The epidemiology of Lp-PLA(2): Distribution and correlation with cardiovascular risk factors in a population-based cohort.

Persson, Margaretha; Nilsson, Jan-Åke; Nelson, Jeanenne J; Hedblad, Bo; Berglund, Göran

Published in:
Atherosclerosis

DOI:
10.1016/j.atherosclerosis.2006.02.016

2007

The Epidemiology of $LpPLA_2$: Distribution and Correlation with Cardiovascular Risk Factors in a Population-based Cohort.

Margaretha Persson, BSc,[1] Jan-Åke Nilsson, BSc,[1]
Jeanenne J Nelson, PhD,[2] Bo Hedblad, MD, PhD,[1]
Göran Berglund, MD, PhD[1]

Department of Medicine, University Hospital Malmö, Sweden;[1] Department of Worldwide Epidemiology, GlaxoSmithKline, Research Triangle Park, NC[2]

Correspondence: Margaretha Persson
Clinical Research unit
University Hospital Malmö
SE-205 02 Malmö
Sweden
e-mail: margaretha.m.persson@skane.se

ABSTRACT

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an enzyme that is produced by inflammatory cells (macrophages, T-lymphocytes and mast cells) and hydrolyzes oxidized phospholipids in LDL. Several epidemiology studies indicate that Lp-PLA_2 appears to be an independent marker of cardiovascular risk. This study was conducted to define the distribution of Lp-PLA_2 in a large population-based cohort and to determine associations between Lp-PLA_2 and other risk factors for CVD. The study group consisted of participants from the Malmö Diet and Cancer study (1992-1994). $LpPLA_2$ (activity and mass) was measured from samples obtained at baseline for 5,402 participants (3,167 women). A strong correlation was observed between $LpPLA_2$ activity and mass in this study (r=0.57). Highest correlations were observed between Lp-PLA_2 activity and LDL (r=0.45) and LDL/HDL ratio (r=0.54) and a strong inverse correlation to HDL (r=-0.31). The correlations between Lp-PLA_2 mass and lipids were not as strong as the correlation between activity and lipids. $LpPLA_2$ activity and mass were correlated with increased ultrasound determined carotid intima-media thickness. We conclude that $LpPLA_2$ is strongly correlated with several cardiovascular risk factors, especially lipid fractions, and with the degree of carotid artery atherosclerosis. However, the measured variables accounted for only 19% and 35% of the variation in Lp-PLA_2 mass and activity respectively.

cardiovascular disease, epidemiology, Lp-PLA_2 , risk factor

INTRODUCTION

Intensive research efforts have been underway for the past decade to improve the understanding of vascular biology and the pathogenesis of atherosclerosis and atherothrombosis, which are the underlying culprits associated with myocardial infarction, stroke, and other occlusive vascular diseases. One of the great achievements of these efforts is the recognition of atherosclerosis as an inflammatory disease. Inflammation is present at every stage of atherosclerosis—initiation, progression, and destabilization of atheroma—and mechanistic studies have identified blood-borne inflammatory cells and their products as primary drivers of the inflammatory processes (1, 2). With this information, several epidemiologic studies were initiated to explore components of the inflammatory processes and their association with acute cardiovascular events. Examples of inflammatory risk markers that have been studied include interleukin-1, interleukin-6, tumor necrosis factor, interferon-, serum amyloid A, leukocyte count, fibrinogen, and C-reactive protein (CRP), and the list is growing (1, 3).

Lipoprotein-associated phospholipase A_2 is a novel inflammatory marker that been studied for a potential association with cardiovascular events. The biology of this enzyme in atherosclerosis has been controversial, with initial studies suggesting an atheroprotective effect through its actions in hydrolyzing polar phosphatidylcholines (particularly platelet-activating factor) (4-6). However, more recent reports suggest the actions of Lp-PLA_2 are proatherogenic (7). In humans, Lp-PLA_2 in circulation is bound predominantly to LDL (8), and oxidative modification to the phospholipid component of LDL provides the substrate for

the enzyme (9, 10). Lp-PLA_2 acts very rapidly following LDL oxidation to hydrolyze one of the fatty acid moieties (sn-2) of the phospholipid to produce two inflammatory molecules—oxidized nonesterified fatty acids and lysophosphatidylcholine (lysoPC) (7). Given that the majority of total body oxidized LDL resides within the intima (versus in circulation), the products of Lp-PLA_2 activity are generated within the vessel wall where the inflammatory processes of atherosclerosis occur. Both oxidized nonesterified fatty acids and lysoPC are highly soluble, diffuse throughout atheroma, and affect the various cell types involved in atherosclerosis (9).

Recent epidemiology studies have examined the role of Lp-PLA_2 in predicting incident cardiovascular events (11-15). The majority of these studies have demonstrated that elevated Lp-PLA_2 (mass or activity) is an independent predictor of future events. There is also emerging evidence that elevated Lp-PLA_2 may confer increased risk for recurrent events in patients with clinically manifested CVD (ie, recurrent events in secondary prevention populations) (16, 17).

However, an extensive examination of the associations of demographic and anthropometric characteristics and other CVD risk factors with plasma Lp-PLA_2 has not been well described in a large, population-based cohort. Therefore, the purpose of this study was to examine the cross-sectional associations of Lp-PLA_2 within the Cardiovascular Cohort of the Malmö Diet and Cancer Study.

METHODS

Subjects

The Malmö Diet and Cancer Study is a population-based prospective cohort study designed to explore the associations between dietary habits and cancer (18). All men and women aged 45-69 years living in the city of Malmö, Sweden was eligible for participation and 28,098 individuals were enrolled in the study. At baseline, anthropometry, blood pressure, hereditary, dietary, socioeconomic, and life style factors were determined and whole blood samples were obtained. Between November 1991 and February 1994, every other participant was randomly invited to participate in a sub-study exploring the associations between cardiovascular risk factors and ultrasound-determined degree of atherosclerosis, hereto known as the Cardiovascular Cohort. This cohort consisted of 6,103 subjects (3,531 women and 2,572 men) and has previously been described in detail (19). Age at baseline examination was between 46 and 68 years of age (mean 58 years). Fasting blood samples were not collected at the baseline visit due to practical reasons. Participants instead were asked to return for a subsequent visit (median time of 8.6 months after the baseline visit) to donate whole blood samples in a fasting state. A total 5,540 of the 6,103 subjects returned to donate samples. Of these participants, sufficient stores of plasma were available from 5,402 (3,167 women and 2,235 men) for the purpose of measuring Lp-PLA_2. In the present study demographic and baseline characteristics of the participants providing the 5,402 samples were nearly identical to the Cardiovascular Cohort as a whole (data not shown).

Measurements

At the baseline examination, each subject was seen by a nurse for anthropometrics, supine blood pressure measurement, non-fasting blood sampling and administration of questionnaires

including hereditary, medical condition, dietary, socioeconomic and life style factors. During this visit, ultrasound examination of the right carotid artery was performed. The carotid bifurcation was imaged for the existence of atherosclerotic plaque, defined as focal thickening of the intima-media layer. *Intima-media thickness* was determined in the far wall of the distal common carotid artery (IMT cca) and in carotid bifurcation (IMT bulb), according to the leading edge principle and using a specially designed computer-assisted image analysis system (20). The examination procedure and image analysis was performed by specially trained and certified sonographers *Plaque score* (values of 0, 1, or 2) was constructed in which 0 corresponded to no visual plaque, 1 corresponded to a visual plaque less than 10 mm^2 and 2 corresponded to $\geq 10mm^2$. *Plaque occurrence was* defined as a focal thickening of intima-media wall more than 1.25mm.

Diabetes mellitus was defined as self-reported physician-diagnosis per questionnaire and current diabetic treatment. *Smoking* habits were classified as current smoker, ex-smoker and never smoker. *Education* was obtained from the questionnaire and classified into one of three groups—9 years or less, 9 to 12 years, or more than 12 years of formal education. In some analyses, a 2 grade scale was used for classifying education, which included 10 years or less or more than 10 years of formal education. *Physical activity* was calculated from questions adapted from the Minnesota Leisure Time Physical Activity Questionnaire including 18 different physical activities, separately for the four seasons. The number of minutes per week of each activity was multiplied with an intensity coefficient (21). *Blood pressure* was

measured once after a 10 min rest in the supine position. *Hypertension* was defined as self-reported physician-diagnosis per questionnaire and current hypertensive treatment. *Blood glucose*, *insulin, HbA1C*, *triglycerides*, *total cholesterol and HDL-cholesterol*, were measured from fasting blood samples, according to standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. The *LDL-cholesterol* concentration was calculated according to Friedewald's formula (22). *HOMA (Homeostasis model Assessment) value* (23) was calculated as (p-insulin x p-glucose)/22.5. The assessment of CRP was performed using the Tina-quant® CRP latex high sensitivity assay (Roche Diagnostics, Basel, Switzerland) on an ADVIA® 1650 Chemistry System (Bayer Healthcare, NY, USA). Study samples were analyzed as discrete samples and results were read in 6-second intervals for a 1-minute time period following incubation for 5 minutes. The mean value of these measurements was the reported result.

Measurement of LpPLA$_2$ mass and activity

Plasma aliquots prepared from fasted blood samples were collected and stored at -80°C. Lp-PLA$_2$ activity was measured using [3H] PAF (platelet activating factor) as substrate using methodology described previously (15). Briefly, plasma (5uL) or assay buffer (for determination of background and total dpm) were transferred into a 96 well flat-bottomed polystyrene plate (Costar) and allowed to equilibrate to room temperature. A 100uL aliquot of [3H] PAF substrate working solution (prepared fresh daily), consisting of 100uM [3H] PAF (0.4M [3H] PAF (Specific Activity 21.5 Ci/mmol, Perkin Elmer Life Sciences) plus 99.6M C16-PAF (Avanti Polar Lipids Inc) in assay buffer (100mM HEPES, 150mM NaCl, 5mM

EDTA, pH7.4) was added to each well and the plate was vortexed and incubated at room temperature for 5 min. The reaction was terminated by addition of 50L ice-cold aqueous bovine serum albumin (BSA) solution (50mg/mL) followed by vortex mixing and incubation for 5min at 4C. Ice-cold trichloroacetic acid (TCA) (56% aqueous solution; 25L) was added to each well, vortexed and incubated for 15min at 4C. Plates were then sealed and centrifuged at ~6,000 x g for 15 min at 4°C, and aliquots of supernatant (45L) were transferred to a picoplate (Perkin Elmer). To determine total dpm added, 10L [3H] PAF substrate working solution was added to wells containing buffer instead of plasma. Some wells were left blank to determine background hydrolysis. Microscint-20 (200L; Perkin Elmer Life Sciences) was added to all wells, plates were sealed and vortex mixed for 10 min. The plates were counted in a Topcount liquid scintillation counter (Perkin Elmer Life Sciences) and LpPLA$_2$ activity values (nmol PAF hydrolysed/min/mL) were derived from the raw data. Lp-PLA$_2$ mass measurements were performed using the PLAC™ Test (diaDexus Inc., South San Francisco, CA, USA). In brief, the test resembles a sandwich enzyme immunoassay with two specific monoclonal antibodies as described by Caslake et al (24) combined with a horseradish-peroxidase (HRP) – tetramethylbenzidine (TMB) detection system. The change in absorbance resulting from the enzymatic turnover of the substrate was measured spectrophotometrically (Wallac, now Perkin Elmer Inc, Boston, MA, USA) and is directly proportional to the concentration of Lp-PLA$_2$ present in the study sample. A 6-point standard curve with known Lp-PLA$_2$ quantities, provided by the manufacturer, was employed to determine Lp-PLA$_2$ concentration from the study samples. All samples were analyzed in

duplicates and a duplicate was expected to show a coefficient of variation of less than 20 percent.

Statistical Methods

The distributions of triglycerides, glucose, insulin, HOMA, HbA_1C, hsCRP and physical activity were markedly skewed and therefore were log-transformed. Means (medians for skewed variables) and percentages for baseline demographic and clinical characteristics were computed for the entire cohort and by sex. For continuous factors, we assessed correlation of Lp-PLA_2 with variables three ways with use of Pearson's or Spearman's correlation coefficient: partially adjusted for age and sex, partially adjusted for age, sex and LDL-level, and partially adjusted for age, stratified by sex. Further, we divided continuous factors into tertiles and computed mean Lp-PLA_2 level within each tertile, stratified by sex. Mean Lp-PLA_2 was computed and adjusted for age and sex by analysis of covariance for key categorical risk factors. Differences between categories were calculated and expressed with 95% confidence intervals. A general linear model was used to examine the incremental influence (cumulative R^2) of risk factors for the degree of explanation of the variation in Lp-PLA_2. All other analyses were performed using SPSS 11.0.

RESULTS

The baseline demographics of the Cardiovascular Cohort are listed in Table 1. Fifty-nine percent of participants were women and participants were between 46 and 68 years of age (mean 58). There was a low prevalence of CV events at baseline (4 percent). LDL levels were high (mean 4.2 mmol/L (161 mg/dL)) and HDL levels were moderately high (mean 1.4

mmol/L (54 mg/dL)), as is common in other northern European populations. Of the participants who self-reported having diabetes, hypertension, or hypercholesterolemia, 1.6 percent, 13 percent, and 2.4 percent, respectively, received pharmacologic therapy at baseline. Fourteen percent of women participating in this study were receiving hormone replacement therapy at baseline.

The distributions of Lp-PLA_2 activity and mass in the study population were approximately normal with a slight right skewness (Figures 1A and 1B). Mean (SD) Lp-PLA_2 activity was 45.5 (13.1) nmol/min/mL; mean Lp-PLA_2 mass was 269.8 (80.7) ng/mL. The correlation between Lp-PLA_2 activity and mass was r=0.57 (p<.0001), with a tendency toward greater variability at higher levels (Figure 2).

Associations between LpPLA_2 Activity/ Mass and Cardiovascular Risk Factors

The correlations between Lp-PLA_2 activity and mass and cardiovascular risk factors are listed in Table 2 and Table 3A, 3B. Lp-PLA_2 activity was positively associated with age (r=0.11; p<0.0001) (Table 2) a correlation that was stronger in women (r=0.15) (Table 3A) than in men (r=0.06) (Table 3B). However, in an LDL-adjusted model, the association between Lp-PLA_2 and age was approximately r=0.03, which suggests that the association is dependent on age-related changes in LDL. Both Lp-PLA_2 activity and mass were significantly higher in men than in women (49.6 ± 13.3 versus 42.5 ± 12.0 nmol/min/ml; p<0.0001 and 287.7 versus 257.2 ng/mL; p<0.0001); therefore, the description and associations with other cardiovascular risk factors were analyzed separately by sex.

Of note, Lp-PLA_2 activity was not correlated with hsCRP in either men or women. Plasma Lp-PLA_2 activity increased with a greater extent of ultrasound-determined atherosclerosis. For women, Lp-PLA_2 activity was approximately 41 nmol/min/mL in the lowest tertile of IMT in the common carotid artery or bulb, 42 nmol/min/mL in the second tertile and 44 nmol/min/mL in the highest tertile. There was a monotonic increase in mean Lp-PLA_2 activity values across plaque score, with mean Lp-PLA_2 activity of 41 nmol/min/mL, 43 nmol/min/mL, and 45 nmol/min/mL among participants with a plaque score of 0, 1 or 2, respectively. A similar pattern of IMT, plaque score and Lp-PLA_2 was observed for men (Table 3A and 3B). Lp-PLA_2 mass also increased with a greater extent of ultrasound-determined atherosclerosis with almost the same pattern as Lp-PLA_2 activity (data not shown).

Lp-PLA_2 Activity in Clinical Subgroups

After adjusting for age and sex, current smokers had significantly higher Lp-PLA_2 activity compared with non-smokers, and enzyme activity also was higher in participants with plaque (Table 4). Participants with more years of formal education had significantly lower Lp-PLA_2 activity compared to those with less education. Mean Lp-PLA_2 activity was higher among the 216 participants with a history of CVD (heart disease or stroke) compared to those without prevalent CVD (48 vs. 45 nmol/min/mL; p=0.006) but this difference was eliminated after accounting for age and gender. Lp-PLA_2 activity was higher among participants with compared to without diabetes and higher among obese compared to non-obese participants, but the differences were not statistically significant. There was only marginal difference in

Lp-PLA_2 activity between hypertensives and normotensives. The findings for Lp-PLA_2 mass are similar to those for Lp-PLA_2 activity with significant higher mass levels among smokers compared to non-smokers, in participants with plaque and in those with lower education (data not shown).

Lp-PLA_2 Variability

To assess how completely known CV risk factors explain the overall variability of Lp-PLA_2, parameters were fit in a generalized linear model and the R^2 was calculated. The measured variables explained significantly more of the variation in Lp-PLA_2 activity than in Lp-PLA_2 mass (cumulative R^2=0.34 versus R^2=0.19) (Table 5). The strongest factors explaining variation in the Lp-PLA_2 activity were LDL, HDL, and sex. For mass the corresponding factors were LDL and sex, but not HDL. Sex explained 7 percent of the variation in Lp-PLA_2 activity and 5 percent of the variation in Lp-PLA_2 mass.

DISCUSSION

Lp-PLA_2 has been demonstrated to independently predicted future cardiovascular events in several epidemiology studies (11-15), and the present findings provide valuable insight regarding correlations between this novel inflammatory marker and conventional risk factors. Lower Lp-PLA_2 (mass and activity) among women (compared with men) has been a consistent finding among these epidemiology studies, including the present study. When we further adjusted mean Lp-PLA_2 levels for its primary carrier, LDL, Lp-PLA_2 levels remained lower among women compared with men. There is evidence to suggest that estrogen down-

regulates Lp-PLA_2 expression and subsequently activity (25). Clearly, there is a need to identify the factors that regulate the expression of Lp-PLA_2 in men and women.

Another important finding from the present study is the strong correlation between Lp-PLA_2 and LDL, which is not unexpected, given that LDL acts as a functional reservoir for Lp-PLA_2 in circulation. A strong positive correlation has been demonstrated consistently with LDL, total cholesterol, or non-HDL cholesterol in epidemiology studies. In the present study, an inverse correlation with HDL was observed, which is similar to what was observed in the Women's Health Study (12), ARIC (13), and Rotterdam (15) studies but not in WOSCOP (11) and MONICA (14). These differences could be explained, in part, by gender composition in the different study populations, with WOSCOP and MONICA comprised entirely of male participants and in the restricted LDL range in WOSCOP. It is noteworthy that Lp-PLA_2 mass but not activity appears to be weakly correlated with hsCRP. This bears further elucidation. The lack of correlation between Lp-PLA_2 activity and hsCRP is consistent with other studies (11-14), suggesting that the two risk markers may have an additive effect on predicting cardiovascular risk for individual patients.

In at least one study, a strong correlation between enzyme mass and activity has been reported ($r \geq 0.8$) (26), but the observed correlation was much lower in the present study ($r=0.57$). The stronger degree of explanation from the measured variables on Lp-PLA_2 activity than mass might indicate that activity is more closely linked to the mechanisms that ultimately lead to atherosclerosis and atherothrombosis. Alternatively, the discrepancy could be due to differences in the methodology for measuring Lp-PLA_2 activity and mass. Future studies

should try to identify the factors (eg genetic, biomolecular, or assay) that could explain discrepancies between mass and activity measurements, especially at higher values (Figure 2). Another intriguing finding is that, after adjusting for age and gender, a weak correlation was observed between Lp-PLA_2 and cardiovascular risk factors associated with an excessive inflammatory burden. For example, a correlation was observed between certain markers of Type 2 diabetes (eg, insulin, HOMA, and glucose, but not HbA_{1c}) in both men and women. Body mass index was correlated with Lp-PLA_2 in men, but such a correlation was not observed in WOSCOP (11) or MONICA (14), studies that included only men. A relationship between Lp-PLA_2 and body mass index was not apparent in women in the present study. Smoking—which is also associated with excessive inflammatory burden—also was positively correlated with Lp-PLA_2 activity. These data are confirmed by findings from the ARIC study (13) but not by the WOSCOP (11), MONICA (14) or Rotterdam (15) studies.

Whether the oxidative stress associated with diabetes and smoking is directly attributable, in part, to Lp-PLA_2 activity is not known. In a recent study involving an animal model of diabetes and hypercholesterolemia, the expression of Lp-PLA_2 by bone-marrow derived leukocytes was significantly up-regulated in the presence of advanced glycation endproducts (27). However, the contribution of components of the metabolic syndrome to the variation in Lp-PLA_2 needs to be explored further. Our findings with continuous parameters of metabolic disorders are compatible with an impact of deteriorated glucose metabolism increasing the level of the Lp-PLA_2 enzyme activity. However, in our study, participants with diabetes did not markedly differ in their Lp-PLA_2 levels from participants without diabetes, possibly due to how we defined diabetics as self-reported physician-diagnosis (questionnaire) and currently

receiving treatment, which may be a conservative definition. Thus, additional studies are needed to explore associations with metabolic disorders.

In the present study, both plasma Lp-PLA_2 activity and mass was correlated with the extent of carotid artery atherosclerosis. This was also observed in the CARDIA study in which plaque burden was measured by electron-beam computer topography coronary artery calcification (28). Although a causal relationship between enzyme activity and atherosclerosis is not possible to establish in this cross-sectional study, it is conceivable that Lp-PLA_2 activity is involved in the very complex processes leading to atherosclerosis and atherothrombosis. In the present study, the measured variables could only explain 35 percent of the variation in plasma Lp-PLA_2 activity and only 19 percent in plasma Lp-PLA_2 mass, suggesting that Lp-PLA_2 may be linked to atherosclerosis and cardiovascular events by different pathophysiologic mechanisms than with conventional cardiovascular risk factors. However, as mentioned above, the putative proatherogenic effects of Lp-PLA_2 are the result of enzyme activity within the intima and not within plasma.

From an epidemiologic viewpoint, this study has several advantages—it is a large, cross-sectional cohort comprising 5,402 apparently healthy men and women with a homogeny ethnic composition. Other studies measuring Lp-PLA_2 have included fewer subjects or have used a nested case-control or case-cohort design for the purpose of evaluating whether Lp-PLA_2 could predict incident cardiovascular events. In the present study we have measured both Lp-PLA_2 activity and mass, which gives a broader basis for understanding how Lp-PLA_2 interacts with other cardiovascular risk factors. Subjects with diabetes, hypertension, or

prevalent cardiovascular disease participated in this study, including those treated with statins, antihypertensive drugs and diuretics.

The primary limitation of this study is that the attendance rate that was only 41 percent, and the study participants were healthier and were at substantially lower risk compared with non-participants (29, 30). Patients were identified as having hypertension and diabetes through self-reporting (ie, questionnaires) and description of current medications. Blood glucose and blood pressure were obtained at only one visit. However these shortcomings tend to decrease the associations studied.

In conclusion, Lp-PLA_2 is a novel cardiovascular risk marker that is correlated with other factors associated with an increased risk for cardiovascular events, particularly LDL. Although the measured variables accounted for only a small percentage of the variation in plasma Lp-PLA_2 activity and mass, Lp-PLA_2 was positively correlated with the extent of carotid artery atherosclerosis. Clearly, more studies are needed to better define a potential causal role for Lp-PLA_2 in atherosclerosis and atherothrombosis. However, this enzyme may have clinical implications as a means to help identify high-risk individuals and as a potential therapeutic target.

Acknowledgment

This study was supported by grants from The Swedish Scientific Council, The Swedish Cancer Society, The Region of Skane, The Swedish Heart and Lung Foundation, and GlaxoSmithKline.

REFERENCES

1. Hansson G. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685-1695.

2. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation.* 2002;105:1135-1143.

3. Wilson PW. CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice. Ability of inflammatory markers to predict disease in asymptomatic patients: a background paper. *Circulation*. 2004;110:e568-571.

4. Tjoelker LW, Wilder C, Eberhardt C, et al. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature*. 1995;374:549-553.

5. Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor acetylhydrolases. *J Biol Chem*. 1997;272:17895-17898.

6. Watson AD, Navab M, Hama SY, et al. Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. *J Clin Invest*. 1995;95:774-782.

7. Macphee CH, Moores K, Boyd H et al. The lipoprotein-associated phospholipase A2 generates two bioactive products during the oxidation of low density lipoprotein. Studies using a novel inhibitor. *Biochem J.* 1999.338;479-487.

8. Stafforini DM, Tjoelker LW, McCormick SP, et al. Molecular basis of the interaction between plasma platelet-activating factor acetylhydrolase and low density lipoprotein. *J Biol Chem*. 1999;274:7018-7024.

9. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis. Biology, epidemiology, and possible therapeutic target. *Arterioscler Thromb Vasc Biol*. 2005;24;[Epub ahead of print].

10. Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J*. 2001;15:2073-2084.

11. Packard CJ, O'Reilly DSJ, Caslake MJ, et al. Lipoprotein-associated phospholipase A2, as an independent predictor of coronary heart disease. *N Eng J Med* 2000; 343:1148-1155.

12. Blake GJ, Dada N, Fox JC, et al. 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.

13. Ballantyne C, Hoogeveen R, Bank H, et al. Lipoprotein-associated phospholipase A2, high sensitive c-reactive protein and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation.* 2004;109:837-842.

14. Koenig W, Khuseyinova N, Löwel H, Trischler G, Meisinger C. Lipoprotein-Associated Phospholipase A2 Adds to Risk Prediction of Incident Coronary Events by C-Reactive Protein in Apparantly 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.

15. 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(5):570-5.

16. Blankenberg S, Stengel D, Rupprecht HJ, et al. Plasma PAF-acetylhydrolase in patients with coronary artery disease: results of a cross-sectional analysis. *J Lipid Res*. 2003;44:1381-1386.

17. 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.

18. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. *J Intern Med.* 1993 Jan;233(1):45-51.

19. Rosvall M, Östergren P O, Hedblad B, Isacsson S-O, Janzon L, Berglund G. Occupational Status, Education Level, and the Prevalence of carotid Athesclerosis in a General Population Sample of Middle-aged Swedish Men an Women: Results from the Malmö Diet and Cancer Study. *Am J Epidemiol.* 2000;152:334-46.

20. Wendelhag I, Gustavsson T, Suurkûla M, Berglund G, Wikstarnd J. Ultrasound measurement of wall thickness in the Carotid artery. Fundamental principles and description of a computerized image analyzing system. *Clin Physiology* 1991;11:565-7.

21. Taylor HL, Jacobs DR, Jr., Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. *J Chronic Dis* 1978;31:741-55.

22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultra centrifuge. *Clin Chem.* 1971;18:499-502.

23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turn RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetiologia.* 1985 Jul;28(7):412-9.

24. Caslake MJ, Packard CJ, Sucking KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydralase: a potential new risk factor for coronary artery disease. *Athesclerosis*. 2000 Jun;150(2):413-9.

25. Miyaura S, Maki N, Byrd W, Johnston JM. The hormonal regulation of platelet-activating factor acetylhydrolase activity in plasma. Lipids. 1991;26:1015-1020.

26. Dada N, Kim NW, Wolfert RL. LpPLA2: an emerging biomarker of coronary heart disease. *Expert Rev Mol Diagn.* 2002;2:17-22.

27. Zhang P, Zhang L, Wilensky RL, et al. Advanced glycation endproducts upregulate expression of lipoprotein-associated phospholipase A2 in peripheral blood mononuclear cells [abstract 736-P]. Presented at: 65th Annual Scientific Sessions of the American Diabetes Association. June 10-14, 2005. San Diego, California..

28. Iribarren C, Gross MD, Darbinian JA, et al. Association of Lipoprotein-Associated Phospholipase A2 Mass and Activity with Calcified Coronary Plaque in Young Adults: the CARDIA Study. *Arterioscler Thromb Vasc Biol*, 2005;25:1-7.

29. Manjer J, Elmståhl S, Janzon L, Berglund G. Invitation to a population-based cohort study: differences between subjects recruited using various strategies. *Scand J Public Health*. 2002;30:103-112.

30. Manjer J, Carlsson S, Elmståhl S, Gullberg B, Janzon L, Lindström M, Mattisson I, Berglund G. The Malmo Diet and Cancer Study: represantativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev.* 2001;10:489-499.

LEGENDS

Figure 1A: Distribution of Lp-PLA2 activity in the cohort.

Mean = mean value of Lp-PLA2 activity in the cohort (nmol/min/mL)

SD = standard deviation

n =number included

Figure 1B: Distribution of Lp-PLA2 mass in the cohort.

Mean = mean value of Lp-PLA2 mass in the cohort (ng/mL)

SD = standard deviation

n =number included

Figure 2: Correlation between Lp-PLA2 activity and mass.

n =number included

r = correlations coefficient

p = p-value

Table 1. Baseline Characteristics in the Total Cohort and By Gender

Variable	Total (n=5402)	Men (n=2235)	Women (n=3167)
Age (years)	57.6	57.7	57.5
BMI (kg/m^2)	25.7	26.2	25.4
SBP (mmHg)	141	143	140
DBP (mmHg)	87	89	86
Cholesterol (mmol/L)	6.2	6.0	6.3
LDL (mmol/L)	4.2	4.1	4.2
HDL (mmol/L)	1.4	1.2	1.5
LDL/HDL ratio	3.2	3.6	3.0
Triglycerides (mmol/L)*	1.2	1.3	1.1
Blood glucose (mmol/L)*	4.9	5.1	4.8
Insulin (mlU/L)*	7.0	7.0	6.0
HbA1C (%)*	4.8	4.8	4.8
HOMA*	1.3	1.5	1.3
hsCRP (mg/mL)*	0.14	0.14	0.14
IMT cca (mm)	0.74	0.77	0.72
IMT bulb (mm)	1.42	1.50	1.35
LpPLA2 (nmol/min/mL)	45.5	49.6	42.5
LpPLA2 mass (ng/mL)	269.8	287.7	257.2
Smoker (%)	26	27	25
Hypertension (%)	13	15	13
Diabetes (%)	2	2.4	1.5
Plaque (%)	59	62	57
Hx of MI or Stroke (%)	4.1	6.6	2.4
Hx of MI (%)	2.7	4.6	1.4
Hx of Stroke (%)	1.7	2.5	1.1

* Median values; otherwise, mean values or percentages

Table 2. Correlation of Lp-PLA2 Activity or Mass with Cardiovascular Risk Factors

Variable	Age, gender-adjusted r		Age, gender, LDL-adjusted r	
	Lp-PLA_2 Activity	Lp-PLA_2 Mass	Lp-PLA_2 Activity	Lp-PLA_2 Mass
Age (years)	0.11[a]	0.12[a]	0.03[c]	0.06[a]
Sex	0.27[a]	0.19[a]	0.32[a]	0.21[a]
BMI (kg/m^2)	0.04[b]	0.01[c]	0.00[c]	-0.02[c]
Smoking	0.06[a]	0.12[a]	0.06[a]	0.12[a]
Education	0.07[a]	0.07[a]	0.05[b]	0.06[a]
Physical activity	-0.04[c]	-0.04[c]	-0.03[c]	-0.04[c]
SBP (mmHg)	0.03[c]	0.02[c]	0.01[c]	0.01[c]
DBP (mmHg)	0.05[a]	0.01[c]	0.03[c]	0.00[c]
Cholesterol (mmol/L)	0.46[a]	0.30[a]	----	----
LDL (mmol/L)	0.48[a]	0.33[a]	----	----
HDL (mmol/L)	-0.24[a]	-0.09[a]	-0.21[a]	-0.06[a]
LDL/HDL ratio	0.50[a]	0.27[a]	-----	-----
Triglycerides (mmol/L)*	0.26[a]	0.09[a]	0.15[a]	0.00[c]
Blood glucose (mmol/L)*	0.06[a]	0.04[b]	0.02[c]	0.01[c]
Insulin (mlU/L)*	0.10[a]	0.06[a]	0.07[a]	0.03[c]
HbA1C (%)*	0.00[c]	0.03[c]	-0.07[a]	-0.01[c]
HOMA*	0.10[a]	0.07[a]	0.06[a]	0.04[b]
hsCRP (mg/mL)*	0.02[c]	0.10[a]	0.01[c]	0.07[a]
IMT cca (mm)	0.06[a]	0.06[a]	0.02[c]	0.03[c]
IMT bulb (mm)	0.08[a]	0.05[b]	0.06[b]	0.04[c]
Plaque score	0.07[a]	0.08[a]	0.03[c]	0.06[c]

* Log-transformed, Spearman correlation
[a] p-value<0.0001
[b] 0.0001≤p-value<0.01
[c] p-value≥0.01

Table 3A. Mean LpPLA2 Activity by Tertile of Cardiovascular Risk Factors; Age-adjusted Correlation: Women

Risk Factor	Cut Offs	Tertile 1		Tertile 2		Tertile 3		Age-adjusted Correlation	
		Mean	Std	Mean	Std	Mean	Std	r	p-value
Age year	54.2 , 61.1	40.4	11.1	42.3	12.1	44.9	12.5	0.15	<0.0001
Smoking (a)	1,2,3	42.6	12.2	40.9	11.5	44.4	12.1	0.08	<0.0001
Education (b)	1,2,3	43.9	12.1	41.7	12.2	41.0	11.4	-0.08	<0.0001
BMI kg/m^2	23.2 , 26.4	42.3	12.2	41.9	11.6	43.4	12.3	0.02	0.281
Physical activity	4920 , 8800	42.3	11.8	42.9	12.4	42.2	11.9	-0.02	0.239
SBP mmHg	130 , 146	41.0	11.6	43.1	12.5	43.2	11.8	0.01	0.691
DBP mmHg	80 , 90	41.7	11.6	42.7	12.0	43.3	12.5	0.03	0.056
vB-gluc mmol/L	4.6 , 5.0	41.5	11.5	42.3	11.9	44.0	12.7	0.05	0.010
P-Insulin mU/L	5.0 , 7.0	41.3	11.7	41.9	11.3	44.3	13.0	0.09	<0.0001
HOMA	0.96 , 1.56	41.4	11.6	41.8	11.3	44.2	13.1	0.09	<0.0001
HbA1C %	4.6 , 5.0	41.8	12.1	42.4	12.1	43.6	11.9	0.01	0.425
Chol mmol/L	5.7 , 6.7	36.4	9.2	42.2	10.9	49.1	12.4	0.46	<0.0001
LDL mmol/L	3.7 , 4.5	35.5	9.1	42.0	10.2	49.6	12.1	0.51	<0.0001
HDL mmol/L	1.3 , 1.6	46.3	13.0	41.4	11.0	40.0	11.2	-0.23	<0.0001
LDL/HDL ratio	2.4 , 3.3	36.3	9.7	41.6	10.1	49.6	12.3	0.50	<0.0001
Tg mmol/L	0.9 , 1.3	39.6	10.8	41.7	11.0	46.4	13.2	0.24	<0.0001
CRP mg/mL	0.08 , 0.21	41.9	11.9	42.9	12.0	42.8	12.3	0.01	0.603
IMT CCA mm	0.65 , 0.75	41.2	11.8	42.0	11.9	44.4	12.3	0.07	<0.0001
IMT bulb mm	1.04 , 1.41	40.4	11.3	42.3	11.8	44.2	12.4	0.10	<0.0001
Plaque score c)	0,1,2	41.1	11.4	42.9	12.2	45.0	12.5	0.07	<0.0001

a) 1= never, 2= former, 3= current smoker

b) 1= < 9 year 2= 9-12 year, 3= > 12 year education

c) 0= no visual plaque, 1= plaque less than 10mm², 2= >10mm²

Table 3A. Mean LpPLA2 Activity by Tertile of Cardiovascular Risk Factors; Age-adjusted Correlation: Men

Risk Factor	Cut Offs	Tertile 1		Tertile 2		Tertile 3		Age-adjusted Correlation	
		Mean	Std	Mean	Std	Mean	Std	r	p-value
Age year	54.3, 61.1	48.8	13.7	49.1	12.9	50.8	13.4	0.06	<0.0001
Smoking (a)	1, 2, 3	48.5	13.0	49.7	13.5	50.5	13.2	0.06	0.006
Education (b)	1, 2, 3	50.5	14.1	49.2	12.8	47.9	12.1	-0.06	0.007
BMI kg/m^2	24.5, 27.2	48.4	13.0	49.8	12.8	50.4	14.1	0.07	<0.0001
Physical activity	5116, 9268	50.2	14.0	49.7	13.0	49.0	13.0	-0.04	0.051
SBP mmHg	134, 150	48.5	13.0	49.5	13.4	50.6	13.5	0.04	0.033
DBP mmHg	85, 92	48.3	12.8	50.0	13.7	50.5	13.4	0.06	0.007
vB-gluc mmol/L	6, 9	47.3	13.0	49.8	12.5	51.3	14.0	0.12	<0.0001
P-Insulin mU/L	4.8, 5.3	48.5	12.6	50.1	12.6	50.2	14.8	0.06	0.009
HOMA	1.15, 1.85	47.1	12.7	49.9	12.7	51.2	13.5	0.13	<0.0001
HbA1C %	4.6, 5	50.2	13.8	49.4	13.0	49.3	13.2	0.01	0.701
Chol mmol/L	5.6, 6.4	43.4	12.3	49.8	11.9	55.6	13.0	0.42	<0.0001
LDL mmol/L	3.7, 4.5	43.1	11.9	49.9	12.1	55.9	12.7	0.45	<0.0001
HDL mmol/L	1.1, 1.3	53.6	14.2	49.5	12.5	45.6	11.9	-0.26	<0.0001
LDL/HDL ratio	3, 4	42.3	10.7	49.3	11.1	57.1	13.7	0.51	<0.0001
Tg mmol/L	1.1, 1.6	45.0	11.3	49.5	12.4	54.3	14.5	0.29	<0.0001
CRP mg/mL	0.09, 0.22	48.5	12.8	49.6	12.7	50.7	14.5	0.04	0.056
IMT CCA mm	0.68, 0.8	48.5	13.8	49.8	13.1	50.4	13.1	0.05	0.028
IMT bulb mm	1.11, 1.6	47.8	12.2	49.6	12.7	50.5	14.4	0.06	0.017
Plaque score c)	0.1, 2	48.7	12.8	49.3	12.5	51.5	14.9	0.05	0.029

a) 1= never, 2= former, 3= current smoker
b) 1= < 9 year 2= 9-12 year, 3= > 12 year education
c) 0= no visual plaque, 1= plaque less than 10mm², 2= >10mm²

Table 4. Lp-PLA2 Activity by Cardiovascular Disease Risk Factor Status, Adjusted for Age and Gender

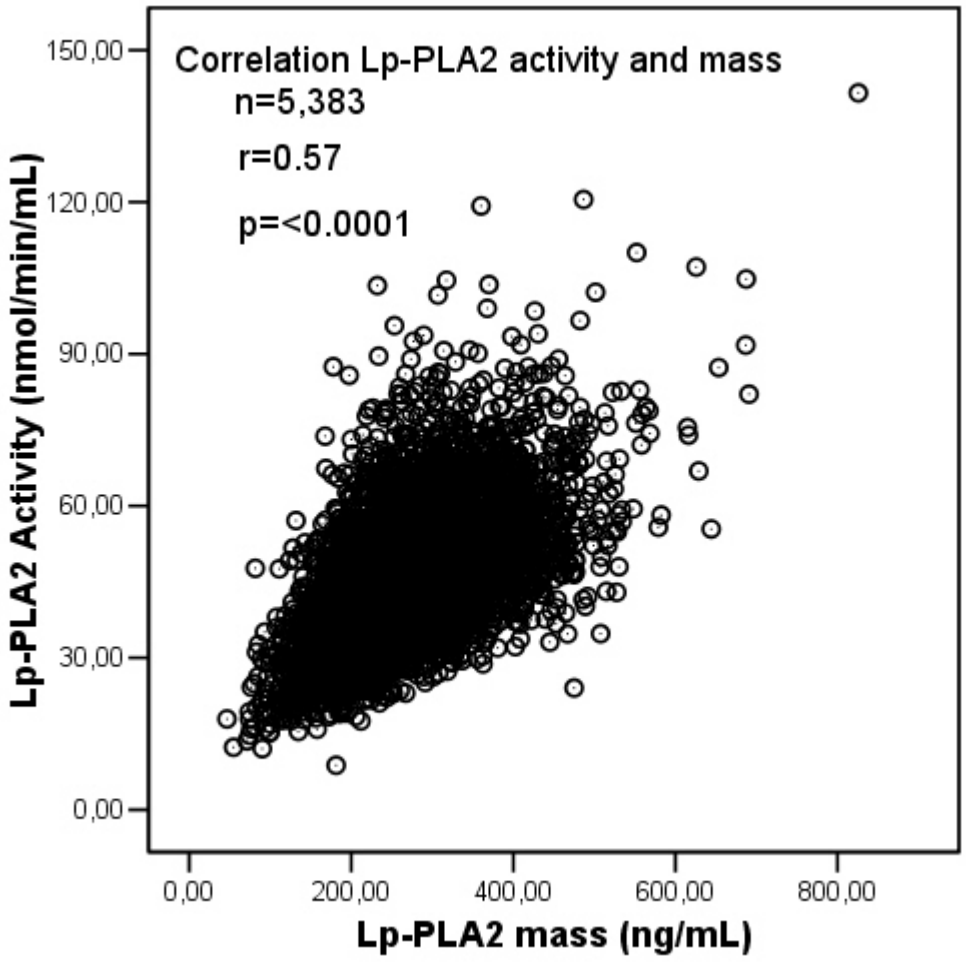
	YES			NO			Diff	CI (95%)
	n	Mean	SE	n	Mean	SE		
Smoker (current)	1396	47.7	0.34	3857	45.4	0.20	2.3	(1.5 to 3.1)
Hypertension a)	719	46.3	0.47	4580	46.0	0.47	0.3	(-0.7 to 1.3)
History of stroke or MI b)	216	46.0	0.86	5186	46.1	0.18	-0.1	(-1.8 to 1.6)
Diabetes c)	97	47.1	1.3	5111	46.0	0.18	1.1	(-1.4 to 3.6)
Obesity >30 in BMI kg/m²	707	46.8	0.47	4690	45.9	0.18	0.9	(-0.1 to 1.9)
Plaque occurrence d)	3182	46.6	0.22	2006	45.2	0.29	1.4	(0.7 to 2,1)
Education e)	1458	44.9	0.33	3790	46.5	0.21	-1.7	-2.4 to -0.9

a) Self-reported diagnosis of hypertension and treatment
b) Diagnosed or register verified history of ischemic stroke or myocardial infarction at baseline
c) Self-reported diagnosis of Diabetes Mellitus and treatment.
d) A focal thickening of intima-media, more than 1.25 mm
e) Education Yes= more than 10 year, No= less than 10 year

Table 5. Degree of Explanation of Variation (R^2) in Lp-PLA2 Activity and Mass Due to Cardiovascular Risk Factors Using Multiple Linear Regression

LpPLA2 activity		
Risk Factor	Cumulative R^2	Significance of change
LDL	0.218	<0,001
Gender	0.295	<0,001
HDL	0.323	<0,001
HbA1C	0.328	<0,001
Triglycerides	0.332	<0,001
BMI	0.336	<0,001
Education	0.338	<0,001
Smoking	0.339	0.001
HOMA	0.341	0.001
Age	0.342	0.014
hsCRP	0.342	0.076
SBP	0.342	0.517

LpPLA2 mass		
Risk Factor	Cumulative R^2	Significance of change
LDL	0.115	<0,001
Gender	0.155	<0,001
Smoking	0.166	<0,001
hsCRP	0.172	<0,001
Education	0.175	<0,001
Age	0.177	0.010
HDL	0.178	0.004
BMI	0.181	<0,001
HOMA	0.182	0.001
Triglycerides	0.184	0.004
Physical act	0.185	0.017
HbA1C	0.185	0.024


Correlation Lp-PLA2 activity and mass
n=5,383
r=0.57
p=<0.0001
Lp-PLA2 Activity (nmol/min/mL)
150,00
120,00
90,00
60,00
30,00
0,00
0,00
200,00
400,00
600,00
800,00
Lp-PLA2 mass (ng/mL)