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Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease

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Abstract

A specific and robust immunoassay for the lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$), platelet-activating factor acetylhydrolase, is described for the first time. The immunoassay was used to evaluate possible links between plasma Lp-PLA$_2$ levels and atherosclerosis risk amongst susceptible individuals. Such an investigation was important because Lp-PLA$_2$ participates in the oxidative modification of low density lipoprotein by cleaving oxidised phosphatidylcholines, generating lysophosphatidylcholine and oxidised free fatty acids. The majority of Lp-PLA$_2$ was found associated with LDL (approximately 80%) and, as expected, enzyme levels were significantly positively correlated to LDL cholesterol. Plasma Lp-PLA$_2$ levels were significantly elevated in patients with angiographically proven coronary artery disease (CAD) when compared with age-matched controls, even though LDL cholesterol levels did not differ significantly. Indeed, when included in a general linear model with LDL cholesterol and other risk factors, Lp-PLA$_2$ appeared to be an independent predictor of disease status. We propose, therefore, that plasma Lp-PLA$_2$ mass should be viewed as a potential novel risk factor for CAD that provides information related to but additional to traditional lipoprotein measurements. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Various dyslipidaemias exist that increase the predisposition of an individual to develop atherosclerotic coronary artery disease (CAD). Elevated plasma levels of low density lipoprotein (LDL) cholesterol have been shown in many studies to be associated with an increased incidence of CAD whereas plasma high density lipoprotein (HDL) levels are inversely related to risk [1,2]. Not surprisingly, plasma concentrations of apolipoprotein (apo) B and A-1, the major protein components of LDL and HDL, respectively, are also accepted risk factors for CAD. The precise pathophysiological mechanisms through which dyslipidaemias increase the likelihood of atherosclerosis remain poorly understood, but the oxidative modification of LDL within the arterial wall appears to play an important role [3,4].

Attention has recently focused on three lipoprotein-associated enzymes that can participate in the LDL oxidation process and as a consequence modulate the many pro-inflammatory activities of oxidised LDL (oxLDL). For instance, much of the protective activity of HDL against oxLDL has been attributed to the presence of paraoxonase and lecithin-cholesterol acyltransferase (LCAT), two enzymes found exclusively on HDL. Paraoxonase hydrolyses and inactivates pro-inflammatory lipid peroxides present in oxLDL [5,6],

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and it was recently demonstrated that paraoxonase-null mice were more susceptible to atherosclerosis than wild-type littermates [7]. LCAT, known for many years to be responsible for plasma cholesteryl ester production, has also been shown to degrade both platelet activating factor (PAF) and oxidised polar phospholipids [8], two proposed atherogenic mediators [9].

The third enzyme thought to be intimately involved in LDL oxidation is the lipoprotein associated phospholipase A_2 (Lp-PLA$_2$), PAF acetylhydrolase. This enzyme, unlike the other two, is found predominately on LDL [10] and as such is better placed to participate directly in any oxidative modification process. Previous work has demonstrated that this phospholipase A_2 is responsible for much if not all of the increased lysophosphatidylcholine content of oxidatively modified LDL [11–13]. It is also now recognised as a subtype of the growing phospholipase A_2 superfamily [14] with a broad substrate preference because it can hydrolyse both PAF as well as more complex polar phosphatidyl-cholines [12,13,15]. The role of Lp-PLA$_2$ in atherogenesis is currently under some debate because it can apparently play a dual function. Since much of the pro-atherogenic activity of oxidised LDL has been attributed to its increased lysophosphatidylcholine content, this PLA$_2$ would appear to be pro-inflammatory in nature [16]; paradoxically, the ability of Lp-PLA$_2$ to hydrolyse PAF and related phospholipids also suggests an anti-inflammatory role [9,17]. More recently, Lp-PLA$_2$ has also been shown to release bioactive oxidised free fatty acids during the oxidation of LDL [13] thus providing further support for the pro-inflammatory argument.

To help resolve this apparent paradox we have developed a specific ELISA to measure Lp-PLA$_2$ mass to evaluate the potential link between plasma Lp-PLA$_2$ levels and individuals with atherosclerosis. Previous studies have shown that the plasma degradation of PAF is increased in patients with atherosclerosis [18–20] and stroke [21], but there was no information on the identity of the enzyme involved. By directly measuring Lp-PLA$_2$ mass we demonstrate that plasma Lp-PLA$_2$ is an independent predictor of CAD and provides information additional to LDL cholesterol.

2. Methods

2.1. Subjects

A total of 148 male subjects, aged 39–74 years, from Glasgow, UK were recruited into the study. They were drawn from to one of three groups. (1) Subjects ($n = 48$) with CAD were identified from those who attended for coronary angiogram at the Glasgow Royal Infirmary and met the entry criteria of a positive angiogram

at age < 55 years. To be referred for angiography in local practice, individuals have symptoms of angina and a positive angiogram is recorded if stenotic disease is present. The severity of disease was not recorded in this study. The mean age at blood sampling was 55 ± 8 years. (2) Post myocardial infarction (PMI) patients ($n = 46$) were recruited from the coronary rehabilitation classes at the Glasgow Royal Infirmary. They were at least 1 year post event which had taken place at age < 55 years. The mean age at sampling was 54 ± 8 years. (3) Fifty-four normal aged matched subjects (53 ± 10 years) were recruited from the local community. Blood pressure measurements were taken and a record of history of smoking and medication was collected by questionnaire. No patients in the CAD and PMI group had diabetes but one in the control group was receiving insulin. Treatment with nitrates, aspirin and β-blockers were similar in diseased subjects, with nine in each group undergoing treatment. One subject in the PMI and two in the CAD groups were being treated with simvastatin and one in the CAD was taking ciprofibrate.

Blood samples (30 ml) were drawn after an overnight fast into K$_2$ EDTA at a final concentration of 1 mg/ml and the plasma was analysed for lipids, lipoproteins and apolipoproteins immediately. Aliquots (1 ml) of plasma were fast frozen in liquid nitrogen and stored at $-70°C$. These frozen samples were transported monthly on dry ice to SmithKline Beecham Laboratories in Welwyn, Herts. for analysis of Lp-PLA$_2$. The identity of the samples was blind to the workers analysing Lp-PLA$_2$ levels.

2.2. Lipoprotein, lipid, and apolipoprotein measurements

Assays of plasma cholesterol, triglyceride, VLDL cholesterol, LDL cholesterol and HDL cholesterol were performed by a modification of the standard Lipid Research Clinic's protocol [22]. Apo AI and B were measured by immunoturbidimetry using kits purchased from Orion Diagnostics, Espoo, Finland.

2.3. Monoclonal antibody (Mab) generation

Lp-PLA$_2$ was purified to homogeneity from human LDL exactly as described previously [12]. Mice were immunised with 25 μg of purified, non-denatured, human Lp-PLA$_2$ in Freund's complete adjuvant followed by booster doses of 25 μg in Freund's incomplete adjuvant after 4 weeks as well as two doses of 20 μg intraperitoneally in saline. Spleen cells were harvested one day after the last dose and fused with myeloma cells according to the method of Zola [23]. Positive hybridomas were cloned twice by the limiting dilution method and the clones expanded to 1-l spinner flasks

for large scale production. Monoclonal antibodies (50–100 mg) were purified from the tissue culture medium by Protein A chromatography (Prosep-A, Bioprocessing, Consett, UK) and isotyped using a standard kit produced by Amersham (Amersham, UK). The monoclonal antibodies used in the assay were IgG and were named Mab 2C10 (first antibody) and Mab 2B4 (second antibody).

2.4. Reagents for immunoassay

Microtitre plates, the plate washer and fluorimeter were from Wallac (Turku, Finland). The plate-shaker incubator was purchased from Amersham. The coating buffer was 50 mM NA_2HPO_4, 150 mM NaCl, and 0.02% Kathon (Rohm and Haas, London, UK), pH 7.4. The blocking buffer was 50 mM Tris–HCl, 150 mM NaCl, 1% bovine serum albumin and 0.02% Kathon, pH 7.4. The wash solution contained 10 mM Tris–HCl, 150 mM NaCl, 0.05% Tween 20, and 0.02% Kathon, pH 7.4. The assay buffer used was 50 mM NA_2HPO_4, 150 mM NaCl, 20 mM CHAPS, 0.5% bovine γ-globulin, and 0.02% Kathon, pH 7.4. The Eu buffer consisted of 50 mM Tris–HCl, 150 mM NaCl, 0.5% bovine serum albumin, 0.05% bovine γ-globulin, 0.01% Tween 40, 20mM diethylenetriaminepentaacetic acid, and 0.02% Kathon, pH 7.4. Biotinylated anti-Lp-PLA_2 Mab 4B4 was prepared using a kit supplied by Amersham.

2.5. Lp-PLA_2 two site immunoassay procedure

Microtitre plate wells were coated with the first Mab 2C10 (4 µg/ml, 100 µl/well) overnight at 4°C after which the contents of the well were aspirated and 250 µl per well of blocking buffer added for 60 min at 22°C. The wells were washed four times and 50 µl biotin labelled second monoclonal antibody 4B4 (2 µg/ml in assay buffer) and 50 µl purified recombinant Lp-PLA_2

Fig. 1. Lp-PLA_2 ELISA standard curve, 0.3–30 ng/ml (correlation coefficient = 0.9997). Recombinant Lp-PLA_2 was added to microtitre plates coated with anti-Lp-PLA_2 Mab, 2C10. Quantitation was performed using a Wallac Delfia system as outlined in Section 2.

standards (0.3–30 ng/ml), plasma (diluted 1/100 in assay buffer) or SMART fraction (diluted 1/10 in assay buffer), was added and the wells incubated for 60 min at 22°C. The plate wells were washed four times and 100 µl per well of Eu-streptavidin (200 ng/ml, Wallac) was added and incubated for 30 min at 22°C. After the immunoreaction was complete the wells were washed four times and 200 µl per well of enhancement solution (Wallac) added and after a 5 min incubation the fluorescence was measured. The fluorimeter software was used for curve fitting and result calculations.

2.6. Lipoprotein fractionation

Plasma samples were thawed, placed on ice, and filtered using a 0.3 µM sterile filter. Plasma was fractionated into its lipoprotein components by gel filtration on a Pharmacia FPLC SMART system. Routinely 30 µl of filtered plasma was analysed using a Superose 6PC 3.2/30 column equilibrated at 15°C with 150 mM NaCl, 1 mM EDTA, and 0.05% Na azide, pH 8. Lipoproteins were eluted at a flow rate of 30 µl/min and the fractions corresponding to VLDL, LDL, and HDL pooled based upon protein (absorbance at 280 nm), cholesterol and Lp-PLA_2 activity profiles.

2.7. Lp-PLA_2 assays

Either 50 µl aliquots of whole plasma or 30 µl of the fractionated lipoprotein pools were assayed using 50 µM PAF as described previously [12].

2.8. Statistics

Group differences were explored by ANOVA using Tukey's and Fishers method for pairwise comparison. Regression analysis and correlation coefficients were obtained for continuous variables. All statistical tests were performed using Minitab version 10.0. The ability of a parameter to predict disease was tested by entering variables into a general linear model.

3. Results

3.1. Lp-PLA_2 two-site immunoassay

Fig. 1 shows that the Lp-PLA_2 calibration curve was linear over the range 0.3–30 ng/ml. The lowest calibrator had counts approximately five times above background. Excellent linearity of dilution was observed and the within- and between-assay coefficient of variation was 4 and 9%, respectively ($n = 27$). The recovery of Lp-PLA_2 added to 10 different patient samples was $95 \pm 8\%$. Zero cross-reactivity in the assay was noted at a 1000-fold excess with three different human recombi-
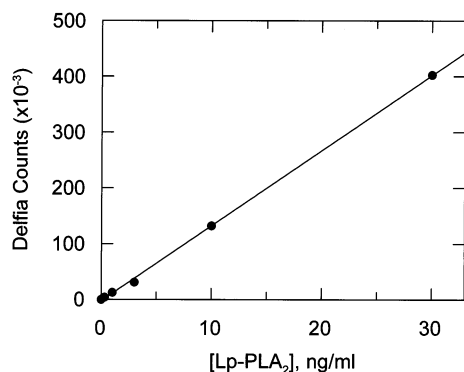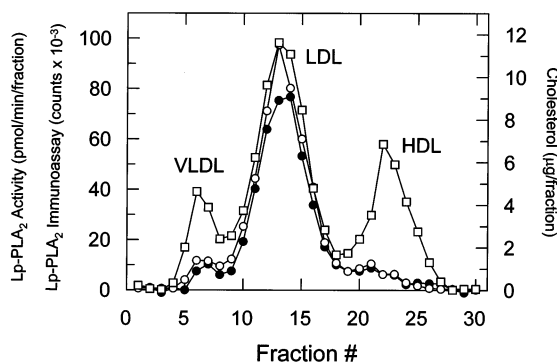
Fig. 2. Distribution of Lp-PLA$_2$ activity ($\bigcirc$), Lp-PLA$_2$ mass ($\bullet$) and cholesterol ($\square$) following gel filtration of human male plasma. Plasma (30 μl) was applied to a Superose 6 column and eluted with 150 mM NaCl, 1 mM EDTA, and 0.05% Na azide, pH 8. Fractions were collected and assayed for cholesterol content, Lp-PLA$_2$ mass using the new two-site immunoassay, and Lp-PLA$_2$ activity as described in Section 2.

Table 1
Differences between groups[a]

Parameters	Control	CAD	PMI	P
N	52	43	43	
Age (years)	53.6 (1.4)	55.1 (1.2)	53.0 (1.3)	0.491
BP systolic	131.9 (2.7)	132.4 (3.2)	128.5 (2.0)	0.553
BP diastolic	83.3 (1.3)	80.5 (2.1)	80.8 (1.5)	0.375
Smokers (%)	20	25	42	0.014

[a] Values are mean (S.E.M.).

Table 2
Lipids and lipoproteins[a]

	Control	CAD	PMI	P
Cholesterol (mmol/l)	5.61 (0.13)	5.92 (0.16)	5.46 (0.15)	0.084
Triglyceride (mmol/l)	1.58 (0.10)	2.18 (0.26)	1.88 (0.15)	0.053
VLDLc (mmol/l)	0.725 (0.05)	1.00 (0.12)	0.85 (0.06)	0.052
LDLc (mmol/l)	3.80 (0.13)	3.96 (0.14)	3.65 (0.14)	0.292
HDLc (mmol/l)	1.13 (0.04)	0.99* (0.04)	1.00* (0.05)	0.031
Apo AI (g/l)	1.23 (0.03)	1.20 (0.05)	1.17 (0.07)	0.710
Apo B (g/l)	1.08 (0.04)	1.25* (0.06)	1.20 (0.06)	0.042

[a] Values are mean (S.E.M.), P from ANOVA.
* Significant by Fishers pair wise comparison $P < 0.05$.

nant PLA$_2$'s which were generated at SmithKline Beecham Laboratories (data not shown): 14 kDa PLA$_2$ (CHO-derived), 85 kDa PLA$_2$ (baculovirus-derived), and a recently described related serine-dependent PLA$_2$ (baculovirus-derived). Both the 14 kDa PLA$_2$ and 85 kDa PLA$_2$ are calcium-dependent arachidonic acid selective enzymes [14], whereas the serine-dependent en-

zyme is calcium-independent and has 40% amino acid identity with Lp-PLA$_2$ [24].

Fig. 2 demonstrates, as expected, a clear co-elution of Lp-PLA$_2$ activity with immunoassay derived Lp-PLA$_2$ mass following separation of plasma lipoprotein fractions by FPLC. Most of Lp-PLA$_2$ is in the LDL size range.

3.2. Analysis of patient data

3.2.1. Lipids, lipoproteins, and apolipoproteins

There were no significant differences between normals and the control groups in age or blood pressure (Table 1). However, the number of smokers was higher in the diseased groups compared to control with 42% of the PMI group being smokers (ANOVA $P = 0.014$). There were no significant differences between normals and diseased groups in plasma cholesterol, triglyceride, VLDL cholesterol, LDL cholesterol and apo AI (Table 2). HDL cholesterol was lower in the diseased groups when compared to normal ($P = 0.031$) and both CAD and PMI groups were significantly different from control ($P < 0.01$). Analysis by ANOVA highlighted a significantly higher ($P = 0.042$) apo B in the diseased groups and this was due to the CAD group which gave a significance of $P < 0.01$ when compared to the normal group. The lipoprotein values were intermediate in the PMI group with higher levels in the CAD group except for HDLc which was lowest in CAD.

3.2.2. Lp-PLA$_2$ mass and activity

There was a strong positive correlation ($r = 0.86$, $P < 0.001$) between mass and activity of plasma Lp-PLA$_2$ (Fig. 3A). The same was true in each of the lipoprotein fractions for PLA$_2$ mass versus activity, VLDL ($r = 0.45$, $P < 0.001$), LDL ($r = 0.60$, $P < 0.001$) and HDL ($r = 0.71$, $P < 001$). Of the total Lp-PLA$_2$ mass associated with the lipoproteins, 5.8% was found in VLDL, 83.3% in LDL and 10.9% in HDL. Similar findings were observed for PLA$_2$ activity with 7% in VLDL, 80.4% in LDL and 12.6% in HDL. Recoveries of plasma Lp-PLA$_2$ mass, plasma Lp-PLA$_2$ activity and total plasma cholesterol from the lipoproteins fractionated by FPLC were all 95%.

Plasma Lp-PLA$_2$ mass was significantly correlated with cholesterol ($r = 0.59$, $P < 0.001$), triglyceride ($r = 0.23$, $P < 0.01$). VLDLc ($r = 0.30$, $P < 0.001$), LDLc ($r = 0.57$, $P < 0.001$), HDLc ($r = -0.23$, $P < 0.01$) and apo B ($r = 0.45$, $P < 0.001$) as shown in Table 3. Likewise PLA$_2$ mass in LDL was significantly correlated with cholesterol ($r = 0.56$, $P < 0.001$), LDLc ($r = 0.58$, $P < 0.001$) and apo B ($r = 0.39$, $P < 0.001$). The linear relationship between Lp-PLA$_2$ mass and LDLc is illustrated in Fig. 3B.

Plasma Lp-PLA$_2$ mass differed significantly between the groups (ANOVA $P = 0.009$) with highest levels in CAD which differed significantly from control ($P <$
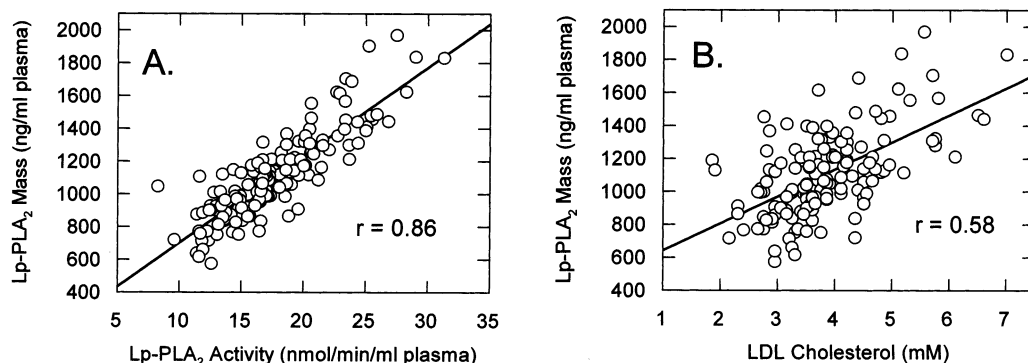
Fig. 3. Relationship between Lp-PLA$_2$ mass and Lp-PLA$_2$ activity (A) or LDL-cholesterol (B) in plasmas from male patients.

0.001) (Table 4). The level of the enzyme in LDL followed the same trend (CAD versus control, $P < 0.01$) but that in VLDL and HDL did not differ between the groups.

General linear models (GLMs) were used to test for predictors of disease. Controls were compared with all disease subjects (CAD and PMI) in model A, with those with CAD in model B and with those with PMI in model C Table 5. Plasma Lp-PLA$_2$ mass was a significant predictor of risk in models A and B and showed a trend in model C. Models A and B indicate that Lp-PLA$_2$ was an independent predictor of risk when the common risk factors, i.e. LDLc, HDLc, smoking and systolic blood pressure, were included in the multivariate models. In a GLM with apo B and Lp-PLA$_2$, the mass of the enzyme was still a significant predictor of CAD ($r2 = 0.91$, $P = 0.047$) but not of PMI status. When HDLc, smoking and systolic BP were added to the model the trend towards higher Lp-PLA$_2$ was still evident but was no longer significant. Apo B, like LDLc, was not a significant predictor in any GLM model when HDLc, smoking and systolic BP were included in the model.

4. Discussion

In view of the known positive correlation between plasma Lp-PLA$_2$ activity and LDLc levels [25–27] we attempted to ensure that the group of male subjects studied were well matched for fasting cholesterol levels (5.5–5.9 mmol/l) and LDLc levels (3.7–4.0 mmol/l). Thus, we intentionally chose PMI patients who were at least 1 year post event because acute MI is associated with depression of plasma lipoprotein concentrations and plasma Lp-PLA$_2$ activity [28,29]. Cholesterol and LDLc did not differ significantly between the three groups although plasma triglycerides (1.6–2.2 mmol/l) did show a tendency to be higher in the two diseased groups, CAD and PMI. HDLc, however, was significantly decreased in both patient groups (1.0 versus 1.13

mmol/l, $P < 0.01$) and apo B significantly elevated in the CAD but not the PMI group. Contrary to earlier findings, PMI subjects were closer to normal than were the CAD group. This we attribute to greater intervention and lifestyle changes in the year following the MI. The CAD subjects studied were attending a risk factor clinic for the first time and so were likely not to have modified their risk factors.

Several workers have reported [10,30–32] that Lp-PLA$_2$ (or PAF-AH) is transported in plasma predominantly associated with LDL and, in agreement, we

Table 3
Correlations between Lp-PLA$_2$ mass and lipoproteins

	Plasma PLA$_2$	VLDL PLA$_2$	LDL PLA$_2$	HDL PLA$_2$
Cholesterol	0.592***	0.306***	0.563***	−0.090
Triglyceride	0.233**	0.374***	0.114	0.234**
VLDLc	0.299***	0.437***	0.174*	0.182*
LDLc	0.574***	0.163	0.584***	−0.256**
HDLc	−0.226**	−0.192*	−0.131	0.084
Apo AI	−0.003	−0.127	−0.018	0.135
Apo B	0.450***	0.266**	0.389***	0.003

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
Lp-PLA$_2$ mass and patient group[a]

	Control	CAD	PMI	P
Plasma PLA$_2$ (ng/ml)	1028.1 (38.5)	1189.3* (40.9)	1100.8 (38.5)	0.009
VLDL-PLA$_2$ (ng/ml)	64.6 (4.6)	68.1 (5.9)	50.7 (6.8)	0.098
LDL-PLA$_2$ (ng/ml)	817.7 (36.8)	932.5* (38.2)	869.4 (32.1)	0.072
HDL-PLA$_2$ (ng/ml)	104.4 (12.8)	119.5 (10.0)	118.2 (6.0)	0.451

[a] Values are mean (S.E.M.), P from ANOVA.
* Significant by Fishers pair wise comparison $P < 0.05$.

Table 5
Predictors of coronary disease using a general linear model

	Model A (CAD and PMI)		Model B (CAD)		Model C (PMI)	
	$r^{2\,a}$	P	r^2	P	r^2	P
LDLc	0.0015	0.207	0.18	0.655	1.32	0.286
HDLc	1.35	0.041	1.31	0.149	1.07	0.042
Lp-PLA$_2$ mass	1.64	0.01	1.38	0.022	0.53	0.081
Smoking	2.13	0.002	1.61	0.007	1.76	0.004
Systolic BP	0.12	0.444	0.02	0.747	0.02	0.765
Overall	26.1		18.3		16.2	

^{a} r^2 is the estimated independent contribution of the variable.

observed that 80% of the PAF-hydrolysing activity within plasma was associated with LDL. Additionally, through the development of a robust and selective immunoassay for Lp-PLA$_2$, we showed for the first time that the lipoprotein distribution of Lp-PLA$_2$ mass matches exactly that of PAF-AH activity. The strong correlation ($r = 0.86$, $P < 0.001$) between Lp-PLA$_2$ mass and activity in plasma clearly shows that Lp-PLA$_2$ contributes the majority of the PAF-hydrolysing capability of plasma.

We found a strong correlation between Lp-PLA$_2$ mass and plasma LDL$_c$ in agreement with earlier studies [25–27] which had based their observations on enzyme activity measurements. Similarly, we noted a strong correlation between plasma Lp-PLA$_2$ mass and plasma cholesterol, triglyceride, VLDLc, and apo B (Table 3) in agreement with Guerra et al. [27] who also demonstrated an association between PAF-AH activity and apo B, cholesterol, and triglyceride. The significant negative correlation observed by others [25–27] between the PAF-degrading capacity of plasma and HDL$_c$ was also corroborated in our Lp-PLA$_2$ mass measurement studies. We therefore propose that the immunoassay developed offers a simple, sensitive, and reliable method for the detection of Lp-PLA$_2$ in plasma compared to activity measurements.

Our observation that plasma Lp-PLA$_2$ mass levels were significantly elevated in the CAD group supports and confirms previous reports [18–20] suggesting a link between this lipoprotein-bound enzyme and individuals with atherosclerosis. This positive association was made all the more striking since LDL cholesterol levels were not significantly different between normals and CAD. A comparison of risk factors in CAD and normal groups indicated that plasma Lp-PLA$_2$ mass was a clearer marker of risk on a case control basis than either plasma cholesterol or LDL cholesterol. Even though the enzyme exhibited a strong relationship with LDL this was not the sole cause of the risk association since it was an independent predictor in the linear model. Indeed, plasma Lp-PLA$_2$ was a stronger a predictor of disease in the CAD group than apo B. Part of

the reason why elevated Lp-PLA$_2$ was such a good predictor of disease may, as suggested by Guerra et al. [27], be due to it reflecting the presence of LDL particles that are cleared slowly from the circulation. They speculated that the rate of removal of LDL from the circulation would determine the clearance rate of Lp-PLA$_2$ and therefore the plasma mass of the enzyme for any individual. Consistent with this idea has been the observation that Lp-PLA$_2$ is found enriched in the highly atherogenic small dense LDL [33], a LDL subtype known to have a prolonged plasma half-life [34]. Moreover, this association would tend to align Lp-PLA$_2$ with a predominantly pro-inflammatory role since these particles are considered pro-atherogenic because they preferentially seed in the arterial intima and, being older, they are prone to oxidative modification [35].

In summary, the studies strongly support plasma Lp-PLA$_2$ as a potential novel risk factor for CAD which provides information related to and additional to that obtained from traditional lipid analyses.

References

[1] Assmann G, Schulte H. Relation of high density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease. Am J Cardiol 1992;70:733–7.

[2] Kwiterovich PO. The antiatherogenic role of high density lipoprotein cholesterol. Am J Cardiol 1998;82:13Q–21Q.

[3] Witztum JL, Steinberg DJ. Role of oxidised low density lipoprotein in atherogenesis. Clin Invest 1991;88:1785–92.

[4] Berliner JA, Heinecke JW. The role of oxidised lipoproteins in atherogenesis. Free Radic Biol Med 1996;20:707–27.

[5] Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low density lipoprotein. FEBS Lett 1991;286:152–4.

[6] Mackness MI, Mackness B, Durrington PN, Connelly PW, Hegele RA. Paraoxonase: biochemistry, genetics, and relationship to plasma lipoproteins. Curr Opin Lipidol 1996;7:69–76.

[7] Shih DM, Gu L, Xia YR, et al. Nature 1998;394:284–7.

[8] Goyal J, Wang K, Liu M, Subbaiah PV. Novel function of lecithin-cholesterol acyltransferase. J Biol Chem 1997;272:16231–9.

[9] 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.

[10] Stafforini DM, McIntyre TM, Carter ME, Prescott SM. Human plasma platelet-activating factor acetylhydrolase: association with lipoprotein particles and role in the degradation of platelet-activating factor. J Biol Chem 1987;262:4215–22.

[11] Steinbrecher UP, Pritchard PH. Hydrolysis of phosphatidyl-choline during LDL oxidation is mediated by platelet activating factor acetylhydrolase. J Lipid Res 1989;30:305–15.

[12] Tew DG, Southan C, Rice SQJ, et al. Purification, properties, sequencing and cloning of a lipoprotein associated, serine dependent phospholipase which is involved in the oxidative modification of low density lipoproteins. Arterioscler Thromb Vasc Biol 1996;16:591–9.

[13] Macphee CH, Moores K, Boyd H, et al. The lipoprotein-associated phosphilipase A_2 generates two bioactive products during the oxidation of low density lipoprotein: Studies using a novel inhibitor. Biochem J 1999;338:479–87.

[14] Dennis EA. The growing phospholipase A_2 superfamily of signal transduction enzymes. TIBS 1997;22:1–2.

[15] Stremler KE, Stafforini DM, Prescott SM, McIntyre TM. Human platelet-activating factor acetylhydrolase: oxidatively fragmented phospholipids as substrates. J Biol Chem 1991;266:11095–103.

[16] Macphee CH, Milliner K, Moores K, Tew DG. The involvement of LDL-associated phospholipase A_2 in atherogenesis. Pharmacol Rev Commun 1996;8:309–15.

[17] Tjoelker LW, Wilder C, Eberhardt C, et al. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature 1995;374:549–53.

[18] Ostermann G, Ruhling K, Zabel-Langhennig R, Winkler L, Schlag B, Till U. Plasma from atherosclerotic patients exerts an increased degradation of platelet-activating factor. Thromb Res 1987;47:279–85.

[19] Ostermann G, Lang A, Holtz H, Ruhling K, Winkler L, Till U. The degradation of platelet-activating factor in serum and its discriminative value in atherosclerotic patients. Thromb Res 1988;52:529–40.

[20] Graham RM, Stephens CJ, Sturm MJ, Taylor RR. Plasma platelet-activating factor degradation in patients with severe coronary artery disease. Clin Sci 1992;82:535–41.

[21] Satoh K, Yoshida H, Imaizumi TA, Takamatsu S, Mizuno S. Platelet activating factor acetylhydrolase in plasma lipoproteins from patients with ischemic stroke. Stroke 1992;23:1090–2.

[22] US Department Health, Education and Welfare. Lipid Research Clinics Program: Manual of Laboratory Operations Vol. 1: Lipid and Lipoprotein Analysis (publication no. (NIH) 75-628). Bethesda, MD: National Institutes of Health, 1975.

[23] Zola H, editor. Monoclonal Antibodies. A Manual of Techniques. Boca Raton, FL: CRC Press, 1987:1987.

[24] Rice S, Southan C, Boyd H, et al. Expression, purification, and characterisation of a human serine dependent phospholipase with high specificity for oxidised phospholipids and platelet activating factor. Biochem J 1998;330:1309–15.

[25] Ostermann G, Kertscher HP, Winkler L, Schlag B, Ruhling K, Till U. The role of lipoproteins in the degradation of platelet-activating factor. Thromb Res 1986;44:303–14.

[26] Carter E, Wu L, Stafforini DM, et al. Platelet-activating factor acetylhydrolase correlates with plasma lipoproteins. Arteriosclerosis 1988;8:712a.

[27] Guerra R, Zhao B, Mooser V, Stafforini D, Johnston JM, Cohen JC. Determinants of plasma platelet-activating factor acetylhydrolase: heritability and relationship to plasma lipoproteins. J Lipid Res 1997;38:2281–8.

[28] Mainard F, Ozanne P, Madec Y. Variations in lipoproteins, hormones and blood glucose during the early acute phase of myocardial infarction. Atherosclerosis 1988;69:225–31.

[29] Stephens CJ, Graham RM, Sturm MJ, Richardson M, Taylor RR. Variation in plasma platelet-activating factor degradation and serum lipids after acute myocardial infarction. Coron Heart Dis 1993;4:187–93.

[30] Pritchard PH, Chonn A, Yeung CCH. The degradation of platelet-activating factor in the plasma of a patient with familial high density lipoprotein deficiency (Tangier disease). Blood 1985;66:1476–8.

[31] Satoh K, Imaizumi T, Kawamura Y, Yoshida H, Takamatsu S, Takamatsu M. Increased activity of platelet-activating factor acetylhydrolase in plasma low density lipoprotein from patients with essential hypertension. Prostaglandins 1989;37:673–82.

[32] Surya II, Mommersteeg M, Erkelens DW, Akkerman J-WN. PAF-acetylhydrolase, predominantly present in LDL in healthy subjects, is associated with HDL in a patient with LDL deficiency. J Lipid Med 1991;3:27–38.

[33] Karabina SP, Liapikos TA, Grekas G, Goudevenos J, Tselepis AD. Distribution of PAF acetylhydrolase activity in human plasma low-density lipoprotein subfractions. Biochim Biophys Acta 1994;1213:34–8.

[34] Packard CJ, Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. Arterioscler Thromb Vasc Biol 1997;17:3542–56.

[35] Chapman MJ, Guerin M, Bruckert E. Atherogenic, dense low density lipoproteins: pathophysiology and new therapeutic approaches. Eur Heart J 1998;19(Suppl. A):A24–30.