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Lipoprotein-Associated and Secretory Phospholipase A_2 in Cardiovascular Disease: The Epidemiological Evidence

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Abstract Among other lipid related biomarkers, lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and type II secretory phospholipase A_2 (sPLA_2) represent emerging candidates for refined assessment of future cardiovascular disease (CVD) risk. Indeed, emerging evidence from more than prospective 15 studies conducted since 2000, clearly demonstrate the prognostic ability of increased Lp-PLA_2 concentrations or elevated activity for risk of future coronary heart disease (CHD) and stroke. Moreover, Lp-PLA_2 might have similar predictive power for both, incident CHD in initially healthy subjects, as well as for recurrent events in those with clinically manifest atherosclerosis. By contrast, to date, there are only few prospective studies that have investigated the relationship of sPLA_2 with future CVD risk. However, most of them show a positive association between increased mass or elevated activity and future atherosclerotic complications. Nonetheless, since inhibitors of Lp-PLA_2 and sPLA_2 have already been developed, these enzymes may be considered as novel therapeutic targets to treat residual risk in certain high risk patient groups. This review summarizes the epidemiologic evidence on the association between increased mass or elevated activity of these two phospholipases and risk of CVD.

Key words Phospholipases · Epidemiology

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Introduction

An atherogenic lipoprotein phenotype characterized by increased levels of total cholesterol (TC), LDL-, non-HDL cholesterol, and low concentration of HDL cholesterol has been well recognized as an important predictor of cardiovascular disease and most of these lipoproteins have been integrated into various scores for coronary heart disease (CHD) risk assessment (e.g. Framingham Risk Score, PROCAM score, ESC SCORE). Despite such circumstantial evidence, various studies during recent years have identified several additional lipid-related markers such as lipoprotein-associated phospholipase A_2 (Lp-PLA_2) or secretory phospholipase A_2 (sPLA_2) as emerging biomarkers that might improve our ability to identify patients at risk for future CHD.

This review summarizes the epidemiological evidence for an association between these biomarkers and the prediction of cardiovascular disease (CVD).

Lipoprotein-associated phospholipase A_2

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a 45.4-kDa protein and a calcium-independent member of the phospholipase A_2 family has recently emerged as a promising biomarker for atherosclerotic disease and is presently under intensive investigation. Due to its unique mechanism of action and its possible causal role in atherogenesis, Lp-PLA_2 might represent a link between lipid metabolism and the inflammatory response. The biology of Lp-PLA_2 is discussed in detail in a review article by Stafforini et al. [1] in this issue of the journal.

Over the past 7 years, a large body of evidence has been accumulated which demonstrates that increased circulating concentrations of Lp-PLA_2 mass or elevated activity of the enzyme are positively associated with various cardiovascu-

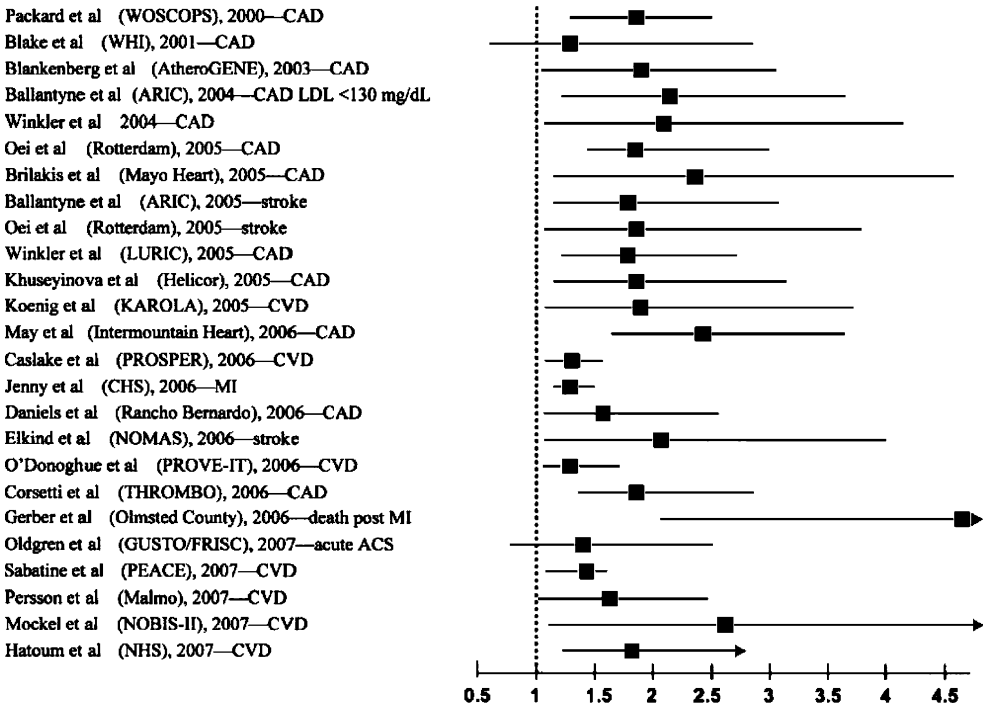

Fig. 1 Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and risk of cardiovascular disease (CVD). Published prospective epidemiologic studies show the association of elevated Lp-PLA_2 (top quartile vs bottom quartile) with cardiovascular risk. A fairly consistent near doubling of risk is associated with elevated Lp-PLA_2. Results are fully adjusted for traditional risk factors, lipids, and often for body mass index and high-sensitivity C-reactive protein. *ACS* acute coronary syndrome, *ARIC* Atherosclerosis Risk in Communities, *CAD* coronary artery disease, *CHS* Cardiovascular Health Study, *GUSTO/FRISC* Global Use of Strategies to Open Occluded Coronary Arteries/Fragmin During Instability in Coronary Artery Disease, *KAROLA* Langzeiterfolge der Kardiologischen Anschlussheil-Behandlung, *LDL* low-density lipoprotein cholesterol, *LURIC* Ludwigshafen Risk and Cardiovascular Health Study, *MI* myocardial infarction, *NHS* Nurse's Health Study, *NOBIS-II* North Wuerttemberg and Berlin Infarction Study—II, *NOMAS* Northern Manhattan Study, *PEACE* Prevention of Events with Angiotensin-Converting Enzyme Inhibition, *PROSPER* Prospective Study of Pravastatin in the Elderly at Risk, *PROVE-IT* Pravastatin or Atorvastatin and Infection Therapy, *THROMBO* Thrombogenic Factors and Recurrent Coronary Events, *WHI* Women's Health Initiative, *WOSCOPS* West of Scotland Coronary Prevention Study (from Corson et al. [2] Copyright Elsevier 2008)

lar endpoints. Such results have been reported in initially healthy subjects from representative population-based studies as well as in patients with manifest CHD (Fig. 1) [2].

Initial evidence for such an association came from WOSCOPS (West of Scotland Coronary Prevention Study), where 580 hypercholesterolemic middle-aged men without pre-existing CHD, who developed a coronary event over a 4.9-year follow-up, served as cases and were compared to 1,160 age- and smoking-matched event-free participants [3]. In this analyses, a one standard deviation (SD) increase in Lp-PLA_2 concentrations was independently associated with a 18% increased risk of future CHD events (relative risk (RR)=1.18; 95% confidence interval (95%CI)=1.05–1.33; after controlling for traditional risk factors and several inflammatory biomarkers). However, in a subsequent study, in a low-risk population for CVD, the Women's Health Study (WHS) [4], a large cohort of middle-age normo-cholesterolemic women, failed to confirm the initially reported association from WOSCOPS. Using a nested case-control design that included 123 cases and 123 controls, the authors found that the RR in the top quartile compared with the bottom quartile was 1.17 (95%CI=0.45–3.05) after adjustment for various risk factors. Such lack of an association, however, might be attributed to potential gender differences for Lp-PLA_2 as a result of the modulatory effect of the hormonal milieu and the low power of this study.

The Atherosclerosis Risk in Communities (ARIC) study served as the database for a case-cohort study of 608 men and women with incident CHD and 740 controls randomly drawn from the remaining cohort and followed for 6–8 years [5]. In age and gender adjusted analysis, Lp-PLA_2 was associated with an increased risk for CHD, but statistical significance was lost after multivariable adjustments. However, in subjects with low LDL-C, Lp-PLA_2 significantly and independently predicted CHD (hazard ratio (HR)=2.08; 95%CI=1.20–3.62), suggesting that it might be a useful marker for identifying high-risk patients with relatively normal levels of LDL-C, a subgroup in whom additional biomarkers may be potentially useful.

Using the MONICA-Augsburg cohort study as a data base, we could demonstrate an independent association of

Lp-PLA_2 with CHD in 934 initially healthy middle-aged men with moderately increased TC, drawn randomly from the general population in 1984 and followed until 1998 [6]. Indeed, in a Cox model, a one SD increase in Lp-PLA_2 was strongly and independently related to a first-ever event (HR=1.23; 95%CI=1.02–1.47), even after controlling for a variety of potential confounders, including the TC/HDL-C ratio as the strongest lipoprotein variable. Importantly, in the ARIC and the MONICA study, the potential additive value of Lp-PLA_2 to C-reactive protein (CRP) in predicting risk has been evaluated. For this purpose, high CRP was defined according to a recent AHA/CDC consensus document as >3.0 mg/L, and for Lp-PLA_2 the upper tertile cut-point was used (422 μg/L in ARIC and 290.8 ng/mL in MONICA). In ARIC, individuals with high Lp-PLA_2 and high CRP exhibited a threefold increased risk for CHD (HR =2.95; 95%CI=1.47–5.94) [5], whereas in the MONICA-Augsburg study the combination of elevated Lp-PLA_2 and elevated CRP resulted in a HR of 1.93 (95%CI=1.09–3.40) compared with both markers not being increased in the fully adjusted model [6].

Data from the Rotterdam Study, a case-cohort study among 7,983 subjects aged 55 years and above, which determined Lp-PLA_2 activity instead of mass [7], were also in accordance with earlier studies. This study included 308 CHD cases and a random sample of 1,820 subjects with a median follow-up of 7.2 years. Similar to results from the MONICA study [6], a one SD increase in Lp-PLA_2 was strongly and independently related to a first-ever CHD event (HR=1.20; 95%CI=1.04–1.39), even after controlling for a variety of potential confounders.

In the recently published Bruneck study [8], a population-based survey of 765 men and women aged 40–79 years, who were followed for incident CV events over a 10-year period, the predictive value of Lp-PLA_2 for future CVD risk could be further demonstrated after multivariate adjustment with a HR of 1.4 per one SD change in enzyme activity. Of interest, this study has also illustrated a complementary role of Lp-PLA_2 and oxPL/apoB in identifying those at highest risk for CVD: the combination of these biomarkers (each in top vs bottom tertiles) lead to an almost fourfold HR for CV events. In addition, an additive effect of risk prediction for the combination of Lp (a) and Lp-PLA_2 was also shown.

Lp-PLA_2 has also been found to be a potent predictor of CVD risk even when conventional lipid measures or other biomarkers often loose their prognostic ability, namely in elderly populations [9–11], although the magnitude of such association seems to be slightly smaller than that observed in middle-aged subjects. Recently, Daniels et al. [9] reported results from the Rancho Bernardo Study, which comprised 1,077 apparently healthy community-dwelling older men and women (mean age 72 years) with no history of CHD at baseline. During 16 years of follow-up, 228 fatal and non-fatal CHD occurred. Measurements of Lp-PLA_2 mass at baseline considered this enzyme as an independent predictor of future CHD events. Thus, in multivariate models a 60% to 90% increased risk for incident CHD across extreme quartiles of Lp-PLA_2 distribution was found. More importantly, the addition of Lp-PLA_2 to the basic model with traditional CV risk factors and CRP demonstrated an incremental benefit in CHD prediction, with an increase in the area under the receiver operating characteristic (ROC) curve (AUC) from 0.595 to 0.617. These results could be partially confirmed by preliminary data from two other studies [10, 11]. Using data from the Cardiovascular Health Study (CHS), an elderly population without a history of vascular disease at baseline, Jenny et al. [10] found in a cohort of 4,318 men and women aged 65 years or older, elevated Lp-PLA_2 mass to be associated with an increased 10-year risk of myocardial infarction (MI) independently of traditional risk factors, including LDL, whereas the association with Lp-PLA_2 activity was only of borderline significance. Almost identical results were obtained by Caslake et al., determining both Lp-PLA_2 mass and activity in 5,657 participants aged 70–82 years of the PROSPER (The Prospective Study of Pravastatin in the Elderly at Risk) trial. After controlling for various confounders, only Lp-PLA_2 mass was found to be significantly related to future CHD risk, with no association found for enzyme activity [11].

Although the association between increased concentrations of Lp-PLA_2 and future CV events seems to be consistent in the primary prevention setting, clinical data on the predictive value of Lp-PLA_2 in the setting of an acute coronary syndrome (ACS) remain to be established, taking into account recent controversial results from various trials [12–15]. The PROVE IT-TIMI 22 trial [12] was the first study that evaluated the prognostic utility of Lp-PLA_2 in patients with ACS. In this study in 3,648 patients with ACS, Lp-PLA_2 activity and mass were measured at baseline and after 30 days (n=3,625). Both, Lp-PLA_2 activity and mass were not predictive for recurrent events when measured at the time of admission to the hospital or early after ACS. However, Lp-PLA_2 activity was able to modify risk prediction when measured some time apart from the acute event e.g. at day 30, showing a 33% increased risk for recurrent events in the top quintile of the Lp-PLA_2 distribution over 24 months (RR=1.33, 95%CI= 1.01–1.74 in the fully adjusted model including CRP and LDL). Results from two further studies in ACS patients from Sweden were also essentially negative [13]. In the first study, Lp-PLA_2 mass measured in 1,362 ACS patients, participating in the FRISC II trial was not associated with mortality or recurrent non-fatal MI or fatal CV events 6 months after randomization and during 2 years of follow-

up. The investigators tried to replicate their findings in another population, in patients with ACS without persistent ST-segment elevation using the GUSTO IV ACS database. Again, increased Lp-PLA_2 concentrations were not related to recurrent MI within 30 days or with cumulative mortality at 1 year, although Lp-PLA_2 concentrations were significantly higher in the high-risk GUSTO IV patients compared to FRISC II patients, who were at moderate risk [13]. However, data from Olmsted County, Minnesota [14] were in contrast to the two above mentioned studies. In 271 patients with acute MI, taken from the general community, Lp-PLA_2 concentrations, measured immediately after symptom onset, were strongly and independently associated with mortality after 1 year. Moreover, Lp-PLA_2 was of incremental value for CHD risk prediction, beyond that of traditional risk factors, since addition of Lp-PLA_2 in a model that already contained traditional risk factors, ejection fraction (EF), Killip class, CRP, and reperfusion or revascularization, resulted in an increase in the AUC from 0.823 to 0.852 (p=0.05) [14]. Yet, the small sample size of the study represents a limitation that should be taken into account. Results from the NOBIS-II study in Germany [15], conducted in 429 unselected consecutive patients admitted to the emergency room with suspected ACS in whom Lp-PLA_2 was measured directly on admission were also in contrast to the FRISC II and GUSTO IV ACS data. Using classification and regression tree (CART) analysis, the Möckel et al. demonstrated for the first time that, Lp-PLA_2 may add incremental information for improved risk stratification, in particular in troponin negative patients with moderately elevated N-terminal proBNP (NT-proBNP) levels.

While data on the prognostic value of Lp-PLA_2 in the ACS need to be further evaluated, the role of Lp-PLA_2 in the prediction of future CV events in patients with manifest, but stable CHD seems to be consistent and rather promising. In a study from the Mayo Clinic [16], 466 consecutive patients scheduled for coronary angiography were followed for a median of 4 years. The relative risk for a future event for a one SD increase in Lp-PLA_2 mass was found to be 1.30 after multivariable adjustments. In the KAROLA (Langzeiterfolge der KARdiOLogischen Anschlussheilbehandlung) study [17], Lp-PLA_2 mass and activity were determined on the average 43 days after the acute event in a cohort of 1,051 patients aged 30–70 years with CHD, who were followed for a mean of 48.7 months for secondary CVD events. An independent prognostic value of Lp-PLA_2 mass was shown in multivariable analyses, whereas Lp-PLA_2 activity became borderline significant in a fully-adjusted model. This study has also quantified the incremental contribution of Lp-PLA_2 mass to risk prediction in the presence of classical risk factors and markers of renal function and hemodynamic stress. ROC analyses showed that the addition of cystatin C and NT-proBNP to a basic model improved the predictive accuracy of the model (AUC from 0.67 to 0.71). After additional inclusion of Lp-PLA2 mass, there was still a further, however smaller, increase (AUC from 0.71 to 0.73). In 766 post-MI patients from the THROMBO (Thrombogenic Factors and Recurrent Coronary Events) study, who were followed for 26 months, increased concentrations of Lp-PLA_2 mass were associated with an approximately twofold increased risk for recurrent coronary events in a multivariate model [18]. In addition, Lp-PLA_2 was able to replace apoB as the most powerful independent predictor of risk in the same study population. More recently, the largest study on the prognostic utility of Lp-PLA_2 for recurrent CV events in patients with stable CHD has been published. Sabatine et al. [19] measured Lp-PLA_2 mass in 3,766 patients with documented CHD, who were enrolled in the PEACE (Prevention of Events with Angiotensin-Converting Enzyme Inhibition) trial. Multivariate analyses clearly showed the significance of elevated Lp-PLA_2 concentrations for the prediction of adverse CV outcomes during a 5-year follow-up period and these effects were more pronounced for the prediction of non-fatal events such as revascularization und unstable angina. Within the large Ludwigshafen Risk and Cardiovascular Health Study (LURIC) [20], including 2,513 patients with angiographically confirmed CHD and 719 without, Lp-PLA_2 activity predicted risk for cardiac and total mortality over 5.5 years. A twofold increased risk for cardiac death was found across extreme tertiles of the Lp-PLA_2 distribution. More importantly, the inclusion of Lp-PLA_2 activity to the model with established risk factors and several biomarkers lead to a significant increase in C statistics, thereby demonstrating the incremental value of this enzyme for prediction of cardiac death over the above mentioned variables. In addition, Lp-PLA_2 added prognostic information in patients with low and medium CRP concentration with regard to 5-year cardiac mortality independently of established risk factors.

Several studies have also considered Lp-PLA_2 as a marker of cerebrovascular risk. Data from the Rotterdam Study [7] demonstrated that increased Lp-PLA_2 activity (quartile (Q) 4) was significantly associated with a 97% increased risk of stroke compared to those with the lowest activity (Q1). For a one SD increase in Lp-PLA_2 activity, a 24% increased risk was seen. These findings are consistent with data from the ARIC cohort [21], in which the relationship between Lp-PLA_2 mass and the incidence of stroke was studied in 194 case-subjects who were compared with a cohort random sample of 766 event-free subjects over a 4.4-year follow-up period. The risk of stroke for individuals in the top tertile was approximately twice as high as that for those in the bottom tertile, even after taking into account other confounders, including lipid variables, diabetes, and CRP. By contrast, LDL-C concentrations did

not predict risk of stroke. In addition, individuals with the highest concentrations of both Lp-PLA_2 and CRP were shown to have a greater than 11-fold increased risk compared to those with the lowest concentrations of both Lp-PLA_2 and CRP [21]. The risk of ischemic stroke was also assessed in postmenopausal women, participants of the Women's Health Initiative (WHI) Observational Study [22]. Using a nested case-control design with 929 incident cases of ischemic stroke and 935 age and race matched controls, the investigators found only an 8% increased risk for ischemic stroke associated with being in the top quartile of the Lp-PLA_2 distribution after adjustment for traditional CV risk factors (OR=1.08, 95%CI=0.74–1.55). An intriguing finding from this study, however, was the higher risk of ischemic stroke among non-hormonal replacement therapy (HRT) users (OR=1.55; 95%CI=1.05–2.28 for Q4 vs Q1), than among current users (OR=0.70; 95%CI=0.42–1.17). This study again suggests that Lp-PLA_2 concentrations might be modulated by direct hormonal regulation.

The role of Lp-PLA_2 in the prediction of stroke in secondary prevention so far has only been assessed in the Northern Manhattan Study (NOMAS), where 467 first ischemic stroke patients have been followed for 4.0 years with 80 recurrent stroke events during this period [23]. Increased concentrations of Lp-PLA_2 mass (Q4 vs Q1) predicted risk of recurrent ischemic stroke resulting in a HR of 2.08 (95%CI=1.04–4.18, after multivariate adjustment for classical CV risk factors and CRP), whereas CRP failed to predict recurrent stroke events in this population. Thus, compared to CHD, the database supporting Lp-PLA_2 as a novel risk marker for stroke is considerably smaller and further prospective studies are clearly needed.

Considering the emerging role of Lp-PLA_2 in CVD risk prediction, meta-analysis seems to be an appropriate tool to provide the most unbiased information on the association between Lp-PLA_2 and disease outcome. Indeed, such meta-analysis including 14 eligible studies with a total number of 20,549 participants has been reported by Garza et al. [24] and confirmed a significant and independent association between elevated Lp-PLA_2 concentrations and risk of CVD, resulting in a summary OR of 1.60 (95%CI=1.36–1.89) after adjustment for conventional CV risk factors. Furthermore, a comprehensive meta-analysis based on individual data from prospective Lp-PLA_2 studies is underway (Emerging Risk Factor Collaboration Study) [25] that might be able to generate even more precise risk estimates including subjects in important subgroups.

Type II secretory phospholipase A_2

Type II secretory phospholipase A_2 (sPLA_2-II) is another well studied member of the phospholipase 2 family which, however, in contrast to Lp-PLA_2 is Ca^{2+}-dependent and belongs to the group of acute phase reactants [26]. Indeed, circulating levels of sPLA_2-II increase greatly during systemic inflammatory conditions, such as sepsis, rheumatoid arthritis, or inflammatory bowel disease [27]. Moreover, sPLA_2-II production is up-regulated in response to pro-inflammatory compounds such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, interferon-γ, and oxidized LDL [26–29]. The biology and mechanism of catalytic activity of this enzyme are discussed in detail in a review article by Webb et al. in this issue of the journal [30].

The existing epidemiological database for sPLA_2-II in atherosclerosis is not as large as for Lp-PLA_2. To date, only a small number of prospective studies are available, which have examined the potential role of sPLA_2-II in CV disorders in initially healthy subjects as well as in subjects with clinically manifest disease and particularly in those with ACS (Table 1).

Elevated plasma levels of sPLA_2 were significant and independent predictors of future CV events in a small study including 142 consecutive patients with angiographically proven, stable CHD and 93 control subjects [31]. At baseline, significantly higher sPLA_2 levels were seen in cases compared with controls. A positive and moderately strong correlation between sPLA_2 and CRP was also observed (r=0.53). Furthermore, CAD patients were followed for a mean duration of 17.2 months, during which 48 coronary events occurred. Kaplan–Meier analysis as well as Cox models revealed that subjects with higher levels of sPLA_2-II (>366 ng/dL) had a significantly higher risk of developing future coronary events such as coronary revascularization, MI, and coronary death, than those with the lowest concentration (246 ng/dL). The prognostic value of sPLA_2-II was independent of traditional cardiovascular risk factors and various biochemical markers, including CRP (OR=3.3; 95%CI=1.3–9.2; p=0.01 for those in the top tertile compared to those in the bottom tertile). In another study from the same group, conducted in 52 patients with unstable angina, 107 stable CHD patients and 96 controls [32], sPLA_2 levels were measured and follow-up was done in the unstable angina group over a 2-year period. First, levels were found to be higher in unstable patients compared to stable patients and controls. Second, in multivariate models, the OR for a coronary event varied between 3.0 (95%CI=1.2–7.4) and 5.1 (95% CI=1.4–18.6) depending upon the underlying coronary anatomy and whether or not patients had suffered a previous MI. The very large confidence intervals reflect the extremely small numbers in the various subgroups studied. Furthermore, in a study with 247 consecutive CHD undergoing percutaneous coronary intervention (PCI) and 100 controls, increased sPLA_2 (>450 ng/dL)

Table 1 Secretory phospholipase A_2 ($sPLA_2$) and risk of cardiovascular disease (CVD)

Author/year	Design	Mass or activity	Cohort	*N*	FU	Endpoint	Risk estimates (95%CI)
Kugiyama et al./ 2000 [32]	Nested case–control	Mass	Pts with sympt. CHD	142/93	17.2 months	PCI,CABG, MI, coronary death	OR=3.46 (1.4–8.3) T3 vs T1[a]
Liu et al./ 2003 [33]	Nested case–control	Mass	Pts with sympt. CHD	247/100	19.3 months	Coronary events	OR=2.1 (1.4–7.0) >450 vs ≤450 ng/dL[a]
Mallat et al./ 2005 [34]	Full cohort	Activity	GRACE: ACS Pts	446	6.5 months	MACE	HR=3.08 (1.37–6.91)[a] T3 vs T1+T2
Koenig et al./ 2008 [35]	Full cohort	Mass and activity	KAROLA: post-MI Pts	1,032	4.1 years	Fatal and non-fatal MI and stroke	Mass HR=2.11 (1.20–3.72) activity HR=1.65 (1.12–2.42) T3 vs T1[a]
Boekholdt et al./ 2005 [36]	Nested case–control	Mass	EPIC-Norfolk: m/w	707/1,396	6 years	Fatal and non-fatal CHD	OR=1.34 (1.02–1.71) Q4 vs Q1[b]
Mallat et al./ 2007 [37]	Nested case–control	Activity	EPIC-Norfolk: m/w	991/1,806	6 years	Incident CHD	OR=1.65 (1.27–2.12) Q4 vs Q1[b]

Pts patients, *CHD* coronary heart disease, *PCI* percutaneous coronary intervention, *CABG* coronary artery bypass graft, *MI* myocardial infarction, *OR* odds ratio, *T* tertile, *GRACE* Global Registry of Acute Coronary Events, *ACS* acute coronary syndrome, *MACE* major adverse coronary events, *HR* hazard ratio, *KAROLA* Langzeiterfolge der Kardiologischen Anschlussheil-Behandlung, *EPIC* European Prospective Investigation of Cancer, *m/w* men/women, *Q* quartile

[a] Adjusted for various traditional CV risk factors

[b] Adjusted for traditional CV risk factors and CRP

measured after the intervention independently predicted outcome after 2 years (OR=2.1; 95%CI=1.4–7.0; p=0.025) [33]. Finally, Mallat et al. [34] studied 446 patients with severe ACS from the Global Registry of Acute Coronary Events (GRACE) and followed them for a median of 5.6 months. At baseline, several biomarkers were measured including $sPLA_2$ activity and antigen. In multivariable analysis, only $sPLA_2$ activity but not mass was strongly associated with the composite endpoint of death or MI (HR=3.08; 95%CI=1.37–6.91). However, the number of hard endpoints was small (n=43), and thus the risk associated with increased $sPLA_2$ activity may have been overestimated.

Assuming that most of the above studies, although being consistent, were relatively small with heterogeneous cohorts, we decided to investigate whether $sPLA_2$ was associated with prognosis in a large cohort of patients with clinically overt CHD. Within KAROLA [35], plasma $sPLA_2$ mass and activity were measured at baseline in a cohort of 1,032 patients aged 30–70 years with CHD participating in an in-patient rehabilitation program after an ACS. During follow-up (mean 4.1 years) 95 patients (9.0%) experienced a secondary CVD event. Baseline levels of $sPLA_2$ mass and activity were higher in subjects who experienced an event compared to event free-subjects (5.11± 6.34 vs 3.96±5.43 ng/mL, p=0.002 and 1.56±1.05 vs 1.33± 0.69 nmol/min/mL p=0.01) for mass and activity, respectively). $sPLA_2$ mass and activity were positively correlated with each other (R=0.63; p<0.001), with leukocyte count, CRP, cystatin C, NT-proBNP, Lp-PLA_2 mass and activity; in general, correlations were stronger for mass than for activity. In a multivariate model, $sPLA_2$ mass and activity were associated with an increased HR of future cardiovascular events. After controlling for age, gender, BMI, smoking, history of MI and diabetes mellitus, initial management of CHD, HDL-C, LDL-C, and statin use, the HR was 2.11 (95%CI=1.20–3.72) and 1.65 (95%CI=1.12–2.42) for mass and activity, respectively, when the top tertiles were compared to the bottom tertiles. Further adjustment for cystatin C, NT-proBNP, CRP, and Lp-PLA_2 attenuated the associations still showing a positive trend for mass but a less clear pattern for activity. However, when $sPLA_2$ mass and activity were analyzed as the continuous variables both still showed a statistically significant increase in risk. Thus, $sPLA_2$ mass and activity appear to be predictive of secondary CVD events in patients with manifest CHD but larger studies are needed to clearly distinguish effects from other biomarkers reflecting inflammation, renal function, and hemodynamic stress.

More recently, the prognostic utility of $sPLA_2$ in asymptomatic subjects at high-risk for the development of future CVD has been evaluated in two nested case-control studies, conducted within the European Prospective Investigation of Cancer (EPIC)-Norfolk cohort. The first study [36], measuring $sPLA_2$ concentrations (mass), comprised 1,105 subjects who subsequently developed fatal and non-fatal CHD events during 6 years of follow-up and 2,209 age-, sex- and enrollment time-matched controls who remained free of disease. The second study [37] included 2,797 subjects (991 subjects with incident CAD and 1,806

event-free controls), among whom activity of this enzyme was determined at baseline. Both, elevated $sPLA_2$ mass and increased activity predicted coronary events in initially healthy subjects: A 34% increased risk for future CHD events in multivariable analyses across extreme quartiles (Q) (OR=1.34; 95%CI=1.02–1.71) was seen in association with elevated $sPLA_2$ concentrations (mass), whereas a stronger 65% increased risk was seen with increased $sPLA_2$ activity (OR=1.65, 95%CI=1.27–2.12 for Q4 vs Q1) after adjustment for the same variables, including CRP. Based on these differing results, the authors concluded that $sPLA_2$ activity, which encompasses several types of $sPLA_2$ including type IIA, V, and X may better reflect the causative role of $sPLA_2$ in the atherogenetic process [37].

However, results in these healthy subjects that came from the same study population have to be replicated in other cohorts until the clinical usefulness of $sPLA_2$ in the prediction of CHD in the primary care setting may be established. Moreover, no studies so far have investigated the potential value of $sPLA_2$ in the prediction of cerebrovascular risk. Thus, larger studies in diverse populations are certainly needed using assays with high precision and an established standard so that results may be compared among studies.

Conclusion

The rapidly increasing literature on Lp-PLA_2 and $sPLA_2$ in CVD has provided us with valuable new information regarding the involvement of these biomarkers in the pathophysiology of this complex disorder. However, before such information can be translated into the clinical setting, a number of criteria have to be fulfilled, as recently put together by Morrow and de Lemos [38]. This list of requirements covers pre-analytical issues, assay methods, costs involved, strength of the association found in various studies, and the potential incremental value over and above existing routinely measured traditional risk factors. Most importantly, rigorous standardization of assays must be carried out to ensure adequate reproducibility of measurements. What cannot be explained at present are the discrepant results in some studies concerning activity and mass of these two enzymes.

Nonetheless, Lp-PLA_2 and $sPLA_2$ represent two promising biomarkers for the prediction of future CVD. Moreover, since specific inhibitors for both enzymes are currently under evaluation in clinical trials (for review please see [39]), lowering Lp-PLA_2 and probably $sPLA_2$ might represent a promising novel strategy for the treatment of residual risk of atherosclerosis complications through direct targeting of vascular inflammation.

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