Modulation of oxidative stress, inflammation, and atherosclerosis by lipoprotein-associated phospholipase A₂

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**ABSTRACT**

Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$), also known as platelet-activating factor acetylhydrolase (PAF-AH), is a unique member of the phospholipase A$_2$ superfamily. This enzyme is characterized by its ability to specifically hydrolyze PAF as well as glycerophospholipids containing short, truncated and/or oxidized fatty acyl groups at the sn-2 position of the glycerol backbone. In humans, Lp-PLA$_2$ circulates in active form as a complex with low- and high-density lipoproteins. Clinical studies have reported that plasma Lp-PLA$_2$ activity and mass are strongly associated with atherogenic lipids and vascular risk. These observations led to the hypothesis that Lp-PLA$_2$ activity and/or mass levels could be used as biomarkers of cardiovascular disease and that inhibition of the activity could offer an attractive therapeutic strategy. Darapladib, a compound that inhibits Lp-PLA$_2$ activity, is anti-atherogenic in mice and other animals, and decreases atherosclerotic plaque expansion in humans. However, disagreement continues to exist regarding the validity of Lp-PLA$_2$ as an independent marker of atherosclerosis and a scientifically justified target for intervention. Circulating Lp-PLA$_2$ mass and activity are associated with vascular risk, but the strength of the association is reduced after adjustment for basal concentrations of the lipoprotein carriers with which the enzyme associates. Genetic studies in humans harboring an inactivating mutation at this locus indicate that loss of Lp-PLA$_2$ function is a risk factor for inflammatory and vascular conditions in Japanese cohorts. Consistently, over-expression of Lp-PLA$_2$ has anti-inflammatory and anti-atherogenic properties in animal models. This thematic review critically discusses results from laboratory and animal studies, analyzes genetic evidence, reviews clinical work demonstrating associations between Lp-PLA$_2$ and vascular disease, and summarizes results from animal and human clinical trials in which administration of darapladib was tested as a strategy for the management of atherosclerosis.

Keywords: Lipoprotein-associated phospholipase A$_2$, PAF-AH, PLA2G7, darapladib, oxidative stress, inflammation, atherosclerosis, cardiovascular disease, stroke, PAF, platelet-activating factor, oxidized phospholipids.

Abbreviations: AA-PC, arachidonoyl phosphatidylcholine; ACS, acute coronary syndrome; apoB100, apolipoprotein B100; CAD, coronary artery disease; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DM-HC, diabetic and hypercholesterolemic; Hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intracellular adhesion molecule-1; IL-6, interleukin-6; Lp-PLA$_2$, lipoprotein-associated PLA$_2$; MCP-1, monocyte chemotactic protein-1; MI, myocardial infarction; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; NASCET, North American Symptomatic Carotid Endarterectomy Trial; OR, odds ratio; OxFA, oxidized fatty acids; OxPL, oxidized phospholipids; OxPL/ApoB, oxidized phospholipid/apolipoprotein B ratio; PAF, platelet-activating factor; PC, phosphatidylcholine; PCI, percutaneous coronary intervention; PLAP, phospholipase A$_2$; RR, relative risk; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; WHHL, Watanabe heritable hyperlipidemic.
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1. INTRODUCTION

Atherosclerosis is characterized by a complex interplay between bloodstream and arterial wall components that leads to a chronic state of vascular oxidative stress and inflammation (1). Oxidative stress is a key feature of atherogenesis as free radical generation is linked to the formation of lipid oxidation products that increase vascular inflammation (2). Vascular inflammation plays a key role in the development of early lesions and fatty streaks and is thought to contribute to complications and life-threatening events such as acute myocardial infarction (MI) and stroke. Thus, inflammation and oxidative stress are hallmarks of atherosclerosis and are thought to contribute to the initiation, progression, and rupture of lipid-rich vascular lesions.

Several strategies have been used to attenuate oxidation and inflammation in the vascular wall. Examples of these include antioxidants (3) and lipooxygenase inhibitors (4) that limit oxidant stress, and secretory phospholipase A$_2$ (sPLA$_2$) inhibitors that inhibit production of pro-inflammatory prostaglandins and leukotrienes (5, 6). Cellular oxidation is controlled through a network of sensors whose function is to ensure that the redox state of the cell remains within viable parameters. In addition to the multiplicity of compensatory responses, individual pathways are biochemically, genetically, and epigenetically regulated by diverse stimuli, environmental cues, receptor ligands, and transcriptional activators whose concentrations are constantly changing to meet homeostatic demands (7). Similarly, a variety of physiological, often redundant, mechanisms regulate the balance between pro- and anti-inflammatory responses. These considerations underscore the challenges associated with strategies that rely on targeting a single pathway of inflammation and/or oxidation (5, 8). Inflammation is a physiologic and highly regulated response: while blocking its onset offers therapeutic benefit in a variety of settings, this mechanism is essential for host defense. Inappropriate blockade of inflammation can have detrimental short-term and/or long-term consequences, and should be carefully assessed in individual cases.

The development of cardiovascular disease (CVD) is associated with altered expression of multiple inflammatory gene products (1). Biomarkers of disease progression sometimes—but not always—offer an opportunity for therapeutic intervention. When mechanistic studies demonstrate that a biomarker is causally linked to disease pathogenesis, a solid and justified opportunity for intervention arises. Circulating levels of Lipoprotein-associated PLA$_2$ (Lp-PLA$_2$) have been proposed to be markers of CVD on the basis of numerous epidemiological studies (9, 10). Initial work conducted at least twenty-five years ago showed good correlations between plasma Lp-PLA$_2$ activity and proven markers of CVD, such as LDL cholesterol and lipoprotein levels (11-15). Thus, the association between Lp-PLA$_2$ and CVD has been recognized for many years.

Lp-PLA$_2$ is a unique member of the PLA$_2$ super-family that currently contains fifteen separate, identifiable groups and numerous subgroups (16-17). These enzymes are characterized by their ability to hydrolyze the sn-2 ester bond of phospholipid substrates and are assigned to groups based on sequence, molecular weight, disulfide bonding patterns, the requirement for Ca$^{2+}$, and other features. There are five main categories of PLA$_2$ activities: secreted small molecular weight sPLA$_2$ enzymes that include groups I-III, V, IX-XIV, larger cytosolic Ca$^{2+}$-dependent cPLA$_2$s (group IV), Ca$^{2+}$-independent iPLA$_2$s (group VI), platelet activating factor (PAF) acetylhydrolases (groups VII and VIII), and lysosomal PLA$_2$s (16-17). Lp-PLA$_2$ has been classified as group VIIA PLA$_2$ (PLA2G7) owing to its sequence, size, calcium independence and substrate specificity (16, 17). This enzyme was discovered based on its ability to catalyze hydrolysis of the acetyl group at the sn-2 position of PAF to generate lyso-PAF and acetate (18). To illustrate the ability of Lp-PLA$_2$ to
inactivate PAF, which has been implicated in a variety of inflammatory diseases (19, 20), the enzyme was referred to as the secreted/plasma form of PAF acetylhydrolase or PAF-AH. Lp-PLA₂ is primarily produced by macrophages (21) and circulates in plasma in active form as a complex with LDL and HDL (22). A small fraction of the enzyme is carried in Lp(a) in subjects that express this particle (23). The biochemical and physiological interactions of the enzyme with lipoproteins gave rise to more descriptive terminology (i.e., Lp-PLA₂) which is commonly used, particularly in clinical settings.

This thematic review presents current knowledge of the structural and biochemical properties of Lp-PLA₂. In addition, we provide a critical discussion of results from genetic, epidemiological, and animal studies and their implication regarding the role of this enzyme in atherosclerosis. Finally, we offer our views on the utility of Lp-PLA₂ as a biomarker and therapeutic target for the treatment of CVD.

2. LIPROTEIN-ASSOCIATED PHOSPHOLIPASE A₂: EXPERIMENTAL STUDIES

2.1. BIOCHEMICAL AND STRUCTURAL PROPERTIES

Lp-PLA₂ is a Ca²⁺-independent, 45 KDa secreted protein that circulates in plasma in active form. Sequence analysis of PLA2G7, the gene encoding Lp-PLA₂, reveals the presence of a GXSXG motif that is characteristic of neutral lipases and serine esterases (24). The enzyme harbors a serine/aspartate/histidine catalytic triad whose linear orientation and spacing is consistent with the α/β hydrolase conformation of neutral lipases and esterases (24). This was confirmed when the solution of the crystal structure of Lp-PLA₂ revealed that the protein indeed has a classic lipase α/β hydrolase fold (25). In contrast to most PLA₂ activities that initiate signal transduction and are regulated by the state of cellular activation, Lp-PLA₂ is not acutely regulated (19). This feature, combined with the independence of the reaction on Ca²⁺, could potentially threaten the integrity of phospholipid components of cellular membranes and lipoproteins (26). However, the ability of the enzyme to recognize only defined types of sn-2 fatty acyl groups insures that undamaged, structural phospholipids are protected from constitutive hydrolysis (27). In addition to PAF, Lp-PLA₂ catalyzes hydrolysis of phospholipids generated from structural components in cellular membranes and lipoproteins (e.g., LDL) in settings of high oxidant stress (28, 29). These substrates, which harbor short- and medium-length sn-2 acyl groups, resemble PAF from structural and functional standpoints (30). The length restriction is relaxed when the sn-2 group contains oxidized functionalities such as fatty alcohols (31) and isoprostanes (32) generated in situ from membrane phospholipids. However, in this case the rate of hydrolysis is much slower. These properties, which were identified using purified substrates (28, 32), were later confirmed in elegant studies using human LDL exhaustively oxidized with CuSO₄ in the presence and absence of SB222657, an irreversible inhibitor of Lp-PLA₂ (33). Lp-PLA₂ lacks a lid region and its active site has been proposed to be accessible to the solvent (34). This open active site conformation may account for the ability of the enzyme to accommodate various sn-2 acyl chains in phospholipid substrates.

A key feature of Lp-PLA₂ is its association with lipoproteins, including HDL, LDL and, to a lesser extent, Lp(a) [reviewed in (26)]. Differential distribution of Lp-PLA₂ in various plasma lipoproteins may impact its function (35-38) and thus much effort has been spent elucidating the molecular basis for these associations. Binding of Lp-PLA₂ to LDL requires W115 and L116, which are located in a region mostly composed of hydrophobic amino acids (39). Recent studies have shown that these residues mediate penetration of the enzyme into the lipid membrane surface, and that they are important for Lp-PLA₂ binding to hydrophobic surfaces (34). Tyrosine-
205, a residue conserved only among Lp-PLA₂ orthologs that associate with endogenous LDL particles, is also required for association with LDL (39). The enzyme appears to bind to apolipoprotein B100 (apoB100) through domains located in a 160 amino stretch comprised between apoB100 residues 4119 and 4279 (39). In recent studies, we found that a carboxyl terminal string of amino acids (H367-K370) unique to human Lp-PLA₂ is required for association with HDL (40); additional regions may be required for this interaction.

### 2.2. Cellular Sources and Regulation of Expression

Multiple inflammatory cells involved in atherogenesis secrete Lp-PLA₂, including monocytes, macrophages, neutrophils, activated bone marrow-derived mast cells, and activated platelets (21, 41). Monocyte/macrophages are key players in both initiation and progression of atherosclerosis, and have been proposed as new potential therapeutic targets for the prevention and treatment of this disease (42). We previously demonstrated that maturation of monocytes into macrophages is accompanied by dramatic increases in Lp-PLA₂ mRNA and protein (21, 43); these observations were recently confirmed (44). Treatment of macrophages with acetylated LDL generates foam cells in vitro and further increases Lp-PLA₂ expression (44), which is consistent with observations in human atherosclerotic plaques (45). The properties of macrophages in atherosclerotic lesions can vary dramatically between two extremes: pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages; these cells can switch from one phenotype to the other depending on micro-environmental cues such as differentiation factors produced in the lesion and T-cell-derived polarizing cytokines (46, 47).

We found that IFN-γ, which has been implicated as a promoter of foam cell formation and an inducer of M1 pro-inflammatory phenotypes (47), decreased expression of Lp-PLA₂ at the transcriptional level (48). Conversely, M-CSF, which induces an M2-like, anti-inflammatory phenotype, increased monocyte Lp-PLA₂ expression to a much higher extent compared with the M1-inducer GM-CSF (49). However, Ferguson and co-workers reported modest increases or no changes in Lp-PLA₂ mRNA levels in M1 and M2 macrophages, respectively, using different induction strategies (44). In addition to M1 and M2, other macrophage phenotypes have been described, including sub-species generated in response to stimulation with oxidized phospholipids [OxPL, (50)]. Agents such as LPS, IL-1β, G-CSF, and TNF-α can significantly increase the synthesis and secretion of functional Lp-PLA₂ by monocytes, RAW264.7, and THP-1 cells (51, 52). These effects appear to be modulated by the state of cellular differentiation (26). It is clear that signals from the microenvironment dynamically regulate Lp-PLA₂ expression and the phenotype of cells of the monocyte/macrophage lineage and that these effects impact key cellular functions.

Independent studies have shown that PAF, which bears structural resemblance to fragmented, short-chain phospholipids, up-regulates expression of Lp-PLA₂ mRNA in monocyte/macrophages (48) and HepG2 cells (53). Consistently, a recent study reported elevated plasma levels of both PAF and Lp-PLA₂ mass in a cohort of coronary heart disease (CHD) patients compared with a control group (54). Increased Lp-PLA₂ mass in this setting may represent in vivo evidence for PAF- and/or OxPL mediated effects on Lp-PLA₂ expression. De Keyzer and colleagues showed that a high-fat diet, which led to increased levels of oxidized LDL, was accompanied by robust increases in Lp-PLA₂ activity (55). When this response was modeled in a cellular system, the investigators found that oxidized LDL robustly increased expression of Lp-PLA₂ mRNA in THP1 cells and, to a lesser extent, human monocyte-derived macrophages (55). These combined results suggest that oxidized LDL, PAF, and perhaps OxPL, up-regulate expression of Lp-PLA₂ in vivo, possibly by increasing mRNA synthesis or stability, resulting in
higher protein expression. This response likely reflects a homeostatic, negative-feedback mechanism. Additional studies should further investigate changes in PLA2G7 mRNA expression in response to stimulation with various stimuli such as OxPL generated during oxidative modification of LDL and in settings of elevated oxidant stress. The impact of the cellular phenotype on these responses should also be investigated.

In addition to transcriptional regulation, Lp-PLA2 expression is genetically controlled. A recent a genome-wide association study that included five population-based reports comprising 13,664 subjects identified eight genetic loci associated with variation in Lp-PLA2 mass and activity (56). Lp-PLA2 mass was found to be regulated mainly by PLA2G7, consistent with another report (57). Lp-PLA2 activity was strongly associated with genetic variants involved in lipid metabolism (56). The findings of this interesting study suggest that genetic regions known to affect lipoprotein levels also influence Lp-PLA2 expression.

2.3. Functional Consequences of Lp-PLA2 Action

A number of studies have concluded that the in vivo action of Lp-PLA2 in settings of elevated oxidant stress, allergy and acute inflammation is beneficial [see (26) for a recent review]. The mechanism accounting for these effects is thought to involve Lp-PLA2-mediated decreases in the bioactivity of phospholipid substrates such as PAF and OxPL. However, an issue that has eluded clarification despite considerable effort over the years is whether the in vivo action of Lp-PLA2 on endogenous substrates, generates products [i.e., lyso-phosphatidylcholine (lyso-PC) plus short and/or oxidized fatty acids] that increase inflammatory responses in the vascular wall. Specifically, it has not been determined whether Lp-PLA2 is beneficial or detrimental, and whether the substrates are more, less, or equally atherogenic than the byproducts (58). This issue has been explored using in vitro approaches to characterize biological properties of relevant substrates and products of the reaction. Both pro- and anti-inflammatory activities have been ascribed to glycerophospholipids harboring fragmented and/or oxidized sn-2 fatty acyl groups (59-69). Similarly, lyso-PC (66, 70-74) and short and/or oxidized fatty acids (75-80) have been reported to elicit both detrimental and beneficial responses. In general, in vitro approaches are strategically useful initially, but they have the usual limitations of studies that do not recapitulate essential in vivo features. This is particularly relevant in studies that involve lipids and lipid metabolites. For example, relatively low (nanomolar) concentrations of lyso-PC recruit monocytes and induce pro-inflammatory cytokine production in vitro (81). However, the concentration of lyso-PC in blood plasma of healthy persons usually ranges from 200 to 400 μM (82, 83) and similar levels have been reported in atherosclerotic tissues (45). Since lyso-PC, unlike PC, is membrane lytic, it is predominantly confined to lipoproteins or plasma proteins, such as albumin or immunoglobulins (83, 84). As a result, only a fraction of lyso-PC is functionally active and it is unclear how total concentration relates to bioavailability (73, 85). Similarly, the bioactivity of fatty acids appears to be modulated by the carrier, again underscoring the importance of location on biological activity (86, 87). Thus, whether results generated using lyso-PC and oxidized fatty acids in vitro realistically reflect in vivo responses remains to be rigorously determined.

In view of these limitations, rather than characterizing functional effects of either Lp-PLA2 substrates or Lp-PLA2 products, a more informative approach is to assess net changes that result when the action of Lp-PLA2 alters substrate-to-product ratios. Examples of this strategy include investigations conducted in the presence and absence of Lp-PLA2 activity, or studies that compare the effect of supplementing active and inactive enzyme. Both pharmacologic and genetic depletion of Lp-PLA2 in minimally modified lipoproteins enhanced monocyte adhesion to
aortic endothelial cells compared with minimally modified lipoproteins that expressed normal activity levels (88). Similarly, exogenous Lp-PLA₂ reduced cellular uptake of oxidized LDL and Lp(a), and cholesterol accumulation by monocyte-derived macrophages, compared with parallel assays conducted with inactive enzyme (89). These results suggest that the enzymatic activity of Lp-PLA₂ limits monocyte recruitment and foam cell formation within atherosclerotic lesions (89). Chen and colleagues found that exogenous Lp-PLA₂, but not vehicle, inhibited endothelial cell apoptosis induced by minimally modified LDL particles (90). These approaches, which have also been used in animal models (91) provide much-needed rigorous, well-controlled comparisons to assess the net effect of altering the balance of metabolites controlled by Lp-PLA₂.

3. LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A₂: EPIDEMIOLOGICAL AND CLINICAL STUDIES

WOSCOPS (West of Scotland Coronary Prevention Study) was the first study to report that circulating Lp-PLA₂ mass levels had a strong, positive association with risk of coronary events that was not confounded by classic cardiovascular risk factors (92). Since then, plasma and serum Lp-PLA₂ concentration and activity have been determined in a great number of epidemiological studies aimed at determining whether these measurements can be used in patient stratification and/or to predict the likelihood of future cardiovascular events such as MI and stroke. Several reviews and meta-analyses have recently addressed this issue [see, for example, refs. (10, 93-95)]. This article will not comprehensively review all the evidence gathered thus far. Instead, we will highlight key features. With few exceptions, we will emphasize studies that assessed the enzymatic activity of Lp-PLA₂. Investigations relying exclusively on Lp-PLA₂ mass determinations should be interpreted within the context of serious methodological concerns regarding the performance of the leading commercially available assay (96-98). This includes long-term consistency in the hands of the manufacturer.

3.1. LP-PLA₂ EXPRESSION IN APPARENTLY HEALTHY SUBJECTS

The initial association between Lp-PLA₂ levels and future risk of CVD reported by WOSCOPS (92) was, in general, confirmed in smaller studies (99-102). In contrast, additional trials failed to reproduce these observations (95, 103-106). The Lp-PLA₂ Studies Collaboration examined associations between circulating Lp-PLA₂ concentration and activity and cardiovascular events from 79,036 subjects who participated in 32 prospective studies (94). Individuals with no history of vascular disease at baseline (n=35,945) were included in the analyses, which were adjusted for age, sex, baseline history of vascular disease and other non-lipid and lipid risk factors. In this group, no association was found between Lp-PLA₂ activity and CHD or ischemic strokes. In summary, Lp-PLA₂ activity levels do not seem to be a useful predictor of future CHD in apparently healthy individuals.

Two studies that merit mention include investigations that evaluated plasma Lp-PLA₂ activity, levels of oxidized phospholipids (OxPL) in a subset of apolipoprotein B-100 (apoB) particles (OxPL/apoB), and future vascular events in apparently healthy participants (102, 107). The first prospective study was relatively small and showed that OxPL/apoB levels were elevated in those who developed coronary, carotid, and femoral artery disease, and acute coronary syndrome [ACS, (102)]. OxPL/apoB measured at baseline predicted the development of cardiovascular events independently of traditional risk factors and high-sensitivity C-reactive protein (hs-CRP). In addition, increased risk was augmented with increasing Lp-PLA₂ activity. The second study [European Prospective Investigation of Cancer (EPIC)-Norfolk] also included a cohort of 45-79 year-old, apparently healthy, men and women (107). This analysis confirmed the
increased risk of cardiovascular events with increasing levels of oxPL/apoB but only a weak poteniation of risk was observed with increasing Lp-PLA2 activity. In sum, the two studies suggest that combined assessment of these two mechanistically related biomarkers (Lp-PLA2 activity and oxPL/apoB ratio) provides modest incremental information to predict risk of future cardiovascular events in apparently healthy individuals.

A nested case-control analysis evaluated whether increased Lp-PLA2 activity correlates with MI in 1,221 apparently disease-free women matched for age, smoking status and date of blood draw. During a 14-year follow-up period, there were 421 cases of incident MI (108). Lp-PLA2 activity was associated with incident MI [RR=1.75; 95% CI (1.09-2.84)] even after multivariable adjustment for clinical, lipid and inflammatory risk factors. The differences in the risk of CHD events associated with increased Lp-PLA2 activity in this observational study of participants without known vascular disease may relate to sex-specific disease associations and longer average duration of follow-up compared with results included in the Lp-PLA2 Studies Collaboration (94).

### 3.2. Lp-PLA2 Expression in Subjects with Stable Cardiovascular Disease

Several studies reported that Lp-PLA2 mass predicts future cardiovascular events in patients with stable CVD (95, 109-111). Since Lp-PLA2 is carried in the circulation as a complex with lipoproteins such as apoB-containing LDL, there is a strong relationship between Lp-PLA2 and LDL concentrations (96). Thus, the strength of the association of Lp-PLA2 with CVD depends substantially on apoB or its associated lipid moieties (96). It is therefore essential to evaluate whether the predictive power of Lp-PLA2 is independent of common risk factors such as high apoB/LDL levels. In one study, adjustment for multiple risk factors [e.g., hypertension, smoking, family history of coronary artery disease (CAD), and prior MI] and medication use did not affect the ability of Lp-PLA2 to predict CAD risk, but abolished the potential to predict all-cause mortality, non-CAD CV death, new MI, nonfatal MI, or a composite end point of all-cause mortality, MI, cerebrovascular accident, and revascularization at follow-up (112). In another study, however, the association remained significant (111). Lp-PLA2 activity levels also appear to predict future cardiovascular events and cardiac mortality in patients with stable CVD (109, 113). The Lp-PLA2 Studies Collaboration analyzed 32 prospective analyses in 35,494 patients with a history of stable vascular disease (94). Risk ratios for CHD and ischemic strokes increased progressively for every 1-standard deviation higher Lp-PLA2 activity or concentration in models adjusted for age, sex, baseline history of vascular disease and other non-lipid and lipid risk factors. In summary, the evidence available thus far indicates that patients with stable CVD have increased risk for MI, ischemic stroke and cardiac death with progressively increased Lp-PLA2 activity or mass.

### 3.3. Lp-PLA2 Expression in Patients with Acute Coronary Syndrome

Initial epidemiological analyses in patients with ACS led to promising results that failed to be confirmed in subsequent work (114). Two small studies suggested that elevated Lp-PLA2 levels may help in the identification of patients who will develop cardiovascular complications (114) or cardiac mortality (115). However, the Fragmin and Fast Revascularization During Instability in Coronary Artery Disease II [FRISC II (116)], the Global Utilization of Strategies to Open Occluded Arteries IV [GUSTO IV (116)] and Pravastatin or Atorvastatin Evaluation and Infection Therapy [PROVE-IT (117)] studies reported that baseline levels of Lp-PLA2 mass and/or activity did not predict recurrent events such as MI, stroke, or death. Analysis of patients diagnosed with an
acute ischemic event in the Lp-PLA\textsubscript{2} Studies Collaboration (n=10,638) revealed no significant associations between Lp-PLA\textsubscript{2} mass or activity and recurrent vascular outcomes. In this meta-analysis, it could be argued that lack of association with recurrence among patients presenting with acute ischemic events could be related to the smaller size of the cohort and the shorter follow-up time (1.1 years vs. 5.8 years). The fact that Lp-PLA\textsubscript{2} measurements were conducted during the acute phase, however, does not appear to explain the observed lack of correlation as a study from the Myocardial Ischemia Reduction With Acute Cholesterol Lowering (MIRA\textsubscript{CL}) trial in patients presenting with ACS reported no significant changes in Lp-PLA\textsubscript{2} mass or activity after ACS, suggesting that Lp-PLA\textsubscript{2} does not participate to any substantial extent in the acute-phase response of this syndrome (118). In addition, the authors found no discernible relationship between Lp-PLA\textsubscript{2} levels and risk of future ischemic events or death in placebo-treated patients (118). These combined studies strongly suggest that measurement of Lp-PLA\textsubscript{2} levels is unlikely to improve stratification or risk prediction in patients presenting with ACS.

### 3.4. Lp-PLA\textsubscript{2} Expression in Statin-Treated Patients

The Heart Protection Collaboration Group evaluated whether baseline Lp-PLA\textsubscript{2} activity was associated with CVD in a randomized, placebo-controlled clinical trial of simvastatin that included individuals at high risk of developing vascular disease (119). While a modest association was initially observed in analyses that adjusted for non-lipid risk factors, after adjustment for apoB, the association with activity became non-significant (120). However, the study population was diverse and confounding factors from cardiovascular medications and prior vascular disease may have affected the results. The JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial compared rosuvastatin to placebo in 17,802 men and women without CVD or diabetes at study entry (98). This study found only modest associations with risk in subjects allocated to the placebo arm and whose cholesterol levels were, therefore, not managed (98). Importantly, the JUPITER trial showed that Lp-PLA\textsubscript{2} did not predict risk or clinical outcomes in rosuvastatin-treated patients. The MIRACL trial, which included placebo-treated and atorvastatin-treated individuals whose Lp-PLA\textsubscript{2} mass and activity were significantly reduced, found no discernible relationship between Lp-PLA\textsubscript{2} levels and risk of future ischemic events or death in either atorvastatin-treated or placebo-treated patients (118). The studies show that Lp-PLA\textsubscript{2} levels are unlikely to predict future risk when cholesterol levels are adequately managed by statin therapy. These observations have important clinical implications that will be discussed in 3.6. An additional important observation from the MIRACL (118) and JUPITER (98) trials is that the data are consistent with conclusions reached in 3.1 above, as in placebo-treated, apparently healthy subjects, Lp-PLA\textsubscript{2} levels did not predict future CHD.

### 3.5. Lp-PLA\textsubscript{2} Expression in Atherosclerotic Plaques

A number of studies have reported increased Lp-PLA\textsubscript{2} expression in macrophages from human and rabbit atherosclerotic lesions (121). A prospective study of 167 carotid endarterectomy patients reported increased local expression of Lp-PLA\textsubscript{2} and lyso-PC in the vulnerable plaques of symptomatic compared with asymptomatic patients (45). Lp-PLA\textsubscript{2} expression was associated with plaque vulnerability in experimental animals and in humans (121, 122). Early lesions stained weakly for Lp-PLA\textsubscript{2} but necrotic cores and surrounding macrophages of vulnerable and ruptured plaques displayed robust Lp-PLA\textsubscript{2} expression (122). Interestingly, Lp-PLA\textsubscript{2} was detected in apoptotic macrophages. This observation may represent
events mediated by accumulated OxPL which are known to be potent inducers of apoptosis (65). Elevated plaque Lp-PLA$_2$ expression is a feature that appears to be reflected in the circulation. Among 42 patients with high-grade carotid stenoses (≥70 percent by the NASCET criteria), circulating Lp-PLA$_2$ levels were higher in patients with unstable plaque compared with those who had stable lesions (123). A larger study that included 467 patients with a first ischemic stroke reported that increased Lp-PLA$_2$ concentration was predictive of recurrent ischemic neurological events (124). Finally, in a study of 40 ACS patients with successful percutaneous coronary intervention (PCI), changes in the volume of non-culprit lesions after 6 months were significantly associated with changes in both LDL cholesterol levels ($r=0.444$) and Lp-PLA$_2$ concentration [$r=0.496$, (125)]. While Lp-PLA$_2$ expression is elevated in advanced atherosclerotic plaques and in the circulation, it is not evident from these results that Lp-PLA$_2$ levels would be a more useful clinical marker of plaque instability than simple determination of LDL cholesterol levels.

It is important to emphasize that protein expression patterns in vascular lesions reflect cellular populations present at the time of lesion collection, along with differential regulation in response to the cellular and physical environment (126). Thus, differential gene/protein expression in atherosclerotic plaques reflects both damage occurring in the tissue and mechanisms engaged to limit additional adverse responses (126). While molecular characterizations of diseased tissues such as atherosclerotic plaques have a proven record of success for biomarker purposes, this approach cannot be used to determine causal versus consequential links. The tissue-protective nature of some of the genes up-regulated in atherosclerotic plaques suggests that their regulation is a result, rather than a cause, of atherosclerosis (126). Examples include up-regulated expression of antioxidant proteins such as superoxide dismutase-2 and glutathione peroxidase (127). The expression of these proteins may be up-regulated to counteract overproduction of reactive oxygen species in atherosclerotic lesions (128). Thus, while multiple studies report high Lp-PLA$_2$ expression in advanced atherosclerotic lesions, this feature cannot be used as surrogate evidence for active contribution of this enzyme to the disease phenotype.

3.6. Implications for Cardiovascular Risk Assessment and Therapeutic Intervention

The strength of the association between Lp-PLA$_2$ mass and activity with vascular risk is markedly reduced after adjustment for basal concentrations of lipids and apolipoproteins (96, 129). Cohort stratification (94) combined with individual analyses of the most rigorously designed studies (96, 98) indicate that Lp-PLA$_2$ activity and risk of future cardiovascular events or death are not significantly associated in individuals without a history of vascular disease, in patients treated with effective statin doses (96), or in patients presenting with ACS. In view of the findings outlined above, it is unlikely that measurement of plasma or serum Lp-PLA$_2$ activity would yield additional power to predict risk of CHD, or help in risk stratification, beyond that already provided by assessment of traditional risk factors (130). A second issue to consider is whether these results impact the suitability of Lp-PLA$_2$ as a target for intervention. Aside from mechanistic considerations that are discussed elsewhere in this article, the epidemiological evidence summarized above does not support the hypothesis that targeting Lp-PLA$_2$ would improve outcomes above and beyond what is already achieved by current strategies to manage CVD.
4. GENETIC STUDIES IN HUMAN POPULATIONS

4.1. GENETIC DEFICIENCY OF Lp-PLA₂

Much information can be gleaned from genetic analyses in human populations combined with molecular studies in biochemical, cellular, and animal models. In addition, merging results from genetic and biomarker studies may help establish disease causality (131). If the relationship between biomarker and outcome is causal, then genotype should associate with outcome (131). This approach has been used to elucidate the real function of Lp-PLA₂ in vivo and its impact in human physiology and disease. Fourteen years ago, one of us identified the molecular basis of Lp-PLA₂ deficiency as a missense mutation at position 994 (G>T) of the PLA2G7 gene, which results in a valine to phenylalanine transversion near the active site of the enzyme [V279F, (132)]. Homozygous carriers of this variant express virtually no enzymatic activity in plasma and heterozygous subjects have a 50% decrease in activity compared with those expressing two copies of the wild-type allele (132, 133). The V279F allele is common in Japanese populations: 25-30% of healthy subjects express one copy of this allele and 3-4% carry two copies (134-136). The mutation has been reported in Korean (137), Taiwanese (138), and Chinese (139, 140) populations, and in subjects from Turkey, Azerbaijan, and Kyrgyzstan (141). Allele frequency varies widely in these populations: the highest prevalence was reported in Japan and in Taiwan, followed by Korea and China [reviewed in (142)]. In contrast, this variant has been observed rarely in Europeans (142, 143); thus far, only one individual of Swiss ancestry has been shown to be a carrier of the V279F allele (143). Other functionally validated null alleles [i.e., Q281R, I317N and Ins 191] have been observed in Caucasian and Japanese populations with extremely low frequencies (143-146).

4.2. Lp-PLA₂ DEFICIENCY AND CORONARY ARTERY DISEASE

Diverse studies have reported that the V279F null allele is associated with increased risk of developing atherosclerosis, CAD, stroke, and MI in Japanese subjects (134, 147-150). A study using strict criteria for statistical significance (p<0.005) showed that the V279F allele is significantly associated with CAD in Japanese men with nonfamilial hypercholesterolemia (151). (151). Based on the same criteria, the presence of the mutated allele did not increase (or decrease) CAD risk in females or normolipidemic men (151). V279F was associated with increased plasma oxLDL/LDL ratio in a recessive manner, but had no effect on intima media thickness (152). A recent meta-analysis involving predominantly Asian individuals analyzed seven case-control studies involving 3,614 patients with CHD and 4334 controls (139). While the crude ORs were not significant, analyses stratified by study size, ethnicity, case definition, and source of controls revealed strong associations with Japanese ethnicity and other parameters, and suggested that V279F contributes to CHD. Similarly, in a study of Chinese Han men and women, the frequency of the V279F allele was more common in CAD patients compared with healthy controls (13.5% vs. 9.3% in controls, p=0.024). Importantly, the severity of atherosclerosis was greater in V279F carriers; these associations remained significant after multivariate adjustment [OR=1.922; 95% CI (1.146-3.224); p=0.013, (153)].

Interestingly, two studies in Korean men concluded that incidence of the V279F allele is lower in CVD patients compared with apparently healthy controls [OR=0.646; 95% CI (0.490-0.850), p=0.002], even after adjustments for age, body mass index, waist circumference, waist to hip ratio, cigarette smoking and alcohol consumption (137). However, these Korean cohorts had unique clinical characteristics. In the initial study, LDL and total cholesterol levels were
lower in patients, including those not treated with lipid-lowering drugs, compared with healthy subjects (137). The second study included mostly patients whose LDL cholesterol levels were pharmacologically decreased by statin therapy (154). In healthy Koreans, homozygosity for the V279F allele is associated with decreased LDL-cholesterol levels [V/V: 120.9 ± 0.69; F/F: 109.2 ± 4.84 mg/dl, p=0.025, (155)]. In contrast, no such relationship has been observed in Japanese cohorts [V/V: 126.0 ± 1.56; F/F: 128.8 ± 0.77 mg/dl, p=0.77, (136, 152)]. These observations point at potentially important differences in Japanese compared with Korean subjects, assuming these findings are re-capitulated in independent Korean cohorts. Thus, with the exception of these two studies by a single Korean group, the evidence available to date strongly suggests that deficiency of Lp-PLA₂ is associated with increased risk of CAD.

4.3. Lp-PLA₂ POLYMORPHISMS AND CORONARY ARTERY DISEASE

In contrast to the V279F allele associated primarily with Asian populations, three relatively well-studied non-synonymous Lp-PLA₂ polymorphisms [R92H, I198T, and A379V] have been observed in all populations studied so far (142, 156). The variants have been functionally characterized using molecular approaches (157). The purified A379V variant displays decreased substrate affinity and increased rate of hydrolysis (157), but these features are not always recapitulated in whole plasma activity determinations (142). In a case-control study of Chinese Han, the risk of MI was higher among CAD patients harboring two VV alleles compared with AA (wild-type) homozygotes (153). In a Taiwanese cohort, A379V was associated with increased risk of MI and increased severity of CAD (138). In contrast, a study of European Caucasians revealed reduced risk of MI in homozygotes for A379V (158) but meta-analyses of European Caucasian populations reported no association with CHD risk (56, 156). In South Koreans, a similar lack of association between A379V and CVD disease was reported (137). These observations suggest that Lp-PLA₂ polymorphisms that modestly impact enzymatic activity are unlikely to be associated with cardiovascular risk.

5. Lp-PLA₂ INHIBITORS

Epidemiological and correlative studies showing that Lp-PLA₂ expression is elevated in CVD, combined with in vitro analyses have led to the proposition that the enzyme has a predominantly pro-inflammatory role in atherogenesis (75, 159). Mechanistically, proponents of this model argue that Lp-PLA₂-mediated lyso-PC and oxidized fatty acid generation contribute to several aspects of plaque formation, and actively contribute to the pathogenesis of vascular disease. In turn, this led to the proposition that inhibiting the enzymatic activity of Lp-PLA₂ could be potentially beneficial for the prevention and/or treatment of atherosclerosis and related disorders (160). A number of azetidinone inhibitors that target the active-site serine residue of the enzyme have been developed (161). One agent, darapladib (SB480848) is being tested in two large Phase III clinical trials to assess clinical benefit in patients diagnosed with CHD (9, 162) and following ACS (163).

5.1. PHARMACOLOGY, BIOLOGICAL PROPERTIES, AND METABOLIC EFFECTS OF DARAPLADIB

Darapladib reversibly and non-covalently binds to human recombinant Lp-PLA₂ with a Ki of 110 pM and an off-rate of 27 min (161). It is lipophilic and has good membrane permeability (164). Darapladib has minimal interaction with cytochrome P450 isozymes and has an oral bioavailability of 11 ± 2% and ≈21% in fed and fasted rats, respectively, and 28 ± 4% in dogs.
Darapladib has been repeatedly shown to inhibit hydrolysis of Lp-PLA₂ substrates \([i.e., \text{PAF, 1-myristoyl-2-(4-nitrophenylsuccinyl) PC, and others}]\). Darapladib inhibits Lp-PLA₂-mediated \textit{exogenous} substrate hydrolysis in plasma and LDL \textit{in vitro} (161). In addition, plasma and atherosclerotic plaque lysates from darapladib-treated Watanabe heritable hyperlipidemic (WHHL) rabbits (161) and diabetic and hypercholesterolemic swine (165) display highly impaired (up to 95% in plaques) \textit{exogenous} substrate hydrolysis (Table 1). However, it is essential to assess effects on \textit{endogenous} phospholipids (Table 1). A number of studies evaluated the impact of darapladib-related compounds on endogenous substrate levels using \textit{ex vivo} and \textit{in vivo} approaches. Exhaustive \textit{in vitro} oxidation of LDL with CuSO₄ in the absence and presence of SB222657, confirmed previous work (31, 32) showing that truncated PC oxidation products are the predominant endogenous substrates for Lp-PLA₂ (33). PCs that undergo oxidative modification without chain fragmentation are clearly much less efficient substrates for Lp-PLA₂, but since they are quantitatively the most abundant PC products of LDL oxidation (33), the enzyme is likely to impact their levels to a certain extent. In fact, lipopolysaccharide-stimulated whole blood specimens from metabolic syndrome patients treated with various dosages of the Lp-PLA₂ inhibitor SB677116, a compound structurally related to darapladib, attenuated the production of C-18 (unfragmented) oxidized fatty acids from endogenous precursors during a 6-h incubation period (166) (Table 1).

\textit{In vivo} studies showed that darapladib treatment reduced the content of lyso-PC in pig atherosclerotic lesions, owing to inhibition of hydrolysis of endogenous glycerophospholipids (165). The nature of possible endogenous substrates was evaluated in studies that compared lipid profiles in darapladib-treated versus placebo-treated animals. In Yorkshire domestic farm pigs, the induction of a diabetic-hypercholesterolemic (DM-HC) phenotype decreased the content of \textit{sn}-2 arachidonoyl-containing PCs (165), possibly owing to increased oxidation and/or activation of PLA₂ activities other than Lp-PLA₂ (see Section 2). Surprisingly, darapladib treatment significantly reduced this response (Table 1), which points at anti-oxidant properties or inhibitory effects of darapladib on long chain PLA₂ activities with specificity for arachidonoyl-containing substrates. While darapladib treatment attenuated the elevated arterial lyso-PC abundance, in a manner similar to its \textit{in vitro} effects on LDL lipids, the compound had no effect on the levels of truncated oxidized PC species known to be Lp-PLA₂ substrates (33). Notably, darapladib failed to alter serum PAF levels in two murine models of atherosclerosis (167, 168). The possibility that other circulating PLA₂ activities are targets of darapladib was evaluated in \textit{in vitro} experiments showing modest (8.7%) inhibition of sPLA₂GX and no effect on sPLA₂GIIa or sPLA₂GV [reviewed in (164)]. However, it is unknown whether darapladib inhibits other PLA₂ activities such as sPLA₂GIII, for example (73).

Aside from effects on lipid metabolism, darapladib treatment decreased caspase-3 and caspase-8 activity \textit{in vivo} (52, 169), and a compound related to darapladib (SB222657) inhibited macrophage apoptosis induced by oxidized LDL \textit{in vitro} (170). These observations may account for the ability of darapladib to limit necrotic core expansion in humans (169), but it is not clear whether these effects occur as a consequence of Lp-PLA₂ inhibition. Inhibition of Lp-PLA₂ activity (and substrate accumulation) is predicted to \textit{increase} apoptosis through caspase activation, based on multiple rigorous laboratory investigations [see (65) for a recent comprehensive review], which is opposite to the observed effect of darapladib on caspase-3 and caspase-8 inhibition (52, 169).

In conclusion, while darapladib and related compounds inhibit Lp-PLA₂ activity as determined using exogenous substrates \textit{in vitro}, it is not clear whether enzyme inhibition accounts for observed \textit{in vivo} effects. An ideal way to rigorously investigate the mechanism of darapladib action would be to test the effect of this agent in patients genetically deficient in Lp-
PLA₂. Efficacy in this patient population would strongly suggest Lp-PLA₂-independent effects. Clinical studies of this nature are difficult to implement, although safety/tolerability trials of darapladib in healthy Japanese subjects have been completed (NCT00551317 & NCT00622830). An excellent alternative would be to test this issue in mice. Since darapladib limited atherosclerosis in ApoE⁻/⁻ and Ldr⁻/⁻ mice (167, 168), these models appear adequate for future investigation. Specifically, comparing the effect of darapladib in control and Lp-PLA₂-deficient mice in a model of atherosclerosis (e.g., ApoE⁻/⁻ or Ldr⁻/⁻) could provide definitive answers regarding the real mechanism of darapladib action.

5.2. In vivo Studies of Lp-PLA₂ Over-Expression versus Darapladib Administration

Similarly to what is observed in humans, enhanced levels of sPLA₂s and Lp-PLA₂ correlate with increased atherosclerosis in animal models (95), with the exception of a study by Forte and co-workers (171). Secretory PLA₂s, unlike Lp-PLA₂, have been demonstrated to actively contribute to atherogenesis, and so correlate with disease status (85). An excellent review by Öörni and colleagues highlights multiple studies showing that development of atherosclerosis is decreased in mice lacking sPLA₂ expression, and increased with over-expression (85). Parallel studies addressing the role of Lp-PLA₂ have clearly shown that over-expression of Lp-PLA₂ diminishes atherosclerosis in mice (172-175) and rabbits (172, 173, 176) (Table 2). This series of studies suggested that Lp-PLA₂ may play a protective role in vascular and inflammatory diseases. Lp-PLA₂-deficient mice displayed decreased intestinal inflammation in vivo (177) but the impact of Lp-PLA₂ deletion on vascular inflammation in mice has not yet been reported.

Studies in which Lp-PLA₂ was over-expressed in mice have recently been complemented by preclinical trials of darapladib, also in mice (167, 168). The effects of darapladib administration were similar to those observed when Lp-PLA₂ was over-expressed (Table 2). Darapladib had multiple anti-inflammatory and anti-atherogenic effects in mice fed atherogenic diets (167, 168), in diabetic and hypercholesterolemic swine (165), and in humans (169) (Table 2). These data suggest that darapladib may have important effects mitigating key pro-inflammatory pathways relevant to atherogenesis. However, as mentioned previously, it is not clear whether the mechanism that mediates the action of darapladib in vivo occurs via Lp-PLA₂. The comparisons provided in this section strongly argue against this possibility.

5.3. Biomarker and Imaging Studies of Lp-PLA₂ Inhibition in Humans and in Animal Models

Two Phase II clinical trials of darapladib, including a biomarker study and an atherosclerosis imaging trial, have been completed. Among stable CHD patients with achieved LDL cholesterol concentrations lower than 115 mg/dL on atorvastatin, treatment with darapladib reduced plasma Lp-PLA₂ activity (43, 55, and 66 % inhibition at doses of 40 mg, 80 mg, and 160 mg, respectively [Table 3, (178)]. Interleukin-6 (IL-6) levels and hs-CRP were reduced by 12.3 % and 13 %, respectively, suggesting a reduction in inflammatory burden. The Integrated Biomarker and Imaging Study (IBIS-2) found no effect of darapladib on coronary atheroma deformability, plasma hs-CRP levels and other endpoints in patients with angiographically documented CAD. [Table 3, (169)]. However, assessment of lipid necrotic core by virtual histology revealed increased size in the placebo group (4.5 ± 7.9 mm³; p=0.009) but not in darapladib-treated patients (0.5 ± 13.9 mm³; p=0.71), resulting in a significant treatment difference of -5.2 mm³ [Table 3, (169)]. These findings are consistent with results from the swine model [Table 3, (165)] and suggest that darapladib stabilizes rupture-prone plaques.
5.4. **Darapladib and Cardiovascular Events: Ongoing Clinical Trials**

The clinical efficacy of darapladib is currently being examined in two large-scale trials that investigate its effects on nonfatal MI, nonfatal stroke, and cardiovascular death in CHD patients. The STABILITY trial (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy) is a multicenter, randomized, double blind, placebo-controlled trial that compares the effect of darapladib and placebo on cardiovascular events in 15,500 patients with stable CHD (162). In addition, SOLID-TIMI 52 (Stabilization of plaques using darapladib thrombolysis in myocardial infarction) is designed to investigate the effects of darapladib on major recurrent cardiovascular events in 11,500 MI/stroke patients (163). In these two secondary prevention trials, it will be important to investigate relationships between cardiovascular events and baseline/on-trial Lp-PLA₂ activity, lipid profiles, and changes in the extent of pro- and anti-inflammatory responses (6, 8). These measurements will provide key information to establish the therapeutic utility of darapladib and help determine whether its effects are mediated through Lp-PLA₂ or other mechanisms.

5.5. **Safety Issues to Consider**

In phase II clinical studies, darapladib was well tolerated at dosages of 40, 80 and 160 mg daily (169, 178). The most common adverse reactions, reported in 16-36 % of study subjects, included diarrhea and malodor of the urine and feces. In addition, CD40L levels and mean systolic blood pressure were higher in the darapladib group during treatment (169, 178). Regardless of the mechanism whereby darapladib affects events relevant to atherosclerosis, there is no doubt that this compound inhibits Lp-PLA₂ activity. Thus, an immediate issue that merits consideration is the potential impact of widespread use of this drug in populations with a large spectrum of conditions. A research team in Korea has reported that decreased Lp-PLA₂ activity is beneficial in certain settings (137, 154) and it is clear that partial/complete deficiency of Lp-PLA₂ does not by itself cause disease (26, 27). However, several independent studies have shown that decreased Lp-PLA₂ expression in humans increases the severity of inflammatory, vascular and other conditions [reviewed in (26, 142, 179)]. It has been argued that these concerns are “theoretical” (9), but it is important to point out that studies in relatively small populations are unlikely to include patients with underlying acute and/or chronic inflammatory conditions (e.g., asthma, anaphylaxis, sepsis) in which decreased or absent Lp-PLA₂ may increase disease severity (135, 180). An example of this paradigm was reported in a clinical trial focused on type II diabetes subjects undergoing hemodialysis (181). This study serendipitously found that patients whose Lp-PLA₂ activity decreased by 25 % or more over a 6-month period suffered from a dramatic increase in mortality [HR=2.48; CI (1.56–3.95), P<0·001, (181)]. This observation is only correlative and, therefore, does not demonstrate a causal role for decreased Lp-PLA₂ in mortality. However, it provides an example of a patient population in which inhibiting Lp-PLA₂ may not be advisable. In cardiovascular medicine, therapies directed at surrogate end points can be associated with excess deaths (182). The safety and efficacy of darapladib should be very carefully evaluated before potential harmful effects become widespread.

6. **Conclusions**

It has been proposed that one of the strongest arguments in favor of a pro-atherogenic role of Lp-PLA₂ is the substantial number of epidemiological studies (66). While it is clear that there is a positive association between Lp-PLA₂ activity and CAD, the strength of this association appears
to be almost exclusively accounted for by the fact that most of the plasma Lp-PLA₂ activity associates with LDL. These observations raise questions regarding the utility of Lp-PLA₂ as a biomarker, particularly when one considers that current guidelines direct clinicians to treat at-risk patients with cholesterol-lowering drugs and that for these patients Lp-PLA₂ levels no longer predict vascular risk (96, 98, 120).

It is unlikely that strategies based on inhibiting the enzymatic activity of Lp-PLA₂ will be efficacious for the prevention or treatment of CVD in humans. The evidence currently available, which includes the majority of genetic analyses in humans and all over-expression studies in mice and rabbits, do not mechanistically support this rationale. Darapladib has been shown to reduce development of experimental atherosclerosis and prevent the expansion of necrotic core lesions in humans, but these effects may not be mediated by Lp-PLA₂. Our ability to establish meaningful links between Lp-PLA₂ and CVD depends on our willingness to go well beyond observational studies that show associations between expression and outcome.
**Table 1. Darapladib and Related Compounds Inhibit Hydrolysis of Exogenous Phospholipid Substrates but Have Varied Effects on the Metabolism of Endogenous Phospholipids**

<table>
<thead>
<tr>
<th>Source of Lp-PLA₂</th>
<th>Agent</th>
<th>Study</th>
<th>Substrate source</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant Lp-PLA₂ (human) LDL particles (human)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma and extracts from atherosclerotic plaques (WHHL rabbits)</td>
<td>Darapladib (SB480848)</td>
<td>In vitro</td>
<td>Exogenous</td>
<td>Inhibition of hydrolysis</td>
<td>(161)</td>
</tr>
<tr>
<td>Plasma and iliac artery tissue (DM-HC male Yorkshire pigs)</td>
<td></td>
<td>In vivo</td>
<td>Endogenous</td>
<td>Increased AA-PC; no change in short chain OxPL</td>
<td>(165)</td>
</tr>
<tr>
<td>LDL exposed to CuSO₄ (human)</td>
<td>SB222657</td>
<td>In vitro</td>
<td>Exogenous</td>
<td>Increased truncated OxPL</td>
<td>(33)</td>
</tr>
<tr>
<td>LPS-treated blood (human)</td>
<td>SB677116</td>
<td></td>
<td>Exogenous</td>
<td>Decreased C18-OxFA content</td>
<td>(166)</td>
</tr>
<tr>
<td>Serum (ApoE⁻/⁻ mice)</td>
<td>Darapladib (SB480848)</td>
<td>In vivo</td>
<td>Exogenous</td>
<td>Inhibition of hydrolysis</td>
<td>(168)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Endogenous</td>
<td>No change in PAF levels</td>
<td></td>
</tr>
<tr>
<td>Serum (Ldlr⁻/⁻ mice)</td>
<td></td>
<td>Exogenous</td>
<td>Inhibition of hydrolysis</td>
<td>(167)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endogenous</td>
<td>No change in PAF levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (human)</td>
<td></td>
<td>Exogenous</td>
<td>Inhibition of hydrolysis</td>
<td>(169)</td>
<td></td>
</tr>
<tr>
<td>Experimental model</td>
<td>Intervention</td>
<td>In vivo model</td>
<td>Effects</td>
<td>Ref.</td>
<td></td>
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<tr>
<td>--------------------</td>
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<td></td>
</tr>
</tbody>
</table>
| Ischemia reperfusion injury induced by coronary ligation | Administration of recombinant Lp-PLA₂ | New Zealand white rabbits | • ↓ Necrotic area  
• ↓ Systolic shortening and wall thickness  
• ↓ Neutrophil infiltration | (172) |
| Aortic injury induced by balloon denudation | Cholesterol-fed New Zealand white rabbits | • ↓ Intima/media ratio  
• ↓ Apoptosis | (173) |
| Atherosclerosis | Adenovirus-mediated Lp-PLA₂ gene transfer | ApoE −/− mice | • ↓ Modified LDL autoantibodies  
• ↓ Neointimal area  
• ↓ Atherosclerosis  
• ↓ Macrophage homing to endothelium  
• ↓ Aortic wall thickness | (174) (175) (176) |
| Balloon-mediated injury of carotid arteries | Normolipidemic New Zealand white rabbits | • ↓ Accumulation of oxidized lipoproteins  
• ↓ Intima/media ratio  
• Anti-inflammatory effects  
• Anti-thrombotic effects  
• Anti-proliferative effects | (176) |
| Atherosclerosis | Darapladib | Ldlr −/− mice | • ↓ Inflammatory burden  
• ↓ Plaque area | (167) |
| | | ApoE −/− mice | • ↓ Inflammatory burden  
• ↓ Plaque area | (168) |
| | | DM–HC swine | • General anti-inflammatory action  
• ↓ Plaque and necrotic core area  
• ↓ Medial destruction | (165) |
| | | Coronary disease patients | • Halted necrotic core volume expansion  
• ↔ coronary atheroma deformability  
• ↔ Plaque volume | (169) |
### Table 3. Effect of Darapladib Treatment on Markers of Inflammation in Animal Models of Atherosclerosis and in Human Patients

<table>
<thead>
<tr>
<th>In vivo model</th>
<th>Biomarker effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ldlr</em>&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>• ↓Serum Lp-PLA&lt;sub&gt;2&lt;/sub&gt; activity</td>
<td>(167)</td>
</tr>
<tr>
<td></td>
<td>• ↓Hs-CRP, IL-6, MCP-1, VCAM-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↔ Serum PAF</td>
<td></td>
</tr>
<tr>
<td><em>ApoE</em>&lt;sup&gt;−/−&lt;/sup&gt; mice</td>
<td>• ↓Serum Lp-PLA&lt;sub&gt;2&lt;/sub&gt; activity</td>
<td>(168)</td>
</tr>
<tr>
<td></td>
<td>• ↓Hs-CRP, IL-6, MCP-1, VCAM-1 and TNF- α</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↔ Serum PAF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↓ Macrophage content in atherosclerotic lesions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↑ Collagen content in atherosclerotic lesions</td>
<td></td>
</tr>
<tr>
<td>DM-HC swine</td>
<td>• ↓Plasma Lp-PLA&lt;sub&gt;2&lt;/sub&gt; activity</td>
<td>(165)</td>
</tr>
<tr>
<td></td>
<td>• ↓Lesion Lp-PLA&lt;sub&gt;2&lt;/sub&gt; activity and lyso-PC content</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↓24 genes associated with macrophage and T lymphocyte functions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↓ Macrophage content in atherosclerotic lesions</td>
<td></td>
</tr>
<tr>
<td>Healthy men</td>
<td>• ↔ ADP-induced platelet aggregation</td>
<td>(184)</td>
</tr>
<tr>
<td></td>
<td>• ↔ Collagen-induced platelet aggregation</td>
<td></td>
</tr>
<tr>
<td>CHD patients (LDL cholesterol &lt; 115 mg/dL on atorvastatin)</td>
<td>• ↓Plasma Lp-PLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>(178)</td>
</tr>
<tr>
<td></td>
<td>• ↓Hs-CRP, IL-6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↔ MPO, MMP-9 activity, P-selectin, CD40L</td>
<td></td>
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<td></td>
<td>• ↔ HDL &amp; LDL cholesterol</td>
<td></td>
</tr>
<tr>
<td>Patients with angiographically documented CHD (IBIS-2)</td>
<td>• ↓Plasma Lp-PLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>(169)</td>
</tr>
<tr>
<td></td>
<td>• ↔ Hs-CRP, MPO, ICAM-1, MMP-9 activity, OxPL/apoB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• ↔ HDL &amp; LDL cholesterol</td>
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7. References


PLA₂ is protective from coronary artery disease in South Korean males. PLoS One 6:e18208.


