Lipoprotein-Associated Phospholipase A_2: A Risk Marker or a Risk Factor?

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Multiple cardiovascular biomarkers are associated with increased cardiovascular disease (CVD) risk. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) appears to be relatively unique in its high specificity for and the causal pathway of plaque inflammation. In both primary and secondary prevention study populations, Lp-PLA_2 was consistently associated with higher cardiovascular risk, and the risk estimate appears to be relatively unaffected by adjustment for conventional CVD risk factors. Risk ratios were similar, whether the mass concentration or activity of the enzyme was measured. The purpose of this article is to review the evidence for the clinical utility of Lp-PLA_2, both as a risk marker and as a risk factor involved in the causal pathway of plaque inflammation and the formation of rupture-prone plaque. **(Am J Cardiol 2008;101[suppl]: 11F–22F)**

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is among the multiple cardiovascular biomarkers that have been associated with increased cardiovascular disease (CVD) risk. Lp-PLA_2 appears, however, to be relatively unique in its high specificity for vascular inflammation as opposed to systemic inflammation, its low biologic variability, and its direct role in the causal pathway of plaque inflammation. In a recent meta-analysis by Garza et al,[1] 14 prospective epidemiologic studies of >20,000 patients were pooled in an examination of Lp-PLA_2 as an independent risk factor for cardiovascular events. For quantile-based (upper quantile vs bottom quantile) comparisons, the risk ratios were 1.86 (95% confidence interval [CI], 1.47–2.34) and for risk per 1 change in standard of deviation in the marker, the relative risk was 1.21 (95% CI, 1.11–1.32). In both primary and secondary prevention study populations, Lp-PLA_2 was consistently associated with a higher cardiovascular risk, and as stated by the investigators, "the risk estimate appears to be relatively unaffected by adjustment for conventional CVD risk factors." Risk ratios were similar, whether the mass concentration or activity of the enzyme was measured. They found that Lp-PLA_2 levels may be useful to further stratify patients with an intermediate probability of developing cardiovascular events by the Framingham score. The purpose of this article is to review the evidence for the clinical utility of Lp-PLA_2 both as a risk marker and as a risk factor involved in the causal pathway of plaque inflammation and the formation of rupture-prone plaque. Thus, it may be speculated that specific therapies after Lp-PLA_2 measurement may be of beneficial effect to decrease cardiovascular risk.

Pathophysiology of Lipoprotein-Associated Phospholipase A_2 and Mechanism of Action

A cardiovascular biomarker should be directly involved in the causal pathway of plaque formation and inflammation. Lp-PLA_2 is a member of a family of intracellular and secretory phospholipase enzymes that are capable of hydrolyzing the sn-2 ester bond of phospholipids of cell membranes and lipoproteins.[2] In fact, Lp-PLA_2 attached to low-density lipoprotein (LDL) is the enzyme solely responsible for the hydrolysis of oxidized phospholipid (oxPL) on the LDL particle.[3] It differs from the other phospholipase enzymes in that its activity is calcium independent and it lacks activity against the naturally occurring phospholipids present on the cellular membrane.[4,5] Thus, Lp-PLA_2 hydrolyzes oxPL on the surface of lipoproteins but has weak activity against non-oxPL. In terms of location on apolipoprotein B–containing lipoproteins, Stafforini et al[6] demonstrated that Lp-PLA_2 interacts directly with apolipoprotein B-100, and that the carboxyl terminus of this apolipoprotein is required for such interaction.

Earlier reports found that approximately 80% of Lp-PLA_2 (a subtype of the PLA_2 family) circulates bound to LDL, whereas the other 20% is bound to high-density lipoprotein (HDL) and remnant lipoprotein particles. However, the distribution of Lp-PLA_2 between HDL and LDL particles may be more variable than previously reported.[7] It has been shown that the distribution of Lp-PLA_2 between LDL and HDL depends on the extent of its glycosylation,

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which can affect plasma Lp-PLA_2 activity.[8] Lp-PLA_2 is also the enzyme that hydrolyzes oxPL on HDL particles, where it may play a role in the antioxidant function of HDL.[9] Interestingly, in mice, in which the main component of cholesterol is HDL, Lp-PLA_2 is protective against the development of atherosclerotic disease and is bound almost exclusively to HDL particles.

The activity of the Lp-PLA_2 enzyme is increased in small, dense LDL and electronegative LDL species.[10] Gazi et al[11] reported that 1 in 100 small/dense LDL particles are associated with the Lp-PLA_2 enzyme, but only 1 in 4,000 large LDL particles are associated with the enzyme. Electronegative LDL is toxic to endothelial cells (because of its oxidized lipid content), and elevated levels of electronegative LDL have been associated with an increased risk of atherosclerosis.[12,13] Similarly, oxidized LDL has also been shown to be associated with atherosclerosis. If oxidized LDL, or rather its oxPL, is the substrate for the Lp-PLA_2 enzyme, then it would be expected that patients with a high concentration of oxPL and high levels of Lp-PLA_2, might be at higher cardiovascular risk. In fact, this finding was reported recently in a study of 765 men and women aged 40–79 years observed prospectively for incident cardiovascular events (cardiac death, myocardial infarction [MI], stroke, and transient ischemic attack) in a 10-year prospective follow-up study in Bruneck, Italy.[14] The investigators found that Lp(a) may be equivalent to the oxPL measurement determined with the antibody to oxPL, and they speculated that Lp(a) acts as a sink or trap for oxPL. In the Bruneck study, elevated Lp(a) and elevated Lp-PLA_2 (top tertiles vs bottom tertiles) exhibited an almost 4-fold hazard ratio (HR) for cardiovascular events.

When an LDL particle enters the intimal space, it may be oxidized by a number of enzymes, including myeloperoxidase and nicotinamide adenine dinucleotide phosphate oxidase. Macphee and colleagues[15] demonstrated that as the oxPL surface of LDL particles becomes oxidized, the Lp-PLA_2 begins to hydrolyze oxPL, forming 2 molecular triggers of the inflammation cascade: oxidized fatty acids and lysophosphatidylcholine (lysoPC). Using a potent azetidinone to inhibit the activity of Lp-PLA_2, these same investigators demonstrated that Lp-PLA_2 was solely responsible for the increase of lysoPC in oxidized LDL. Oxidized fatty acids and lysoPC, in turn, stimulate expression of adhesion molecules and cytokines by endothelial cells and plaque-based macrophages and other leukocytes.[16] As monocytes marginate along and adhere to the endothelium, cytokines, such as monocyte chemoattractant protein, attract them into the subendothelial space where they become activated and differentiate into macrophages. An apparent role of the macrophage is to phagocytose LDL particles, with a preference for oxidized LDL. Lipids from the engulfed lipoproteins accumulate in the macrophage, forming lipid-laden macrophages known as foam cells. Studies of Lp-PLA_2 messenger RNA expression have shown that these same intimal-based macrophages and foam cells are the primary source of Lp-PLA_2 production.[17] Although in vitro studies have shown that peripheral mononuclear cells and platelets may produce Lp-PLA_2, in vivo, it is almost exclusively produced by macrophages and foam cells based in the intima.[18] The fact that Lp-PLA_2 is produced locally within atherosclerotic plaque itself likely accounts for its high specificity for vascular as opposed to systemic inflammation. A proposed mechanism of action was originally illustrated in a review article by Sudhir[19] and is presented here in a modified version (Figure 1).

Confirmation of this hypothetical mechanism of action of Lp-PLA_2 was recently reported in an in vivo study showing the net production of Lp-PLA_2 in blood flowing across an atherosclerotic versus nonatherosclerotic coronary vascular bed.[20] In 30 patients with minimal ($<0\%$) angiographic coronary stenosis, Lavi and colleagues[20] obtained blood samples from the aorta at the coronary os, and simultaneously, blood on the venous side of the coronary circulation in the coronary sinus. The difference between levels of Lp-PLA_2 at the entrance to the coronary circulation and in the coronary sinus reflects the gradient of either net production or net absorption of Lp-PLA_2 as blood traverses the coronary circulation. As reviewed previously, when Lp-PLA_2 hydrolyzes the sn-2 fatty acids of oxPL, both lysoPC and oxidized fatty acids are produced. Because lysoPC is associated with endothelial dysfunction (ED), the gradient of lysoPC across the coronary vascular bed was also examined. To separate the cases (modest atherosclerosis) from controls (no atherosclerosis), 3-dimensional intravascular ultrasound was used. The results showed that atherosclerotic plaque in human coronary arteries in vivo is associated with local coronary production of both Lp-PLA_2 and lysoPC (Figure 2).

There was net production of Lp-PLA_2 in patients with coronary atherosclerosis versus patients with no atherosclerosis ($p = 0.001$). This study supports the notion that the Lp-PLA_2 enzyme is present in the early stage of the disease. There was also a statistically significant correlation between the amount of Lp-PLA_2 produced in atherosclerotic arteries and the percent atheroma volume measured by 3-dimensional intravascular ultrasound. There was no net coronary production of C-reactive protein (CRP), a finding consistent with the hypothesis that CRP may be a systemic inflammatory marker. When the patients were categorized according to their epicardial artery endothelial function (based on a decrease in the coronary artery diameter in response to acetylcholine infusion), the group with ED showed a net production of lysoPC ($+87$ ng/min) versus a net reduction in lysoPC (-590 ng/min, $p = 0.03$) for the group without ED. LysoPC net production correlated statistically significantly with the degree of ED. Thus, it is the lysoPC product of Lp-PLA_2-mediated hydrolysis of oxidized LDL particles that may participate in driving ED. Possible mechanisms by which lysoPC may affect endothelial function include (1) downregulation of endothelial nitric oxide, (2) enhanced production of reactive oxygen species and oxidative stress,

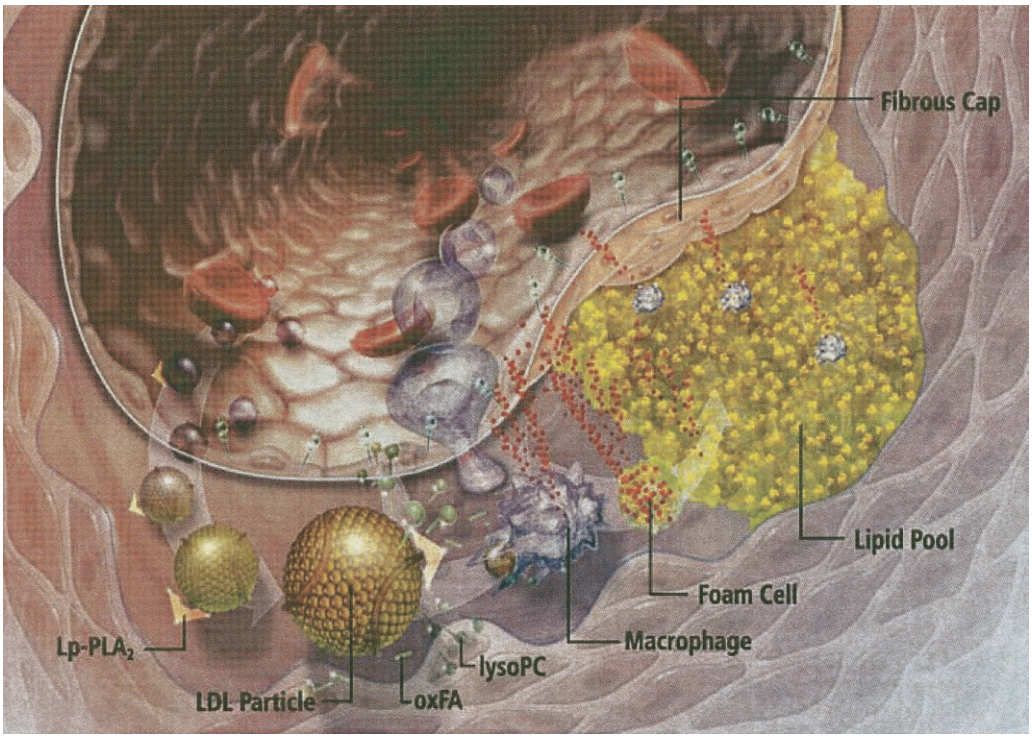

Figure 1. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) as a trigger of inflammation and rupture-prone plaque formation. The Lp-PLA_2 enzyme is produced by intraplaque macrophages and foam cells and is released by plaque into the circulation, where it binds to lipoproteins. Approximately 1 in 500 low-density lipoprotein (LDL) particles has an Lp-PLA_2 enzyme bound to it. On entry into the intimal space, the phospholipid surface of the LDL particle can oxidize. Lp-PLA_2 hydrolyzes oxidized phospholipids into oxidized fatty acids (oxFA) and lysophosphatidylcholine (lysoPC) products. These products stimulate adhesion molecule expression and release of cytokines, which recruit more leukocytes to the lesion. A vicious cycle of more Lp-PLA_2 production appears to ensue. (illustration by Scott Barrows, medical illustrator, University of Illinois at Chicago.)

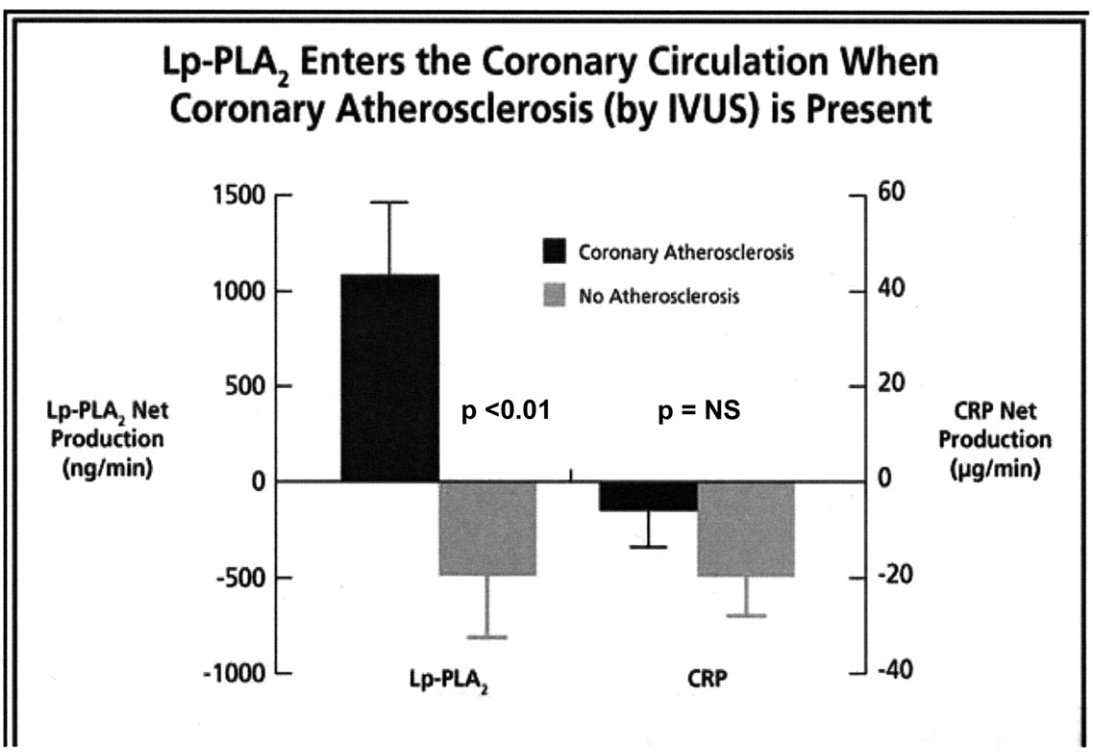

Figure 2. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) enters the coronary circulation when coronary atherosclerosis (by intravascular ultrasound [IVUS]) is present. A total of 15 patients with early coronary atherosclerosis by IVUS were compared with 15 controls. Lp-PLA_2 levels in the coronary os (aorta) were compared with levels in the coronary sinus (vein), the difference reflecting either coronary production versus absorption of Lp-PLA_2. When coronary atherosclerosis was present, there was net production of Lp-PLA_2 as blood flowed across the coronary vascular bed. CRP = C-reactive protein; NS = not significant. (Courtesy of Amir Lerman, MD.)

(3) induction of endothelial cell apoptosis, and (4) blocking of the repair mechanism by inhibition of endothelial cell migration to sites of endothelial damage.[21–24] In summary, this study in human subjects provides important confirmation of the mechanism of action and the functional significance of Lp-PLA_2 in that early atherosclerotic plaques appear to produce Lp-PLA_2, and that Lp-PLA_2 appears to trigger an inflammation cascade via production of lysoPC.

Evidence for the Role of Lipoprotein-Associated Phospholipase A_2 in the Causal Pathway of Plaque Inflammation and Endothelial Dysfunction

Evidence implicating the role of Lp-PLA_2 in the formation of inflamed, rupture-prone plaque has emerged in the last decade. This evidence includes histopathologic studies of atherosclerotic plaque for Lp-PLA_2, in vitro and in vivo application of a novel inhibitor of the Lp-PLA_2 enzyme, and finally, studies of endothelial function and its association with Lp-PLA_2. Kolodgie et al[25] demonstrated that whereas rupture-prone and ruptured plaques using monoclonal antibodies showed intense Lp-PLA_2 staining, early plaques with fatty streaks or thick fibrous caps showed minimal Lp-PLA_2 staining. Similarly, Vickers et al[26] stained carotid plaques using Lp-PLA_2 antibodies and found that Lp-PLA_2 was most abundant in the shoulder region of fibrous caps and that Lp-PLA_2 co-localized with extracellular pools of oxidized LDL, as well as in the cytosol of foam cells. Both of these histopathologic studies of Lp-PLA_2 suggest that intense concentration of the enzyme is found in the vulnerable shoulder region of thin fibrous cap atheromas, where plaque rupture typically occurs.

A further support for the role of Lp-PLA_2 in the formation of atherosclerotic plaque arises from the use of inhibitors of the enzyme. Azetidinones have been shown to inhibit Lp-PLA_2 activity. Recently, a novel azetidinone (SB0848) was shown to inhibit 80% of the enzyme activity of atherosclerotic plaques within 14 days of treatment.[27] Subsequently, Shi et al[28] studied human leukocytes in vitro to examine their production of cytokines when either native LDL or oxidized LDL were added. The cytokines, interleukin-6 and interleukin-1β, and tumor necrosis factor–α were expressed by the leukocytes when oxidized LDL was added, but not when normal LDL was added. However, when an azetidinone was added along with oxidized LDL, the Lp-PLA_2 hydrolysis of oxPL was abolished, and cytokine production was abrogated. In an ex vivo culture of the carotid plaque tissue, interleukin-18 expression was increased when oxidized LDL was added.[29] The addition of an Lp-PLA_2 enzyme inhibitor decreased the production of the interleukin-18 cytokine by about one third. These Lp-PLA_2 inhibitor studies provide further evidence of the role of the enzyme in the causal pathway of cytokine production and plaque inflammation.

Most MIs and cases of sudden death are attributable to plaque rupture at the sites of moderate stenosis, so it would be highly useful clinically to have a means to assess the nonobstructive atherosclerosis disease activity in these patients.[25,30] Atherosclerosis is a gradual process generally involving decades of plaque formation and progression. Thus, a noninvasive test identifying persons whose atherosclerosis has progressed to the point where their plaque is beset by inflammation and ED would be valuable. To identify and implement a clinically relevant biomarker, a focus on markers present in the early stages of the disease will prove more productive than relying on those at the end stages of the disease when patients present with cardiovascular events.

An approach to identify the activity of atherosclerotic disease is to measure endothelial function. The endothelium lines the arteries and serves as the physical and metabolic barrier between the blood and the underlying vascular intima. It may be measured peripherally by evaluating the response to ischemia induced in the brachial artery with an inflated sphygmomanometer. The normal response of a brachial artery with healthy endothelium to postischemic restoration of blood flow is vasodilation, which may be assessed quantitatively with ultrasound. A more direct approach to the assessment of coronary artery endothelial function is to infuse intracoronary acetylcholine, which causes vasoconstriction in the presence of ED. This approach is, by definition, invasive and uses both coronary angiography and intracoronary Doppler. Measurement of both peripheral and coronary artery ED provides an important means of assessing atherosclerosis disease activity and has been shown to predict both coronary events and stroke.[31,32] The utility of a simple laboratory test as a marker of ED would be practical. In a clinical study by Yang et al,[33] 172 patients with $<30\%$ stenosis were evaluated for coronary artery ED with an intracoronary infusion of acetylcholine. Patients in the high tertiles 2 and 3 for Lp-PLA_2 mass concentration had a significantly lower attenuated change in coronary blood flow and greater epicardial coronary artery vasoconstriction in response to acetylcholine (Figure 3). The odds ratio for coronary ED in patients with Lp-PLA_2 in the highest tertile was 3.3 (95% CI, 1.6–6.6). This finding was independent of other cardiac risk factors (Figure 4).

As has been reported previously, although there were modest associations of Lp-PLA_2 to serum lipids, Lp-PLA_2 remained an independent predictor of coronary ED after full multivariate adjustment. Thus, by identifying which patients have coronary ED despite having only minimal-to-modest ($<30\%$) coronary artery stenosis, Lp-PLA_2 may help resolve an important clinical need.

Plaque Burden May Not Reflect Plaque "Quality"

It is clinically reasonable to shift our focus to identifying persons with highly active atherosclerotic disease instead

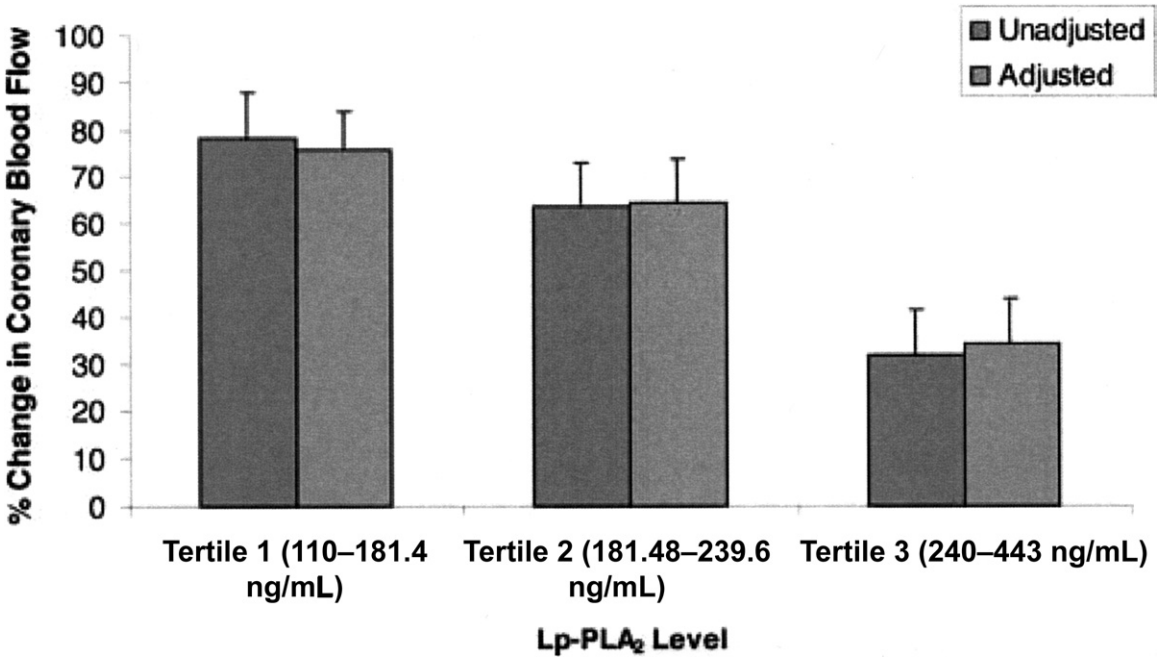

Figure 3. Endothelial dysfunction in coronary arteries worsens as lipoprotein-associated phospholipase A_2 (Lp-PLA_2) increases. Unadjusted and adjusted association between percent change (±SE) in coronary blood flow in response to the maximal dose of intracoronary acetylcholine (microvascular endothelial function) and Lp-PLA_2 is shown. Values are adjusted for age, sex, body mass index, serum creatinine, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and the use of lipid-lowering medication. Analysis of variance for trend: $p < 0.001$ for unadjusted and $p = 0.008$ for adjusted data. (Reprinted with permission from *Arterioscler Thromb Vasc Biol*.[33])

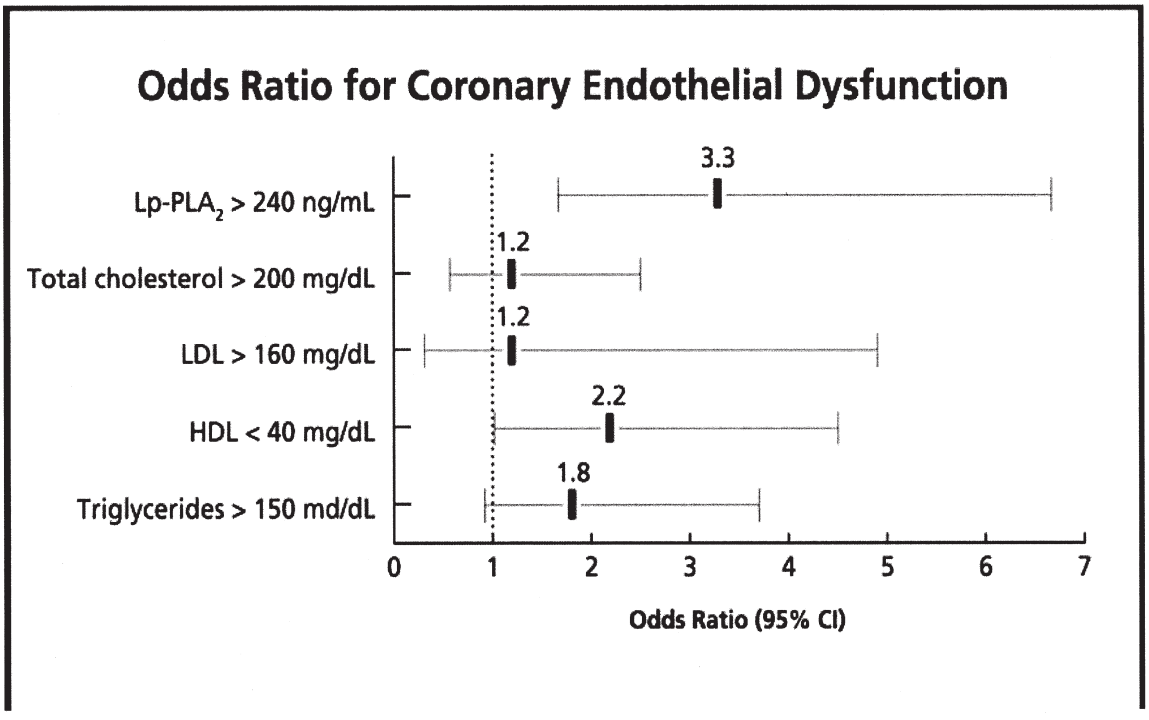

Figure 4. Odds ratio for coronary artery endothelial dysfunction for different lipid markers and lipoprotein-associated phospholipase A_2 (Lp-PLA_2). Elevated Lp-PLA_2 in the top tertile (>240 ng/mL) was associated with a statistically significant 3.3 odds ratio for coronary endothelial dysfunction (post acetylcholine challenge). Commonly measured lipids did not achieve significance as predictors of endothelial dysfunction in this cohort of 172 patients with <30% angiographic coronary stenosis. CI = confidence interval; HDL = high-density lipoprotein; LDL = low-density lipoprotein. For cholesterol, 1 mg/dL = 0.02586 mmol/L; for triglycerides, 1 mg/dL = 0.0113 mmol/L. (Courtesy of Amir Lerman, MD.)

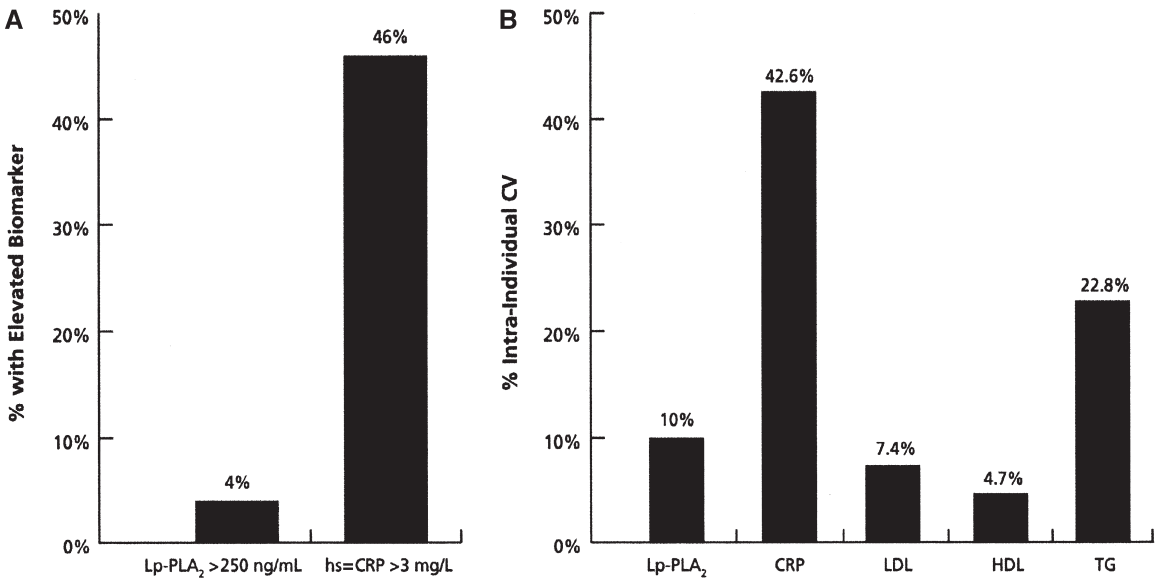

Figure 5. Specificity and biologic variability of cardiac risk markers. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has a higher specificity and lower biologic variability than other inflammatory markers and is specific to vascular inflammation. (*A*) Inflammatory markers were drawn in 90 young, healthy volunteers (average age, 34 years). Using roughly the top tertile of each biomarker, lipoprotein-associated phospholipase A_2 (Lp-PLA_2) was rarely elevated in these healthy individuals, whereas 46% of the individuals had elevated high-sensitivity C-reactive protein (hs-CRP). (*B*) Blood was drawn from 43 healthy adults 7 times for a 4-week period. Lp-PLA_2 is shown to exhibit low biologic variability (fluctuation within an individual over time) comparable to commonly measured lipids but in contrast to high biologic variability for hs-CRP. CV = cardiovascular; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TG = triglycerides. (Adapted from *Circulation.*[37])

of those with luminal narrowing. It should be further noted that many patients with a high degree of stenosis but whose plaques have been stabilized with medical management will often have low levels of Lp-PLA_2. Iribarren et al[34] found a significant association between Lp-PLA_2 mass concentration and coronary artery calcification, and Kardys et al[35] did not find an association between Lp-PLA_2 activity and coronary artery calcification.[35] In the latter study, Lp-PLA_2 was not associated significantly with other measures of extracoronary atherosclerosis, including carotid intima-media thickness and the ankle-arm index.[35] In a study performed at the Mayo Clinic, Brilakis and colleagues[36] found a strong and independent association between Lp-PLA_2 and coronary artery disease (CAD) events. However, although Lp-PLA_2 levels correlated with the extent of angiographic coronary disease on univariate analysis, the association was no longer significant after adjustment for clinical and traditional lipid risk factors.

Thus, there is a dichotomy between Lp-PLA_2 as reflective of atherosclerosis disease activity versus Lp-PLA_2 as reflective of plaque burden. Another inflammatory risk marker (CRP) that has been associated consistently with CAD events also has not been associated consistently with atherosclerotic burden (angiographic stenosis or coronary calcification). This suggests that risk markers for CAD events that may be predictive of plaque rupture are different from markers of atherosclerotic progression or plaque burden.

Clinical Laboratory Characteristics of Lipoprotein-Associated Phospholipase A_2 Testing

To have practical clinical utility, a biomarker of atherosclerosis disease activity should have high specificity and low biologic variability. Most inflammatory markers are increased in the presence of infections, rheumatologic disorders, or the inflammation that accompanies excessive mesenteric adipose tissue and insulin resistance. Wolfert et al[37] have reported that Lp-PLA_2 is not increased in patients with rheumatoid arthritis, osteoarthritis, chronic bronchitis (chronic obstructive pulmonary disease), or sinusitis. In addition, a study of patients with systemic lupus erythematosus found that patients with CVD had elevated Lp-PLA_2 activity and that lupus patients without a history of CVD had low Lp-PLA_2 activity.[38] Koenig et al[39] reported that Lp-PLA_2 was significantly associated with CAD independently from 24 other cardiac risk markers, including a multitude of inflammatory and hemostatic markers. Also, elevated Lp-PLA_2 is not associated with the severity of insulin resistance in subjects without diabetes mellitus.[40] As depicted in Figure 5, Wolfert et al[37] reported that although systemic inflammation (as reflected by high-sensitivity CRP [hs-CRP] >3 mg/L) may be present in young, healthy individuals (average age, 34 years), the increase of Lp-PLA_2 is relatively rare in this demographic.

Figure 5A shows that in 90 apparently healthy young adults, only 4% had elevated Lp-PLA_2 levels >250 ng/mL, whereas 46% had a CRP level >3 mg/L. Both cut points

reflect approximately the top tertile for each biomarker. As shown in Figure 5B, blood was drawn in the same 43 healthy individuals 7 times each over a 4-week period. The percent coefficient of variation within an individual was 10%, similar to LDL cholesterol, whereas CRP varied as much as 42.6% within the same individual. For this reason, Lp-PLA_2 can be reliably followed serially over time, whereas CRP cannot.[41] Thus, the corollary of being highly specific for vascular inflammation is that Lp-PLA_2 levels are stable over time.

The selection of the clinically useful biomarkers and their incremental value in risk stratification over traditional, established predictors has been the subject of much study and debate. Part of the debate and controversy is a result of the assessment of biomarkers as "screening tests" in primary prevention populations using the area under the curve (or c statistic) to evaluate biomarkers in a receiver operating characteristic analysis. Basically, this kind of analysis shows how often a given test scores higher in cases than in controls, and a c statistic reflecting a "worthless" test would be 0.50 whereas a "perfect" test would be 1.00. In the Women's Heart Study, age alone yielded a c statistic of 0.70, meaning that 70% of the time, age would be higher in a case with a cardiovascular event. Adding LDL cholesterol to age only increased the c statistic from 0.70 to 0.71, underscoring the limitation of the c statistic in predicting the future importance of a cardiac biomarker.[42] More importantly, this example illustrates that it is difficult to increase the c statistic above age alone in a screening population. In fact, in a recent review of the Framingham Heart Study of 10 novel biomarkers in the general population, age and sex together produced a c statistic of 0.75, and the addition of traditional risk factors increased it to 0.80, versus the 10 biomarkers together only increasing it to 0.79.[43] This again illustrates that novel biomarkers are probably not going to be very useful in low-risk persons with only 0 or 1 traditional risk factor. However, biomarkers should not be discounted based on their failure to predict events in a low-risk population. The utility of these markers improves when intermediate- or high-risk persons are evaluated. Because by definition intermediate- and high-risk persons typically have $\geq$2 traditional risk factors including older age, these risk factors do not increase the c statistic by much in these non–low-risk groups. In fact, in the setting of intermediate- or high-risk patients, several biomarkers have been shown to significantly increase the c statistic, including troponin, N-terminal pro–brain natriuretic peptide, and Lp-PLA_2.[44–46] In addition, these biomarkers provide useful and specific clinical information about the pathophysiology of the cardiovascular system (elevated troponin signals myocardial damage, N-terminal pro–brain natriuretic peptide signals myocardial stretch and early heart failure, and Lp-PLA_2 may signal that atherosclerotic plaque has become inflamed and rupture-prone).

There are now >25 prospective epidemiologic studies that consistently show that elevated Lp-PLA_2 levels predict cardiovascular events.[14,36,39,44–69] Of these, 2 studies showed that adjustment for LDL cholesterol significantly attenuated risk prediction for the biomarker: the Women's Health Study (WHS) and the Atherosclerosis Risk in Communities (ARIC) study.[46,47] In the WHS trial, there were a small number of cases, and the small size of this study likely explains the lack of statistical significance. The ARIC study comprised a larger cohort, and although LDL significantly attenuated the ability of Lp-PLA_2 to predict coronary events in the whole cohort, examination of the subcohort with LDL cholesterol values <130 mg/dL (1 mg/dL = 0.02586 mmol/L) resulted in reestablishment of the typical HR of an approximate doubling of risk for coronary events.

For stroke, LDL is not a reliable predictor of risk,[53] whereas Lp-PLA_2 has been shown to significantly predict ischemic stroke in both primary and secondary prevention studies.[52,53,58,67–69] Because LDL does not predict stroke, it is less likely to attenuate the stroke risk associated with high levels of Lp-PLA_2. Such is the case in the ARIC study, where Lp-PLA_2 predicts the risk of stroke even after adjustment for LDL and all traditional risk factors (top tertile to bottom tertile HR, 1.91; 95% CI, 1.15–3.18).[47] The ARIC stroke results have been further analyzed to see if Lp-PLA_2 and CRP together would lead to a significant reclassification of persons deemed at only moderate risk of ischemic stroke based on traditional risk factors. Ballantyne et al[70] reported that 37% of persons at moderate risk for stroke would have been correctly reclassified to either higher- or lower-risk categories when Lp-PLA_2 and hs-CRP were added to traditional risk assessment.

Although there appears to be a strong positive correlation between Lp-PLA_2 mass and activity in general white populations, this correlation may theoretically be weakened if an individual has a polymorphism or mutation that influences activity of the gene product.[2] Overall, both Lp-PLA_2 mass concentration and Lp-PLA_2 enzyme activity seem to predict higher cardiovascular risk to a similar magnitude.[1]

A panel of national experts recommended a cut point for circulating Lp-PLA_2 mass concentrations for risk stratification.[71] Levels >235 ng/mL in an intermediate-risk patient should prompt the clinician to consider treating that patient as if they were in the next higher cardiovascular risk category (high risk instead of intermediate risk). An Lp-PLA_2 mass concentration of 235 ng/mL is the 50th percentile in the healthy, adult population. Lp-PLA_2 values in the general population are not normally (Gaussian) distributed. Instead, 80% of healthy adults have Lp-PLA_2 values <290 ng/mL, with the upper 20% found in a long tail of higher Lp-PLA_2 values. This cut point has been confirmed by 2 subsequent studies: the Mayo Olmsted County study[44] and the North Wuerttemberg and Berlin Infarction Study–II (NOBIS-II).[66] In the Mayo Olmsted County study, the risk of death 1 year after MI was examined. They concluded that an Lp-PLA_2 level of 225 ng/mL was consistently associated with increased mortality, regardless of the model used, and can therefore be supported by the present data. Similarly, in the

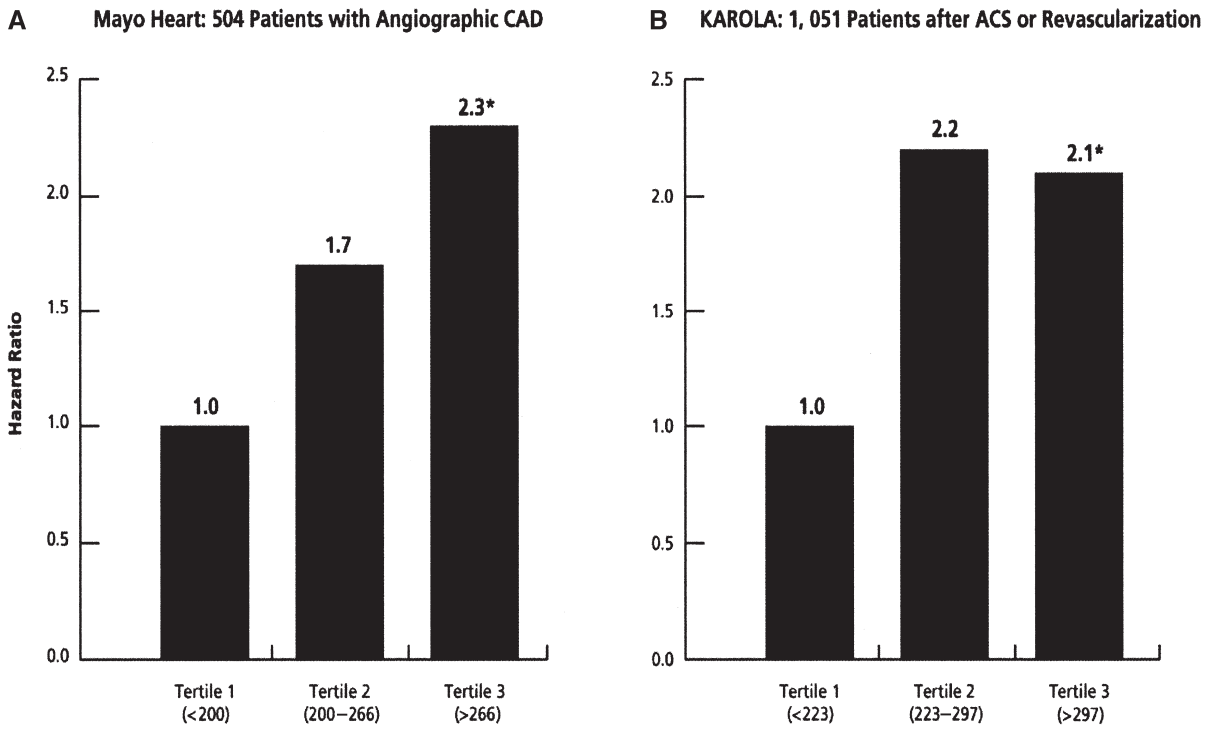

Figure 6. Apparent cardiovascular risk threshold above the bottom tertile for the elevation of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) in high-risk patients. In 504 patients with angiographic coronary artery disease in the Mayo Heart Study (*A*), and in 1,051 patients post acute coronary syndromes or coronary revascularization in the Langzeiterfolge der Kardiologischen Anschlussheil-Behandlung (KAROLA) study (*B*), the risk for subsequent hard cardiovascular events increases steeply at the middle tertile for the Lp-PLA_2 biomarker. (Adapted from *Eur Heart J*[36] and *Arterioscler Thromb Vasc Biol*.[39])

NOBIS-II study, risk of cardiovascular events within 42 days of presentation to the emergency room with chest pain consistent with acute coronary syndrome was assessed. Classification and regression tree analysis was used to determine that the ideal cut point for risk stratification for Lp-PLA_2 was 210 ng/mL, which was associated with an HR of 2.6 (95% CI, 1.1–1.6). There appears to be a risk threshold for Lp-PLA_2 mass concentration around the lowest tertile, based on data from the Mayo Heart Study and the Langzeiterfolge der Kardiologischen Anschlussheil-Behandlung (KAROLA) study (Figure 6).[36,39]

Whether Lp-PLA_2 mass concentration is in the middle or top tertile, the cardiovascular risk ratios are similar, suggesting that a risk threshold for this biomarker is equal to or slightly above 200 ng/mL.

Lipoprotein-Associated Phospholipase A_2 is Lowered by Lipid-Modifying Medications

Lp-PLA_2 is implicated in the formation of rupture-prone plaque, and its inhibition is associated with decreased cytokine production. However, prospective outcome studies using a specific Lp-PLA_2 enzyme inhibitor are not available at this time. Lipid-modifying medications stabilize plaques, with regression of the size of the lipid core, decreased macrophage infiltration, and thickening of the fibrous cap. This has been shown in coronary plaque with statins, as well as with combination lipid-modifying therapy in carotid plaques.[72–74] This raises questions of whether Lp-PLA_2 levels are modifiable, and whether lowering levels of Lp-PLA_2 may lead to improved clinical outcomes. Although it does not prove cause and effect, practically all lipid-modifying medications that have been previously been shown to reduce risk for cardiovascular events also reduce Lp-PLA_2. Pravastatin, fluvastatin, atorvastatin, simvastatin, and rosuvastatin all lower levels of Lp-PLA_2.[51,59,75–78] In the Pravastatin or Atorvastatin and Infection Therapy (PROVE-IT) study, even with intensive LDL cholesterol reduction in the group taking atorvastatin 80 mg/day, the LDL cholesterol reduction only accounted for 25% of the Lp-PLA_2 reduction in Lp-PLA_2 mass concentration.[59] At first glance, this is not intuitive because LDL particles carry most of the Lp-PLA_2 in most humans. However, only 1 in 500 apolipoprotein B–containing particles carry Lp-PLA_2 (based on ratios of the molar concentrations of apolipoprotein B and Lp-PLA_2 in human plasma), so a 1-to-1 correspondence between the reduction in LDL cholesterol (or LDL particle number) and Lp-PLA_2 would not be expected. It seems likely that statins lower Lp-PLA_2 because they stabilize plaques, with a subsequent decreased production of Lp-PLA_2. This is consistent with studies of fenofibrate and omega-3 fatty acids, which lower Lp-PLA_2 although they do not change or even increase LDL cholesterol levels.[76,79] Importantly, lipid-modifying therapies combining a statin with niacin or a statin with omega-3 fatty acids significantly lower Lp-

PLA_2, even when the statin had already lowered LDL cholesterol to optimal levels.[79,80] Kuvin et al[80] added 1 g/day of an extended-release niacin preparation to the regimen of patients with stable CAD who were already receiving a statin. After 3 months, the niacin lowered the Lp-PLA_2 level a significantly additional 20%, although the baseline level of LDL cholesterol of the patients receiving statin monotherapy was already in the therapeutic range. Similarly, in a study of patients with hypertriglyceridemia (triglyceride levels, 200–499 mg/dL [1 mg/dL = 0.0113 mmol/L]) with an achieved LDL cholesterol level of 80 mg/dL while receiving simvastatin 40 mg/day, the addition of a prescription omega-3 fatty acid preparation for 2 months resulted in a highly significant reduction in Lp-PLA_2 mass concentrations.[80] In both of these studies, there appeared to be additional "opportunity" to lower Lp-PLA_2 despite baseline achievement of LDL levels in the range of 70–80 mg/dL with statin monotherapy. Interestingly, the change in small LDL particle concentration in the statin plus omega-3 fatty acids study was significantly but relatively weakly correlated with the reduction in Lp-PLA_2 levels ($r^2 = 0.06$, p = 0.02). In the statin plus niacin study, the 20% decrease in Lp-PLA_2 with niacin exceeded the 8% decrease in small LDL particle number. These findings suggest pleiotropic anti-inflammatory actions of both omega-3 fatty acids and niacin that are uncoupled from their favorable influences on LDL particle size. Finally, in a recent study of patients with statin-intolerant type IIa or type IV dyslipidemia, the cholesterol absorption inhibitor ezetimibe lowered Lp-PLA_2 mass concentration significantly, by 18% (from 397 ng/mL to 325 ng/mL, $p < 0.05$).[77] In summary, statins, fenofibrate, niacin, omega-3 fatty acids, and ezetimibe all lower Lp-PLA_2 mass concentrations.

Conclusion

The independent association between Lp-PLA_2 levels and cardiovascular risk among different populations and across different levels of cholesterol lends support to the hypothesis that Lp-PLA_2 has a causative role in the progression of early, relatively stable atherosclerotic plaque to advanced, rupture-prone plaque. In the last few years, >25 prospective epidemiologic studies have established Lp-PLA_2 as an important aid to improve risk stratification. In addition, histopathology studies show that Lp-PLA_2 is particularly localized to the vulnerable shoulder region of rupture-prone plaques. The biomarker has been demonstrated to reflect atherosclerosis disease activity in studies of ED and in studies showing decreased cytokine production when Lp-PLA_2 enzyme activity is inhibited both in vitro and in vivo. There is a net production of Lp-PLA_2 in the coronary circulation when coronary plaque is present, and its byproduct lysoPC seems to trigger the inflammation cascade along with ED. The Lp-PLA_2 biomarker is highly specific and has low biologic variability, making it attractive relative to other inflammatory markers, which may reflect systemic inflammation more than vascular inflammation. Lp-PLA_2 predicts risk independently of traditional risk factors, including body mass index, and independently from other cardiac risk markers. Its ability to predict future cardiovascular events in higher-risk populations is evidenced by a significant increase of the c statistic (or area under the curve) in receiver operating characteristic analysis.

These recent findings suggest that Lp-PLA_2 may be a risk factor involved in the causal pathway of plaque inflammation and ultimate plaque rupture. The goal of inflammatory markers is to enhance stratification of at-risk patients so that the intensity of treatment can be matched to the appropriate level of risk. Lp-PLA_2 levels are modifiable, but there are no completed prospective treatment studies to establish it as a target of treatment. Although outcome studies will be required before Lp-PLA_2 can be considered as a target of therapy, lipid-modifying treatments that have already been proved to lower primary and secondary cardiovascular events coincidentally lower Lp-PLA_2. Although there is insufficient evidence to support the use of Lp-PLA_2 as a test in screening populations, it is conceivable that Lp-PLA_2 testing may prove to be a practical diagnostic tool in intermediate- and high-risk patients to aid in risk stratification for coronary events and stroke. If Lp-PLA_2 levels are elevated, patients should be closely monitored and their global lipid and nonlipid risk factors should be treated intensively.

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1. Garza CA, Montori VM, McConnell JP, Somers VK, Kullo IJ, Lopez-Jimenz F. Association between lipoprotein-associated phospholipase A_2 and cardiovascular disease: a systematic review. *Mayo Clin Proc* 2007;82:159–165.
2. McConnell JP, Hoefner DM. Lipoprotein-associated phospholipase A_2 [review]. *Clin Lab Med* 2006;26:679–697.
3. Macphee CH, Moores KE, Boyd HF, Dhanak D, Ife RJ, Leach CA, Leake DS, Milliner KJ, Patterson RA, Suckling KE, Tew DG, Hickey DM. Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. *Biochem J* 1999;338:479–487.
4. Tjoelker LW, Wilder C, Eberhardt C, Stafforini DM, Dietsch G, Schimpf B, Hooper S, Le Trong H, Cousens LS, Zimmerman GA, et al. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature* 1995;374:549–553.
5. Min JH, Jain MK, Wilder C, Paul L, Apitz-Castro R, Spleaf DC, Gelb MH. Membrane-bound plasma platelet activating factor acetylhydro-

lase acts on substrate in the aqueous phase. *Biochemistry* 1999; 38:12935–12942.
6. Stafforini DM, Tjoelker LW, McCormick PA, Vaitkus D, McIntyre TM, Gray PW, Young SG, Prescott SM. Molecular basis of the interaction between plasma platelet activating factor acetylhydrolase and low density lipoprotein. *J Biol Chem* 1999;274:7018–7024.
7. Snyder MR, Mullins JL, Ninova D, Hartman SJ, Wolfert RL, Jaffe AS, McConnell JP. Distribution of Lp-PLA2 among lipoprotein fractions and association with atherosclerotic risk markers (Abstract C-132). *Clin Chem* 2006;52:(6 Suppl):A120–1.
8. Tselepis AD, Karabina SA, Stengel D, Piedagnel R, Chapman MJ, Ninio E. N-linked glycosylation of macrophage derived PAF-AH is a major determinant of enzyme association with plasma HDL. *J Lipid Res* 2001;42:1645–1654.
9. Marathe GK, Zimmerman GA, McIntyre TM. Platelet-activating factor acetylhydrolase, and not paraoxonase-1, is the oxidized phospholipids hydrolase of high density lipoprotein particles. *J Biol Chem* 2003;278:3937–3947.
10. Karabina SA, Liapikos TA, Grekas G, Goudevenos J, Tselepis AD. Distribution of PAF-acetylhydrolase activity in human plasma low-density subfractions. *Biochim Biophysics Acta* 1994;1213:34–38.
11. Gazi I, Lourida ES, Filippatos T, Tsimihodimos V, Tselepis EM. Lipoprotein-associated phospholipase A_2 activity is a marker of small, dense LDL particles in human plasma. *Clin Chem* 2005;51:2264–2273.
12. Hodis HN, Kramsch DM, Avogaro P, Bittolo-Ban G, Cazzolato G, Hwang J, Peterson H, Sevanian A. Biochemical and cytotoxic characteristics of an in vivo circulation oxidized low density lipoprotein (LDL). *J Lipid Res* 1994;35:669–677.
13. Yang CY, Raya JL, Chen HH, Chen CH, Abe Y, Pownall HJ, Taylor AA, Smith CV. Isolation, characterization, and functional assessment of oxidatively modified subfractions of circulating low density lipoproteins. *Arterioscler Thromb Vasc Biol* 2003;23:1083–1090.
14. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Widermann CJ, Oberthaler S, Xu Q, Witztum JL, Tsimikas S. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A_2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. *Arterioscler Thromb Vasc Biol* 2007;27:1788–1795.
15. Macphee CH, Tew DG, Rice SQJ, Lawrence GMP, Southan CD, Milliner KJ, Moores KE, Li H, Gloger IS. A novel phospholipase A_2 has been cloned that is responsible for the increased lyso-phosphatidylcholine content of oxidatively modified LDL. *Atherosclerosis* 1995;115:S33.
16. Glass CK, Witztum JL. Atherosclerosis: the road ahead [review]. *Cell* 2001;104:503–516.
17. Hakkinen T, Luoma JS, Hiltunen MO, MacPhee CH, Milliner KJ, Patel L, Rice SQ, Tew DG, Karkola K, Yla-Herttuala S. Lipoprotein-associated phospholipase A_2, platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 1999;19:2909–2917.
18. Tselepis AD, Chapman M. Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A_2, platelet activating factor-acetylhydrolase. *Atheroscler Suppl* 2002;3: 57–68.
19. Sudhir K. Clinical review: lipoprotein-associated phospholipase A_2, a novel inflammatory biomarker and independent risk predictor for cardiovascular disease. *J Clin Endocrinol Metab* 2005;90:3100–3105.
20. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A_2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* 2007;115:2715–2721.
21. Safaya R, Chai H, Kougias P, Lin P, Lumsden A, Yao Q, Chen C. Effect of lysophosphatidylcholine on vasomotor functions of porcine coronary arteries. *J Surg Res* 2005;126:182–188.
22. Inoue N, Takeshita S, Gao D, Ishida T, Kawashima S, Akita H, Tawa R, Sakurai H, Yokoyama M. Lysophosphatidylcholine increases the secretion of matrix metalloproteinase 2 through the activation of NADH/NADPH oxidase in cultured aortic endothelial cells. *Atherosclerosis* 2001;155:45–52.
23. Takahashi M, Okazaki H, Ogata Y, Takeuchi K, Ikeda U, Shimada K. Lysophosphatidylcholine induces apoptosis in human endothelial cells through a p38-mitogen-activated protein kinase-dependent mechanism. *Atherosclerosis* 2002;161:387–394.
24. Chadhuri P, Colles SM, Damron DS, Graham LM. Lysophosphatidylcholine inhibits endothelial cell migration by increasing intracellular calcium and activating calpain. *Arterioscler Thromb Vasc Biol* 2003; 23:218–223.
25. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, Virmani R. Lipoprotein-associated phospholipase A_2 protein expression in the natural progression of human coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;26:2523–2529.
26. Vickers KC, Maguire C, Wolfert RL, Insull W, Holvoet P, Morrisett J. Quantitation and distribution of lipoprotein-associated phospholipase A_2 and oxidized low-density lipoprotein in atherosclerotic lesions of carotid endarterectomy tissues [abstract]. *J Am Coll Cardiol* 2006; 47(suppl A):346A.
27. Johnson A, Zalewski A, Janmohamed S, Sawyer J, Rolfe T, Staszkiewicz W, Alvarez S. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity, an emerging CV risk marker, can be inhibited in atherosclerotic lesions and plasma by novel pharmacologic intervention: the results of a multicenter clinical study [abstract]. *Circulation* 2004;110:III-590.
28. Shi Y, Zhang P, Zhang L, Osman H, Mohler ER, Macphee C, Zalewski A, Postle A, Wilensky RL. Role of lipoprotein-associated phospholipase A_2 in leukocyte activation and inflammatory responses. *Atherosclerosis* 2007;191:54–62.
29. Zhang P, Zhang L, Carabasi A, Diehl J, Dimuzio PJ, Dimatteo C, Lombardi J, Larson R, Zalewski A, Macphee C, O'Brien S, Shi Y. Oxidized LDL-induced upregulation of IL-18 in monocytes/macrophages is modulated by lipoprotein-associated phospholipase A_2: potential mechanism of inflammatory burden in atherosclerosis. *Circulation* 2006;114:II-24. Abstract 258.
30. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation* 1995;92:657–671.
31. Targonski PV, Bonetti PO, Pumper GM, Higano ST, Holmes DR, Lerman A. Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. *Circulation* 2003;107:2805–2809.
32. Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation* 2005;111:363–368.
33. Yang EH, McConnell JP, Lennon RJ, Barsness GW, Pumper G, Hartman SJ, Rihal CS, Lerman LO, Lerman A. Lipoprotein-associated phospholipase A_2 is an independent marker for coronary endothelial dysfunction in humans. *Arterioscler Thromb Vasc Biol* 2006;26:106–111.
34. Iribarren C, Gross MD, Darbinian JA, Jacobs DR Jr, Sidney S, Loria CM. Association of lipoprotein-associated phospholipase A_2 mass and activity with calcified coronary plaque in young adults: the CARDIA study. *Arterioscler Thromb Vasc Biol* 2005;25:216–221.
35. Kardys I, Oei HH, Hofman A, Oudkerk M, Witteman JC. Lipoprotein-associated phospholipase A_2 and coronary calcification: the Rotterdam Coronary Calcification Study. *Atherosclerosis* 2007;191:377–383.
36. Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A_2 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.
37. Wolfert RL, Kim NW, Selby RG, Sarno MJ, Warnick RG, Sudhir K. Biological variability and specificity of lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a novel marker of cardiovascular risk [abstract]. *Circulation* 2004;110(suppl 3):309.

38. Cederholm A, Svenungsson E, Stengel D, Fei GZ, Pockley AG, Ninio E, Frostegard J. Platelet-activating factor-acetylhydrolase and other novel risk and protective factors for cardiovascular disease in systemic lupus erythematosus. *Arthritis Rheum* 2004;50:2869–2876.
39. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A_2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function and hemodynamic stress (KAROLA). *Arterioscler Thromb Vasc Biol* 2006;26:1586–1593.
40. McLaughlin TM, Abbasi F, Wolfert R, Lamendola C, Reaven G, Reaven P. Lipoprotein-associated phospholipase A_2 is not increased in association with insulin resistance [abstract]. *Arterioscler Thromb Vasc Biol* 2004;24:E-67.
41. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, Fadl YY, Fortmann SP, Myers HY, Myers GL, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499–511.
42. Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. *Circulation* 2007;115:928–935.
43. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Shelhub J, Robins SJ, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med* 2006;355:2631–2639.
44. Gerber Y, McConnell JP, Jaffe AS, Weston SA, Killian JM, Roger VL. Lipoprotein-associated phospholipase A_2 and prognosis after myocardial infarction in the community. *Arterioscler Thromb Vasc Biol* 2006; 26:2517–2522.
45. Winkler K, Hoffmann MM, Winkelmann BR, Friedrich I, Schafer G, Seelhorst U, Wellnitz B, Wieland H, Boehm BO, Marz W. Lipoprotein-associated phospholipase A_2 predicts 5-year 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.
46. Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. Prospective evaluation of lipoprotein-associated phospholipase A_2 levels and the risk of future cardiovascular events in women. *J Am Coll Cardiol* 2001;38:1302–1306.
47. Ballantyne C, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A_2, high-sensitivity C-reactive protein, and risk for incident heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2004;109:837–842.
48. Packard CJ, O'Reilly DSJ, Caslake MJ, McMahan AD, Ford I, Cooney J, MacPhee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GDO, for the West of Scotland Coronary Prevention Study Group. Lipoprotein-associated phospholipase A_2 as an independent predictor of coronary heart disease. *N Engl J Med* 2000;343:1148–1155.
49. Blankenberg S, Stengel D, Rupprecht HJ, Bickel C, Meyer J, Cambien F, Tiret L, Ninio E. Plasma PAF-acetylhydrolase in patients with coronary artery disease: results of a cross-sectional analysis. *J Lipid Res* 2003;44:1381–1386.
50. Koenig W, Khuseyinova N, Löwel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A_2 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 (MONICA-Augsburg). *Circulation* 2004;110:1903–1908.
51. Winkler K, Abletshauser C, Friedrich I, Hoffmann MM, Wieland H, März W. Fluvastatin slow-release lowers platelet-activating factor acetyl hydrolase activity: a placebo-controlled trial in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2004;89:1153–1159.
52. Oei H-HS, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MMB, Witteman JCM. Lipoprotein-associated phospholipase A_2 activity is associated with risk of coronary heart disease and ischaemic stroke: the Rotterdam Study. *Circulation* 2005;111:570–575.
53. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A_2, 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.
54. Winkler K, Winkelmann BR, Scharnagl H, Hoffmann MM, Grawitz AB, Nauck M, Bohm BO, Marz W. Platelet-activating factor acetylhydrolase activity indicates angiographic coronary artery disease independently of systemic inflammation and other risk factors. *Circulation* 2005;111:980–987.
55. Khuseyinova N, Imhof A, Rothenbacher D, Trischler G, Kuelb S, Scharnagl H, Maerz W, Brenner H, Koenig W. Association between Lp-PLA_2 and coronary artery disease: focus on its relationship with lipoproteins and markers of inflammation and hemostasis. *Atherosclerosis* 2005;182:181–188.
56. May HT, Horne BD, Anderson JL, Wolfert RL, Muhlestein JB, Renlund DG, Clarke JL, Kolek MJ, Bair TL, Pearson RR, Sudhir K, Carlquist JF. Lipoprotein-associated phospholipase A_2 independently predicts the angiographic diagnosis of coronary artery disease and coronary death. *Am Heart J* 2006;152:997–1003.
57. Caslake MJ, Cooney J, Murray E, Bedford D, Robertson M, Nelson JJ, Packard CJ. Lipoprotein-associated phospholipase A_2 as a risk factor for coronary vascular disease in the elderly (PROSPER). Proceedings from the XIV International Symposium on Atherosclerosis; June 18–22, 2006; Rome, Italy. Abstract page 484.
58. Elkind MS, Tai W, Coates K, Paik MC, Sacco RL. High-sensitivity C-reactive protein, lipoprotein-associated phospholipase A_2, and outcome after ischemic stroke. *Arch Intern Med* 2006;166:2073–2080.
59. O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, Braunwald E. Lp-PLA_2 and its association with cardiovascular outcomes in patients with acute coronary syndromes in the PROVE IT–TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection-Thrombolysis in Myocardial Infarction) trial. *Circulation* 2006;113:1745–1752.
60. Jenny NS, Solomon C, Cushman M, Nelson JJ, Tracy RP, Psaty BM, Furberg CD. Lipoprotein-associated phospholipase A_2 and cardiovascular disease: results from the Cardiovascular Health Study [abstract]. *Circulation* 2006;113:E-332.
61. Corsetti JP, Rainwater DL, Moss AJ, Zareba W, Sparks CE. High lipoprotein-associated phospholipase A_2 is a risk factor for recurrent coronary events in postinfarction patients. *Clin Chem* 2006;52:1331–1338.
62. Sabatine MS, Morrow DA, O'Donoghue M, Jablonski KA, Rice MM, Solomon S, Rosenberg Y, Domanski MJ, Hsia J, for the PEACE Investigators. Prognostic utility of lipoprotein-associated phospholipase A_2 for cardiovascular outcomes in patients with stable coronary artery disease. *Arterioscler Thromb Vasc Biol* 2007;27:2463–2469.
63. Daniels LB, Barrett-Connor E, Sarno M, Laughlin GA, Bettencourt R, Wolfert RL. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) independently predicts incident coronary heart disease (CHD) in an apparently healthy older population: the Rancho Bernardo Study [abstract]. *Circulation* 2006;114:II-870.
64. Oldgren J, James SK, Siegbahn A, Wallentin L. Lipoprotein-associated phospholipase A_2 does not predict mortality or new ischaemic events in acute coronary syndrome patients. *Eur Heart J* 2007;28:699–704.
65. Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA_2 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.

66. Mockel M, Muller R, Vollert JO, Muller C, Danne O, Gareis R, Stork T, Dietz R, Koenig W. Lipoprotein-associated phospholipase A_2 for early risk stratification in patients with suspected acute coronary syndrome: a multi-marker approach: the North Wuerttemberg and Berlin Infarction Study-II (NOBIS-II). *Clin Res Cardiol* 2007;96:604–612.
67. Robins SJ, Collins D, Nelson JJ, Bloomfield HE, Asztalos BF. Cardiovascular events with increased lipoprotein-associated phospholipase A2 and low high-density lipoprotein-cholesterol. The Veterans Affairs HDL Intervention Trial. *Arterioscler Thromb Vasc Biol* 2008; 28. DOI:10.1161/ATVBAHA.107.160739.
68. Furie KL, Parides MK, Greer DM, Camargo ECS, Singhal AB, Lederer M, Hagan N, Dipietro A, Bliss S, McCarthy CJ, et al. Lipoprotein-associated phospholipase A_2 activity predicts early stroke recurrence [abstract]. *Stroke* 2007;382s:458.
69. Wassertheil-Smoller S, Kooperberg C, McGinn AP, Kaplan RC, Hsia J, Hendrix SL, Manson JE, Berger JS, Kuller LH, Allison MA, Baird AE. Lipoprotein-associated phospholipase A2, hormone use, and the risk of ischemic stroke in postmenopausal women. *Hypertension* 2008; 51(4):1115–1122.
70. Ballantyne C, Nambi V, Chambless L, Hu Y, Coresh J, Ni J, Folsom A, Sharrett A, Boerwinkle E, Hoogeveen R. Inflammatory biomarkers Lp-PLA_2 and CRP for prediction of first ischemic stroke [abstract]. *Vasc Med* 2007;12:146.
71. Lanman RB, Wolfert RL, Fleming JK, Jaffe AS, Roberts WL, Warnick GR, McConnell JP. Lipoprotein-associated phospholipase A_2: review and recommendation of a clinical cut point for adults. *Prev Cardiol* 2006;9:138–143.
72. Libby P, Sasiela W. Plaque stabilization: can we turn theory into evidence? *Am J Cardiol* 2006;98(suppl):26P–33P.
73. Taylor AJ, Sullenberger LE, Lee HJ, Lee JK, Grace KA. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* 2004;110: 3512–3517.
74. Zhao XQ, Yuan C, Hatsukami TS, Frechette EH, Kang XJ, Maravilla KR, Brown BG. Effects of prolonged intensive lipid-lowering therapy on the characteristics of carotid atherosclerotic plaques in vivo by MRI: a case-control study. *Arterioscler Thromb Vasc Biol* 2001;21: 1623–1629.
75. Albert MA, Glynn RJ, Wolfert RL, Ridker PM. The effect of statin therapy on lipoprotein associated phospholipase A_2 levels. *Atherosclerosis* 2005;182:193–198.
76. Muhlestein JB, May HT, Jensen JR, Horne BD, Lanman RB, Lavasani F, Wolfert RL, Pearson RR, Yannicelli HD, Anderson JL. The reduction of inflammatory biomarkers by statin, fibrate, and combination therapy among diabetic patients with mixed dyslipidemia. *J Am Coll Cardiol* 2006;48:396–401.
77. 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 A_2. *Arterioscler Thromb Vasc Biol* 2007;27:2236–2243.
78. Schaefer EJ, McNamara JR, Asztalos BF, Tayler T, Daly JA, Gleason JL, Seman LJ, Ferrari A, Rubenstein JJ. Effects of *atorvastatin* versus other statins on fasting and postprandial C-reactive protein and lipoprotein-associated phospholipase A_2 in patients with coronary heart disease versus control subjects. *Am J Cardiol* 2005;95:1025–1032.
79. Shalwitz RA, Maki KV, Doyle RT, Ballantyne CM. Lipoprotein subfraction responses differentially predict changes in lipoprotein-associated phospholipase A_2 during prescription omega-3 therapy [abstract]. *Arterioscler Thromb Vasc Biol* 2007;27:e93–e94.
80. Kuvin JT, Dave DM, Sliney KA, Mooney P, Patel AR, Kimmelsstiel CD, Karas RH. Effects of extended-release niacin on lipoprotein particle size, distribution, and inflammatory markers in patients with coronary artery disease. *Am J Cardiol* 2006;98:743–745.