

Reduction in Inflammatory Biomarkers by Combination Therapy and Diet: A Case Report

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Background: Atherosclerotic cardiovascular heart disease is the number one cause of morbidity and mortality in United States¹. In the last few years, atherosclerosis has become increasingly recognized as an inflammatory disease, and many new inflammatory biomarkers can now predict degree of inflammation in atherosclerotic plaques. Lipoprotein associated phospholipase A2 (Lp-PLA₂) along with high sensitive C-reactive protein (hs-CRP) are two new biomarkers that can identify the high risk individual requiring aggressive lipid lowering therapy independent of and additive to traditional risk factors²⁻⁵. Although, prevalence of elevated levels of both biomarkers increase the predictability of cardiovascular events, the high levels of Lp-PLA₂ is especially appealing in this respect as its highly vascular specific and directly correlates to the pathophysiology of atherosclerotic plaque.

Introduction: Cardiovascular heart disease (CHD) is the leading cause of death in most western nations and extensive national efforts are focused on reducing the morbidity and mortality associated with this disease. Traditional risk factors such as total and Low-density lipoprotein (LDL) cholesterol can predict the CHD risk. However, many epidemiological studies had reported that >50% of CHD events took place in population who were at low to moderate risk as per traditional risk factors. Therefore, indicating better and more effective identification of persons at high cardiovascular risk is needed. In this line of thinking, Lp-PLA₂ and hs-CRP are two new inflammatory biomarkers which have better risk predictability. More than 25 prospective epidemiological studies demonstrate the association of elevated Lp-PLA₂ with future coronary events and strokes. Interestingly, the elevated levels of both biomarkers provide even greater predictability for CHD events⁴. Moreover, ATP III recognized the modest efficacy of traditional risk factors in predicting CHD events, and now proposed that Lp-PLA₂ might be used in practice to refine CHD risk prediction. In present case report, we demonstrate a reduction in Lp-PLA₂ (36%), hs-CRP (98%) along with apolipoprotein B (28%) levels achieved on combination therapy (simvastatin 40mg, ezetimibe 10mg, colsevelam HCL 625mg 3 tab twice daily and omega-3 fatty acid 2000mg twice daily) in conjunction with low saturated fat, low carbohydrate diets.

Case Presentation: We report a case of a 78 year old Caucasian male with a BMI of 24, who came for a cardiac evaluation. His past medical history was significant for paroxysmal atrial fibrillation, hypercholesterolemia, coronary artery disease status post stent, essential hypertension, sick sinus syndrome status post pacemaker, and bleeding problems with coumadin for which he was admitted to the hospital. His review of system revealed a history of persistent fatigue, dyspnea on exertion and arthritis. He was on

amiodarone 200mg once daily for his fibrillations, lisinopril 20mg once a day for hypertension, lovastatin 40mg once a day for hypercholesteremia, metoprolol 50mg, nitroglycerine 30 mg, aspirin 81 mg once daily for coronary artery disease. He stopped taking Coumadin ever since he had INR of 12 and had complication staying on Coumadin.

On physical examination, he was conscious, alert, oriented and was not in any apparent distress. Blood pressure was 130/60 with a heart rate of 60 beats per minute and a respiration rate of 14. Neck examination revealed no elevated JVP, no bruit heard and no lymph nodes were palpable. Chest: clear to auscultation bilaterally. Heart: S1, S2, no S3, positive S4 with a 2/6 systolic murmur heard in the left lower sternal border. Extremities: no cyanosis, clubbing or edema with 2+ pulses in all four extremities.

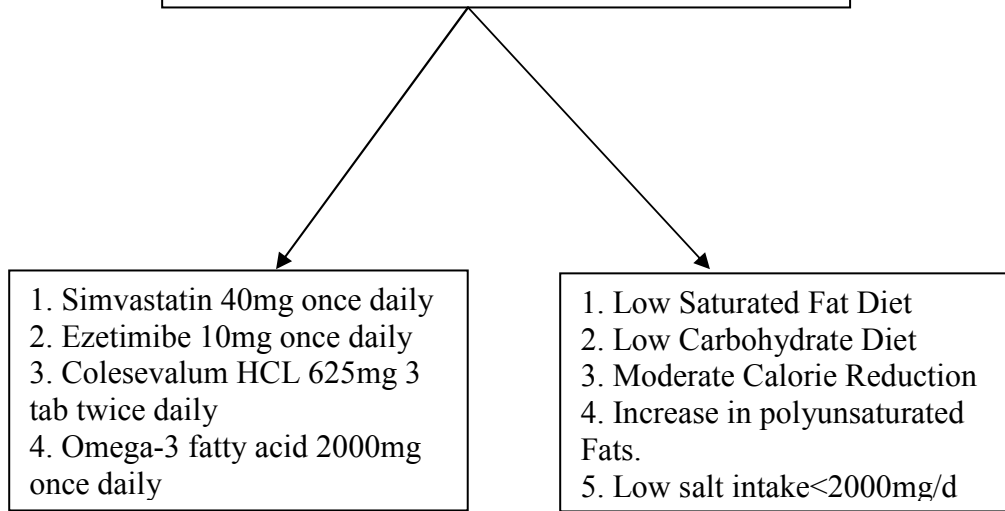
An EKG was done which revealed a normal sinus rhythm with right bundle branch block and left anterior fascicular block which were similar to the previous EKG's. A carotid IMT was done in past which showed a maximum IMT of 1.5. An Echo was also done which showed normal LV ejection fraction, mild tricuspid regurgitation with normal PA pressure.

Considering his status post stent coronary artery disease, hypercholesteremia, abnormal IMT, essential hypertension and strong family history for coronary artery disease, patient underwent Berkeley heart lab advanced lipid profile testing and a coronary calcium score assessment with a 64 slice CT machine. Interestingly, heart scan finding showed a total calcium score of 630 which places him in between 75th and 90th percentile, which correlates to a very high risk for cardiovascular heart disease for next several years. His advanced lipid profile (see tab 1) revealed results which were normal as per traditional risk factors. However, had elevated concentrations of Lp-PLA2 (245ng/ml), hs-CRP (>50mg/l), apolipoproteinB(74mg/dl), insulin(>21 µU/ml), NT-proBNP(1126pg/ml) and fasting glucose(111mg/dl). These elevated levels of non traditional risk factors along with calcium score of 630, places him in a very high risk category for cardiovascular heart disease. Consequently, we decided to stop his lovastatin monotherapy and started him on a combination therapy (fig 1) in conjunction with a low saturated fat low carbohydrate diets focusing on reducing daily calorie intake and total body weight.

Table 1. Baseline Advanced Lipid profile

<i>Lipid Parameters</i>	<i>Lipid values</i>
1. Total cholesterol (mg/dl)	136
2. LDL cholesterol (mg/dl)	59
3. HDL cholesterol (mg/dl)	58
4. Triglycerides (mg/dl)	96
5. ApoB (mg/dl)	74
6. Lp-PLA2 (ng/ml)	245
7. hs -CRP (mg/l)	50
8. Insulin	21
9. Glucose (mg/dl)	111
10. NT-proBNP(pg/ml)	1126

Fig 1. Composition of Combination Therapy and Diet



Follow at 4months

Follow up results at 4 months are shown in table 2. Remarkably, in addition to the further improvement noted in traditional risk factors, we also achieved marked reduction in L-pla₂ by 36%, hs-CRP by 98%, ApoB by 28%, insulin by 71%, NT-proBNP by 64% and glucose by 25% .

Table 2. Post Therapy Advanced Lipid profile

<i>Lipid Parameters</i>	<i>Lipid values</i>	<i>% Change</i>
1. Total cholesterol (mg/dl)	123	-9.5
2. LDL cholesterol (mg/dl)	50	-15.2
3. HDL cholesterol (mg/dl)	63	8.6
4. Triglycerides (mg/dl)	49	-49
5. ApoB (mg/dl)	53	-28.3
6. Lp-PLA ₂ (ng/ml)	156	-36.3
7. hs -CRP (mg/l)	0.8	-98.2
8. Insulin	6	-71
9. Glucose (mg/dl)	83	-25.2
10. NT-proBNP(pg/ml)	400	-64.4

Apo B = apolipoprotein B, LDL= low density lipoprotein, HDL= high-density lipoprotein

Discussion

The present case report demonstrates the superior efficacy of combination therapy along with dietary modifications over standard monotherapy, in lowering lipid levels, stabilizing the plaques and decreasing inflammation in the arteries. At the same time results from present case report also emphasize that the clinician should not be deceived by normal levels of traditional risk factors and should remain more proactive to identify the individuals who are at higher coronary risk on the basis of non traditional biomarkers. Most of the sudden cardiac deaths are secondary to massive clot formation rather than stenotic arteries. Consequently, aiming at determining atherosclerotic disease activity and shifting the present focus from identification of stenosis, which is focal disease, to identification of patients with inflamed and rupture prone plaque should be adopted. Thus, identifying the biomarkers which can really predict the formation of such massive clot could provide an edge over traditional risk factors in reducing cardiovascular heart disease morbidity and mortality.

Lp-PLA₂ is a biomarker which is released from an inflamed rupture prone atherosclerotic plaque and is highly vascular specific. Similar to hs-CRP, an elevated Lp-PLA₂ approximately doubles the risk for the first and recurrent cardiovascular events. Interestingly, when both inflammatory markers are elevated, they have an even greater predictability and several studies have illustrated this complementarity of Lp-PLA₂ and hs-CRP^{3, 4, 5}. However, hs-CRP levels can be affected by other systemic inflammatory diseases such as arthritis and is not specific for vascular inflammation. Therefore, Lp-PLA₂ may serve as a better measure of vascular inflammation and plaque quality. In this context, a study showed that Lp-PLA₂ is highly associated with coronary calcium scores but this association was inconsistent in a second study⁶⁻⁷. Moreover, in our recent presentation, we showed the prevalence of elevated levels of Lp-PLA₂ in individuals with low Framingham scores and coronary calcium scores less than 300⁸.

Lipid modifying therapy stabilizes the plaque, decrease macrophage infiltration; thicken the fibrous cap and eventually lowering the Lp-PLA₂ levels⁹⁻¹⁰. This has been shown in coronary plaque with statins, as well as with combination therapy in carotid plaques¹¹⁻¹². Parvastatin, fluvastatin, Atorvastatin, Simvastatin and Rosuvastatin all lowers levels of Lp-PLA₂. For instance in the Pravastatin or Atorvastatin and Infection Therapy (PROVE-IT) study⁹⁻¹⁰, even with intensive LDL cholesterol in the group taking Atorvastatin 80mg/day, the LDL cholesterol reduction only accounted for 25 % of Lp-PLA₂ reduction in Lp-PLA₂ mass concentration. At first look this change is not impressive as most Lp-PLA₂ is carried by LDL particles. However, only 1 in 500 apolipoprotein B containing particles carry Lp-PLA₂, therefore 100% correlation between reduction in LDL cholesterol (or LDL particles) and Lp-PLA₂ is not expected. It seems likely that statins lower Lp-PLA₂ because they stabilize plaques with subsequent decreased production of Lp-PLA₂. In fact fenofibrates and omega 3 fatty acids which do not change or increase LDL cholesterol, also lowers Lp-PLA₂ suggests stabilizing plaque reduces Lp-PLA₂¹³⁻¹⁴. Moreover, it highlights that Lp-PLA₂ reduction is only partially related to LDL reduction. In this context, lipid modifying therapies combining a statin

with niacin or a statin with omega -3 fatty acids significantly lower the Lp-PLA₂, even when the statin had already lowered LDL cholesterol to optimal levels. Kuvin et al¹⁵ added 1g/day of extended -release niacin preparation to statins in patients with stable coronary artery disease. After 3 months, a significant additional 20% reduction in Lp-PLA₂ was reported with niacin, although the baseline level of LDL cholesterol of patients receiving statin monotherapy was already in therapeutic range. These results indicate that there appeared to be additional opportunity to reduce Lp-PLA₂ when statins are used in combination with other lipid modifying drugs.

Combination therapy with diet in present case report resulted in more than 30% reduction in Lp-PLA₂ and >90% reduction in hs-CRP. In addition to the significant reduction in Lp-PLA₂ and hs -CRP, we also achieved marked reduction in ApoB, NT-proBNP, fasting insulin and glucose levels. These parameters are also considered as independent risk factors for cardiovascular events and studies had demonstrated that coronary risk increases several folds in the presence of elevated insulin, glucose and ApoB levels¹⁶⁻¹⁷. The decrease NT-proBNP on combination therapy with diet could be attributed to change in life style and low salt consumption. Safety of statins was demonstrated in sub analysis of Heart Protection Study (HPS),¹⁸ in which researchers found that Simvastatin resulted in high reduction in major cardiovascular events among the patients with high NT-proBNP. In fact numerous studies demonstrated that NT-proBNP may predict risk of cardiovascular morbidity and mortality in population without prevalent vascular disease¹⁷. Olsen et al¹⁹ showed in a population-based study that NT-proBNP is associated with cardiovascular risk independently of classical risk factors and other markers of cardiac function; the odd ratio was 1.56 (95% CI 1.33-1.83) per 1 SD increase in plasma levels of NT-proBNP. Additionally, in present case report the combination therapy with diet also achieved an absolute HDL cholesterol value (63mg/dl) more than LDL cholesterol value (50mg/dl), as well as HDL/ApoB ratio greater than one. Available literature recognizes that reaching a goal where HDL is more than LDL cholesterol and ApoB, will possibly reverse the cholesterol transport from arteries to the liver.

The dietary intervention still remains the cornerstone for the cardiovascular heart disease (CHD) primary prevention. It is now increasingly recognized that the consumption of total fat per se is less related to higher risk of coronary artery disease than previously thought. Low saturated fat, low carbohydrate diets with sparingly use of polyunsaturated fats is now becoming the primary dietary recommendation for prevention of CHD. Even with studies using combination therapy the degree of reduction achieved in Lp-PLA₂ was not profound. The significant additional reduction in Lp-PLA₂ accomplished in present study could be partly attributed to the life style intervention. Although, no studies have been done illustrating the effects of low saturated fat, low carbohydrate diets on Lp-PLA₂, but considering that such diets can reduce total cholesterol, LDL cholesterol, and LDL particle size and increase HDL concentrations, points out their possible role in reducing the hydrolyzation of oxidized phospholipids and atherogenic particles by Lp-PLA₂ enzymes. In fact a study reported that the activity of Lp-PLA₂ enzyme is increased in small dense LDL and electronegative LDL species²⁰. Gazi et al²¹ reported that 1 in 100 small dense LDL particle are associated with the Lp-PLA₂ compared to 1 in 4000 large LDL particles are associated with the enzyme. Thus, combination therapy with diet decreases the overall production of proinflammatory molecules like lysophosphatidylcholine, oxidized fatty acids, in

the intimal layer of arteries and eventually lowers the inflammation, stabilizes the plaque and rendering it less rupture prone.

Conclusion

In summation, the combination therapy with low saturated fat, low carbohydrate diet is more efficacious in reducing the Lp-PLA₂, hs-CRP levels and other potential relevant biomarkers implicated in advanced cardiovascular risk prediction. However, present study is a case report and limited by its size and design. To establish further usefulness of such combination therapy a larger sample is needed to demonstrate significant reduction in inflammatory biomarkers.

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