

Effects of lifestyle counseling and combination lipid-modifying therapy on lipoprotein-associated phospholipase A2 mass concentration

Kota J. Reddy, MD, Manmeet Singh, MD*, Richard R. Batsell, PhD
Joey R. Bangit, MD, Rekha A. Miraskar, MD, Misbah S. Zaheer, MD
Carol Cockerham, MS, Michael Wegner, PhD

Q1 Reddy Cardiac Wellness, Cardiology, 3519 Town Center BLVD, Suite A, Sugar Land, TX, USA

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BACKGROUND: Lipoprotein-associated phospholipase A2 (Lp-PLA₂) is a novel inflammatory biomarker that is associated with increased cardiovascular disease risk independent of and additive to traditional risk factors. Lp-PLA₂ activity is correlated with the degree of inflammation in the atherosclerotic plaque. In human blood, approximately 80% of Lp-PLA₂ is associated with low-density lipoproteins (LDL). Thus, it is hypothesized that changes in Lp-PLA₂ should imitate the changes in the LDL cholesterol.

OBJECTIVE: In this present study, we examined the efficacy of lifestyle intervention and combination lipid-lowering therapy on reducing the Lp-PLA₂ levels and determined the relationship between changes in LDL-C and Lp-PLA₂.

RESULTS: The study revealed a 32.5% reduction in mean Lp-PLA₂ values (baseline 181.1 ± 41.5 vs 122.1 ± 28.1 ng/mL after treatment; $P < .001$). The change observed in LDL-C was 41%, (baseline 126.2 ± 43 vs 73.9 ± 37.7 mg/dL after treatment), which also was significant ($P < .001$). However, a Pearson correlation test analysis revealed only a weak positive association between changes in Lp-PLA₂ and LDL-C ($r^2 = 0.052$, $P < .001$).

CONCLUSION: Lp-PLA₂ is reduced with the use of life style counseling and combination lipid lowering therapy. Results also revealed that changes in Lp-PLA₂ may be partially explained by the changes in LDL-C.

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Introduction

Cardiovascular disease is the leading cause of death in the United States as well as other industrialized and western nations.¹⁻⁴ Substantial national efforts have been focused on reducing the morbidity and mortality associated with

cardiovascular disease. Available evidence suggests that traditional risk factors such as high total cholesterol, low-density lipoprotein cholesterol (LDL-C), smoking, hypertension, and diabetes are good predictors for cardiovascular disease events. Despite this, these major cardiovascular risk factors account for only approximately half of the variability in coronary heart disease risk in the U.S. population.⁵ A study⁶ reported that approximately 62.4% of individuals Q3 already diagnosed with coronary artery disease (CAD) present with only 0 to 1 of traditional risk factors. Nineteen percent present with absolute no risk factors, and another

* Corresponding author.

E-mail address: manmeetsingh79@yahoo.com

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43% have only 1 risk factor⁶. This finding suggest that a significant number of cardiovascular events can still take place among individuals who are estimated to be at low to moderate risk by the use of traditional risk factors.

Lipoprotein-associated phospholipase A2 (Lp-PLA₂) mass is a novel inflammatory biomarker that independently predicts cardiovascular risk and events.⁷ Moreover, Lp-PLA₂ is a biomarker that is believed to be directly involved in the formation of rupture prone unstable plaques.⁸ Lp-PLA₂, also known as platelet-activating factor acetyl hydrolase, is a 50-kDa Ca-independent phospholipase that is distinct from another macrophage product, secretory PLA₂, a 14-kDa Ca-dependent enzyme.⁹ Lp-PLA₂ is highly vascular specific in terms that it is expressed in atherosclerotic plaques,⁹ in macrophages within the fibrous cap of human rupture prone lesions,¹⁰ and is not affected by any systemic inflammatory diseases. In the blood plasma, approximately 80% usually circulates bound to LDL particles. Thus, it is hypothesized that changes in Lp-PLA₂ should mirror changes in levels of LDL-C.

Lp-PLA₂ is the only plasma enzyme responsible for the hydrolysis of oxidized phospholipid, resulting in the production of lysophosphatidylcholine and the products of oxidized fatty acids.¹¹ The proinflammatory and atherogenic properties of lysophosphatidylcholine are well known.¹² Among various agents used to treat patients with cardiovascular disease, only the drugs that affect lipid metabolism can significantly influence plasma Lp-PLA₂.^{13,14} Thus, several statins as well as nonstatin lipid-lowering drugs can reduce the Lp-PLA₂ plasma concentration in parallel with a reduction in LDL-C levels.¹⁵⁻¹⁹

Although published data exist in which the authors examine the influence of these pharmacologic interventions on reducing Lp-PLA₂ levels, to our knowledge, no studies have examined the effect of multiple drug therapy and lifestyle interventions on changes in Lp-PLA₂ mass in a clinical practice setting. The primary purpose of the present study was to assess the ability of lifestyle and combination lipid drug therapy to reduce levels of Lp-PLA₂ among patients treated for mixed dyslipidemia. The secondary purpose was to examine the relationship between changes observed in Lp-PLA₂ and LDL-C.

Methods

All patients included this study were being treated in a clinical practice setting for their mixed dyslipidemia. Two hundred forty-eight patients (58% men and 42% women) who completed the lifestyle intervention in combination with pharmacologic therapy for an average period of 10.5 months were included in this retrospective chart review study (Table 1). The mean baseline body mass index of 248 patients was 27.7 ± 12 kg/m². The mean age among the group was 59.2 years, 43.6% had stable, angiographically established CAD, 20.6% had type II diabetes mellitus, and 50% were classified as having the metabolic syndrome based on Adult

Table 1 Descriptive characteristics of patients (n = 248)

Parameters	Mean	Standard deviation	Range
Age (years)	59.2	12.1	29-90
Height (in)	66.9	4.2	55-76
Weight (lbs)	179.3	44.5	95-380
BMI (kg/m ²)	27.1	5.1	16-46
Total cholesterol (mg/dL)	210.2	49.1	99-360
LDL-C (mg/dL)	126.8	43.0	32-277
HDL-C (mg/dL)	53.5	15.0	12-103
Triglyceridies (mg/dL)	151.4	109.3	31-750
Apo B (mg/dL)	106.5	30.4	44-196
Apo A1 (mg/dL)	150.3	29.6	79-243
Lp-PLA ₂ (ng/mL)	181.1	41.5	68-359

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; Apo, apolipoprotein; Lp-PLA₂, lipoprotein-associated phospholipaseA₂.

Treatment Panel (ATPIII) criteria. Twelve percent of patients had a history of musculoskeletal inflammatory disease (osteoarthritis), and 18% had received previous lipid-altering therapy. Lifestyle modification included diet and exercise counseling. Combination therapy included omega 3 fish oil (2000 mg/d), extended-release niacin (500 – 1000 mg/d), ezetimibe (10 mg/d), fenofibrate 160 mg/d, and colesvelam HCl (1850 mg/d), as well as statins. The statins used were either simvastatin (20 – 40 mg/d) or rosuvastatin (5 – 20 mg/d). Sixty five percent (n = 161) received low-to-medium doses of simvastatin, whereas 35% (n = 87) received low-to-medium doses of rosuvastatin (Fig. 1).

Measurements and statistical procedures

Lp-PLA₂ mass measurement was determined by a Food and Drug Administration-cleared ELISA assay (PLAC test, diaDexus, Inc., South San Francisco, CA). The PLAC test is based on the principle of a sandwich enzyme immunoassay that uses two specific IgG monoclonal antibodies (2C10 and 4B4) described by Caslake.²⁰ The blood samples were processed to serum for testing. The assay system uses monoclonal anti-Lp-PLA₂ antibody (2C10) directed against Lp-PLA₂ for solid-phase immobilization on the microwell strips. The plasma sample is added to the plate and incubated for 10 minutes at 20–26 °C. A second monoclonal anti-Lp-PLA₂ antibody (4B4) labeled with the enzyme horseradish peroxidase is then added and reacted with the immobilized antigen at 20–26 °C for 180 minutes, resulting in the Lp-PLA₂ molecules being captured between the solid phase and the enzyme-labeled antibodies. The wells are washed with a supplied buffer to remove any unbound antigen. The substrate, tetramethylbenzidine, is then added and incubated at 20–26 °C for 20 minutes, resulting in the development of a blue color. Color development is stopped with the addition of Stop Solution, changing the color to yellow. The absorbance of the enzymatic turnover of the

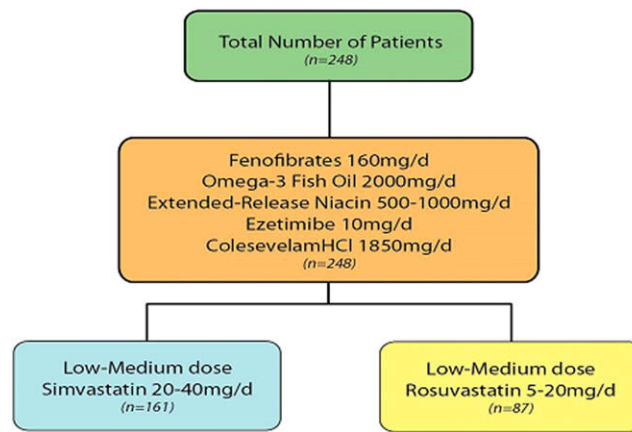


Figure 1 Schematic presentation of combination lipid therapy.

substrate is determined spectrophotometrically at 450 nm and is directly proportional to the concentration of Lp-PLA₂ present. A set of Lp-PLA₂ calibrators are used to plot a standard curve of absorbance vs Lp-PLA₂ concentration from which the Lp-PLA₂ concentration in the test sample can be determined.

The LDL-C was calculated by use of the Friedewald equation from measurements of overnight fasting blood samples. In addition to this, blood samples from all 248 patients were assessed for total cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein B, Apolipoprotein A1, high sensitivity C-reactive protein and were sent to Berkeley Heart Lab for assays of LDL particle size. Pearson Correlation test analysis procedures were used to evaluate the relationship between changes in Lp-PLA₂ and changes in LDL-C. Paired *t*-tests (2-tailed) were used to determine the significance of changes observed for the other measured parameters compared to the baseline.

Lifestyle counseling

As part of their treatment plan, all patients received lifestyle counseling during which each patient participated in an individual session with a registered dietitian. Counseling sessions included a review of their blood test results and an explanation of how lifestyle management along with medications would improve outcomes. Specifically, patients were instructed in the use of a heart healthy diet, low in saturated fat (6% of total calories) and carbohydrates (20% of total calories including all carbohydrates and simple sugars). The daily caloric intake was limited between 1500 and 1800 calories per day. The estimated target for daily calories coming from total fat (unsaturated fat) and proteins was 45% and 25%, respectively, and was determined through diet information provided by patients. The dietitian developed an individual meal plan for each patient based on their personal preferences and lifestyle. During follow-up sessions, the meal plan was reviewed and adjusted to meet the needs of the patient, including a discussion of quality and quantity of food choices, regional

food preferences, and calories in vs calories out. The anticipated calculation of calories in vs calories out was based on each patient's daily routine activity; quantity and quality of food; and duration and type of daily exercise. Daily calorie consumption, percentage intake of saturated fats and carbohydrates were estimated from patient interviews. Patients were provided diet sheets and, based on the self-recorded intake of foods, average daily percentage intake for total fat (saturated vs unsaturated), carbohydrate, and protein was calculated. In addition, the dietitian emphasized the importance of exercise to enhance heart health and encouraged the patients to implement a brisk walking program for up to 30 minutes 5 days per week.

Results

The study revealed a 32.5% reduction in mean Lp-PLA₂ values (baseline 181.1 ± 41.5 vs after treatment 122.1 ± 28.1 ng/mL; $P < .001$). The change observed in LDL-C was 41%, (baseline 126.2 ± 43 vs 73.9 ± 37.7 mg/dL after treatment), which also was statistically significant ($P < .001$). However, a Pearson correlation test analysis revealed only a weak positive association between changes in Lp-PLA₂ and LDL-C ($r^2 = 0.052$, $P < .001$). Thus, suggesting that only 5.2% change in Lp-PLA₂ is explained by the change in LDL-C. A paired sample *t*-test was run on every lipid parameter to determine the significance of changes observed compared with the baseline measurements. The effect of treatment on measured parameters is shown in Table 2. Each measured parameter was significantly different from the baseline measurement except the high-density lipoprotein cholesterol and Apo A1 (Tables 1 and 2).

Discussion

The present study demonstrates that combination therapy comprising low-to-moderate doses of statins (simvastatin or rosuvastatin) and nonstatin lipid-lowering medications (ezetimibe, niacin, omega-3 fish oil, colesvelam, and fenofibrate)

Table 2 Effect of treatment on measured variables

Parameters	Pretreatment, mean ± SD	Posttreatment, mean ± SD	Percent change	P-value
Weight (lbs)	179.6 ± 44.5	177.0 ± 44.6	-1.5	<.00
BMI (kg/m ²)	27.7 ± 5.2	27.4 ± 5.2	-1.1	<.00
Total cholesterol (mg/dL)	210.2 ± 49.1	149.3 ± 44.6	-29.0	<.00
LDL-C (mg/dL)	126.8 ± 43.0	73.9 ± 37.8	-41.7	<.00
HDL-C (mg/dL)	53.5 ± 15.0	54.5 ± 17.0	1.8	.248
Triglycerides (mg/dL)	151.4 ± 109.3	106.7 ± 55.7	-29.5	<.00
Apo B (mg/dL)	106.5 ± 30.3	69.3 ± 28.5	-34.9	<.00
Apo A1 (mg/dL)	150.1 ± 29.3	149.8 ± 33.9	-0.02	.623
Lp-PLA ₂ (ng/mL)	181.1 ± 41.5	122.1 ± 28.1	-32.5	<.00
hs-CRP (mg/dL)	4.0 ± 6.9	2.1 ± 3.1	-47.5	<.00

hs-CRP, high-sensitivity c-reactive protein; other abbreviations as in Table 1.

along with lifestyle counseling can reduce the Lp-PLA₂ mass concentration. In this study, the reduction was 32.5%. This finding was expected based upon a number of well-controlled research studies that have demonstrated the ability of lipid-altering drug therapy to significantly reduce levels of Lp-PLA₂ mass.^{21,22} However, to our knowledge, the present study is the first to demonstrate Lp-PLA₂ reductions with drug therapy and lifestyle intervention in a clinical practice setting. These findings are important in that it substantiates the external validity of the previously published literature.

Another important finding from the present study is that changes in Lp-PLA₂ levels were only moderately correlated with changes observed in LDL-C levels ($r^2 = 0.052$, $P < .001$). It is possible that a stronger correlation would have been observed if we had measured and reported the association between changes in Lp-PLA₂ and the LDL lipoprotein subfractions; in particular the smaller, more dense LDL lipoproteins with which Lp-PLA₂ has been reported to be more closely associated.²³ However, given this study design limitation, our study does provide sufficient evidence to conclude that practitioners cannot rely on a simple examination of LDL-C changes to estimate changes in levels of Lp-PLA₂.

Lp-PLA₂ is an inflammatory biomarker that has been implicated in the formation of unstable rupture prone plaque and is predictive of the atherosclerotic disease activity. Statins and nonstatin lipid-lowering medications have been shown to stabilize the plaque and in some cases produce regression of the lipid core.^{24,25} In addition, these medications have been shown to decrease macrophage infiltration and vascular inflammation as well as increase thickening of fibrous caps in both coronary plaques with statins and carotid plaques with lipid-modifying combination drug therapy.^{24,25} Pravastatin, atorvastatin, lovastatin, simvastatin, or rosuvastatin all lower the Lp-PLA₂ mass concentration,²⁶⁻²⁸ which may be attributed to statin-induced reduction in the plasma concentration of all LDL subfractions.²¹ This finding suggests that Lp-PLA₂ reduction by statins appears to be a result of receptor-mediated removal of LDL particles. Moreover, it has been suggested that statins can reduce Lp-PLA₂ through a receptor-independent clearance of the lipid and enzyme contents of LDL.²⁹ This mechanism could possibly explain the weak

correlation between changes in Lp-PLA₂ and LDL-C observed in the present study. Furthermore, medications such as fenofibrates and omega-3 fatty acids also lower the Lp-PLA₂, although they may not change the LDL-C levels.^{27,30} Thus, the reduction in vascular disease events with several drug classes may be attributable to the reduction in Lp-PLA₂ as well as that of LDL-C.

The degree of reduction achieved in Lp-PLA₂, even with the intensive statin therapy, has been modest. In the Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) trial, intensive therapy with 80 mg/d of atorvastatin compared with 40 mg/day of pravastatin, only a 25% mean reduction in Lp-PLA₂ was observed.^{31,32} However, available literature suggests that combining a statin with a nonstatin lipid-lowering medications may achieve additional reduction in Lp-PLA₂ mass concentration. Kuvini et al²² added 1 g/day of extended-release niacin to the statin-containing regimens of patients with stable CAD and reported a significant 20% further reduction in Lp-PLA₂ after 3 months of therapy. Similarly, in patients with hypertriglyceridemia (200–499 mg/dL) who have achieved a mean LDL-C concentration of 80 mg/dL on stable simvastatin 40 mg/day for 2 months, the addition of prescription omega-3 fatty acids resulted in a significant reduction in Lp-PLA₂ mass concentrations.²² The COMBination of prescription Omega-3 with Simvastati (COMBOS) trial, in which authors assessed the effect on lipid parameters of therapeutic doses of omega-3 fatty acids when added to stable statin therapy, found that a significant additional reduction of 10.7% occurred in the level of Lp-PLA₂.³³ Omega-3 fatty acids appear to decrease overall vascular inflammation, with an associated decrease in proinflammatory cytokines such as interleukin-6, tissue necrosis factor- α , and eventually decreases the levels of Lp-PLA₂.³⁴⁻³⁶ Similarly, additional 20% reduction of Lp-PLA₂ on statin plus niacin therapy may reflect an anti-inflammatory action of niacin that helps in stabilizing plaques.^{24,37}

Ezetimibe is another lipid-lowering drug that has been found to lower the Lp-PLA₂ mass concentration. In a recent study involving type IIa or type Iva, in dyslipidemic statin-intolerant patients Lp-PLA₂ mass concentration was lowered significantly by 18%.²¹ Similarly, in the same study

ezetimibe significantly decreased Lp-PLA₂ activity as well. The decrease in Lp-PLA₂ enzyme activity and mass was associated with all LDL subfractions, suggesting that ezetimibe reduces Lp-PLA₂ by removing LDL particles from plasma.²¹

Combining dietary counseling and advice regarding exercise produced significant weight loss in this study. These lifestyle changes combined with drug therapy may explain the reduction of Lp-PLA₂ by more than 30%. We found no reports of studies demonstrating the effects of low saturated fat, low-carbohydrate diets alone on Lp-PLA₂. However, considering that such dietary regimens reduce LDL-C particles and may reduce vascular inflammation, the dietary changes in this study may have played a major role in lowering Lp-PLA₂ mass concentration. The present study is limited by the fact that it is a retrospective study and does not have a control group. Moreover, we did not use tested and documented questionnaires in the measurement of calorie consumption and percentage intake of saturated fats and carbohydrates. The change in exercise was advised but the quantitative measures of energy expenditure were not used.

In summary, results from the present study indicate that Lp-PLA₂ is significantly reduced by combining lifestyle counseling and lipid-modifying therapy. The changes in Lp-PLA₂ were only weakly correlated with the changes observed in LDL-C.

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