levels of secretory type II phospholipase A<sub>2</sub> predict coronary events in patients with coronary artery disease. *Circulation* 1999;100:1280-4.
- Ivancic B, Castellani LW, Wang X-P, et al. Role of group II phospholipase A<sub>2</sub> in atherosclerosis. 1. Increased atherogenesis and altered lipoproteins in transgenic mice expressing group II<sub>1</sub> phospholipase A<sub>2</sub>. *Arterioscler Thromb Vasc Biol* 1999;19:1284-90.
- Elinder LS, Dumitrescu A, Larsson P, Hedin U, Frostegard J, Claesson H-E. Expression of phospholipase A<sub>2</sub> isoforms in human normal and atherosclerotic arterial wall. *Arterioscler Thromb Vasc Biol* 1997;17:2257-63.
- Sartipy P, Camejo G, Svensson L, Hurt-Camejo E. Phospholipase A<sub>2</sub> modification of low density lipoproteins forms small high density particles with increased affinity for proteoglycans and glycosaminoglycans. *J Biol Chem* 1999;274:25913-20.
- Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase A<sub>2</sub>, platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. *Atherosclerosis* 2000;150:413-9.
- Cao Y, Stafforini DM, Zimmerman GA, McIntyre TM, Prescott SM. Expression of plasma platelet-activating factor acetylhydrolase is transcriptionally regulated by mediators of inflammation. *J Biol Chem* 1998;273:4012-20.
- Tew DG, Southan C, Rice SQJ, et al. Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins. *Arterioscler Thromb Vasc Biol* 1996;16:591-9.
- Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995;333:1301-7.
- Macphee CH, Moores KE, Boyd HF, et al. Lipoprotein-associated phospholipase A<sub>2</sub>, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. *Biochem J* 1999;338:479-87.
- Berliner J, Leitinger N, Watson A, Huber J, Fogelman A, Navab M. Oxidized lipids in atherogenesis: formation, destruction and action. *Thromb Haemost* 1997;78:195-9.
- Lowe GDO, Rumley A, Norrie J, et al. Blood rheology, cardiovascular risk factors and cardiovascular disease in the West of Scotland Coronary Prevention Study. *Thromb Haemost* (in press).
- The WOSCOPS Study Group. Screening experience and baseline characteristics in the West of Scotland Coronary Prevention Study. *Am J Cardiol* 1995;76:485-91.
- Yarnell JWG, Baker IA, Sweetnam PM, et al. Fibrinogen, viscosity and white blood cell count are major risk factors for ischaemic heart disease: the Caerphilly and Speedwell collaborative heart disease studies. *Circulation* 1991;83:836-44.
- The West of Scotland Coronary Prevention Study Group. Baseline risk

factors and their association with outcome in the West of Scotland Coronary Prevention Study. *Am J Cardiol* 1997;79:756-62.

21. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA* 1998;279:1477-82.
22. Danesh J, Muir J, Wong YK, Ward M, Galleore JR, Pepys MB. Risk factors for coronary heart disease and acute-phase proteins: a population-based study. *Eur Heart J* 1999;20:954-9.
23. Hak AE, Stehouwer CDA, Bots ML, et al. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. *Arterioscler Thromb Vasc Biol* 1999;19:1986-91.
24. Tracy RP. Inflammation markers and coronary heart disease. *Curr Opin Lipidol* 1999;10:435-41.
25. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppel SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endo-

thelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999;19:972-8.

26. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor  $\alpha$ , in vivo. *J Clin Endocrinol Metab* 1997;82:4196-200.
27. Bhakdi S, Torzewski M, Klouche M, Hemmes M. Complement and atherogenesis: binding of CRP to degraded, nonoxidized LDL enhances complement activation. *Arterioscler Thromb Vasc Biol* 1999;19:2348-54.
28. Snyder F. Platelet-activating factor and its analogs: metabolic pathways and related intracellular processes. *Biochim Biophys Acta* 1995;1254:231-49. [Errata, *Biochim Biophys Acta* 1995;1257:297, 1259:121.]
29. Hakkinen T, Luoma JS, Hiltunen MO, et al. Lipoprotein-associated phospholipase A<sub>2</sub>, platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 1999;19:2909-17.