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CONTENTS	PAGE	ENTRIES

Lp-PLA$_2$ Selected Annotated Bibliography

REVIEWS

1. Toth PP, McCullough PA, Wegner MS, Colley KJ. Lipoprotein-Associated Phospholipase A$_2$: Role in Atherosclerosis and Utility as a Cardiovascular Biomarker. *Expert Rev Cardiovasc Ther.* 2010; 8(3): 425-38.

   This review summarizes the large body of basic scientific and clinical research supporting the conclusion that inflammation plays a significant role in atherogenesis along the entire continuum of its progression. Inflammation adversely impacts intravascular lipid handling and metabolism, and because Lp-PLA$_2$ potentiates intravascular inflammation and atherosclerosis, many epidemiologic studies support the utility of Lp-PLA$_2$ measurements for estimating and further refining cardiovascular disease risk. Also reviewed is a promising drug therapy in development that inhibits Lp-PLA$_2$; darapladib inhibits Lp-PLA$_2$ and reduces the progression of the necrotic core volume of coronary artery atheromatous plaque.

2. Madjid M, Ali M, Willerson JT. Lipoprotein-Associated Phospholipase A$_2$ as a Novel Risk Marker for Cardiovascular Disease: a Systematic Review of the Literature. *Tex Heart Inst J.* 2010; 37(1):25-39.

   In this review the authors sought to critically assess the role of Lp-PLA$_2$ in the prediction of cardiovascular events in primary and secondary prevention settings. The inclusion criteria for this analysis included population-based epidemiologic studies and the presence of clinical outcomes of interest, including atherosclerotic disease, coronary events, stroke, and cardiovascular death, while studies that lacked clinical outcomes or that involved animals were excluded. They included primary and secondary prevention studies of subjects in all ethnic groups and of either sex, with no age limitation. In terms of methodology, the authors searched several standard databases for studies with publication dates from January 1970 through July 2009 and searched major cardiology meeting abstracts from 2000 through 2009. Of 33 studies meeting inclusion criteria, 30 showed a significant association between Lp-PLA$_2$ and cardiovascular events. Most studies had been adjusted for major Framingham risk factors and other variables that might influence the effect under question, and after multivariate adjustments in cohort and nested case-control studies, increased levels of Lp-PLA$_2$ remained a significant predictor of cardiovascular events. Accordingly, the authors conclude that the available body of evidence suggests that Lp-PLA$_2$ is a reliable marker of risk for cardiovascular events.

3. Wilensky RL, Macphee CH. Lipoprotein-Associated Phospholipase A$_2$ and Atherosclerosis. *Curr Opin Lipidol.* 2009; 20(5): 415-20.

   This review summarizes the substantial data from over 50,000 patients providing evidence that increased Lp-PLA$_2$ mass or activity is associated with an increased risk of cardiac death, myocardial infarction, acute coronary syndromes and ischemic stroke. The authors review how only recently have data emerged demonstrating a role of Lp-PLA$_2$ in development of advanced coronary artery disease, and as such, Lp-PLA$_2$ may be an important link between lipid homeostasis and the vascular inflammatory response. They summarize the biology and pathophysiology of Lp-PLA$_2$ as it generates proinflammatory mediators and their role in the development of atherosclerotic necrotic cores and sustained macrophage apoptosis; furthermore, they importantly show how selective inhibition of Lp-PLA$_2$ reduces development of these necrotic cores and may result in stabilization of atherosclerotic plaques. This selective inhibition of Lp-PLA$_2$ has been postulated to reduce necrotic core progression and the clinical sequelae of advanced, unstable atherosclerosis.

4. KJ, Singh M, Bangit JR, Batsell RR. The Role of Lipoprotein-Associated Phospholipase A$_2$ on Cardiovascular Disease Risk Assessment and Plaque Rupture: a Clinical Review. *J Clin Lipidol.* 2009; 3:85-93.

   This clinically-oriented review summarizes how, during the last several decades, reduction in lipids has been the main focus to decrease the risk of coronary heart disease (CHD), although more recently several lines of evidence have indicated that lipids account for <50% of cardiovascular risk in the United States. The authors address the need for a more effective approach for better identification of people at high cardiovascular risk. They review the understanding of atherosclerosis shifting from a focal disease resulting in symptoms caused by severe stenosis to a systemic disease distinguished by plaque inflammation with a potential to rupture, and how Lp-PLA$_2$ can provide much needed information about plaque inflammation and plaque stability. Evidence from previous studies addressing the effect of different therapies on decreasing Lp-PLA$_2$ and the role of a direct Lp-PLA$_2$ inhibitor is reviewed. This work also briefly reviews the evidence of Lp-PLA$_2$ clinical utility as a potential marker of vascular inflammation and formation of rupture prone plaques. Additionally, they also discuss the implication of available evidence in context of current cardiovascular inflammatory biomarkers recommendations and the evidence from epidemiologic studies addressing the relationship of Lp-PLA$_2$ and risk of cardiovascular disease.

5. Lavi S, Herrmann J, Lavi R, McConnell JP, Lerman LO, Lerman A. Role of Lipoprotein-Associated Phospholipase A$_2$ in Atherosclerosis. *Curr Atheroscler Rep.* 2008; 10(3): 230-5.

   This review summarizes the evidence that Lp-PLA$_2$ is a biomarker that can be used to assess the risk for cardiovascular disease and events. The authors also address that, in addition to being a useful marker of a risk factor, several studies suggest that Lp-PLA$_2$ has a pathophysiologic role in the atherosclerotic disease process. In light of this aspect of Lp-PLA$_2$ the authors also review its therapeutic implications.

REVIEWS

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6. Weintraub H. Identifying the Vulnerable Patient with Rupture Prone Plaque. *Am J Cardiol.* 2008; 101[suppl]: 3F–10F.

Dr. Weintraub reviews the clinical dilemma posed by the evidence that most cases of myocardial infarction and sudden cardiac death are not caused by severe stenosis, but rather plaque inflammation, rupture and occlusion secondary to thrombosis. He explains that atherosclerosis is nearly ubiquitous in American adults but that lipid testing, risk factor tabulation, and most imaging studies looking for stenosis fail to identify rupture-prone plaque. He identifies the critical necessity for understanding plaque quality as opposed to plaque volume, and that new diagnostic modalities that directly assess the atherosclerotic disease activity of the arterial wall or endothelium are required for understanding which adults have vascular inflammation and endothelial dysfunction, both precursors to plaque rupture. He concludes that "elevation of the Lp-PLA$_2$ biomarker appears to be specific for high atherosclerotic disease activity, independent and additive to traditional risk factors and the metabolic syndrome. As such, Lp-PLA$_2$ could alert the clinician to initiate proven strategies for coronary event and stroke risk reduction."

7. Corson MA, Jones PH, Davidson MH. Review of the Evidence for the Clinical Utility of Lp-PLA$_2$ as a Cardiovascular Risk Marker. *Am J Cardiol.* 2008; 101[suppl]: 41F–50F.

The authors found that "Lp-PLA$_2$ would appear to meet the proposed criteria of the ATPIII guideline panel to determine the clinical significance of an emerging risk marker. There is substantial evidence published in peer-reviewed journals, including 25 prospective population based studies, supporting Lp-PLA$_2$ as a cardiovascular risk marker that provides incremental predictive ability over traditional risk factors." They also find that "a relatively unique characteristic of Lp-PLA$_2$ is its independence from body mass index (BMI) and insulin resistance", and that it adds to the CV risk associated with the Metabolic Syndrome.

8. Davidson MH, Alberts MJ, Anderson JL, Gorelick PB, Jones PH, Lerman A, McConnell JP, Weintraub H. Consensus Panel Recommendation for Incorporating Lp-PLA$_2$ Testing into Cardiovascular Disease Risk Assessment Guidelines. *Am J Cardiol.* 2008; 101[suppl]: 51F–57F.

An expert consensus panel met to expand on the earlier 2001 ATPIII guidelines for use of emerging risk factors, which included inflammatory markers, as an adjunct to traditional CV risk factor assessment. Their recommendations included using a simplified framework for identification of moderate risk persons, and then using Lp-PLA$_2$ testing in moderate and high risk persons to decide if their LDL-C target should be lowered further along with intensification of statin and lifestyle intervention. In addition, a clinical cutpoint of >200 ng/mL was recommended for considering a patient at higher risk.

9. Gorelick PB. Lp-PLA$_2$ and Risk of Stroke. *Am J Cardiol.* 2008; 101[suppl]: 34F–40F).

Dr. Gorelick provides the first published review of several important prospective epidemiological studies of Lp-PLA$_2$ and risk of stroke. He finds that the "Lp-PLA$_2$ immunoassay may prove to be especially useful for proper risk classification of persons with stroke or cardiovascular diseases who are found to be at moderate risk. It appears useful in overall cardiovascular risk classification and may lead to more aggressive therapeutic approaches with statin agents for lipid control or with other high-risk patient approaches for cardiovascular disease reduction."

10. Lerman A, McConnell JP. Lp-PLA$_2$: A Risk Marker or a Risk Factor? *Am J Cardiol.* 2008; 101[suppl]: 11F–22F.

These Mayo Clinic investigators review the evidence for Lp-PLA$_2$ as a potential risk factor in the direct causal pathway of plaque inflammation and rupture, including pathophysiology, mechanism of action, and histopathological findings. In addition, laboratory issues related to Lp-PLA$_2$ testing are reviewed. The authors state that "the Lp-PLA$_2$ biomarker is highly specific and has low biovariability, 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 significant raising of the c statistic, or area under the curve, in receiver operating characteristic analysis." They conclude that "Lp-PLA$_2$ may be a risk factor involved in the causal pathway of plaque inflammation and ultimate plaque rupture".

11. Anderson JL. Lp-PLA$_2$: An Independent Predictor of CHD Events in Primary and Secondary Prevention. *Am J Cardiol.* 2008; 101[suppl]: 23F–33F.

This is the most comprehensive review of the prospective primary and secondary epidemiological studies examining the associations of elevated Lp-PLA$_2$ levels and risk of coronary and cardiovascular events. The author concludes, "Given its role in plaque pathology and consistent results from clinical trials to date as summarized above, Lp-PLA$_2$ may be hypothesized to be unique as a biomarker of patients with rupture-prone plaque, and conversely, of patients whose plaques have been successfully stabilized therapeutically."

Lp-PLA$_2$ Selected Annotated Bibliography

EPIDEMIOLOGICAL STUDIES

12. Nelson TL, Biggs ML, Kizer JR et al. Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) and Future Risk of Type 2 Diabetes: Results from the Cardiovascular Health Study. J Clin Endocrinol Metab 2012.

Lp-PLA2, as a proinflammatory enzyme, has been linked to diabetes. Lp-PLA2 activity and/or mass are higher among diabetics than among non-diabetics. Plausible mechanisms include an increase in inflammatory activity associated with Lp-PLA2's hydrolysis of oxidized phospholipids that could induce insulin resistance, thereby increasing the risk for type 2 diabetes, and/or an association with increased adipose tissue inflammation and adipokine abnormalities that lead to insulin resistance and eventual pancreatic -cell failure. In this study, the authors investigated whether baseline Lp-PLA2 mass and activity were associated with the risk of incident type 2 diabetes among older adults, independent of other diabetes risk factors.

The authors conducted analyses of Lp-PLA2 and prevalent and incident diabetes among 5,474 men and women from the Cardiovascular Health Study, a population-based cohort study of risk factors for CVD in older adults (1989-2007). Subjects with known diabetes at baseline and no diabetes beyond baseline were excluded, leaving 4,508 participants for analysis. Lp-PLA2 mass and activity were measured at baseline. Diabetes status was ascertained annually with medication inventories and repeated blood glucose measurements. Generalized linear and Cox proportional hazards models were used to adjust for confounding factors. Over a median follow-up of 11.7 years, 419 cases of incident diabetes were ascertained.

Mean Lp-PLA2 concentrations at baseline were 344.9 ± 118.1 ng/ml for mass and 39.3 ± 13.0 nmol/min/ml for activity. At baseline, the top two quintiles of Lp-PLA2 activity were significantly associated with prevalent type 2 diabetes with a multivariable relative risk = 1.35 [95% confidence interval (CI) = 1.11-1.63] for quintile 4, and relative risk = 1.33 (95% CI = 1.07-1.66) for quintile 5. Among participants free of diabetes at baseline, there was a significant positive association with both the homeostatic model assessment for insulin resistance (HOMA-IR) and the homeostatic model assessment for -cell function (HOMA-) per sd increase in Lp-PLA2 activity (P values for both <0.01). In prospective analyses, the risk of incident type 2 diabetes was significantly higher among those in the highest quintile of Lp-PLA2 activity [multivariable hazard ratio = 1.45 (95% CI = 1.01-2.07)] compared with the lowest quintile. Lp-PLA2 mass was not significantly associated with incident type 2 diabetes.

Lp-PLA2 activity with incident diabetes remained significant after controlling for CRP, fibrinogen, white blood cells, and body mass index (BMI), suggesting that Lp-PLA2's interaction reflected diabetogenic pathways independent of these other accepted inflammatory markers. In conclusion, Lp-PLA2 activity was positively associated with insulin resistance and predicted incident type 2 diabetes among older adults, independent of multiple factors associated with recognized diabetes pathogenesis.

13. Massot A, Pelegri D, Penalba A, Arenillas J, Boada C, Giralt D, Ribó M, Molina CA, Rosell A, Alvarez-Sabín J, Chacón P, Rovira A, Delgado P, Montaner J. Lipoprotein-Associated Phospholipase A$_2$ Testing Usefulness Among Patients with Symptomatic Intracranial Atherosclerotic Disease. Atherosclerosis online 5 May 2011 doi:10.1016/j.atherosclerosis.2011.04.031.

The aim of this study was to investigate the relationship of Lp-PLA$_2$ mass and activity levels in a selected cohort of first-ever transient ischemic attack (TIA) or ischemic stroke patients having intracranial atherosclerotic disease (ICAD), with the presence of classical vascular risk factors, response to secondary prevention treatments and risk of recurrent vascular events. The investigators measured Lp-PLA$_2$ mass and activity levels three months after TIA or stroke in 75 patients. During the median follow-up time of 23 months, transcranial Doppler ultrasonography (TCD) was performed and the presence of a new vascular event was assessed every six months. Key findings: Several preventive treatments, including statins and clopidogrel, were significantly associated with lower Lp-PLA$_2$ mass and activity, and 18 patients (24%) suffered a new vascular event. Baseline factors associated with new vascular events were: history of CAD, number of intracranial stenoses detected by TCD, and Lp-PLA$_2$ activity, which was the only independent predictor for new vascular events (HR=2.89, 95%CI 1.029-8.096, p=0.044) after multivariate analysis. The authors conclude that Lp-PLA$_2$ activity could be a useful tool to identify intracranial large-artery occlusive disease patients at higher risk of suffering new vascular events.

14. Nelson TL, Kamineni A, Psaty B, Cushman M, Jenny NS, Hokanson J, Furberg C, Mukamal KJ. Lipoprotein-Associated Phospholipase A$_2$ and Future Risk of Subclinical Disease and Cardiovascular Events in Individuals with Type 2 Diabetes: the Cardiovascular Health Study [CHS]. *Diabetologia*. 2011; 54: 329–33.

The aim of this CHS substudy was to investigate whether increased levels of inflammatory factors such as Lp-PLA$_2$ are associated with type 2 diabetes (T2DM), an established risk factor for cardiovascular disease (CVD), and to determine whether Lp-PLA$_2$ levels help explain the greater prevalence of subclinical CVD and greater incidence of CVD outcomes associated with T2DM in this study population. The investigators conducted a cross-sectional and prospective study of 4,062 men and women without previous CVD, measuring Lp-PLA$_2$ mass and activity at baseline, and determining subclinical disease at baseline. Incident CVD was ascertained annually over an average follow-up of almost 13 years. Key findings: At baseline, Lp-PLA$_2$ mass did not differ significantly by T2DM status, while Lp-PLA$_2$ activity was significantly higher among T2DM. Baseline

subclinical disease was significantly associated with baseline DM status, with this association being similar in models unadjusted or adjusted for Lp-PLA$_2$ (OR 1.68 [95% CI 1.31-2.15] vs OR 1.67 [95% CI 1.30-2.13]). Baseline T2DM was also significantly associated with incident CVD events, including fatal CHD, fatal and non-fatal MI in multivariable analyses, but there were no differences in these estimates after further adjustment for Lp-PLA$_2$ activity. The authors conclude that in this older cohort, differences in Lp-PLA$_2$ activity did not explain any of the excess risk for subclinical disease or CVD outcomes related to diabetes.

15. Hatoum IJ, Cook NR, Nelson JJ, Rexrode KM, Rimm EB. Lipoprotein-Associated Phospholipase A$_2$ Activity Improves Risk Discrimination of Incident Coronary Heart Disease Among Women. *Am Heart J.* 2011; 161:516-22.

The aim of this study was to determine the relationship between, and discriminative capability of, Lp-PLA$_2$ and coronary heart disease (CHD) in a large population of disease-free women. From the Nurses' Health Study, investigators examined 421 cases of incident myocardial infarction (MI) during 14 years of follow-up, and controls were matched to cases 2:1 using risk set sampling based on age, smoking, and blood draw date. They found that after conditioning on the matching factors, Lp-PLA$_2$ activity was significantly associated with MI (RR=2.86 for extreme quartiles, 95% CI 1.98-4.12), and after additional adjustment for lipid, inflammatory, and clinical risk factors, the RR remained statistically significant (RR=1.75, 95% CI 1.09-2.84). The discriminative capability of Lp-PLA$_2$ was assessed by comparing the area below the receiver operating characteristic curves (AUROC) for models with and without Lp-PLA$_2$ and by calculating the net reclassification improvement index (NRI). Addition of Lp-PLA$_2$ activity to the adjusted model increased the AUROC curves from 0.720 to 0.733 and significantly improved the NRI (P=0.004). The authors conclude that levels of Lp-PLA$_2$ activity were significantly associated with incident CHD among women and additionally that Lp-PLA$_2$ activity added significantly to CHD risk discrimination.

16. Kinney GL, Snell-Bergeon JK, Maahs DM, Eckel RH, Ehrlich J, Rewers M, Hokanson JE. Lipoprotein-Associated Phospholipase A$_2$ Activity Predicts Progression of Subclinical Coronary Atherosclerosis. *Diabetes Technology & Therapeutics.* 2011; 13(3): 381-7.

The aim of this study was to determine the relationship between Lp-PLA$_2$ and progression of coronary calcium (CAC) in the Coronary Artery Calcification in Type 1 Diabetes study (CACTI). CAC was measured in the CACTI study by electron beam CT twice over a 2.6 year interval, and Lp-PLA$_2$ mass and activity were measured at baseline in 1,097 subjects, 506 with and 591 without type 1 diabetes. Investigators found that in type 1 diabetes, Lp-PLA$_2$ mass was marginally higher (285±79 vs. 278±78 ng/mL, P=0.1), and Lp-PLA$_2$ activity was significantly lower (137±30 vs. 146±36 nmol/min/mL, P<0.0001) than in those without

diabetes. There was a greater proportion of those with progression of CAC in type 1 diabetes compared with those without diabetes (24% vs. 10%, P<0.0001). Lp-PLA$_2$ activity was independently associated with CAC progression in multivariate analysis (4th quartile verses bottom three quartiles, OR=1.77 [1.08-2.91], P=0.02). Lp-PLA$_2$ mass was not significantly associated with CAC progression in this cohort (P=0.09). The authors conclude that Lp-PLA$_2$ activity predicts progression of subclinical atherosclerosis in individuals with and without type 1 diabetes.

17. Constantinides A, van Pelt LJ, van Leeuwen JJ, de Vries R, Tio RA, van der Horst IC, Sluiter WJ, Dullaart RPF. Carotid Intima Media Thickness is Associated with Plasma Lipoprotein-Associated Phospholipase A$_2$ Mass in Nondiabetic Subjects but not in Patients with Type 2 Diabetes. *Eur J Clin Invest.* 2011; 41(8):820-7.

The aim of this study was to determine whether the relationship of carotid intima media thickness (cIMT), a measure of subclinical atherosclerosis, with Lp-PLA$_2$ mass differs between diabetic and non-diabetic subjects, since it had been previously shown that Lp-PLA$_2$ activity but not mass was found to be a determinant of cardiovascular outcome in type 2 diabetes mellitus (T2DM). Investigators examined Lp-PLA$_2$ mass and cIMT relationships by comparing 74 patients with type 2 diabetes with 64 non-diabetic subjects. They found that cIMT was increased (P=0.016) while plasma Lp-PLA$_2$ mass was decreased in patients with diabetes compared to non-diabetic subjects (277 ± 66 vs. 327 ± 62 ng/mL, P<0.001). In non-diabetic subjects, cIMT was correlated positively with Lp-PLA$_2$ (r=0.325, P<0.009), and regression analysis confirmed an independent association of cIMT with Lp-PLA$_2$ (=0.192, P=0.048). In contrast, cIMT was unrelated to Lp-PLA$_2$ in patients with diabetes (r=0.021, P=0.86), and the relationship of cIMT with Lp-PLA$_2$ was different in diabetic and control subjects (P<0.001). The relationship of Lp-PLA$_2$ with the total cholesterol/HDL ratio also differed between diabetic and non-diabetic subjects (P<0.001). The authors conclude that plasma Lp-PLA$_2$ may relate to early stages of atherosclerosis development, whereas in T2DM the association of cIMT with plasma Lp-PLA$_2$ mass is abolished. They suggest this could be partly ascribed to redistribution of Lp-PLA$_2$ mass from apolipoprotein B-containing lipoproteins towards HDL, and they bring into question the usefulness of plasma Lp-PLA$_2$ mass measurement as a marker of subclinical atherosclerosis in T2DM.

18. Gong HP, Yi-meng Du, Li-na Zhong, Zhao-qiang Dong, Xin Wang, Yong-jun Mao, Qing-hua Lu. Plasma Lipoprotein-Associated Phospholipase A$_2$ in Patients with Metabolic Syndrome and Carotid Atherosclerosis. *Lipids in Health and Disease.* 2011; 10(13):1-8.

The aim of this study was to determine the relationship and correlation between Lp-PLA$_2$ activity and carotid atherosclerosis in patients with metabolic syndrome (MetS). The investigators

Lp-PLA$_2$ Selected Annotated Bibliography

evaluated the potential role of Lp-PLA$_2$ as a MetS biomarker in individuals with and without carotid atherosclerosis by examining 118 consecutive patients with MetS and 70 age- and sex-matched healthy subjects as controls. The patients were further divided into two groups: 39 with carotid plaques and 79 without carotid plaques to help elucidate the role of Lp-PLA$_2$ in carotid atherosclerosis, and Lp-PLA$_2$ activity and carotid intimal-media thickness (cIMT) by ultrasound was measured in all participants. The investigators found that Lp-PLA$_2$ activity was significantly increased in MetS subgroups vs. controls and was higher in patients with carotid plaques than those without plaques (P<0.05). They also found a significant difference in Lp-PLA$_2$ activity between patients with three and four disorders of MetS (P<0.01). Age ($=0.183$, P=0.029), LDL-cholesterol ($=0.401$, P<0.001) and waist-hip ratio ($=0.410$, P<0.001) emerged as significant and independent determinants of Lp-PLA$_2$ activity. Regression analysis revealed that LDL-C ($=0.309$, P<0.001), systolic blood pressure ($=0.322$, P=0.002) and age ($=0.235$, P=0.007) significantly correlated with cIMT, and Lp-PLA$_2$ was not an independent predictor for cIMT. The authors conclude that Lp-PLA$_2$ may be a modulating factor for cIMT via age and LDL-C, but not an independent predictor in the pathophysiological process of carotid atherosclerosis in patients with MetS.

19. Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hofman A, Packard C, Thompson SG, Collins R, Danesh J. Lp-PLA$_2$ Studies Collaboration, Lipoprotein-Associated Phospholipase A$_2$ and Risk of Coronary Disease, Stroke, and Mortality: Collaborative Analysis of 32 Prospective Studies. *Lancet*. 2010; 375(9725): 1536-44.

The Lp-PLA$_2$ Studies Collaboration (LSC) investigated the associations of Lp-PLA$_2$ mass and activity with cardiovascular disease (CVD) risk in over 79,000 individuals, with over 17,000 outcomes, across 32 prospective clinical studies on Lp-PLA$_2$. This meta-analysis included risk of coronary heart disease (CHD), stroke and mortality in various clinical populations. The LSC analyzed approximately 36,000 individuals with no history of vascular disease, about 35,000 patients with history of stable vascular disease and approximately 10,000 patients with recent acute ischemic events. Key findings: 1) Lp-PLA$_2$ levels (mass and activity) were found to be significantly associated with each other and with pro-atherogenic lipid markers. 2) Lp-PLA$_2$ levels are significantly related to CVD risk in a continuous, log-linear association. While not a binary association around 200 ng/mL, Lp-PLA$_2$ elevated above this level warrants more aggressive patient management due to the elevated CVD risk. 3) CVD risk due to elevated Lp-PLA$_2$ levels in this LSC analysis (10% per 1-SD) is comparable to the elevated CVD risk associated with two other well established risk markers: non-HDL-C and blood pressure. 4) Lp-PLA$_2$ levels provide independent CVD risk assessment from

other biomarkers and could provide distinct insight into the relationship between inflammation, atherosclerosis and cardiovascular outcomes.

20. Jenny NS, Solomon C, Cushman M, Tracy RP, Nelson JJ, Psaty BM, Furberg CD. Lipoprotein-Associated Phospholipase A$_2$ (Lp-PLA$_2$) and Risk of Cardiovascular Disease in Older Adults: Results from the Cardiovascular Health Study. *Atherosclerosis*. 2010; 209: 528–32.

This study examined associations between Lp-PLA$_2$ antigen level (mass) and enzymatic activity and cardiovascular disease (CVD), including incident myocardial infarction (MI, n = 508), stroke (n = 565) and CVD death (n = 665), in 3949 older adults (≥65 years at baseline) from the Cardiovascular Health Study (CHS). Lp-PLA$_2$ mass and activity were associated with incident CVD events in older adults in CHS. Lp-PLA$_2$ and CRP were independent and additive in prediction of events. For MI, both mass and activity added excess risk to elevated CRP alone (20% excess risk) and activity added excess risk for CVD death (~12%).

21. Caslake MJ, Packard CJ, Robertson M, Cooney J, Nelson JJ, Ford I, Gaw A, Jukema JW, Macfarlane PW, Stott DJ, Shepherd J. PROSPER Study Group. Lipoprotein-Associated Phospholipase A$_2$, Inflammatory Biomarkers, and Risk of Cardiovascular Disease in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). *Atherosclerosis*. 2010; 210(1): 28-34.

This study investigated whether Lp-PLA$_2$ mass and activity were related to risk over 3.2 years for vascular events (definite or suspected death from CHD, non-fatal MI, fatal or non-fatal stroke) in the 2804 men and 3000 women age 70-82 years in the Prospective Study of Pravastatin in the Elderly (PROSPER). The authors evaluated the association of Lp-PLA$_2$ with vascular events in the elderly where the importance of LDL is diminished as a risk factor for coronary disease. Lp-PLA$_2$ showed a moderate, positive association with risk of a vascular event with hazard ratios of 1.25 (CI 1.02-1.54) for mass and 1.39 (CI 1.14-1.70) for activity for top versus bottom quartile. The inclusion of classical risk factors, lipids and inflammatory markers, including C-reactive protein (CRP) and white cell count, in the models attenuated the risk associations. Inclusion of all three inflammatory markers in multivariate models negated the association of HDL cholesterol with risk (HR 0.98; CI 0.88-1.10), but increased the prediction of coronary events, as the C statistic rose from 63.2% to 64.4% (P<0.001). The authors conclude that in elderly people, Lp-PLA$_2$ alongside other inflammatory indices, has potential as a biomarker for vascular events, particularly CHD.

22. Hatoum IJ, Hu FB, Nelson JJ, Rimm EB. Lipoprotein-Associated Phospholipase A$_2$ Activity and Incident Coronary Heart Disease Among Men and Women with Type 2 Diabetes. *Diabetes*. 2010; 59(5): 1239-43.

    This study investigated the association of Lp-PLA$_2$ with future coronary events among diabetic men and women, measuring Lp-PLA$_2$ activity among 740 men and 777 women with confirmed diabetes from those enrolled in two prospective cohort studies – the Health Professionals Follow-up Study (HPFS, n=51,529 men) and Nurses' Health Study (NHS, n=121,700 women). Participants were free of all CVD and cancer at baseline. During 10 years of follow-up among men and 14 years among women, the authors documented 178 and 146 cases of CHD, respectively (CHD was defined as coronary artery bypass graft, angioplasty, non-fatal myocardial infarction (MI), and fatal CHD). After adjustment for age, smoking, medical history, and biomarkers including CRP, HDL, and LDL, the relative risk of total CHD comparing extreme tertiles of Lp-PLA$_2$ was 1.39 (95% CI: 1.01-1.90; p-trend=0.03). Restricting analyses to only non-fatal MI and fatal CHD, the relative risk was 1.75 (95% CI: 1.05-2.92; p-trend=0.001). LDL, HDL, CRP, HRT use, and diabetes duration did not modify these relationships. In summary, levels of Lp-PLA$_2$ activity were significantly associated with incident CHD among men and women with type 2 diabetes, independent of traditional and inflammatory risk factors, and this positive association was strongest for more severe clinical endpoints.

23. Anuurad E, Ozturk Z, Enkhmaa B, Pearson TA, Berglund L. Association of Lipoprotein-Associated Phospholipase A$_2$ with Coronary Artery Disease in African-Americans and Caucasians. *J Clin Endocrinol Metab*. 2010; 95(5): 2376-83.

    This study investigated the association between Lp-PLA$_2$ and CAD in a bi-ethnic population. Lp-PLA$_2$ mass, activity, and "index" (activity per mass – an integrated measure of mass and activity), and other cardiovascular risk factors were determined in 224 African-Americans and 336 Caucasians undergoing coronary angiography. Levels of Lp-PLA$_2$ mass and activity were higher among Caucasians compared with African-Americans (293±75 vs. 232±76 ng/ml, P=0.001 for mass and 173±41 vs. 141±39 nmol/min/ml, P=0.001 for activity, respectively). However, Lp-PLA$_2$ index was similar in the two groups (0.61±0.17 vs. 0.64±0.19, P=NS). In both ethnic groups, Lp-PLA$_2$ activity and index was significantly higher among subjects with CAD, but African-American subjects with CAD had significantly higher Lp-PLA$_2$ index than corresponding Caucasian subjects. While Lp-PLA$_2$ activity and index was associated with presence of CAD among African-Americans and Caucasians undergoing coronary angiography, these findings suggest an independent impact of vascular inflammation among African-Americans as contributory to CAD risk and underscore the importance of Lp-PLA$_2$ as a cardiovascular risk factor.

24. Hatoum IJ, Nelson JJ, Cook NR, Hu FB, Rimm EB. Dietary, Lifestyle, and Clinical Predictors of Lipoprotein-Associated Phospholipase A$_2$ Activity in Individuals Without Coronary Artery Disease. *Am J Clin Nutr*. 2010; 91(3): 786-93.

    This study aimed to determine dietary, lifestyle, and clinical measures associated with Lp-PLA$_2$ activity by measuring this biomarker in 853 female participants of the Nurses' Health Study (NHS, n=121,700 women) and 878 male participants of the Health Professionals Follow-Up Study (HPFS, n=51,529 men) who were free of cancer and cardiovascular disease. Multivariable linear regression models were utilized to assess the relation between potentially modifiable factors and Lp-PLA$_2$. The authors found that, while smoking, being overweight, aspirin use, hypercholesterolemia, and age were associated with elevated Lp-PLA$_2$ activity, not smoking, use of postmenopausal hormones, having a lower body mass index, increased alcohol consumption, and increased protein consumption all represent potential modifiable factors that may favorably influence Lp-PLA$_2$ activity.

25. Elkind MS, Tai W, Coates K, Paik MC, Sacco RL. Lipoprotein-Associated Phospholipase A$_2$ Activity and Risk of Recurrent Stroke. *Cerebrovasc Dis*. 2009; 27(1): 42-50.

    This study investigated whether Lp-PLA$_2$ activity levels would predict risk of stroke recurrence, given that Lp-PLA$_2$ mass levels are associated with prognosis after stroke. In the population-based Northern Manhattan Stroke Study (NOMAS), first ischemic stroke patients ≥40 years old were followed for recurrent ischemic stroke. Lp-PLA$_2$ activity levels were assessed in 467 patients, categorized by quartile, and adjusted for demographics, vascular risk factors, and high sensitivity C-reactive protein (hsCRP). Over the median follow-up time of 4 years, there were 80 recurrent ischemic strokes. Compared to the lowest quartile of Lp-PLA$_2$ activity, those in the highest had an increased risk of recurrent stroke (adjusted HR=2.54, 95% CI 1.01-6.39), demonstrating that stroke patients with Lp-PLA$_2$ activity levels in the highest quartile, compared to those in the lowest quartile, had an increased risk of recurrence after first ischemic stroke.

26. Elkind MS, Leon V, Moon YP, Paik MC, Sacco RL. High-sensitivity C-reactive Protein and Lipoprotein-Associated Phospholipase A$_2$ Stability Before and After Stroke and Myocardial Infarction. *Stroke*. 2009; 40(10): 3233-7.

    This study investigated the stability over time of the inflammatory biomarkers Lp-PLA$_2$ and high-sensitivity C-reactive protein (hsCRP), and in particular, the effects of acute vascular events on these marker levels. Serum samples were collected at 4 annual intervals in 52 stroke-free participants from the Northern Manhattan Study (NOMAS) and assayed for hsCRP and Lp-PLA$_2$ mass and activity levels, and additional samples from 37 initially stroke-free participants with stroke (n=17) or MI (n=20) were measured before and after the vascular event (median 5 days, range 2-40 days). While hsCRP and Lp-PLA$_2$ activity levels were

Lp-PLA$_2$ Selected Annotated Bibliography

stable over time, Lp-PLA$_2$ mass levels decreased on average 5% per year; although, using accepted clinical risk threshold categories of Lp-PLA$_2$ mass, there was no significant change over time. Acutely after stroke and MI, Lp-PLA$_2$ mass and activity levels both decreased significantly (for Lp-PLA$_2$ mass, from median 210.0 ng/mL to 169.4 ng/mL post-stroke, P=0.0348, and from median 233.0 ng/mL to 153.9 post-MI, P=0.0001), whereas hsCRP increased after stroke and MI. These changes imply that measurements made soon after stroke and MI are not reflective of pre-stroke levels and measurements taken for assessment of long-term risk stratification would be more appropriately performed outside of the acute post-event window.

27. Cucchiara BL, Messe SR, Sansing L, MacKenzie L, Taylor RA, Pacelli J, Shah Q, Kasner SE. Lipoprotein-Associated Phospholipase A$_2$ and C-reactive Protein for Risk-stratification of Patients with TIA. *Stroke*. 2009; 40(7): 2332-6.

In this prospective study of 167 patients presenting to the ED with acute TIA. Investigators determined clinical risk score using the ABCD^2 method, and they measured Lp-PLA$_2$ mass (-M) and activity (-A) as well as high-sensitivity C-reactive protein (hsCRP). The primary outcome measure was a composite end point consisting of stroke or death within 90 days or identification of a high-risk stroke mechanism requiring specific early intervention (>50% stenosis in a vessel referable to symptoms or a cardioembolic source warranting anticoagulation). This composite outcome end point occurred in 25% of study patients. Lp-PLA$_2$ mass and activity levels were higher in end point-positive compared to end point-negative patients, while there was no relationship between CRP and outcome. Subgroup analysis showed that both Lp-PLA$_2$-M (P=0.04) and Lp-PLA$_2$-A (P=0.06) but not CRP (P=0.36) were elevated in patients with >50% stenosis. In multivariate analysis using cut-off points defined by the top quartile of each marker, predictors of outcome included Lp-PLA$_2$-A and ABCD^2 score. In contrast to CRP, both Lp-PLA$_2$ mass and activity were associated with the composite end point of stroke or death within 90 days after TIA, and Lp-PLA$_2$-A appears to provide additional prognostic information beyond the ABCD^2 clinical risk score alone.

28. Nambi V, Hoogeveen RC, Chambliss L, et al. Lipoprotein-Associated Phospholipase A$_2$ and High-Sensitivity C-Reactive Protein Improve the Stratification of Ischemic Stroke Risk in the Atherosclerosis Risk in Communities (ARIC) Study. *Stroke*. 2009; 40: 376-81.

This study examined the relationship of Lp-PLA$_2$ and hs-CRP to determine these biomarkers ability to improve the area under the curve (AUC) of receiver operating characteristic curves (ROC) and their ability to alter the classification of individuals into low-, moderate-, or high-risk categories compared to traditional risk factors for 5-year ischemic stroke risk. The study revealed a significant improvement in the AUC (from 0.732 to 0.774) when both hs-CRP and Lp-PLA$_2$ and the interaction between the two

variables were added to traditional risk factors. Moreover, Lp-PLA$_2$ and hs-CRP levels altered the risk classification of individuals compared to traditional risk factors. Most revealing, the addition of hs-CRP and Lp-PLA$_2$ reclassified approximately 39% of the intermediate-risk category (28% reclassified to a lower risk and 11% reclassified to a higher risk).

29. Brilakis ES, Khera A, McGuire DK, See R, Banerjee S, Murphy SA, de Lemos JA. Influence of Race and Sex on Lipoprotein-Associated Phospholipase A$_2$ Levels: Observations from the Dallas Heart Study. *Atherosclerosis*. 2008; 199(1): 110-5.

This study investigated whether Lp-PLA$_2$ levels differ according to race and sex, since previously most Lp-PLA$_2$ studies included mainly white men. Lp-PLA$_2$ mass and activity levels were measured in 3332 subjects age 30-65 participating in the Dallas Heart Study, a multiethnic, population-based, probability sample. Lp-PLA$_2$ levels were compared between different race and sex groups: mean age was 45±9 years and 44% were men; 30% were white, 17% hispanic, and 53% black. Mean Lp-PLA$_2$ activity and mass were 146±40 nmol/min/mL and 191±60 ng/mL, respectively. Lp-PLA$_2$ activity was lower in women compared with men (134±35 vs. 161±40, p=0.001) and was lowest in black (136±38), intermediate in hispanic (151±36), and highest in white subjects (161±39) (trend p=0.0001). In multivariable linear regression models, after adjusting for age, body mass index (BMI), smoking, total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, triglycerides and high sensitivity C-reactive protein (hsCRP), Lp-PLA$_2$ activity was 19 nmol/min/mL higher in men vs. women (p<0.001); compared with black subjects, adjusted Lp-PLA$_2$ activity was 11 and 20 nmol/min/mL higher in white and hispanic subjects, respectively (both p<0.001). Similar race and sex differences were observed for Lp-PLA$_2$ mass. Therefore the authors concluded that race and sex independently influence Lp-PLA$_2$ activity and mass, and suggested that thresholds to define Lp-PLA$_2$ elevation may need to be sex and race specific.

30. Gerber Y, Shannon D. Dunlay, Allan S. Jaffe, Joseph P. McConnell, Susan A. Weston, Jill M. Killian, Veronique L. Roger. Plasma Lipoprotein-Associated Phospholipase A$_2$ Levels in Heart Failure: Association with Mortality in the Community. *Atherosclerosis*. 2008;.07.035. [Epub ahead of print]

The authors evaluated the association of Lp-PLA$_2$ with mortality in patients with heart failure and assessed its incremental value for risk discrimination over established risk factors and biomarkers. 646 residents of Olmsted County, MN, diagnosed with heart failure (HF) between September 2003 and April 2007 (mean age 76 years, 51% women) were prospectively enrolled and followed-up. Plasma Lp-PLA$_2$ levels were measured at baseline and evaluated along with known risk indicators. Results of this study showed that Lp-PLA$_2$ was positively associated with male gender and low-density lipoprotein cholesterol and inversely associated with statin use and diabetes. During

follow-up (median 21 months), 213 deaths occurred. Elevated Lp-PLA$_2$ was associated with an increased risk of mortality (hazard ratio [HR] = 1.57; 95% CI: 1.03-2.37; p=0.035). The relationship differed markedly by age, with a strong association in patient's under 80 years of age. For these patients under 80 years of age, an improvement in the model's discriminatory power was obtained by adding Lp-PLA$_2$ to established risk indicators and biomarkers (area under the receiver operating characteristic curve improved from 0.709 to 0.744, p=0.008).

31. Daniels LB, Laughlin GA, Sarno MJ, Bettencourt R, Wolfert RL, Barrett-Connor E. 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. *J Am Coll Cardiol.* 2008; 51: 913-9.

    577 women and 500 men, average age 72, and all apparently healthy were followed for 16 years. 228 had CHD events of which 38% were fatal. The hazard ratio for Q4:Q1 was 1.64 [1.05-2.55], p <0.05, adjusted for age, sex, smoking, systolic blood pressure, LDL, HDL, and diabetes. Elevated Lp-PLA$_2$ raised the AUC significantly over traditional risk factors as well. This finding is particularly important since LDL cholesterol does not predict incident CHD or stroke in the elderly.

32. 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 A$_2$, Hormone Use, and the Risk of Ischemic Stroke in Postmenopausal Women. *Hypertension.* 2008; 51(4): 1115-22.

    This is the largest study of the association of ischemic stroke with elevated Lp-PLA$_2$ in older women. Among 1,137 nonusers of hormone therapy at baseline, the corresponding odds ratio was 1.55 (95% CI: 1.05 to 2.28), whereas there was no significant association among 737 hormone users (odds ratio: 0.70; 95% CI: 0.42 to 1.17; P for interaction = 0.055). Moreover, among non-hormone users, women with high C-reactive protein and high Lp-PLA$_2$ levels had more than twice the risk of stroke (odds ratio: 2.26; 95% CI: 1.55 to 3.35) compared to women with low levels of both biomarkers. Women on hormone replacement therapy had lower Lp-PLA$_2$ levels, a finding consistent with earlier reports that estrogen lowers Lp-PLA$_2$.

33. Persson M, Berglund G, Nelson JJ, Hedblad B. Lp-PLA$_2$ Activity and Mass are Associated with Increased Incidence of Ischemic Stroke: A Population-Based Cohort Study from Malmo, Sweden. *Atherosclerosis.* 2008; 200(1): 191-8.

    The first Malmo publication found elevated Lp-PLA$_2$ to significantly predict combined CV events, and to do so additively to Metabolic Syndrome as a CV risk factor. This study examined risk for incident stroke and CHD events separately. In all, 347 subjects had an event (195 CHD and 152 ischemic strokes) during the over ten-year follow-up period. In an age-, sex- and CV risk factors-adjusted Cox regression analysis, comparing top to bottom tertile of Lp-PLA$_2$ mass, the relative risk [RR; 95% confidence interval (CI)] for incident CHD and ischemic stroke events were 0.95; 0.65–1.40 and RR: 1.92; 1.20–3.10. The authors concluded that "elevated levels of Lp-PLA$_2$ activity and mass, respectively, were in this study, independently of established risk factors related to the incidence of ischemic stroke but after adjustment for lipids not significantly related to incident CHD". Reasons why it was not positive for incident CHD events may be related to the relatively high LDL-C in this study (median LDL cholesterol was 170 mg/dL). However, multivariate adjustment for Lp-PLA$_2$ for stroke risk was not attenuated by the high LDL-C (likely because LDL-C does not predict stroke like it does heart attack).

34. Garza CA, Montoir VM, McConnell JP, Somers VK, Jullo IJ, Lopez-Jimenez F. Association Between Lipoprotein-Associated Phospholipase A$_2$ and Cardiovascular Disease: A Systematic Review. *Mayo Clin Proc* 2007:82(2):159-165.

    This is the first meta-analysis of 14 epidemiological studies of Lp-PLA$_2$, "the meta-analytic odds ratio adjusted for conventional CVD risk factors was 1.60 (95% confidence interval, 1.36 - 1.89). Differences in study methods explained differences in results across studies. The risk estimate appears to be relatively unaffected by adjustment for conventional CVD risk factors. In addition, Lp-PLA$_2$ may represent a potential therapeutic target for CVD risk reduction." No stroke studies were included in the meta-analysis, however.

35. 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(8): 1788-95.

    82 of 765 men and women ages 40-79 years old developed incident CV events (cardiac death, MI, stroke and TIA) in a ten year, prospective follow-up study in Bruneck, Italy. The fully adjusted relative risk for CV death, MI, stroke and TIA per standard deviation of Lp-PLA$_2$ = 1.4 (95% CI 1.1-1.4), p = 0.008. When both the Lp-PLA$_2$ enzyme and its substrate, oxidized phospholipid (expressed as the oxPL to apoB ratio), were in the top tertile, the two CV risk markers were additive with a hazard ratio of 3.9 (p = 0.018). After substituting Lp(a) for oxPL/apoB the investigators achieved the same results, suggesting that Lp(a) acts as a sink or trap for oxPL, and is essentially equivalent to the latter. The oxPL to apoB ratio correlated very highly with Lp(a), r = 0.87.

EPIDEMIOLOGICAL STUDIES

Lp-PLA$_2$ Selected Annotated Bibliography

EPIDEMIOLOGICAL STUDIES

36. 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(9): 604-12.

In The North Wuerttemberg and Berlin Infarction Study-II (NOBIS-II) study, 429 consecutive patients presented to the ER with ACS symptoms. Blood was drawn within 7 hours (median) of symptom onset. Via Classification and Regression Tree (CART) analysis, three biomarkers were significant and independent predictors of major adverse cardiac events (MACE) in the ensuing 42 days: troponin, NTproBNP and Lp-PLA$_2$ mass. hsCRP did not add prognostic information. Troponin negative patients with moderately elevated NTproBNP but an Lp-PLA$_2$ >210 ng/ml had a risk ratio of 2.6 (95% CI 1.1-6.6) for MACE which occurred in 56/429 patients. This study suggests that there may be a "window" in the first few hours post-MI when Lp-PLA$_2$ is not yet suppressed and may provide important prognostic information.

37. 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: 1411-6.

The Malmo Diet and Cancer Study followed 4,480 non-diabetics who developed 261 CV events after ten years of follow-up. High Lp-PLA$_2$ levels and the presence of the Metabolic Syndrome were additive predictors of those who had a cardiovascular event. This finding supports the recommendation that Lp-PLA$_2$ be used for further CV risk stratification in moderate risk, Metabolic Syndrome patients.

38. 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: 2463-9.

3,766 patients with stable CAD (and blood sampled over 90 days post-ACS) in the PEACE trial (a study which was designed to look at already well managed CAD patients to see if trandalopril would further reduce risk), an Lp-PLA$_2$ in the fourth quartile had an adjusted HR for CV death, MI, UA, stroke and revascularization Q4:Q1 adj. HR 1.41, 95% CI 1.17 to 1.70. Interestingly, Lp-PLA$_2$ was a highly significant predictor of need for revascularization, adj. HR 1.29 (1.04–1.61) p = 0.01, whereas hsCRP did not predict revascularization procedures. The authors noted similar findings in the PROVE-IT trial, where they found a strong trend for Lp-PLA$_2$ as a predictor of revascularization Q5:Q1 adj. HR 1.33 (0.97–1.82).

39. Winkler K, Hoffman 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(8):1440-1447.

2513 patients with angiographically confirmed coronary atherosclerosis (>20% stenosis) and 719 patients with <20% stenosis were followed for a median of 5.5 years. Risk for cardiac death doubled in the second and third tertiles for Lp-PLA$_2$ activity. These hazard ratios were maintained despite adjustment for traditional risk factors, NTproBNP and hsCRP. In patients with hsCRP <3 mg/L, Lp-PLA$_2$ above the bottom tertile continued to predict a doubling in cardiac death rates. Persons with coronary atherosclerosis but in the bottom tertile for Lp-PLA$_2$ activity demonstrated remarkably low 5% cardiac mortality in up to seven years of follow-up.

40. 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: 2073-80.

467 patients with first-ever ischemic stroke were followed for four years to determine whether levels of hs-CRP and Lp-PLA$_2$ drawn in the setting of acute stroke (84% drawn within 72 hours of stroke) predict risk of stroke recurrence. Levels of Lp-PLA$_2$ and hs-CRP were weakly correlated. After multivariate analysis, patients with the highest Lp-PLA$_2$ levels had double the risk for recurrent stroke and for the combined outcome of stroke, MI, or vascular death. Lp-PLA$_2$ identifies stroke patients who require the most aggressive treatment to prevent a second event.

41. 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: 2517-22.

271 post-MI patients in Olmsted County, Minnesota, had Lp-PLA$_2$ checked during their acute MI. When Lp-PLA$_2$ was in the top tertile the risk of death one year after MI increased five-fold. The high rate of death is consistent with Lp-PLA$_2$ acting as a marker of advanced, rupture-prone plaque and the implication that near-term risk is therefore high. Conversely, a baseline Lp-PLA$_2$ below 219 ng/ml was associated with only a 5% rate of death one year after MI. This is the third study (Mayo Heart, KAROLA) which suggests that a Lp-PLA$_2$ below the low 200's ng/ml has a high negative predictive value of about 95% for future CV event rates – suggesting plaques have been stabilized by treatment and that there is low (5%) residual CV risk.

To view the *Lp-PLA$_2$ Expanded Annotated Bibliography*, please visit **www.plactest.com/AB**

42. 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: 1586-93.

    The purpose of this study was to evaluate whether Lp-PLA$_2$ is associated with four year prognosis in 1,051 patients followed after ACS or revascularization with a baseline LDL cholesterol of 100 mg/dl. Patients above the lowest tertile of Lp-PLA$_2$ (>223 ng/ml) had a significant doubling of risk for CV events, fully adjusted for traditional risk factors, lipids and also cystatin C and NT-pro-BNP. As in the Mayo Heart Study (Brilakis), there appeared to be a risk threshold in the low 200's ng/ml for Lp-PLA$_2$ mass concentration.

43. 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.

    1,493 consecutive coronary angiography patients drawn from the Intermountain Heart Collaborative Study were followed for 6.7 years. Lp-PLA$_2$ was found to independently predict the severity of obstructive coronary artery disease (CAD) at the time of coronary angiography (hazard ratio for Q4 vs. Q1 2.44, p <0.001). High Lp-PLA$_2$ levels also uniquely predicted cardiac death after full adjustment for traditional risk factors and CRP. Lp-PLA$_2$ was found to improve risk stratification for patients receiving coronary angiography and the authors concluded, "Our results, together with previous reports, suggest that Lp-PLA$_2$ represents a vascular-specific inflammatory biomarker of clinical CV risk, independently and complementary to CRP."

44. 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: 2479-84

    The ARIC study assessed Lp-PLA$_2$ and CRP levels from 194 stroke cases and from a cohort random sample of 766 non-cases from the apparently healthy 45-64 year olds (1/3 of strokes nationally now occur in persons under age 65). After adjusting for traditional cardiovascular risk factors, lipids and hsCRP, elevated levels of Lp-PLA$_2$ were associated with a doubling of risk for ischemic stroke. As in other stroke epi studies, LDL cholesterol (LDL-C) did not differentiate stroke cases from controls in ARIC. Interestingly, statins lower risk of ischemic stroke (and levels of Lp-PLA$_2$), even though LDL-C is not a reliable predictor of stroke.

45. 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: 137-44.

    This study evaluated 504 patients with clinically indicated angiography to determine the association of Lp-PLA$_2$ levels with coronary heart disease risk factors, with severity of angiographic CAD, and with major cardiovascular events. Lp-PLA$_2$ in the top tertile vs. the bottom tertile was associated with a hazard ratio for cardiovascular events of 2.29, p = 0.023, but did not correlate with degree of angiographic disease. After 4-year follow-up, 95% of patients with Lp-PLA$_2$ <200 ng/mL were event-free. There appeared to be a risk threshold for Lp-PLA$_2$ at the bottom tertile, i.e. <200 ng/ml.

46. Iribarren C, Gross MD, Darbinian JA, et al. 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: 211-6.

    In this nested case-control study of 266 young adults with significant coronary artery calcium and 266 controls, a statistically significant association remained for Lp-PLA$_2$ mass (OR, 1.28 per standard deviation; 95% CI, 1.03 to 1.60) after adjusting for multiple covariates including LDL-C, HDL-C, triglycerides, and C-reactive protein. The authors conclude that Lp-PLA$_2$ mass may be a useful marker of subclinical cardiovascular risk.

47. Khuseyinova N, Imhof A, Rothenbacher D, Trischler G, Kuelb S, Scharnagl H, Maerz W, Brenner H, enk W. Association Between Lp-PLA$_2$ and Coronary Artery Disease: Focus on its Relationship with Lipoproteins and Markers of Inflammation and Hemostasis (HELICOR). *Atherosclerosis.* 2005; 182: 181-8.

    Lp-PLA$_2$ concentrations, as well as a wide range of lipid, inflammatory, and hemostatic parameters, were measured in 312 patients with coronary artery disease and 479 age- and gender-matched controls. After adjustment for all traditional risk factors, patients in the top vs. bottom quartile had an odds ratio for severe angiographic CAD of 1.91; 95% CI, (1.12 - 3.28). Lp-PLA$_2$ was independent of hsCRP, serum amyloid A, PAI-1, interleukin 6, TNF-α, ICAM-1, WBC count, fibrinogen, d-Dimer, Lp(a) and other inflammatory markers. These results provide strong support for Lp-PLA$_2$ as a biomarker for CAD risk which is independent of markers of systemic inflammation and hemostasis.

48. 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 Ischemic Stroke: The Rotterdam Study. *Circulation.* 2005; 111: 570-5.

PATHOPHYSIOLOGY AND GENETICS STUDIES

Lp-PLA$_2$ Selected Annotated Bibliography

Over 2000 subjects were studied to determine whether Lp-PLA$_2$ predicts coronary heart disease and stroke in men and women >55 years old over a mean follow-up of 7.2 years. Lp-PLA$_2$ was found to be an independent predictor of both future coronary events and ischemic stroke across all ranges of cholesterol. The stroke risk associated with elevated Lp-PLA$_2$ was not attenuated after adjustment for CV risk factors. This fills an important unmet need as lipids (here non-HDL cholesterol and HDL cholesterol) are not reliable predictors of ischemic stroke risk. These findings are consistent with the ARIC study, where LDL-C did not predict stroke risk, but Lp-PLA$_2$ did.

49. Ballantyne CM, 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: 837-42.

Nearly 13,000 apparently healthy middle aged subjects in the ARIC study were followed over 6 years to determine the relation between Lp-PLA$_2$, CRP, traditional risk factors and risk for CHD events, including fatal and non-fatal MIs. For individuals with LDL-C <130 mg/dL, elevated Lp-PLA$_2$ conferred nearly a two-fold risk increase for incident CHD following adjustment for all traditional risk factors and hsCRP. Lp-PLA$_2$ and CRP were complementary in identifying individuals at high CHD risk who may have otherwise been missed by traditional risk assessment alone.

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: 1903-8.

934 apparently healthy but moderately hypercholes-terolemic men aged 45-64 were followed for 14 years. After full adjustment for traditional risk factors, TC/HDL ratio, BMI and hsCRP, Lp-PLA$_2$ was associated with increased risk for coronary events (relative risk per standard deviation 1.21 (95% CI 1.01 - 1.45).

51. Packard CJ, O'Reilly DS, Caslake MJ, McMahan AD, Ford I, Cooney J, MacPhee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GDO. Lipoprotein-Associated Phospholipase A$_2$ as an Independent Predictor of Coronary Heart Disease. West of Scotland Coronary Prevention Study Group. *N Engl J Med.* 2000; 343: 1148-55.

Levels of Lp-PLA$_2$, CRP, and other markers of inflammation were measured in 1740 patients (580 with events, 1160 without events) to determine the association of these variables with the risk for coronary events. High levels of Lp-PLA$_2$ were associated with a two-fold increased risk for a CHD event. After multivariate analysis, including adjustment for traditional risk factors and other inflammatory markers being evaluated, Lp-PLA$_2$ was the only marker whose association with CHD event remained statistically significant. Lp-PLA$_2$ may have a direct role in atherogenesis and may be a valuable addition to cardiovascular risk assessment.

PATHOPHYSIOLOGY AND GENETICS STUDIES

52. Herrmann J, Mannheim D, Wohlert C, Versari D, Meyer FB, McConnell JP, Gössl M, Lerman LO, Lerman A. Expression of Lipoprotein-Associated Phospholipase A$_2$ in Carotid Artery Plaques Predicts Long-term Cardiac Outcome. *Eur. Heart J.* 2009 Dec; 30(23): 2930-8.

This study investigated whether carotid artery plaque expression of Lp-PLA$_2$ predicts cardiac events. In this prospective cohort study of 162 consecutive patients undergoing elective carotid endarterectomy (CEA), several molecular features of the tissue were evaluated: Lp-PLA$_2$ content was quantified by immunoblotting, lysophosphatidylcholine (lysoPC, a pro-inflammatory enzymatic product of Lp-PLA$_2$) by LC/MS/MS, and additionally by immunoblotting: CRP, p67phox, and MMP-2 and -9. Macrophage plaque content and plaque collagen content were determined by staining techniques. The follow-up period for cardiac death and non-fatal acute myocardial infarction was 48 ± 14 months. It was found that expression of Lp-PLA$_2$ and lysoPC was higher in carotid plaques of patients with than without cardiac events; and smoking and point increase in carotid Lp-PLA$_2$ expression but no other traditional cardiovascular risk factor, histological or molecular marker remained predictive of cardiac events in the multivariate Cox proportional hazard analyses [respectively: HR 3.65 (1.36-9.83), p=0.01 and HR 1.34 (1.01-1.77), p=0.039]. Carotid plaque Lp-PLA$_2$ expression above the median constituted >3X higher risk for cardiac events [HR 3.39 (1.13-10.17), p=0.03]. The authors concluded that Lp-PLA$_2$ expression in carotid artery plaques is a predictor of long-term cardiac outcome, with this study supporting the concept of atherosclerosis as a systemic disease with multi-focal complications.

53. Vickers KC, Maguire CT, Wolfert R, Burns AR, Reardon M, Geis R, Holvoet P, Morrisett JD. Relationship of Lipoprotein-Associated Phospholipase A$_2$ and Oxidized Low Density Lipoprotein in Carotid Atherosclerosis. *J Lipid Res.* 2009; 50(9): 1735-43.

Because plasma levels of Lp-PLA$_2$ and oxLDL have been identified as risk factors for cardiovascular disease, and because Lp-PLA$_2$ is the sole enzyme responsible for the hydrolysis of oxidized phospholipids on LDL particles in atherosclerotic plaques, the authors evaluated the relationship between Lp-PLA$_2$ and oxLDL in carotid endarterectomy (CEA) tissues and in matched plasmas. In extracts from CEA anatomical segments, levels of oxLDL were significantly associated with the levels of Lp-PLA$_2$ protein mass (r=0.497) and activity (r=0.615). OxLDL and Lp-PLA$_2$ mass/activity were most abundant in the carotid

bifurcation and internal segments where plaque was most abundant. In extracts from CEA atheroma, levels of oxLDL and Lp-PLA$_2$ were significantly correlated (r=0.634), and in matched plasma and atheroma extracts, Lp-PLA$_2$ levels were negatively correlated (r=-0.578). The ratio of Lp-PLA$_2$ to oxLDL was higher in atheromatous tissue (277:1) than in normal tissue (135:1) and plasma (13:1). Immunohistochemical experiments indicated that in plaques, oxLDL and Lp-PLA$_2$ existed in overlapping but distinctly different distribution, and fluorescence microscopy showed both oxLDL and Lp-PLA$_2$ epitopes on the same LDL particle in plasma but not in plaque. The authors conclude that these results suggest the relationship between Lp-PLA$_2$ and oxLDL in the atherosclerotic plaque is different from that in the plasma compartment.

54. Lenzini L, Antezza K, Caroccia B, Wolfert RL, Szczech R, Cesari M, Narkiewicz K, Williams CJ, Rossi GP. A Twin Study of Heritability of Plasma Lipoprotein-Associated Phospholipase A$_2$ (Lp-PLA$_2$) Mass and Activity. *Atherosclerosis*. 2009; 205(1): 181-5.

This study investigated heritability of plasma levels of Lp-PLA$_2$ mass and activity. The authors evaluated 54 healthy twins pairs and estimated genetic variance and heritability of Lp-PLA$_2$ mass and activity; they estimated intra-class correlation (ICC) and proportion of additive genetic variance from a model comprising additive genetic influence, environmental effect common to co-twins and individually unique environmental influence (ACE model). They found that 26 twin pairs were monozygotic (MZ) and 28 dizygotic (DZ), and that Lp-PLA$_2$ mass and activity showed a significant correlation (r=0.87, p<0.001), with mean values similar in MZ and DZ. ICC estimates of heritability for Lp-PLA$_2$ were 0.27 (mass) and 0.28 (activity); ACE model-based estimates of heritability were 0.37 (mass) and 0.54 (activity). Heritability estimates were not significant for Lp-PLA$_2$ mass, but significant for Lp-PLA$_2$ activity. The authors concluded that these results suggest heritability for activity, but not for mass, in healthy Caucasians.

55. Mannheim D, Herrmann J, Versari D, Gössl M, Meyer FB, McConnell JP, Lerman LO, Lerman A. Enhanced Expression of Lp-PLA$_2$ and Lysophosphatidylcholine in Symptomatic Carotid Atherosclerotic Plaque. *Stroke*. 2008; 39: 1448-55.

In carotid tissue harvested by endarterectomy (CEA), the expression of Lp-PLA$_2$ in 167 carotid artery plaques was determined by immunoblotting and immunostaining. The intensity of staining for Lp-PLA$_2$ correlated with risk of a cerebrovascular event within 3 months of CEA, with most of the association powered by higher risk of TIA versus stroke.

56. Khuseyinova N, Greven S, Rueckerl R, Trischler G, Loewel H, Peters A, Koenig W. Variability of Serial Lp-PLA$_2$ Measurements in Post-Myocardial Infarction Patients: Results from the AIRGENE Study Center Augsburg. *Clin Chem*. 2008; 54(1): 124-30.

Lp-PLA$_2$ mass was measured in 200 post-myocardial infarction (post-MI) patients aged 39-76 years, from whom 6 blood samples were collected in monthly intervals. The nearly 1,200 samples showed stability and reproducibility of Lp-PLA$_2$ within subjects similar to those for lipid measurements (15.9% within subject, including 4.4% analytic variation (CV). In contrast, the within subject plus analytic variation for hsCRP <10 mg/L was 58.3%. Also Lp-PLA$_2$ appeared to be depressed in months 1 and 2 post-MI then returned to apparent baseline in months 3-6.

57. 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: 2715-21.

Coronary angiography, blood flow, flow reserve, endothelial function assessment, and intravascular ultrasound with volumetric analysis were performed in 15 patients with mild coronary atherosclerosis and in 15 control subjects. Plasma samples were collected simultaneously from the left main coronary artery (coronary os) and coronary sinus for measurement of Lp-PLA$_2$, lysophosphatidylcholine (lysoPC is an inflammatory end product of the reaction of Lp-PLA$_2$ with oxidized LDL), and C-reactive protein. Across the coronary vascular bed there is a net increase (production) of Lp-PLA$_2$ and lysoPC in patients with early atherosclerosis, while there is a modest net decrease (absorption apparently by healthy intima) of Lp-PLA$_2$ in controls without evidence of atherosclerosis. An increase in lysoPC across the coronary circulation was associated with endothelial dysfunction. Taken together, these findings confirm the causal role of Lp-PLA$_2$ and oxidized LDL in triggering the inflammation cascade, as first promulgated by Macphee et al.

58. 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(1): 54-62.

Adding oxidized LDL to human leukocytes in vitro stimulates cytokine production, but normal LDL does not. The addition of a small molecular weight Lp-PLA$_2$ inhibitor along with the oxidized LDL abrogates the leukocyte production of cytokines. This suggests that inactivating the Lp-PLA$_2$ enzyme with a small molecular inhibitor may suppress the vascular inflammatory cascade.

59. 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: 2523-9.

Plaques in coronary segments taken from 25 sudden coronary death patients were classified as pathologic intimal thickening, fibroatheromas, thin-cap fibroatheromas (rupture-prone), and

THERAPEUTIC MODULATION STUDIES

Lp-PLA$_2$ Selected Annotated Bibliography

ruptured. The segments were stained for Lp-PLA$_2$ using the same antibodies used in the PLAC Test for Lp-PLA$_2$. Minimal Lp-PLA$_2$ staining was detected in early stage plaques, while later stage plaques showed intense staining for Lp-PLA$_2$. In addition, Lp-PLA$_2$ co-localized with apoptotic macrophages. The authors concluded, "Lp-PLA$_2$ is strongly expressed within the necrotic core and surrounding macrophages of vulnerable and ruptured plaques, with relatively weak staining in less advanced lesions." In addition, 72 autopsy specimens from sudden cardiac death were studied. "Sudden death secondary to plaque rupture and thrombosis occurred in 55 cases (76%) with the remaining cases (24%) associated with severe luminal narrowing." In other words, rupture-prone plaques appeared to be three times as likely to cause sudden death as were stenotic lesions.

60. 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(1): 106-11.

   Utilizing the gold standard for measurement of coronary artery endothelial dysfunction, acetylcholine was infused into the LAD and coronary artery diameter and rebound in blood flow was assessed. Lp-PLA$_2$ was a significant predictor of endothelial dysfunction whereas lipids were not.

61. 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: 2909-17.

   Using combined in situ hybridization and immunocytochemistry, researchers detected Lp-PLA$_2$ mRNA and protein in the macrophages of both human and rabbit atherosclerotic lesions. In addition, about a six-fold higher Lp-PLA$_2$ activity was detected in atherosclerotic aortas of Watanabe heritable hyperlipidemic rabbits compared with normal aortas from control rabbits. The authors conclude that Lp-PLA$_2$ is expressed by macrophages in atherosclerotic lesions.

THERAPEUTIC MODULATION STUDIES

62. Ridker PM, Macfadyen JG, Wolfert RL, Koenig W. Relationship of Lipoprotein-Associated Phospholipase A2 Mass and Activity with Incident Vascular Events among Primary Prevention Patients Allocated to Placebo or to Statin Therapy: An Analysis from the JUPITER Trial. Clin Chem 2012.

   The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial compared rosuvastatin 20 mg to placebo among 17,802 apparently men and women without cardiovascular disease or diabetes at study entry, but with unexplained elevations of hs-CRP (≥2mg/dL). While it is widely accepted that Lp-PLA2 levels are associated with an increased risk for cardiovascular events and that Lp-PLA2 is physically linked to LDL-C, the authors asked whether Lp-PLA2 mass or activity could continue to predict risk after aggressive LDL-C reduction by powerful statin therapy. In this new analysis from JUPITER, the authors analyze the subjects, relationships of Lp-PLA2 mass and activity with risk of future vascular events in both the placebo and rosuvastatin-treatment groups.

   This analysis included 10,439 study participants with data for both baseline and 1 year on treatment. Median age was 66 years 36% were female. Median baseline labs included LDL-C =109 mg/dL, HDL=49 mg/dL, and hs-CRP=4.1 mg/L. In the JUPITER population overall, LDL-C concentrations on treatment were decreased to approximately 50mg/dL. Rosuvastatin therapy reduced Lp-PLA2 mass by 33.8%, Lp-PLA2 activity by 33.2%, and LDL-C by 48.7% (all P < 0.0001). In the placebo group, the baseline Lp-PLA2 levels predicted incident vascular events, the primary endpoint of the study. A significant increase in risk was observed across increasing quartiles of Lp-PLA2 activity [p=0.001]. Baseline Lp-PLA2 predicted risk in quartile 3 and 4 which was maintained even after fully adjusting for all traditional risk factors (including LDL-C, HDL-C and hs-CRP). The prediction was statistically significant (HR Q4 v Q1 = 2.15, fully adjusted). For placebo, increasing quartiles of Lp-PLA2 activity (Ptrend = 0.04) but not Lp-PLA2 mass (Ptrend = 0.92) were associated with incident cardiovascular events after adjustment for LDL-C and conventional risk factors. Comparable analyses conducted among those allocated to rosuvastatin revealed no significant relationship between Lp-PLA2 levels and subsequent vascular events. The ability of rosuvastatin to reduce vascular events was not significantly modified by baseline Lp-PLA2 level.

   The authors conclude that levels of Lp-PLA2 activity, but not mass, were modestly associated with cardiovascular risk among those study participants randomly allocated to placebo. However, among those randomly allocated to rosuvastatin 20 mg daily, Lp-PLA2 levels no longer predicted risk nor modified clinical outcomes. It is important to note that in the treated group, incident vascular deaths per person in the treatment group were low, as expected in a healthy population with only elevated hs-CRP levels. As such, clinical events were not sufficiently powered in quartile analysis to reach statistical significance, which is not noted in the paper's discussion.

   Importantly, the Lp-PLA2 mass assay referred to in this publication was a research-grade turbidimetric assay (TIA) never cleared for commercial use, nor commercially available. The FDA-cleared mass assay in use in the USA is the PLAC Test, an ELISA method.

63. Delgado P, Chacon P, Penalba A et al. Temporal profile and prognostic value of Lp-PLA2 mass and activity in the acute stroke setting. Atherosclerosis 2012;220(2):532-536.

Physicians are actively searching for biomarkers that can help in the guidance of stroke thrombolysis. Due to its enhanced expression in unstable atherosclerotic carotid lesions and its role in vascular-specific inflammation, Lp-PLA2 may play a role in the pathophysiology of cerebrovascular disease, particularly in strokes of atherosclerotic origin. In fact, Lp-PLA2 has been shown to predict risk of first-ever or recurrent stroke and myocardial infarction. These investigators studied the relationship of Lp-PLA2 to early outcome after stroke in a population of t-PA-treated stroke patients. This study reports for the first time Lp-PLA2 mass and activity in acute ischemic stroke patients treated with t-PA, their temporal profile within the first 24 h from stroke onset and their relationship with the efficacy and safety of intravenous thrombolysis. Patients with higher Lp-PLA2 mass are more likely to be resistant to iv t-PA, with very low early recanalization rates.

Lp-PLA2 mass and activity were measured in 135 healthy controls and also in stroke patients treated with t-PA at baseline (n = 99) and serially thereafter (n = 34). Clinical examinations were performed on admission and at 12, 24 and 48 h from stroke onset by means of National Institutes of Health Stroke Scale (NIHSS). To evaluate vessel status, transcranial Doppler (TCD) measurements were performed before t-PA administration and serially (1, 2, 6 and 24 h). Outcome was defined according to early neurological status, the presence of arterial recanalization and functional outcome at third month.

Lp-PLA2 mass was increased as compared to controls, whereas Lp-PLA2 activity was significantly decreased at baseline as compared with controls and with 1 and 24 h determinations. Lp-PLA2 mass and activity were not related with early (48 h) neurological status. Regarding recanalization, higher mass and activity were found among patients who did not achieve complete recanalization by the end of t-PA treatment (p = 0.029 for mass, p = 0.044 for activity). Lp-PLA2 mass and the existence of a proximal occlusion at baseline were the most powerful predictors for persistent occlusions [OR for proximal occlusion 6.8 (p = 0.036); OR for Lp-PLA2 mass 7.2 per standard deviation increase (p = 0.008)].

Lp-PLA2 is a known marker of atherosclerotic disease; consistent with that is the data from this study showing higher Lp-PLA2 mass and activity in patients with large-artery atherosclerotic disease as stroke etiology. This study also documents the significant changes in Lp-PLA2 concentrations that occur early after stroke onset. Acute vascular events alter the concentrations and activity of Lp-PLA2 likely as a consequence of lipid profile modifications in the acute phase of stroke. These changes can be observed as early as 3 h from stroke onset. A significant increase of Lp-PLA2 mass in acute stroke and a baseline decrease in Lp-PLA2 activity were found. The authors explain this by the fact that the Lp-PLA2 mass assay is measuring accessible Lp-PLA2 on the lipoprotein particle surfaces, whereas the activity assay is measuring total Lp-PLA2 activity dissociating from lipoprotein particles under denaturing conditions. As such, the 2 assays provide different and complementary information. Both the differential effect of Lp-PLA2 mass and activity with outcome after t-PA treatment and the different temporal profile exhibited by mass and activity may relate to the differential distribution of Lp-PLA2 mass and activity across LDL and HDL subfractions. Since more Lp-PLA2 mass resides on HDL, but more Lp-PLA2 activity is associated with LDL, any acute event modifying LDL/HDL might also have a differential effect on Lp-PLA2 mass and activity measurements.

The authors conclude that Lp-PLA2 mass may add relevant information regarding early arterial recanalization in intravenous t-PA-treated stroke patients.

64. White HD, Simes J, Barnes, E et al. Changes in Lp-PLA2 activity in secondary prevention predict coronary events and treatment effect by pravastatin in long term intervention with pravastatin in ischemic disease (LIPID) Trial. Circulation, abstract 14857, AHA 2011

Effects of statins on CHD are largely explained by LDL-C lowering, but some of the treatment effect may also be explained by an effect on Lp-PLA2 levels. The LIPID trial randomized 9014 patients with total cholesterol levels 155-217 mg/dL to placebo or pravastatin (40mg) after an MI or admission with unstable angina. The original LIPID report showed that pravastatin treatment resulted in a 29% reduction in all cardiovascular outcomes.

In this study, the authors analyzed the relationship of Lp-PLA2 in a lipid-lowering setting to determine whether baseline Lp-PLA2 (per quartile) was predictive of coronary events (CHD death or nonfatal MI) during follow-up a one year, to determine the effect of pravastatin treatment on Lp-PLA2 levels (per quartile), and to determine the extent of the pravastatin treatment effect explained by changes (reductions) in Lp-PLA2. This study suggests that—prior to therapeutic intervention with statin, and during statin treatment—beyond simply targeting LDL-lowering, Lp-PLA2 should be monitored to assess the potential efficacy of statin therapy to help direct ongoing cardiovascular disease risk reduction management.

The median baseline Lp-PLA2 value was 262 nmol/min/mL, consistent with the elevated median LDL-C level (152 mg/dL) in the study cohort. The average reduction of Lp-PLA2 on pravastatin treatment was 16%. All analyses were adjusted for pravastatin treatment and gender. Effect of changes in Lp-PLA2 on subsequent CHD events used a landmark analysis with Cox regression. Multivariate analyses were performed adjusted for treatment, gender, 23 other traditional risk factors and 7 novel biomarkers. Similar relative effects of pravastatin were observed within each subgroup defined by baseline Lp-PLA2 quartiles (and with greater absolute benefit among those with higher baseline levels). When adjusted for gender and treatment,

THERAPEUTIC MODULATION STUDIES

Lp-PLA₂ Selected Annotated Bibliography

baseline Lp-PLA2 predicted CHD death and MI, but after full adjustment for all baseline risk factors, only CHD death was predicted. The extent of reduction of Lp-PLA2 levels by pravastatin predicted CHD death and MI and total CVD events, even after adjustment for all baseline factors. The subjects in the highest Lp-PLA2 quartile, having the greatest reductions in Lp-PLA2 after pravastatin treatment, had a 35% reduction in CHD death and MI (HR = 0.65) and a 30% reduction in total CVD events (HR = 0.70). Even after adjustment for all other factors, including baseline LDL-C and the extent of reduction attributed to LDL-C, the reduction in Lp-PLA2 was determined to account for 57% of the pravastatin treatment effect. By comparison, change in LDL-C levels accounted for less than half (43%) of the treatment effect.

In conclusion, the reduction of Lp-PLA2 with statin therapy, independent of baseline Lp-PLA2 levels, can help determine the effectiveness of statin therapy and predict the reduction in CVD events. Tracking the reduction in Lp-PLA2 and LDL-C in response to therapy is a better indicator of future CVD events than the reduction of LDL-C levels alone. In fact, the reduction of Lp-PLA2 accounted for more than half of the pravastatin treatment effect.

65. Iwase M, Sonoki K, Sasaki N, Ohdo S, Higuchi S, Hattori H, Iida M. Lysophosphatidylcholine Contents in Plasma LDL in Patients with Type 2 Diabetes Mellitus: Relation with Lp-PLA₂ and Effects of Simvastatin Treatment. *Atherosclerosis*. 2008; 196(2): 931-6.

    Lysophosphatidylcholine (lysoPC) content of LDL particles was found to be higher in diabetics than in healthy matched controls. It was also higher in those patients with retinopathy and nephropathy. LysoPC content was shown to be highly correlated with Lp-PLA₂ content (r = 0.56, p <0.0001). Simvastatin reduced LDL associated lysoPC and LDL associated Lp-PLA₂. The authors suggested that Lp-PLA₂ has proatherogenic activity mediated by production of lysoPC.

66. Robins SJ, Collins D,   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(6): 1172-8.

    In this large study of 1,451 men with low LDL-C (110 mg/dL) and low HDL-C (32 mg/dL), an increase in Lp-PLA₂ activity (measured at baseline and six months on gemfibrozil treatment or placebo) also predicted an increase in CV events (320 cases of MI, stroke or CV death) in fully adjusted models over a tertile range (1.85 HR, 95% CI 1.38 to 2.50, P <0.0001, comparing tertile 3 to 1). Furthermore, an increase in Lp-PLA₂ coupled with either highest levels of LDL-C or lowest levels of HDL-C was related to the highest rates of CV events. Finally gemfibrozil modestly lowered Lp-PLA₂ and this reduction was significantly associated with 23% and 30% relative risk reductions for CV events in quartile, 3 and 4 of higher Lp-PLA₂, respectively, but non-significant reductions in the patients in the bottom 2 quartile of Lp-PLA₂.

Therefore this is the first treatment outcome study where elevation in Lp-PLA₂ is associated with significant event reductions with a fibrate.

67. Rosenson RS. Fenofibrate Reduces Lipoprotein Associated Phospholipase A₂ Mass and Oxidative Lipids in Hypertriglyceridemic Subjects with the Metabolic Syndrome. *Am Heart J.* 2008; 155(3): 499.e9-16.

    In 55 non-diabetic patients with metabolic syndrome, fenofibrate for 3 months reduced Lp-PLA₂ mass concentration by 13.2% and oxidized fatty acids (oxFA) by 15.5%. This reduction in Lp-PLA₂ is smaller than in the DIACOR (Muhlestein et al) study but baseline Lp-PLA₂ levels were much lower in this study. Adjustment for LDL-C or LDL-P did not affect the results, but the reduction in small LDL-P particles was significantly associated with the reduction in Lp-PLA₂, suggesting that fenofibrate may lower Lp-PLA₂ via plaque stabilization mediated by lowering small LDL-P.

68. Schmidt EB, Koenig W, Khuseyinova N, Christensen JH. Lipoprotein-Associated Phospholipase A₂ Concentrations in Plasma are Associated with the Extent of Coronary Artery Disease and Correlate to Adipose Tissue Levels of Marine n-3 Fatty Acids. *Atherosclerosis.* 2008; 196: 420–4.

    Lp-PLA₂ was measured in patients (n = 301) admitted to elective coronary angiography because of suspected coronary artery disease (CAD). In a multiple linear regression analysis, the degree of CAD (0-, 1-, 2- or 3-vessel disease) and plasma LDL cholesterol significantly correlated to Lp-PLA₂ levels. Also the content of the marine n-3 fatty acid, eicosapentaenoic acid (EPA) in adipose tissue, a measure of long-term intake of seafood independently and inversely correlated with plasma levels of Lp-PLA₂(r =-0.18, p < 0.01). The results support that Lp-PLA₂ may relate to CAD and that intake of marine n-3 fatty acids might reduce plasma Lp-PLA₂ suggesting another mechanism by which n-3 fatty acids could reduce the risk of cardiovascular disease.

69. Saougos VG, Tambaki AP, Kalogirou M, Kostapanos M, Gazi IF, Wolfert RL, Elisaf M, Tselepis AD. Differential Effect of Hypolipidemic Drugs on Lipoprotein-Associated Phospholipase A₂. *Arterioscler Thromb Vasc Biol.* 2007; 27: 2236-43.

    This is the first study to demonstrate that ezetimibe and rosuvastatin both lower Lp-PLA₂ mass. Statin intolerant Type IIa dyslipidemics had an 18% reduction in Lp-PLA₂ mass with ezetimibe 10 mg/day, and Type IIa dyslipidemics had a 29% reduction in Lp-PLA₂ mass with rosuvastatin 10 mg/day. It also showed that fenofibrate 200 mg/day lowered Lp-PLA₂ mass 32%, a finding similar to fenofibrate's effect on Lp-PLA₂ mass in Type 2 DM.

THERAPEUTIC MODULATION STUDIES

70. O'Donoghue M, Morrow DA, Sabatine MS, et al. Lipoprotein-Associated Phospholipase A$_2$ and Its Association With Cardiovascular Outcomes in Patients With Acute Coronary Syndromes in the PROVE IT-TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction) Trial. *Circulation*. 2006; 113: 1745-52.

In this study of ACS patients with aggressively treated LDL cholesterol, "Lp-PLA$_2$ activity is significantly lowered with high-dose statin therapy and is associated with an increased risk of CV events independent of C-reactive protein and LDL cholesterol levels." Thus, Lp-PLA$_2$ helped identify coronary atherosclerosis patients with very low LDL who still appeared to have unstable, rupture-prone plaque. The intensive reduction in LDL cholesterol only explained 25% of the Lp-PLA$_2$ reduction, suggesting a pleiotropic benefit from atorvastatin therapy.

71. Kuvin JT, Dave DM, Sliney KA, Mooney P, Patel AR, Kimmelstiel 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: 743-5.

This study evaluated the effect on Lp-PLA$_2$ of adding niacin to stable coronary heart disease patients with well-managed baseline LDL levels of 76 mg/dL. While there was no significant change in baseline LDL levels after three months, niacin significantly lowered Lp-PLA$_2$ by 20%. It appears from this study that Lp-PLA$_2$ lowering was independent of LDL (which did not change) and that there appears to be residual opportunity to lower Lp-PLA$_2$ in patients with low achieved LDL cholesterol, consistent with the concept that low achieved LDL alone may not assure that plaque has stabilized.

72. 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.

This study evaluated the effect of simvastatin 20 mg and fenofibrate 160 mg on Lp-PLA2 and CRP in type 2 diabetic patients with mixed dyslipidemia. Fenofibrate, simvastatin and the combination each lowered Lp-PLA2, and the effect was greatest among patients with baseline levels greater than the median. In this study, lipid-modifying agents lowered Lp-PLA2 by more than 25% (fenofibrate: 27%; simvastatin: 35%).

73. Albert MA, Glynn RJ, Wolfert RL, Ridker PM. The Effect of Statin Therapy on Lipoprotein Associated Phospholipase A$_2$ Levels. *Atherosclerosis*. 2005; 182: 193-8.

The researchers evaluated the effect of pravastatin 40 mg daily vs. placebo on Lp-PLA2 levels in a cardiovascular disease free population derived from the PRINCE trial. After 12 weeks, Lp-PLA2 levels decreased by 22.1% among treated patients (vs. 7.8% among placebo group). Only 6% of the lowering of Lp-PLA2 by pravastatin could be accounted for by the lowering of LDL-C.

74. 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: 1025-32.

The impact of various statins at the 40 mg/day dosage on Lp-PLA2 was compared. The study found that "atorvastatin is more effective than fluvastatin, lovastatin, pravastatin, or simvastatin for decreasing not only low density lipoprotein cholesterol but also hs-CRP and Lp-PLA2".

75. Winkler K, Abletshauser C, Friedrich I, Hoffman 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: 1153-9.

This placebo-controlled study assessed the ability of fluvastatin XL 80 mg to lower lipid parameters and Lp-PLA2 activity in patients with type II diabetes. This is also the first study to evaluate Lp-PLA2's association with coronary artery disease risk in a diabetic population. After adjustment for all traditional risk factors, and also adjusting for apoB in small/dense LDL and non-HDL cholesterol, Lp-PLA2 remained significantly associated with a doubling of risk for CAD. After eight weeks, fluvastatin XL lowered Lp-PLA2 activity by 23%. "Due to its lowering of Lp-PLA2 activity, the antiatherogenic potential of fluvastatin in type II diabetics may exceed its effects on LDL-C and triglycerides alone."

EMERGING INHIBITORS

76. Karakas M, Koenig W. Review: Lp-PLA$_2$ Inhibition - The Atherosclerosis Panacea? *Pharmaceuticals* 2010; 3(5): 1360-73.

This review summarizes the evidence surrounding how the biomarker Lp-PLA$_2$ in particular has gained considerable interest recently, in the context of there being a large number of biomarkers that relate to lipids, inflammation, immunity, thrombosis and hemostasis that have been investigated experimentally, in epidemiologic studies and in clinical trials. Based on the complex pathophysiology of atherosclerosis, such interest focuses on the potential role to aid in risk stratification, as possible surrogate markers of atherosclerosis, and potential targets for therapy. In addition to a plausible pathophysiological role by generating pro-inflammatory and pro-atherogenic compounds from oxidized LDL in the vessel wall, there is a large, fairly consistent epidemiological database indicating that increased levels of Lp-PLA$_2$ mass or activity are associated with increased risk for cardiovascular outcomes and that such data improves risk stratification. In addition, clinical studies indicate that increased Lp-PLA$_2$ levels are associated with endothelial dysfunction. Furthermore, it may

EMERGING INHIBITORS

Lp-PLA$_2$ Selected Annotated Bibliography

also serve as a therapeutic target, since a specific inhibitor of the enzyme is available with initial positive data. The authors also review the recent experimental data from: 1) a pig model strongly suggesting that increased Lp-PLA$_2$ in the vessel wall is associated with a more vulnerable plaque phenotype which can be modulated by inhibiting Lp-PLA$_2$ activity; 2) a biomarker study in more than 1,000 patients with CHD over three months demonstrating a positive effect on various inflammatory molecules; and 3) an imaging study in more than 300 patients with CHD treated over 12 months using IVUS based modalities (greyscale, virtual histology, and palpography) together with a panel of biomarkers (IBIS-2 Study), indicating that the progression of the necrotic core of the plaque can be retarded. Inhibition of the pro-atherogenic and pro-inflammatory effects of Lp-PLA$_2$ may therefore contribute to decreasing the residual risk in high risk patients already on polypharmacotherapy. This hypothesis is now being tested in two large phase 3 clinical trials (STABILITY and SOLID). The authors therefore conclude that Lp-PLA$_2$ indeed represents a biomarker and promising target for intervention.

77. Braun LT, Davidson MH. Lp-PLA$_2$: A new target for statin therapy. *Curr Atheroscler Rep.* 2010; 12(1): 29-33.

This review summarizes the important role inflammation plays in atherogenesis and plaque vulnerability. Inflammatory-type markers have been evaluated for their association with atherosclerotic vascular disease and their ability to improve cardiovascular disease (CVD) risk stratification. In light that Lp-PLA$_2$ is a vascular-specific inflammatory enzyme that increases the risk of CVD events and stroke approximately twofold, a consensus panel recently recommended the measurement of Lp-PLA$_2$ in moderate-risk and high-risk patients for improved risk stratification and modification of low-density lipoprotein target levels. Lipid lowering agents, particularly statins, lower Lp-PLA$_2$ mass and activity; therefore, Lp-PLA$_2$ may represent an important target of lipid-lowering therapy for reducing the inflammatory nature of atherosclerosis and plaque vulnerability. A large morbidity and mortality trial was recently initiated to evaluate the long-term safety and efficacy of darapladib, an Lp-PLA$_2$ inhibitor, in patients with high-risk coronary heart disease.

78. Bui QT, Wilensky RL. Darapladib. *Expert Opin Investig Drugs.* 2010; 19(1): 161-8.

The authors provide an overview of the selective Lp-PLA$_2$ inhibitor darapladib by reviewing the studies (1990-2009) that have provided the rationale for its development, with discussion of its potential merit as a new therapeutic drug to target high-risk atherosclerosis. Darapladib represents a new class of therapeutic agents that target cardiovascular inflammation. In discussing the role of selective inhibitors of Lp-PLA$_2$ as new therapeutic agents, they review the importance of inflammation during atherogenesis as well as of the biology of Lp-PLA$_2$ and its proatherogenic role.

79. McCullough PA. Darapladib and Atherosclerotic Plaque: Should Lipoprotein-Associated Phospholipase A$_2$ be a Therapeutic Target? *Curr Atheroscler Rep.* 2009; 11(5): 334-7.

The author reviews the evidence that the orally available, specific inhibitor of Lp-PLA$_2$ activity, darapladib, holds promise as an anti-atherosclerotic therapy. Darapladib has been shown to reduce lysophosphatidylcholine content and expression of multiple genes associated with macrophage and T-lymphocyte functioning, causing considerable decrease in plaque and necrotic core area. The discussion addresses the great interest in developing a reliable measure of atherosclerotic disease activity that can serve as an index of response to anti-atherosclerotic therapies. Because Lp-PLA$_2$ is part of a family of lipases involved in the modification of lipids within the atheroma and may be a complementary therapeutic target to the reduction of LDL-C in patients with advanced atherosclerosis, darapladib is posited to hold the hope of being a bona fide anti-atherosclerotic therapy that can be gauged through blood measurement of Lp-PLA$_2$ activity.

80. Mohler ER, Ballantyne CM, Davidson MH, et al. The Effect of Darapladib on Plasma Lipoprotein-Associated Phospholipase A$_2$ Activity and Cardiovascular Biomarkers in Patients with Stable Coronary Heart Disease or Coronary Heart Disease Risk Equivalent: the Results of a Multicenter, Randomized, Double-Blind, Placebo-controlled Study. *Journal of the American College of Cardiology*, 2008; 17: 1632-41.

This study was a multi-centre, 12 week treatment study examining the use of various doses of the Lp-PLA$_2$ inhibitor darapladib, also known as (SB-480848) on sustained inhibition of Lp-PLA$_2$ activity as well as changes in selective cardiovascular biomarker levels among high CHD risk persons concomitantly treated with either 20 mg or 80 mg of atorvastatin. Baseline (prior to inhibitor treatment) observational findings from this study concluded that activity levels of Lp-PLA$_2$ were greater among men compared to women; and that the level of Lp-PLA$_2$ activity measured paralleled the CHD risk profile of the individual (eg. higher levels were witnessed among those with vascular disease and among individuals with elevated traditional risk factor levels such as age, high LDL-C and low HDL-C concentrations). Results from the dose ranging study showed no changes in Lp-PLA$_2$ activity among those given placebo; however, a sustained dose response effect of Lp-PLA$_2$ activity inhibition was seen among persons randomly assigned to either the 40 mg (43%), 80 mg (55%) or 160 mg (66%) respectively. There was no significant interaction between the dose of statin administered and the level of Lp-PLA$_2$ inhibition. Finally, darapladib 160 mg produced a significant reduction in hs-CRP (13%) and in IL-6 (12%) compared to placebo without any significant safety issues.

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To view the *Lp-PLA$_2$ Expanded Annotated Bibliography*, please visit **www.plactest.com/AB**

81. Wilensky RL, Shi Y, Mohler ER, Hamamdzic, D, et al. Inhibition of Lipoprotein-Associated Phospholipase A$_2$ Reduces Complex Coronary Atherosclerotic Plaque Development. *Nature Medicine*. 2008; 14:1059-66.

This study was designed to examine whether a causal relationship exists between Lp-PLA$_2$ and coronary atherosclerosis. Because Lp-PLA$_2$ is produced predominantly by macrophages within the necrotic core and fibrous cap of vulnerable and ruptured plaques, it is postulated Lp-PLA$_2$ may have a direct role in establishing plaque instability. To test the hypothesis, diabetes and hypercholesterolemia induced swine were randomly assigned to either control or 28 weeks of treatment with the Lp-PLA$_2$ inhibitor darapladib, also known as (SB-480848). This study specifically examined the influence of Lp-PLA$_2$ inhibition on gene expression associated with vascular inflammation and coronary lesion development. Swine serve as good subjects for this type of evaluation based upon the fact that they possess a plasma lipoprotein profile similar to humans (which Lp-PLA$_2$ associates with) and these animals develop coronary lesions similar to that observed in humans. Wilinsky and colleagues concluded that when compared to controls, the inhibition of Lp-PLA$_2$ activity resulted in an anti-inflammatory effect; thus, reducing the expression of a number of genes associated with atherosclerosis and an improvement in the phenotypic coronary lesion.

82. Serruys PW, Garcia-Garcia HM, Buszman P, Erne P, Verheye S, Aschermann M., et al. Effects of the Direct Lipoprotein-Associated Phospholipase A$_2$ Inhibitor Darapladib on Human Coronary Atherosclerotic Plaque. *Circulation*. 2008; 118: 1172-82

This study compared the effects of 12 months of treatment with darapladib (an oral Lp-PLA$_2$ inhibitor) 160 mg daily or placebo on coronary deformability (as measured by intravascular ultrasound palpography), changes in necrotic core size (measured by intravascular ultrasound radiofrequency), atheroma size (measured by intravascular ultrasound gray scale) and plasma hs-CRP in 330 patients with angiographically documented coronary disease. Lp-PLA$_2$ activity was inhibited by 59% with darapladib (p<0.001 versus placebo). Other biomarkers of inflammation (i.e. interleukin-6, myeloperoxidase, intracellular adhesion molecule-1, oxidized phospholipid, apolipoprotein B, and matrix metalloproteinase-9 activity) did not change significantly. In the placebo-treated group, necrotic core volume increased significantly (4.5+17.9 mm3; p=0.009), whereas in the darapladib group, necrotic core volume was essentially halted (-0.5+13.9 mm3; p=0.71) resulting in a significant treatment difference of -5.2 mm3 (p=0.012). These intra-plaque compositional changes occurred despite a non-significant treatment difference in total atheroma volume, plaque deformability or plasma hs-CRP. Background therapy was comparable between groups, with no difference in LDL-C (placebo, 88 mg/dL; darapladib 84 mg/dL).

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