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CLINICAL RESEARCH
Prevention and epidemiology

Lipoprotein-associated phospholipase A2 activity, ferritin levels, metabolic syndrome, and 10-year cardiovascular and non-cardiovascular mortality: results from the Bruneck study

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Aims To identify factors that influence plasma levels and assess the prognostic value of lipoprotein-associated phospholipase A2 (Lp-PLA2) activity in a prospective, population-based survey of the epidemiology and pathogenesis of atherosclerosis.

Methods and results The Bruneck study is a prospective, population-based survey initiated in 1990. Lp-PLA2 activity and baseline variables for the current analysis were measured in 765 subjects aged 45–84 years in 1995. Incident cardiovascular disease (CVD) (cardiovascular death, myocardial infarction, stroke, and transient ischaemic attack) and rates of non-CVD mortality were assessed between 1995 and 2005.

Subjects with incident CVD had higher levels of Lp-PLA2 activity (884 ± 196 vs. 771 ± 192 μmol/min/L, $P < 0.001$). Increased Lp-PLA2 activity was significantly related to incident CVD [age- and sex-adjusted hazard ratio (95%CI) 2.9 (1.6–5.5); third vs. first tertile group; $P < 0.001$] and with vascular mortality but not with non-CVD mortality. Lp-PLA2 activity was enhanced in subjects with the metabolic syndrome and showed highly significant positive associations with LDL-C, apoB-100, ferritin, and HOMA-IR, and inverse associations with HDL-C and anti-oxidant levels.

Conclusion Increased Lp-PLA2 activity is associated with metabolic syndrome and incident fatal and non-fatal CVD, but not with non-CVD mortality. Furthermore, Lp-PLA2 activity is strongly influenced by ferritin levels, LDL-C, and apoB-100 supporting its integral role in lipid peroxidation. Clinical utility of Lp-PLA2 activity for prediction of cardiovascular risk has to be explored in future studies.

Keywords Lipoprotein-associated phospholipase A2 • Myocardial infarction • Oxidation • Atherosclerosis • Anti-oxidants

Introduction

Oxidative stress is a key mechanism through which atherosclerosis and cardiovascular disease (CVD) develops. It is mediated by reactive oxygen species that alter the fundamental properties of cholesterol, cholesteryl esters, and phospholipids on lipoproteins, as well as other proteins, to make them dysfunctional, immunogenic, and pro-atherogenic.[1,2] Oxidative stress can be enhanced by non-enzymatic pathways, such as by copper and iron cations, as well as by enzymatic pathways, such as by lipoxygenases, myeloperoxidase, and NADPH oxidase.[3]

These pro-oxidant pathways are balanced by anti-oxidant mechanisms, such as anti-oxidant vitamins (alpha-tocopherol and carotenoids) present within lipoproteins, and anti-oxidant enzymes,

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such as superoxide dismutase and glutathione peroxidase.[4,5] Many of these enzymes and products of oxidation can be measured in the circulation, including oxidized low-density lipoprotein, oxidized phospholipids, isoprostanes, and myeloperoxidase, and have been shown to predict the presence of CVD and incident cardiovascular events (reviewed by Tsimikas[6]).

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme with broad specificity in cleaving oxidized fatty acids at the sn-2 positions of oxidized phospholipids thereby generating lysophosphatidylcholine and free oxidized fatty acids. Based on studies in Lp-PLA2-deficient individuals and overexpression studies in animals, it was proposed that Lp-PLA2 has anti-atherogenic properties.[7] However, studies in animals with Lp-PLA2 inhibitors[8] and most clinical evaluations,[9–19] but not all,[20–22] involving plasma measurements of Lp-PLA2 mass or activity, suggest that elevated levels of Lp-PLA2 enhance cardiovascular risk.

The Bruneck study is a prospective, population-based survey on the epidemiology and pathogenesis of atherosclerosis. The cohort consists of men and women 45–84 years old (1995 evaluation) and was followed for 10 years. The subjects are extremely well characterized in terms of demographic, vascular, coagulation, lipid and biochemical factors, and life-style parameters.[23] The Bruneck cohort represents a unique opportunity to identify factors that predict new cardiovascular events in an unselected population. Therefore, the aim of this study was to assess the predictive value of Lp-PLA2 activity for incident CVD, vascular and non-vascular mortality and to identify potential determinants of Lp-PLA2 activity.

Methods

Baseline demographics, laboratory, and life-style measure of the study subjects

The study population was recruited in 1990 as a sex- and age-stratified random sample of all inhabitants of Bruneck, Italy (125 women and 125 men in the fifth to eighth decades each, $n = 1000$). The current study focuses on the 1995 re-examination of 765 subjects and a 10-year follow-up period (1995–2005).[23] Detailed information about incident cardiovascular events was available in all of these subjects (100% follow-up). The study protocol was approved by the appropriate ethics committees and all study subjects gave their written informed consent before entering the study.

All risk factors were assessed by validated standard procedures. Study methodology and laboratory methods for factors listed in *Table 1* were previously described in detail[23,24] and are summarized in the Supplementary material online. The metabolic syndrome was defined as having three out of five of the following parameters: waist circumference in men >102 cm (>40 in.) and women >88 cm (>35 in.), triglycerides ≥150 mg/dL, HDL cholesterol in men <40 and women <50 mg/dL, blood pressure ≥130/≥85 mm, and fasting glucose ≥110 mg/dL.[25]

Determination of lipoprotein-associated phospholipase A2 activity

Lp-PLA2 activity was measured with a commercially available kit (Azwell Inc., Osaka, Japan) as previously described.[24] Reference normal values provided by the manufacturer are <800 IU/L (μmol/min/L).

Assessment of future cardiovascular events

Assessment of non-cardiovascular death and future cardiovascular events in the Bruneck population was described previously[24] and detailed in the Supplementary material online. In brief, the primary analysis was comprised of composite CVD, which included all incident cases of cardiovascular death, myocardial infarction (WHO definition), ischaemic stroke, and transient ischaemic attack (TIA) (criteria of the National Survey of Stroke) ($n = 82$). Extended composite endpoints additionally included revascularization procedures which increased the number of individuals affected from 82 to 98 and new onset symptomatic peripheral arterial disease which further increased the number to 108. Ascertainment of events was based on a careful review of medical records provided by the general practitioners and files of the Bruneck Hospital and the extensive clinical and laboratory examinations performed as part of the study protocols.

Statistics

Calculations were performed using the SPSS 15.0 and BMDP software packages. Continuous variables were presented as means ± SD or medians (interquartile range), and dichotomous variables as percentages. Differences in baseline levels of vascular risk attributes between subjects with and without subsequent CVD (1995–2005) were analysed with the Student's *t*-test and χ^2-test. Variables with a skewed distribution were $\log_e$-transformed. Correlations between Lp-PLA2 activity and potential determinants were estimated by calculation of partial (age-/sex-corrected) Pearson correlation coefficients. The multivariable association was analysed by means of linear regression analysis (see Supplementary material online). Cox proportional hazard models were used to assess whether baseline Lp-PLA2 activity levels were independently related to incident CVD and both vascular und non-vascular mortality (see Supplementary material online). For this purpose, Lp-PLA2 activity was either modelled as a categorical (tertile or sextile groups) or as a continuous variable. Two models were run: the first one included age and sex; the second model was additionally adjusted for numerous established and putative vascular risk factors. Added predictive ability of Lp-PLA2 as a novel risk marker for CVD (model discrimination and calibration, reclassification characteristics) was assessed by standard procedure, see Supplementary material online. A two-sided *P*-value <0.05 was considered statistically significant except for *Table 1* in which *P*-values <0.00147 indicate significance (Bonferroni correction for multiple comparisons).

Results

Baseline demographic, clinical, and laboratory characteristics of the study subjects

Baseline demographic, clinical, and laboratory characteristics of study subjects (all of Caucasian origin) are shown in *Table 1*. Data are presented for subjects with (CVD+) and without (CVD−) incident CVD over the 10-year follow-up period. Those with incident CVD had significantly higher levels of Lp-PLA2 activity (884 ± 196 vs. 771 ± 192 μmol/min/L, $P < 0.001$ for difference) and were more likely to be older,

Table 1 Baseline characteristics of study subjects and age-/sex-adjusted correlations with Lp-PLA2 activity ($n = 765$)

Variable	Mean ± SD, Median (IQR)[a] or %		*P*-value[†]	*R*[b]
	CVD− ($n = 683$)	CVD+ ($n = 82$)		
Age (year)	61.8 ± 10.9	70.2 ± 10.3	<0.001	—
Female sex (%)	51.0	37.8	0.024	—
Lp-PLA2 activity (μmol/min/L)	770.7 ± 192.1	884.1 ± 196.0	<0.001	—
Vascular risk factors				
Hypertension (%)	67.5	69.5	0.712	—
Systolic BP (mmHg)	147.4 ± 20.3	152.3 ± 22.4	0.041	0.104
Diastolic BP (mmHg)	86.9 ± 9.0	87.1 ± 9.5	0.832	0.090
Current smoking (%)	20.1	18.3	0.711	—
Smoking (cigarettes/day)	2.6 ± 6.1	2.8 ± 7.1	0.705	0.043
Diabetes (WHO) (%)	8.8	14.6	0.087	—
Fasting glucose (mmol/L)	5.64 ± 1.37	5.99 ± 1.57	0.033	0.107
Ferritin (pmol/L)	295.7 ± 344.2	368.1 ± 384.2	0.076	**0.163**
C-reactive protein (mg/L)	2.7 ± 7.1	3.7 ± 6.6	0.030	0.033
HOMA-IR[a]	1.98 (1.41–2.96)	2.4 (1.65–4.05)	0.022	**0.163**
Microalbuminuria (g/L)[a]	9.0 (7.0–16.0)	11.5 (8.0–54.8)	0.003	−0.006
Coagulation				
Fibrinogen (μmol/L)	7.61 ± 2.14	9.03 ± 2.08	0.013	0.006
Antithrombin III (%)	99.6 ± 11.5	97.3 ± 11.6	0.094	−0.058
Activated protein C ratio	3.2 ± 0.6	3.1 ± 0.6	0.119	−0.014
Lipids and lipoproteins				
Total cholesterol (mmol/L)	5.90 ± 1.09	6.22 ± 1.13	0.013	**0.424**
Triglycerides (mmol/L)[a]	2.74 (2.07–4.06)	3.05 (2.33–4.24)	0.226	**0.170**
HDL cholesterol (mmol/L)	1.53 ± 0.42	1.47 ± 0.47	0.238	**−0.132**
LDL cholesterol (mmol/L)	3.72 ± 0.97	4.03 ± 1.02	0.007	**0.450**
Apolipoprotein A-I (g/L)	1.66 ± 0.27	1.65 ± 0.30	0.698	−0.036
Apolipoprotein B-100 (g/L)	1.15 ± 0.31	1.25 ± 0.34	0.005	**0.442**
OxPL/apoB[a]	0.05 (0.03–0.13)	0.070 (0.04–0.22)	0.008	0.052
Lipoprotein(a) (μmol/L)[a]	0.41 (0.16–1.23)	0.74 (0.28–1.80)	0.010	0.058
Nutrition, activity, and body composition				
Sports index (Beacke)	2.4 ± 0.9	2.1 ± 0.8	0.001	0.007
Energy intake (kJ)	11874 ± 3647	11648 ± 3391	0.591	−0.037
Fat intake (g/day)	148.0 ± 48.6	145.2 ± 45.3	0.615	−0.049
Alcohol consumption (g/day)	24.0 ± 31.4	23.9 ± 29.4	0.986	0.022
Body-mass index (kg/m^2)	25.6 ± 3.7	26.0 ± 4.5	0.307	0.068
Waist–hip ratio (cm/cm)	0.928 ± 0.071	0.948 ± 0.075	0.017	0.071
Levels of vitamins and antioxidants (available in a subgroup of 390 subjects)				
Retinol[c]	0.018 ± 0.009	0.014 ± 0.007	0.031	**−0.206**
Tocopherol[c]	0.179 ± 0.081	0.160 ± 0.065	0.275	−0.150
Carotene[c]	0.010 ± 0.005	0.007 ± 0.004	0.022	−0.110

[a]Median and interquartile range (IQR) is presented for skewed variables.
[b]Partial Pearson correlation coefficients between Lp-PLA2 activity and given variable, corrected for age and sex. When accounting for the multiple comparisons performed, *P*-values <0.00147 (in bold) are considered statistically significant (Bonferroni correction).
[c]Ratio of retinol, carotene, and tocopherol plasma levels and LDL level.
[†]*P*-values for difference in variable levels between subjects with and without incident CVD (1995–2005).

male, physically inactive, and had higher levels of systolic blood pressure, fasting glucose, C-reactive protein, homeostasis model of insulin resistance (HOMA-IR), microalbuminuria, fibrinogen, total cholesterol, LDL-C, apoB-100, OxPL/apoB, Lp(a), and lower levels of retinol and carotene (normalized for LDL-C) (*Table 1*). Lp-PLA2 activity was higher in men than in women (817 ± 205 vs. 749 ± 179 μmol/min/L, $P < 0.001$) and modestly increased with age ($r = 0.073$, $P = 0.045$).

Association of Lp-PLA2 activity with vascular, coagulation, lipid and lifestyle variables, and antioxidant levels

Distribution of Lp-PLA2 activity approximates a normal distribution. Level of Lp-PLA2 activity was strongly correlated with ferritin, HOMA-IR, total cholesterol, triglycerides, LDL-C, and apoB-100, and inversely with HDL-C and anti-oxidant levels (*Table 1* and Supplementary material online, *Figure S1A*). Moreover, level of Lp-PLA2 activity steadily increased with an increasing number of components of the metabolic syndrome clustering in individuals (age-/sex-adjusted Lp-PLA2 activity in subjects with 0 to 5 components: 736, 755, 769, 837, 869, and 851 μmol/min/L; $P < 0.001$) (Supplementary material online, *Figure S1B*). No association was noted with life-style variables including smoking, alcohol, diet, body mass index (BMI), and sports activity.

In a multivariable stepwise linear regression analysis (forced entry of age and sex), LDL-C or apoB-100 and ferritin level emerged as highly significant, independent determinants of Lp-PLA2 activity ($P < 0.001$ each). The metabolic syndrome added significantly to the model composed of age, sex, LDL-C, and ferritin (*Table 2*). In this model, metabolic syndrome could be substituted by the number of metabolic abnormalities clustering in individuals ($P = 0.028$). When allowing for antioxidant levels (available in 390 subjects), retinol levels additionally showed an independent relation with Lp-PLA2 activity ($P = 0.002$).

Relationship of Lp-PLA2 levels with cardiovascular disease and mortality

In Cox regression analysis, the risk of incident composite CVD gradually increased across tertile groups for Lp-PLA2 activity (*Table 3*). This finding applied to base models adjusted for age and sex [hazard ratio (HR) (95%CI) 1.7 (0.9–3.4) and 2.9 (1.6–5.5) for comparison between the middle and highest vs. lowest tertile group; $P < 0.001$ for trend], and to multivariable models [HR 1.6 (0.8–3.2) and 2.2 (1.1–4.8), $P = 0.019$ for trend]. Similar HR was noted for the extended composite CVD endpoints, which included revascularization and peripheral arterial disease, and for individual disease endpoints (stroke and TIA, myocardial infarction) (*Table 2*). Cox models calculated for a 1-SD unit increment in Lp-PLA_2 activity showed similar and consistent results (*Table 3*, right columns). Additional adjustment for statins and other standard cardiovascular therapies had no influence on the results (data not shown). Interestingly, the findings extended to vascular mortality [HR 2.0 (0.8–5.2) and 2.7 (1.1–6.5) for comparison between the middle and highest vs. lowest tertile group; $P = 0.044$ for trend] but not to non-CVD death [HR 0.9 (0.5–1.4) and 0.9 (0.5–1.6), $P = 0.56$ for trend]. Cumulative hazard plots are depicted in *Figure 1*.

Supplementary analysis using sextiles of Lp-PLA2 activity indicate a gradual increase in CVD risk (data not shown). When using orthogonal polynomials, the linear term was highly significant ($P = 0.001$) with no evidence of additional non-linear components.

Table 2 Multivariable association of Lp-PLA2 activity with potential determinants in the Bruneck Study ($n = 765$)

Variables	Regression coefficient	95% Confidence interval	*P*-value
Age	8.98	−3.11 to 21.08	0.146
Male gender	69.29	42.96 to 95.62	<0.001
LDL cholesterol	83.72	71.40 to 96.04	<0.001
Ferritin	29.53	16.43 to 42.63	<0.001
Metabolic syndrome	35.49	8.40 to 62.57	0.010

Regression coefficients (95%CI) were calculated for a 1-SD unit increment of continuous variables. Metabolic syndrome was defined according to AHA-NHLBI criteria.

Lp-PLA2 activity and cardiovascular risk in subgroup analysis

Associations were consistent in subgroups, including men vs. women [HR per 1-SD unit increment in Lp-PLA2, 1.62 (1.28–2.06) in men and 1.28 (0.90–1.81) in women; interaction $P = 0.25$], diabetics vs. non-diabetics, BMI ≤25 vs. BMI >25, no prior CVD vs. prior CVD, non-smokers vs. current/ex-smokers, and in subjects with different LDL-C, C-reactive protein, and Framingham Risk Score (FRS) (*Figure 2*). There was a non-significant trend towards a stronger association in groups with lower LDL-C. However, there was a significant interaction with Lp(a) level (*Figure 2*).

Added predictive ability of Lp-PLA2 as a risk marker for cardiovascular disease

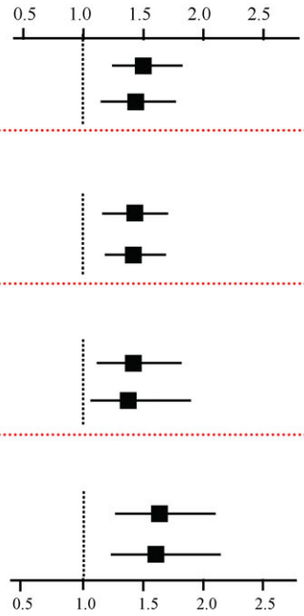
Addition of Lp-PLA2 activity level to equations based on the Framingham risk function resulted in a modest increase in the area under the receiver–operating characteristic (ROC) curve (0.737 vs. 0.717, Δ 0.020, $P = 0.31$) (*Figure 3*), but a significant improvement in the likelihood function (Δ likelihood ratio Chi-square 10.2; $P = 0.001$). *P*-values derived from the modified Hosmer–Lemeshow calibration statistic (comparing observed and predicted risk using decile categories of predicted probabilities) amounted to 0.54 and 0.61 for the models not including and including Lp-PLA2 indicating a marginal gain in model calibration. The net reclassification improvement for 10-year risk categories <10%, 10–20%, and >20% was 9.5% ($P = 0.11$). Among individuals with outcome events, the proportions of subjects reclassified to a higher and lower risk category were 19.5 and 4.9%, respectively. Corresponding proportions among individuals free of CVD during follow-up were 11.3 and 6.1%.

Discussion

This prospective epidemiological study of unselected subjects followed for 10 years confirms that increased baseline Lp-PLA2 activity is associated with increased risk of future cardiovascular events. Importantly, it documents several novel observations consistent with the specific pathophysiological role of Lp-PLA2 in atherogenesis: first, it demonstrates a significant relationship with vascular death but not with non-CVD mortality consistent with a

Table 3 Association between Lp-PLA$_2$ activity and cardiovascular disease risk in the Bruneck study cohort (1995–2005)

Model	Hazard ratio (95% CI) Lp-PLA2 tertile groups I	II	III	*P*-value for trend	Hazard ratio (95% CI) per 1-SD unit increase in Lp-PLA$_2$	*P*-value
Primary composite CVD endpoint						
Age/sex-adjusted Cox model ($n = 82$)	1.0	1.7 (0.9–3.4)	2.9 (1.6–5.5)	<0.001		<0.001
Multivariable Cox model[a] ($n = 82$)	1.0	1.6 (0.8–3.2)	2.2 (1.1–4.8)	0.019		0.004
Extended composite CVD endpoints						
Multivariable Cox model[†] ($n = 98$)	1.0	1.4 (0.8–2.7)	1.9 (1.0–3.5)	0.036		0.008
Multivariable Cox model[‡] ($n = 108$)	1.0	1.6 (0.8–2.9)	2.0 (1.1–3.7)	0.022		0.002
Stroke / TIA						
Age/sex-adjusted Cox model ($n = 45$)	1.0	1.2 (0.5–2.9)	2.2 (1.0–4.9)	0.029		0.013
Multivariable Cox model[a] ($n = 45$)	1.0	1.2 (0.5–2.9)	2.0 (0.8–4.8)	0.107		0.045
Myocardial infarction						
Age/sex-adjusted Cox model ($n = 43$)	1.0	2.3 (0.8–6.6)	4.0 (1.5–10.5)	0.003		0.001
Multivariable Cox model[a] ($n = 43$)	1.0	2.4 (0.8–7.1)	3.6 (1.2–10.3)	0.017		0.005



Squares and lines are hazard ratios (HRs) and 95% confidence intervals. HRs were derived from Cox models and calculated for Lp-PLA$_2$ activity tertile groups (left-hand columns) and for a 1-SD unit increase in Lp-PLA$_2$ activity (right-hand columns). Primary composite endpoint: stroke, TIA, myocardial infarction, and vascular death. In all analysis, only the first outcome event occurring in study participants was considered while potential further or recurrent events were censored.

[a]Multivariable adjustment: age, sex, previous cardiovascular disease, systolic blood pressure, smoking, diabetes, ferritin level, fibrinogen level, LDL and HDL cholesterol, waist-to-hip ratio, alcohol consumption, social status, sports activity, and loge-transformed levels of HOMA-IR, lipoprotein(a), C-reactive protein, and urinary albumin.

[†]Primary composite CVD endpoint + revascularization procedures.

[‡]Primary composite CVD endpoint + revascularization procedures + new onset symptomatic peripheral artery disease.

specific effect on the vessel wall; second, it identifies plasma ferritin levels, a measure of body and macrophage iron content and a potent pro-oxidant,[26,27] as a potential modulator of Lp-PAL2 activity; third, it demonstrates an inverse correlation between anti-oxidant levels (normalized to LDL-C) and HDL-C with Lp-PLA2 activity; fourth, it confirms a significant relationship of Lp-PLA2 with the metabolic syndrome (AHA-NHLBI criteria), the number of metabolic syndrome parameters and level of HOMA-IR. In total, these data support an integral role of Lp-PLA2 activity in lipid peroxidation and cardiovascular risk.

The totality of published reports on Lp-PLA2, measured either as mass or activity, suggest that it is significantly related to future CVD with hazard ratios of 1.5–2.0, but with a modest attenuation of risk following multivariable analysis. The current study, which adjusted for age, sex, previous CVD, systolic blood pressure, smoking, diabetes, ferritin, fibrinogen, LDL and HDL cholesterol, waist-to-hip ratio, alcohol consumption, social status, sports activity, HOMA-IR, lipoprotein(a), C-reactive protein, and urinary albumin, confirms a similar role of Lp-PLA2 in predicting CVD. Importantly, we also documented that baseline Lp-PLA2 activity was associated with vascular but not non-vascular death (*Figure 1*). The relationship between Lp-PLA2 and CVD risk was noted in most subgroups tested, including patients in various tertile groups for LDL-C and hs-C-reactive protein, and different FRS estimates. These data are consistent with studies in subjects with sudden death or undergoing carotid endarterectomy that have reported prominent expression of Lp-PLA2 within the necrotic core and surrounding macrophages of vulnerable and ruptured plaques and apoptotic cells.[28,29] Furthermore, a correlation of atheroma volume documented by IVUS with Lp-PLA2 mass measured in the coronary sinus has been documented.[30] These findings suggest that Lp-PLA2 is intimately associated with the transition of such plaques to clinical events.

Like with most other novel markers of vascular risk, no significant global increase in model performance (*Figure 3*) was observed upon addition of Lp-PLA2 activity to a standard prediction model (based on the Framingham risk function). The net reclassification improvement was 9.5% but fell short of significance. There is some promise, however, for a clinical utility in subgroups especially subjects at moderate risk or in combination with other novel risk markers. A large-scale meta-analysis is currently underway to resolve this issue.

The determinants of plasma Lp-PLA2 mass and activity levels are not fully known. In this study, variables primarily related to lipoproteins, oxidation, and insulin resistance were correlated with

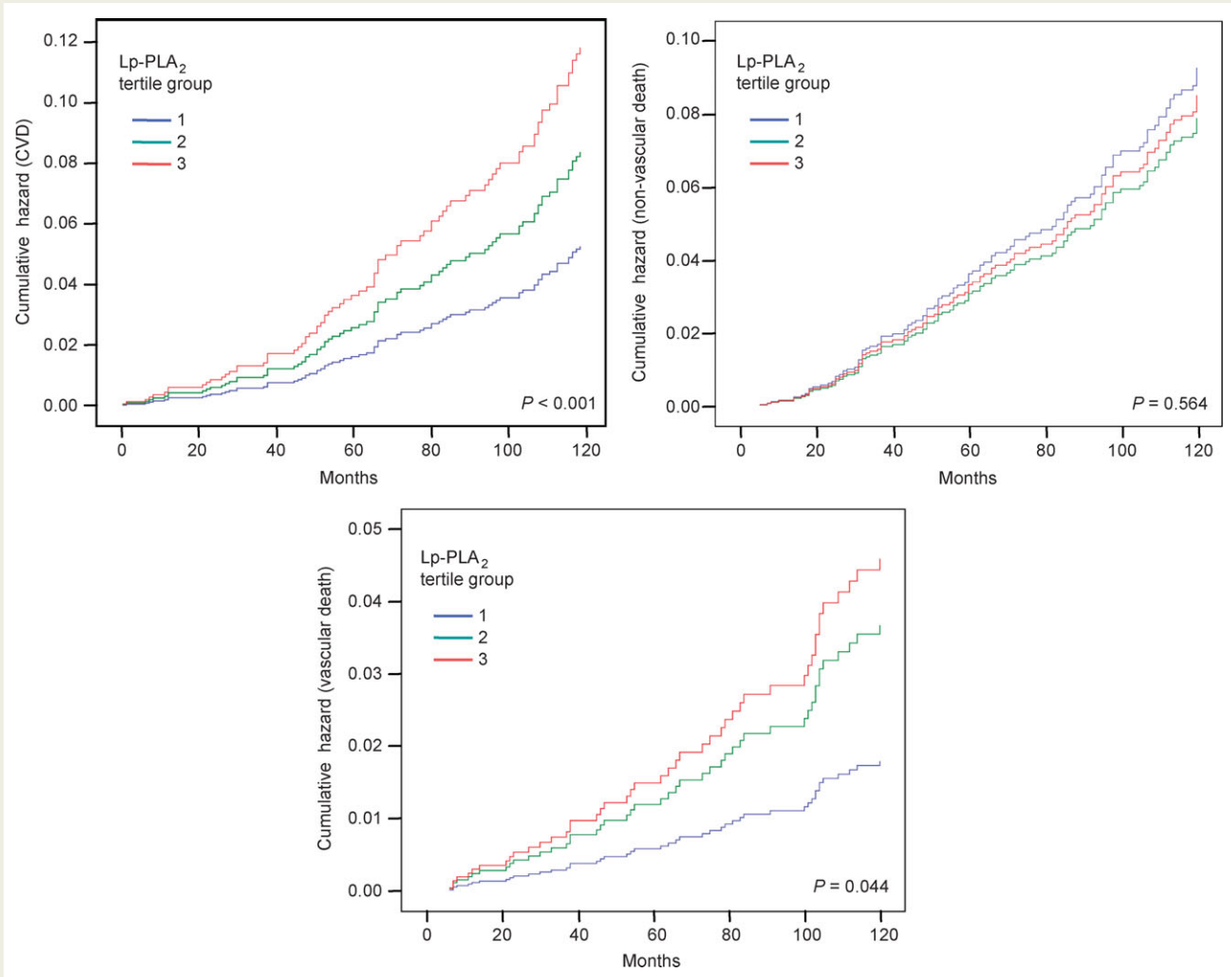


Figure 1 Cumulative hazard curves of cardiovascular disease (CVD), CVD death, and non-CVD death for tertiles of Lp-PLA2 activity (multivariable adjustment).

Lp-PLA2 activity. In particular, a positive association was noted between Lp-PLA2 activity and LDL-C, apoB-100, ferritin, and HOMA-IR, but an inverse association with HDL-C and anti-oxidant levels. Although a cause and effect relationship cannot be determined from this study, it does suggest that Lp-PLA2 activity may be influenced by the underlying atherogenic and pro-oxidant milieu. Interestingly, well-accepted risk factors such as hypertension, diabetes, and smoking and life-style behaviours all were not correlated with Lp-PLA2 activity.

There is compelling evidence that levels of LDL-C correlate with Lp-PLA2 mass and activity. Although it is known that Lp-PLA2 is released by inflammatory cells and then attaches itself to apoB-100 through a specific non-covalent interaction, the factors that mediate changes in Lp-PLA2 have not been determined. In most subjects, the majority of Lp-PLA2 mass is found on LDL-C and a relatively small amount (~5%) is found on HDL.[31] Interestingly, lipoprotein(a) particles carry proportionally more Lp-PLA2 mass (1.5-fold) and activity (up to 7-fold) compared to equimolar amounts of LDL in the same subjects (reviewed by Tsimikas *et al.*[32]). In fact, a recent study from the Bruneck population showed that subjects in the highest tertile of both Lp-PLA2 activity and of oxidized phospholipids on apolipoprotein B-100 particles (OxPL/apoB) have nearly double the risk of future cardiovascular events compared to subjects in the highest tertile of each factor individually.[24] This suggests that a pro-oxidant environment where both substrate (OxPL) and enzyme (Lp-PLA2) activity levels are elevated results in a higher risk compared to each factor alone. Additionally, lipid lowering drugs have differential effects on LDL- and HDL-associated Lp-PLA2 activity. For example, whereas ezetimibe, rosuvastatin, and fenofibrate all reduce non-HDL-associated Lp-PLA2 mass and activity, only fenofibrate increases HDL-associated Lp-PLA2 mass and activity.[33] These data suggest that additional information on the pathophysiological role of Lp-PLA2 may be obtained by evaluating both its interaction with individual lipoproteins and makes a strong rationale for further research in this area.

This is the first study to document that ferritin levels positively correlate with Lp-PLA2 activity and that HDL-C and anti-oxidant

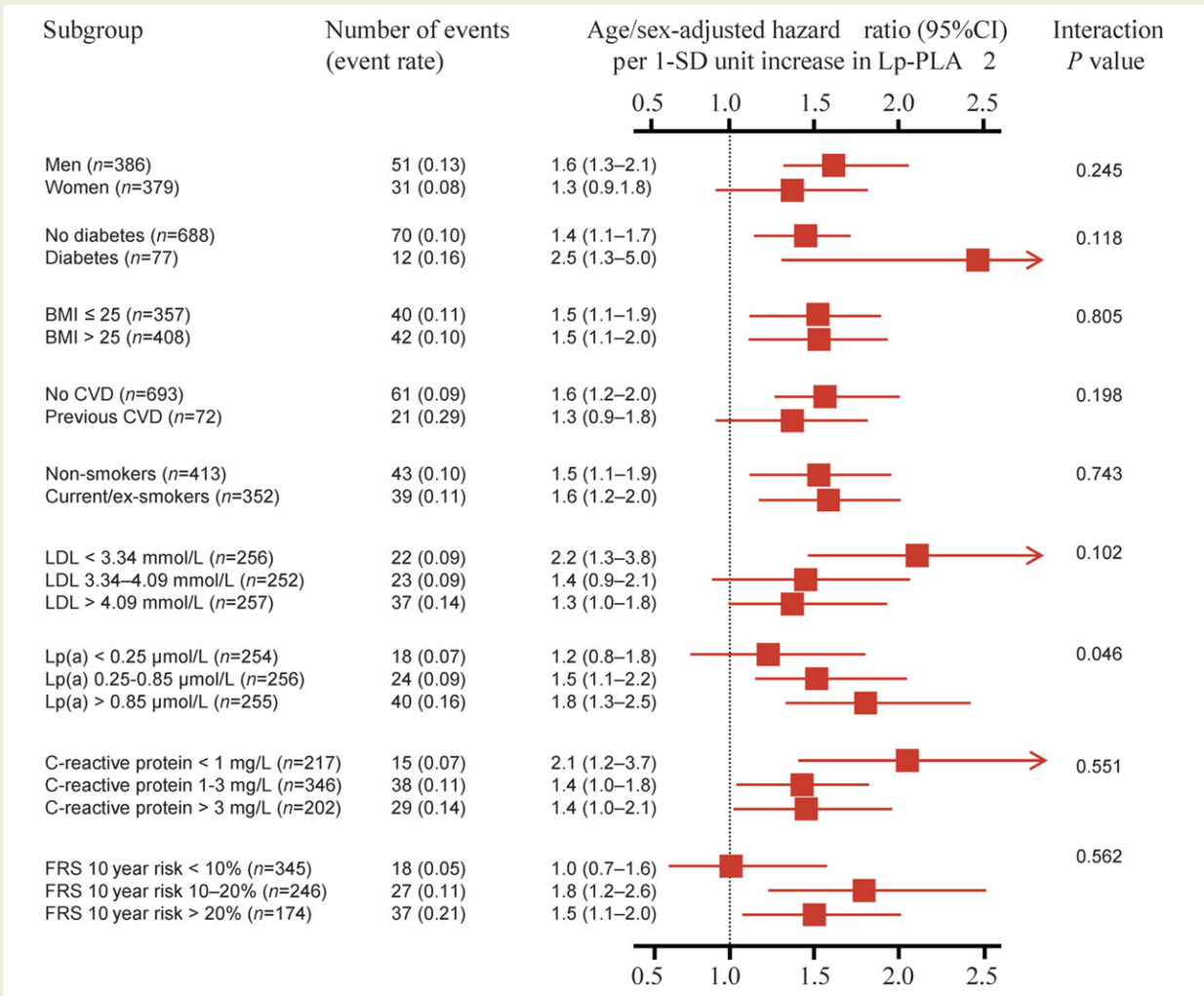


Figure 2 Age- and sex-adjusted hazard ratio (95%CI) per 1-SD unit increment in Lp-PLA2 activity for various subgroups. FRS, Framingham Risk Score for 10-year risk.

levels are inversely associated. Additionally, this study confirms the strong association between Lp-PLA2 levels and the metabolic syndrome.[34,35] Ferritin is the main intracellular storage protein of iron. Heavy metal cations, such as iron and copper, are strong catalysts for oxidation of lipoproteins resulting in a large number of biological properties that could in principle make oxidized LDL proatherogenic.[36] Haem, an iron complex with protoporphyrin IX, is also a strong LDL-oxidizing agent, especially when activated by low concentrations of peroxides. Small amounts of haemoglobin are constantly leaking from damaged erythrocytes, particularly in the vascular regions with turbulent flow, such as vessel bifurcations and aortic curvatures. Haemoglobin-induced LDL oxidation has been suggested to significantly contribute to the increased levels of OxLDL found in the plasma of the patients on haemodialysis.[37]

Prior studies have primarily focused on Lp-PLA2 mass, which is now clinically available as an adjunct to clinical risk prediction.[38] In this study, we measured Lp-PLA2 activity, which is only available as a research assay. It has not been established whether Lp-PLA2 mass or Lp-PLA2 activity provide similar, complementary, or independent information. In published studies where both mass and activity were measured,[15,22,35] only a modest correlation was noted between Lp-PLA2 mass and activity. Future studies will be important to ascertain the role of each measure, particularly with the current clinical trials of Lp-PLA2 inhibitors to reduce cardiovascular risk.[39]

Limitations

Although this is a prospective study with 10-year follow-up, the number of events was limited. However, the cohort is extremely well characterized with 100% ascertainment of follow-up. Many of the associations with Lp-PLA2 were modest in strength, although highly statistically significant. These observations need to be verified in future studies.

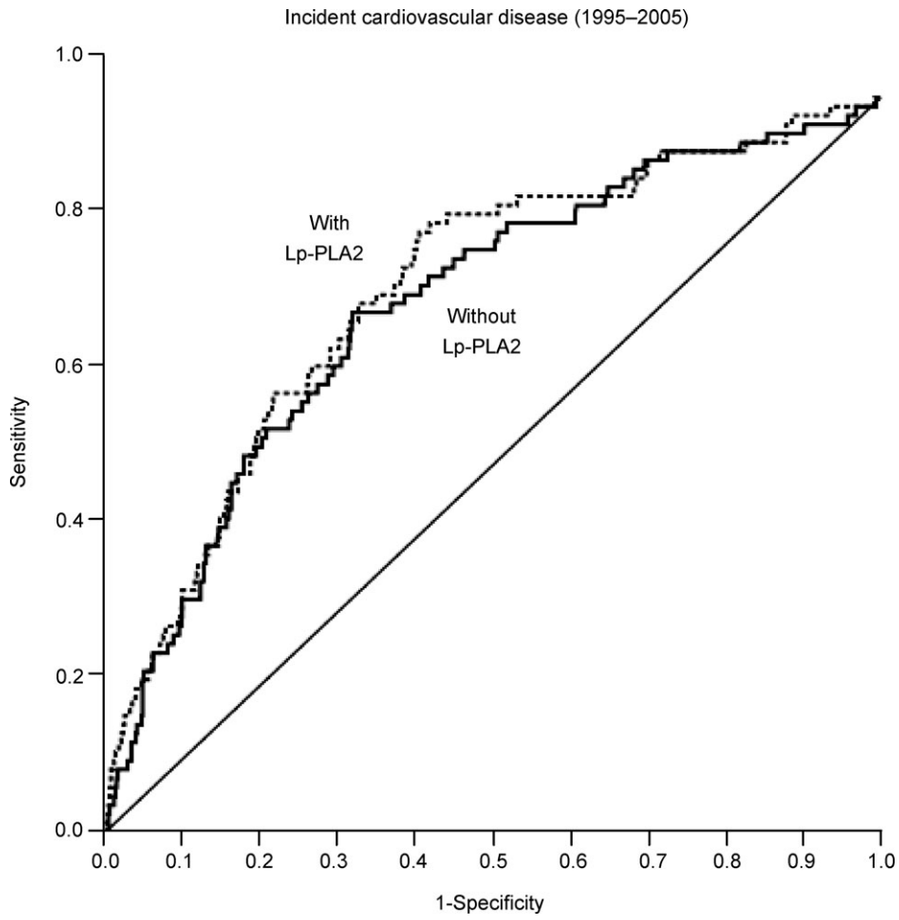


Figure 3 Receiver–operating characteristic (ROC) curves for incident cardiovascular disease (1995–2005). Curves are based on models of prediction of risk using conventional risk variables (Framingham Risk Score) with and without the level of Lp-PLA2 activity.

Conclusions

This study demonstrates that elevated Lp-PLA2 activity levels are associated with CVD and vascular death, but not non-vascular mortality. Our data also suggest an interaction between Lp-PLA2 levels and increased susceptibility to oxidation, as manifested by increased ferritin levels, low HDL-C, and diminished anti-oxidant vitamin levels, in a milieu of pro-oxidant stress manifested by increased substrate (LDL-C) and insulin resistance. It can be hypothesized that this pro-oxidant state may in fact mediate increases in Lp-PLA2 levels noted in these patient subsets. These observations link several pathophysiologically related cardiovascular risk factors and provide a basis in further understanding the role of Lp-PLA2 in CVD. Addition of Lp-PLA2 activity to the standard prediction models of vascular risk resulted in a modest (non-significant) gain in model discrimination and calibration and a net reclassification improvement of 9.5%. Further studies are required to establish the utility of Lp-PLA2 activity for routine prediction of cardiovascular risk.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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References

1. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. *Am J Cardiol* 2003;**91**:12A–16A.
2. Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, Vahabzadeh K, Hama S, Hough G, Kamranpour N, Berliner JA, Lusis AJ, Fogelman AM. Thematic review series: The Pathogenesis of atherosclerosis: The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J Lipid Res* 2004;**45**:993–1007.

3. Glass CK, Witztum JL. Atherosclerosis. The road ahead. *Cell* 2001;**104**:503–516.
4. Blankenberg S, Rupprecht HJ, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ, for the Athero Gene Investigations. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med* 2003;**349**:1605–1613.
5. Torzewski M, Ochsenhirt V, Kleschyov AL, Oelze M, Daiber A, Li H, Rossmann H, Tsimikas S, Reifenberg K, Cheng F, Lehr HA, Blankenberg S, Forstermann U, Munzel T, Lackner KJ. Deficiency of glutathione peroxidase-1 accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2007;**27**:850–857.
6. Tsimikas S. Oxidative biomarkers in the diagnosis and prognosis of cardiovascular disease. *Am J Cardiol* 2006;**98**:9P–17P.
7. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. *Arterioscler Thromb Vasc Biol* 2005;**25**:923–931.
8. MacPhee CH, Nelson JJ, Zalewski A. Lipoprotein-associated phospholipase A2 as a target of therapy. *Curr Opin Lipidol* 2005;**16**:442–446.
9. Packard CJ, O'Reilly DSJ, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GDO, Docherty G, Burczak JD. The West of Scotland Coronary Prevention Study Group. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. *N Engl J Med* 2000;**343**:1148–1155.
10. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Arch Intern Med* 2005;**165**:2479–2484.
11. Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population. Results from the 14-year follow-up of a large cohort from southern Germany. *Circulation* 2004;**110**:1903–1908.
12. Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A2 levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. *Eur Heart J* 2005;**26**:137–144.
13. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. *Circulation* 2005;**111**:570–575.
14. Corsetti JP, Rainwater DL, Moss AJ, Zareba W, Sparks CE. High lipoprotein-associated phospholipase A2 is a risk factor for recurrent coronary events in postinfarction patients. *Clin Chem* 2006;**52**:1331–1338.
15. O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, Braunwald E. Lipoprotein-associated phospholipase A2 and Its association with cardiovascular outcomes in patients with acute coronary syndromes in the PROVE IT-TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction) Trial. *Circulation* 2006;**113**:1745–1752.
16. Gerber Y, McConnell JP, Jaffe AS, Weston SA, Killian JM, Roger VL. Lipoprotein-associated phospholipase A2 and prognosis after myocardial infarction in the community. *Arterioscler Thromb Vasc Biol* 2006;**26**:2517–2522.
17. Winkler K, Hoffmann MM, Winkelmann BR, Friedrich I, Schafer G, Seelhorst U, Wellnitz B, Wieland H, Boehm BO, Marz W. Lipoprotein-associated phospholipase A predicts 5-year cardiac mortality independently of established risk factors and adds prognostic information in patients with low and medium high-sensitivity C-reactive protein (the Ludwigshafen Risk and Cardiovascular Health Study). *Clin Chem* 2007;**53**:1440–1447.
18. Sabatine MS, Morrow DA, O'Donoghue M, Jablonksi KA, Rice MM, Solomon S, Rosenberg Y, Domanski MJ, Hsia J. Prognostic utility of lipoprotein-associated phospholipase A2 for cardiovascular outcomes in patients with stable coronary artery disease. *Arterioscler Thromb Vasc Biol* 2007;**27**:2463–2469.
19. Daniels LB, Laughlin GA, Sarno MJ, Bettencourt R, Wolfert RL, Barrett-Connor E. Lipoprotein-associated phospholipase A2 is an independent predictor of incident coronary heart disease in an apparently healthy older population: the Rancho Bernardo Study. *J Am Coll Cardiol* 2008;**51**:913–919.
20. Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. A prospective evaluation of lipoprotein-associated phospholipase A2 levels and the risk of future cardiovascular events in women. *J Am Coll Cardiol* 2001;**38**:1302–1306.
21. Oldgren J, James SK, Siegbahn A, Wallentin L. Lipoprotein-associated phospholipase A2 does not predict mortality or new ischaemic events in acute coronary syndrome patients. *Eur Heart J* 2007;**28**:699–704.
22. Allison MA, Denenberg JO, Nelson JJ, Natarajan L, Criqui MH. The association between lipoprotein-associated phospholipase A2 and cardiovascular disease and total mortality in vascular medicine patients. *J. Vasc Surg* 2007;**46**:500–506.
23. Kiechl S, Willeit J. The natural course of atherosclerosis. Part I: incidence and progression. *Arterioscler Thromb Vasc Biol* 1999;**19**:1484–1490.
24. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Wiedermann CJ, Oberthaler S, Xu Q, Witztum JL, Tsimikas S. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck Study. *Arterioscler Thromb Vasc Biol* 2007;**27**:1788–1795.
25. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C, for the Conference Participants. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;**109**:433–438.
26. Ziouzenkova O, Asatryan L, Akmal M, Tetta C, Wratten ML, Loseto-Wich G, Jurgens G, Heinecke J, Sevanian A. Oxidative cross-linking of ApoB100 and hemoglobin results in low density lipoprotein modification in blood. Relevance to atherogenesis caused by hemodialysis. *J Biol Chem* 1999;**274**:18916–18924.
27. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. *Circulation* 1997;**96**:3300–3307.
28. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, Virmani R. Lipoprotein-associated Phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;**26**:2523–2529.
29. Mannheim D, Herrmann J, Versari D, Gossl M, Meyer FB, McConnell JP, Lerman LO, Lerman A. Enhanced expression of Lp-PLA2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. *Stroke* 2008;**39**:1448–1455.
30. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* 2007;**115**:2715–2721.
31. Saougos VG, Tambaki AP, Kalogirou M, Kostapanos M, Gazi IF, Wolfert RL, Elisaf M, Tselepis AD. Differential effect of hypolipidemic drugs on lipoprotein-associated phospholipase A2. *Arterioscler Thromb Vasc Biol* 2007;**27**:2236–2243.
32. Tsimikas S, Tsironis LD, Tselepis AD. New insights into the role of lipoprotein(a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2007;**27**:2094–2099.
33. Saougos VG, Tambaki AP, Kalogirou M, Kostapanos M, Gazi IF, Wolfert RL, Elisaf M, Tselepis AD. Differential effect of hypolipidemic drugs on lipoprotein-associated phospholipase A2. *Arterioscler Thromb Vasc Biol* 2007;**27**:2236–2243.
34. Rizos E, Tambaki AP, Gazi I, Tselepis AD, Elisaf M. Lipoprotein-associated PAF-acetylhydrolase activity in subjects with the metabolic syndrome. *Prostaglandins Leukot Essent Fatty Acids* 2005;**72**:203–209.
35. Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. *Arterioscler Thromb Vasc Biol* 2007;**27**:1411–1416.
36. Berliner JA, Watson AD. A role for oxidized phospholipids in atherosclerosis. *N Engl J Med* 2005;**353**:9–11.
37. Ziouzenkova O, Asatryan L, Sevanian A. Oxidative stress resulting from hemolysis and formation of catalytically active hemoglobin: protective strategies. *Int J Clin Pharmacol Ther* 1999;**37**:125–132.
38. Lanman RB, Wolfert RL, Fleming JK, Jaffe AS, Roberts WL, Warnick GR, McConnell JP. Lipoprotein-associated phospholipase A2: review and recommendation of a clinical cut point for adults. *Prev Cardiol* 2006;**9**:138–143.
39. Mohler ER III, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnson JL, Zalewski A, for the Darapladib Investigators. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. *J Am Coll Cardiol* 2008;**51**:1632–1641.