

REVIEW

Open Access

Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A₂ (Lp-PLA₂): a review

Isis T Silva, Ana PQ Mello and Nágila RT Damasceno*

Abstract

The association of cardiovascular events with Lp-PLA₂ has been studied continuously today. The enzyme has been strongly associated with several cardiovascular risk markers and events. Its discovery was directly related to the hydrolysis of the platelet-activating factor and oxidized phospholipids, which are considered protective functions. However, the hydrolysis of bioactive lipids generates lysophospholipids, compounds that have a pro-inflammatory function. Therefore, the evaluation of the distribution of Lp-PLA₂ in the lipid fractions emphasized the dual role of the enzyme in the inflammatory process, since the HDL-Lp-PLA₂ enzyme contributes to the reduction of atherosclerosis, while LDL-Lp-PLA₂ stimulates this process. Recently, it has been verified that diet components and drugs can influence the enzyme activity and concentration. Thus, the effects of these treatments on Lp-PLA₂ may represent a new kind of prevention of cardiovascular disease. Therefore, the association of the enzyme with the traditional assessment of cardiovascular risk may help to predict more accurately these diseases.

Keywords: Lp-PLA₂, Cardiovascular risk, antioxidant, pro-inflammatory

1. Introduction

The physiopathology of cardiovascular disease (CVD) is marked by the presence of atherosclerosis that involves endothelial dysfunction, inflammation, oxidative stress, insulin resistance and dyslipidemia.

Even considering the early diagnosis and the new variety of treatments for CVD, the American College of Cardiology still predicts that there will be 25 million cases only in USA until the end of 2050 [1]. Furthermore, given the current importance of CVD, thanks to its high worldwide prevalence that accounts for nearly 30% of the global deaths [2], the monitoring of the new biomarkers and risk factors represents an important focus of new researches.

In this context, lipoprotein-associated phospholipase A₂ (Lp-PLA₂) represents a potential cardiovascular risk marker, given its correlations with coronary disease and stroke [3-7]. Initially, Lp-PLA₂ was recognized by its action on hydrolyzing platelet-activating factor (PAF);

such characteristic has assigned to it the first name platelet-activating factor acetylhydrolase (PAF-AH) [8].

Despite the other important reviews of Lp-PLA₂ [9-11], the question of whether high activity of Lp-PLA₂ is a causal event or a result of atherosclerosis remains open. Therefore, the main goal of this review is to show the antioxidant and inflammatory role of Lp-PLA₂ and its connection with atherosclerosis, aiming to contribute to the discussions of atherogenic or anti-atherogenic role of Lp-PLA₂. We also discuss possible mechanisms of modulation of Lp-PLA₂.

2. Biochemistry and structural aspects

A brief biological background is necessary to comprehend mechanisms enrolling Lp-PLA₂ and atherosclerosis. Platelet-activating factor (PAF) is an active phospholipid related to many pathologic and physiologic reactions [12]. The PAF is formed through two reactions (Figure 1). Firstly, the cytosolic phospholipase A₂ (cPLA₂) acts on membrane phospholipids producing lysophospholipids; then, the lysophospholipids are modified by PAF acetyltransferase, resulting in the formation of PAF [13].

* Correspondence: nagila@usp.br
Departamento de Nutrição, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, SP, Brasil

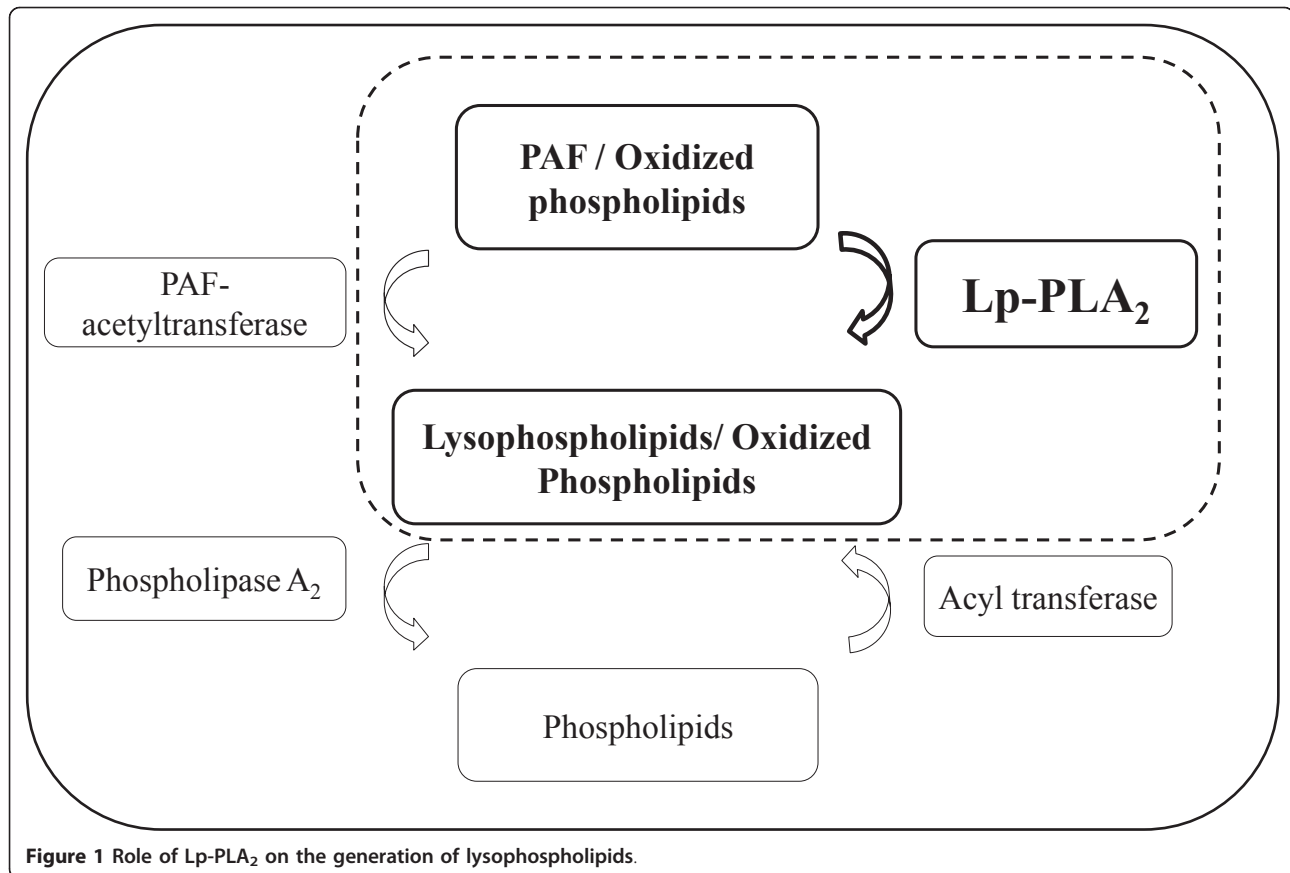


Figure 1 Role of Lp-PLA₂ on the generation of lysophospholipids.

Thus, PAF concentration is modulated by Lp-PLA₂ activity [13,14].

Lp-PLA₂ was discovered in 1980 and it was classified as a Ca²⁺-independent PLA₂ [8], produced by a wide range of inflammatory and non-inflammatory cells [15-17]. It is considered a member of phospholipases family (PLA₂), although exhibits different properties when compared to other PLA₂ [18]. In addition, while Lp-PLA₂ is specific for the breakdown of PAF and oxidized fatty acid residues, PLA₂ is specific for phospholipids containing two long chain acyl groups [18-21].

Another feature of Lp-PLA₂ is that it shows different isoforms, though the more common types are distributed in intracellular [22] and extracellular compartment [8]. Intracellular Lp-PLA₂ shows two variables, I and II [23], while brain tissue exhibits a subtype named Lp-PLA₂-Ib [24]. The Lp-PLA₂ type II consists of a 40-KDa polypeptide chain, and has been associated with antioxidant properties [25]. The extracellular Lp-PLA₂, identified as plasma form, circulates in association primarily with LDL (80-85%) and on minor portion with HDL (15-20%), having its activity strongly correlated with the cholesterol concentrations [26,27]. Lp-PLA₂ has been extracted from human plasma and erythrocytes, bovine brain, liver and seminal plasma, guinea pig

peritoneal fluid and plasma, mouse plasma and platelets, cultured rat Kupffer cell- and hepatocyte-conditioned media, rat bile and the parasite *Nippostrongylus brasiliensis* [28]. On the same hand, it was verified that the different isoforms of Lp-PLA₂ define distinct activities for the enzyme [23,29,30].

3. Antioxidant role of Lp-PLA₂

The oxidative stress is closely associated with inflammation and bioactive lipid formation. These bioactive lipids, such as PAF, PAF-like substances, and oxidized phospholipids, have been identified in atherosclerotic plaque [31]. PAF-like products are formed when the phospholipids of the cellular membrane suffers oxidative damage, resulting in compounds that have structures with shorter peroxidized residues at their second carbon and that mimic the action of PAF [32].

In presence of oxidized phospholipids, Lp-PLA₂ removes these fragments acting as an antioxidant. Matsuzawa *et al.* [33], suggested that the over expression of Lp-PLA₂ protects the cells of reactive oxygen species (ROS)-induced apoptosis through oxidized phospholipids hydrolysis.

In addition, oxidized LDL and LDL(-) are known to be important factors on the atherosclerosis initiation and

development [34-36]. Heery *et al.* [37] demonstrated that the formation of oxidized phospholipids in LDL stimulates Lp-PLA₂ activity. It is most likely that the Lp-PLA₂ hydrolysis of the lipids present in this particle represents an important antiatherogenic role. In this context, Watson *et al.* [38] showed that the Lp-PLA₂, hydrolyzing oxidized phospholipids, minimizes the generation of highly oxidized LDL, increasing the minimally oxidized LDL content. Subsequently, Benitez *et al.* [39] found that the major portion of Lp-PLA₂ was associated with LDL(-) in detriment to LDL(+), suggesting that the release of chemotactic induced by LDL(-) could be a consequence of the high Lp-PLA₂ activity. Indeed, LDL (-) can be generated by Lp-PLA₂, although the origin of this sub-group of LDL could be also compatible with oxidative reaction and other mechanisms such as non enzymatic glycosylation, changes on Apo E (apolipoprotein E) and Apo CII (apolipoprotein CII), non esterified fat acids (NEFAS) enrichment or cross linking with hemoglobin [40].

Lourida *et al.* [41] showed that Lp-PLA₂ activity is important for reducing the immunogenicity of oxLDL, a phenomenon that can be attributed to the decreasing of oxidized phospholipids in patients with coronary artery disease and healthy ones. More recently, Noto *et al.* [42] showed in animals that Lp-PLA₂ protects lipoproteins from oxidation, producing less proatherogenic lipoproteins and preserving HDL functions. In this direction, Bazan [43] proposed that recombinant Lp-PLA₂ could be a potential tool directed to antiatherogenic therapy.

4. Inflammatory action of Lp-PLA₂

Despite the antioxidant potential described above, the association of Lp-PLA₂ with inflammatory reactions represents the majority of the studies in literature in the last years.

When Lp-PLA₂ hydrolyzes bioactive lipids, reducing their biological activity, the most generated metabolites are the lysophospholipids. These lipids are involved with atherosclerotic process and show a deleterious role of Lp-PLA₂, contributing to the inflammatory response against oxidized lipoproteins [39,44,45]. These compounds generated by phospholipases A₂ during cell activation, injury, or apoptosis, are known to affect the function of neutrophils and of a diversity of cell types [46], and can be also produced by phospholipase A1 and by the action of lecithin-cholesterol acyltransferase (LCAT) or endothelial lipase. There are many different lysophospholipids, but the main product of Lp-PLA₂ action is lysophosphatidylcholine [47]; these metabolic processes occur in physiological conditions.

Furthermore, lysophospholipids from apoptotic cells contribute to attract monocytic cells and primary macrophages [48,49]. In this context, Steinbrecher &

Pritchard [45] showed that oxLDL, on the presence of phenylmethanesulphonylfluoride (PMSF), an inhibitor of Lp-PLA₂, has lower values of lysophospholipids. In this fashion, Muller *et al.* [50] proposed that lysophosphatidylcholine represents a biomarker of the intensity of the reactive oxygen species production at the inflammatory site. Accordingly, Lavi *et al.* [51] found that patients with early coronary atherosclerosis had higher lysophosphatidylcholine when compared with control subjects. This profile was confirmed by Herrmann *et al.* [52], who showed that carotid artery plaques of patients with cardiac events presented higher Lp-PLA₂, lysophospholipids, macrophage and collagen content when compared to patients without events.

Studying the effects of oxLDL, Kuniyasu *et al.* [53] demonstrated that oxLDL, and particularly, the lysophosphatidylcholine present in this particle, enhances the plasminogen activator inhibitor-1 expression. Vickers *et al.* [54] demonstrated also that lysophosphatidylcholine can contribute to calcify vascular cells on the atherosclerotic plaque, through up-regulation of osteogenic genes and proteins. Hence, many events present in atherosclerotic process involve directly Lp-PLA₂ or its products.

Figure 2 summarizes the possible atherogenic mechanisms involving Lp-PLA₂. In this context, there can be an individual with dyslipidemia, obesity, hypertension, insulin resistance and oxidative stress, and therefore, highly prone to atherosclerosis. These factors contribute initially to the endothelial dysfunction, characterized by the expression of more adhesion molecules and by larger spaces between endothelial cells. Thus, the LDL, macrophages and T lymphocytes can transmigrate more easily to arterial intima. This LDL particle shows a phenotype more atherogenic, being dense and small, characteristics that make it more susceptible to oxidation. In this site, the reduced content of antioxidants favors the high production of free radicals, and consequently oxidative modifications of LDL. Thus, the Lp-PLA₂ will be activated by oxidized phospholipids present in OxLDL.

The enzyme minimizes modifications of OxLDL, hydrolyzing its oxidized phospholipids; this may be interpreted as an antioxidant action. However, during this process, there are produced high contents of lysophospholipids and oxidized non esterified fat acids (OxNEFAS) that promote adhesion molecules expression and attract macrophages to the arterial intima. The OxLDL, lysophospholipids and OxNEFAS also stimulates cytokines production, like TNF- α and IL-6, which increase the inflammatory profile in the region of the plate. The activated macrophages, through scavenger receptors, phagocyte OxLDL, gradually turning up in foam cells. The muscle cells are also attracted, and migrate to the intima, where they produce collagen,

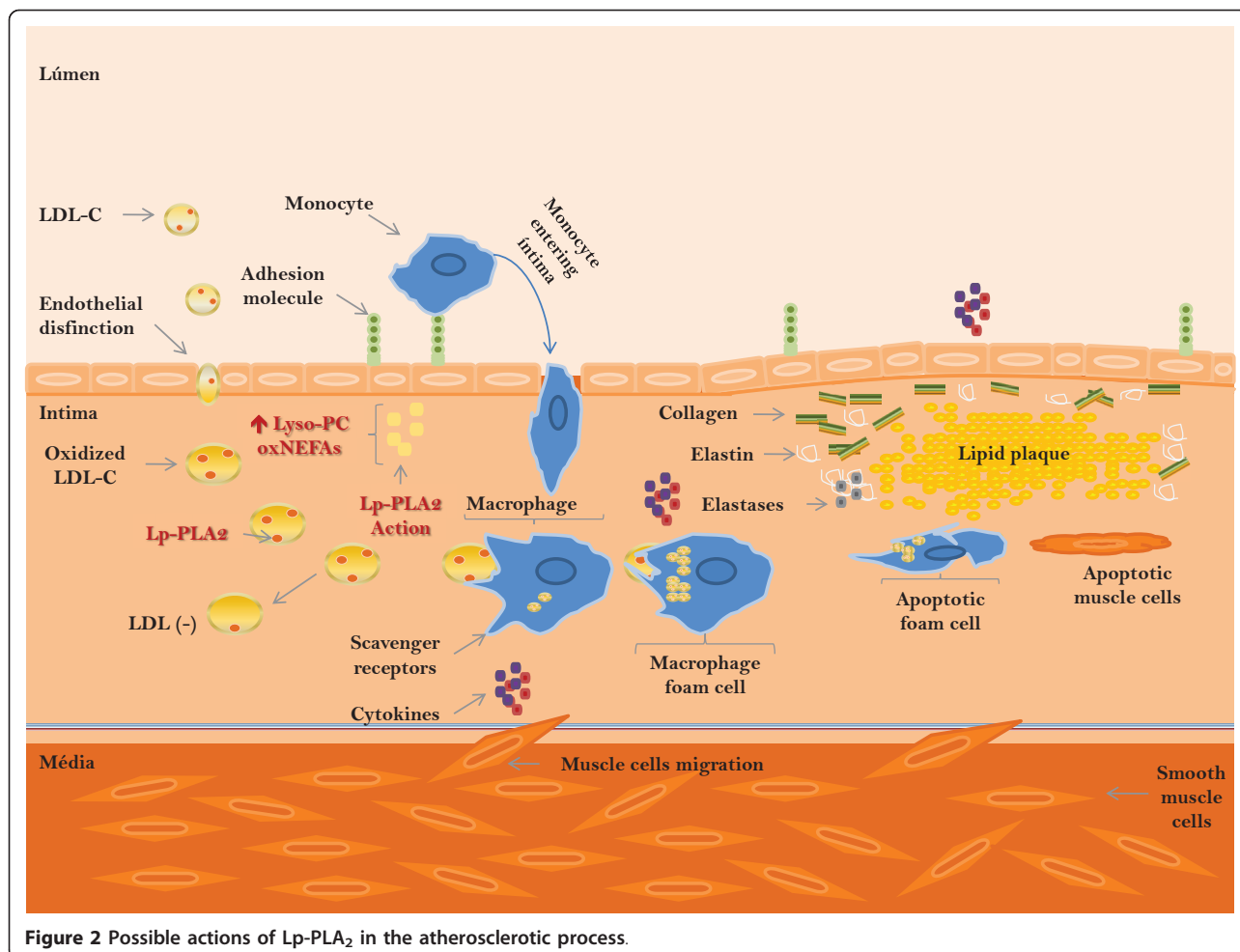


Figure 2 Possible actions of Lp-PLA₂ in the atherosclerotic process.

elastin and elastases, involving and stabilizing the lipid plaque. Subsequently, the macrophages become apoptotic, as well as the muscle cells, causing released of lipids in the plaque. In this process, the presence of OxLDL, as well as lysophospholipids and OxNEFAS produced by Lp-PLA₂, is always stimulating the growth of the plaque; these are factors that can be decisive to plaque rupture susceptibility that can culminate in a cardiovascular event.

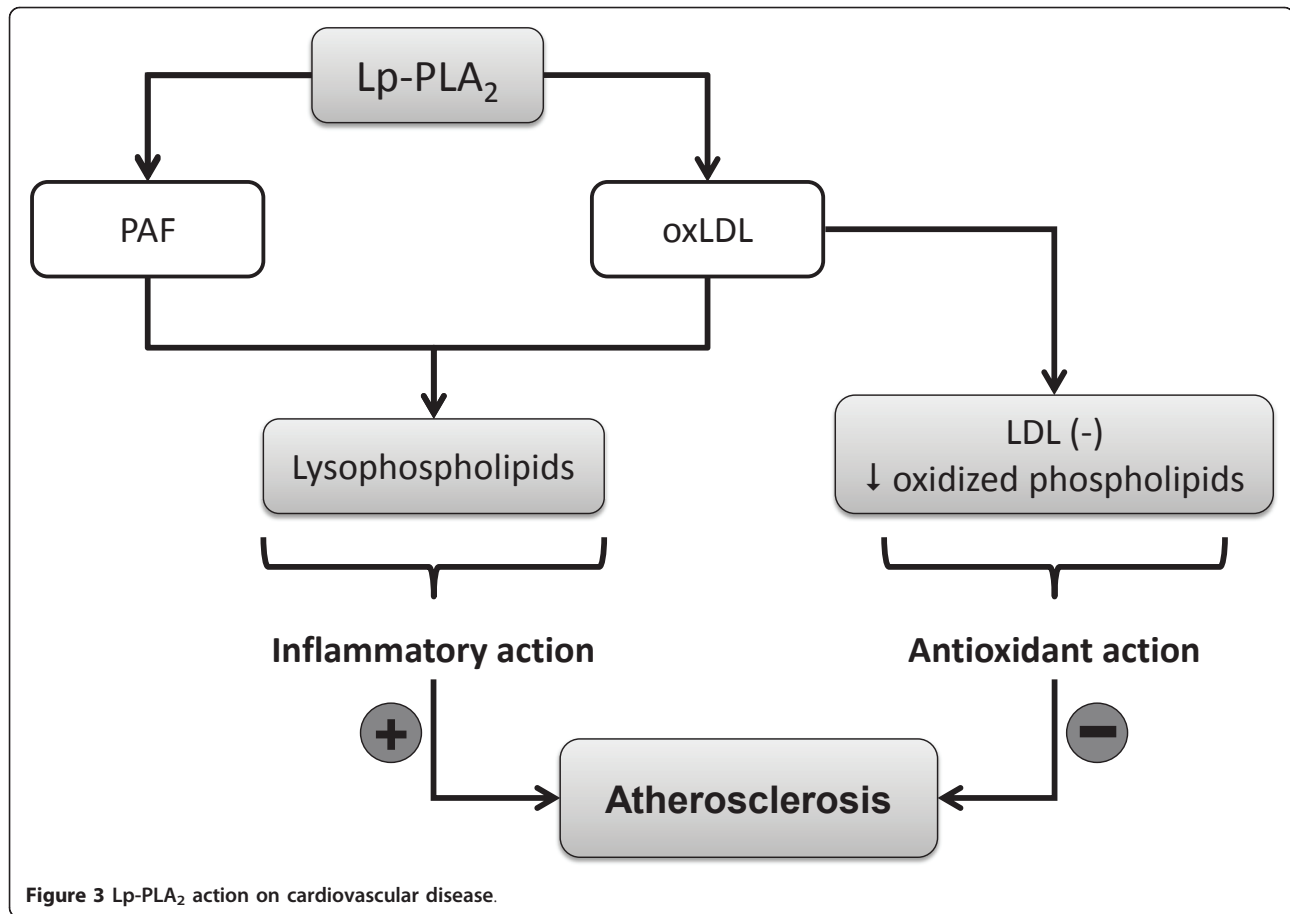
5. Lp-PLA₂ and Cardiometabolic Risk

Taking into account the mechanisms described, Lp-PLA₂ could influence the cardiometabolic risk; the Figure 3 expresses the two main possibilities for action of the enzyme in cardiovascular disease context; the antioxidant action, where these hydrolysis reduce the oxidized phospholipids in plasma and the oxLDL contributing to generation of LDL(-); the inflammatory action, where the hydrolysis of PAF, PAF-like products or oxidized phospholipids generate lysophospholipids

that stimulate inflammation, so the atherogenic process is stimulated.

According to Campo *et al.* [55], Lp-PLA₂ activity was significantly associated with LDL-cholesterol in hypercholesterolemic patients. As a matter of fact, dyslipidemia promotes an increase in plasma Lp-PLA₂ activity and alters the enzyme distribution between apo B- and apo AI-containing lipoproteins, as observed by Tsimihodimos *et al.* [56]. The role of LDL-associated Lp-PLA₂ remains controversial, possibly because of the difficulty in analyzing the actions of the enzyme in the dense LDL particle [57].

On the other hand, studies also have showed that the enzyme activity associated with HDL particles can play an antiatherogenic action. Theilmeier *et al.* [58] demonstrated by *in vitro* and *in vivo* models that HDL-Lp-PLA₂ (HDL-Lipoprotein-associated phospholipase A₂) activity was linked to reduction of endothelial adhesiveness and of macrophage recruitment to lesion prone sites. Afterwards, the same group demonstrated that



atorvastatin induced the increase of HDL-Lp-PLA₂ activity and the reduction of LDL-Lp-PLA₂ (LDL-Lipoprotein-associated phospholipase A₂) activity [56].

Papavasiliou *et al.* [59], investigating chronic kidney disease patients, found an increase in plasma Lp-PLA₂ activity and a reduction of the ratio of HDL-Lp-PLA₂ to plasma when compared to controls. In the same way, Rizos *et al.* [26] demonstrated that patients with metabolic syndrome have higher Lp-PLA₂ activity than controls. Nevertheless, the Lp-PLA₂ content in HDL was lower; these results were confirmed by Lagos *et al.* [60], who observed that the HDL-Lp-PLA₂ activity was lower in patients with metabolic syndrome. Okamura *et al.* [31] suggested that even the Lp-PLA₂ having an important function in atherogenesis, its association with HDL plays the opposite role, as observed by high LDL-Lp-PLA₂ to HDL-Lp-PLA₂ ratio in patients with atrial fibrillation.

In this fashion, Noto *et al.* [61] showed that diabetic patients with metabolic syndrome have significantly higher Lp-PLA₂ activity than those without this disease, reflecting its importance to metabolic risk. Following up, in a cohort with 299 subjects, Allison *et al.* [62]

demonstrated that an increment of one standard deviation in Lp-PLA₂ activity was associated with a higher risk of CVD in five years, but not with mortality. Kiechl *et al.* [63], in a prospective study, demonstrated that oxidized phospholipids/apo B ratio predicted the cardiovascular risk, being the Lp-PLA₂ activity an amplifier of this risk.

Accordingly, Sabatine *et al.* [4] observed that an elevated level of Lp-PLA₂ is a predictor of adverse cardiovascular outcomes, independently of the traditional clinical risk factors in patients with stable coronary artery disease. Persson *et al.* [64] observed that this enzyme was strongly correlated with lipid fractions and the degree of carotid artery atherosclerosis; this study showed that the association with cardiovascular risk is stronger for activity than for mass, reinforcing the impact of activity in atherogenesis [64].

In a prospective population-based survey, which occurred between 1990 and 2005, it was verified that Lp-PLA₂ was higher in subjects with incidence of CVD [5]. In the same year, Jenny *et al.* [65] showed that subjects with heart failure have the elevation of Lp-PLA₂ levels associated with an increase in the mortality risk.

It was detected also that subjects aged > 65 years presented an association between the Lp-PLA₂ and myocardial infarction [65]. An increasing risk of major adverse cardiac events associated with elevated Lp-PLA₂ was also observed in community-based cohort of patients with acute coronary syndrome [6].

More recently, the Lp-PLA₂ Studies Collaboration, analyzing 32 prospective studies, confirmed that the enzyme activity and mass were related to proatherogenic lipids and vascular risk [66]. The study showed also that the association of the enzyme activity with lipid markers is stronger than the association with mass [66]. Recently, the authors of this review verified that the Lp-PLA₂ activity in adolescents is positively associated with total cholesterol, LDL-C, insulin, glucose, HOMA-IR, Apo B (apolipoprotein B)/Apo AI (apolipoprotein AI) ratio and negatively related to HDL size.

In contrast with the studies above, Tsironis *et al.* [67] showed that patients with coronary disease exhibit reduced LDL-Lp-PLA₂ mass and catalytic efficiency, suggesting a diminished ability to degrade pro-inflammatory phospholipids.

Therefore, it is probably that Lp-PLA₂ shows a dual action, directly dependent on its association with LDL (proatherogenic) or HDL (antiatherogenic). Table 1

Table 1 Potential action of the Lp-PLA₂ , according to studies with distinct design.

Study design	Action	Reference
Experimental	Cells ROS protection.	[33]
Experimental	↓ bioactivity phospholipids in oxLDL	[37]
Experimental	↓ oxidized phospholipids in mildly oxLDL	[38]
Case/Control	≈ Oxidized phospholipids and anti-Lp-PLA ₂	[41]
Case/Control	↓ HDL oxidation, foam cell and autoantibodies titers.	[42]
Case/Control	↓ HDL-Lp-PLA ₂ activity	[25]
Case/Control	↓ HDL-Lp-PLA ₂ activity	[60]
Case/Control	↑ LDL-Lp-PLA ₂ to HDL-Lp-PLA ₂ ratio	[31]
Case/Control	↓ HDL-Lp-PLA ₂ and ↑ of LDL-Lp-PLA ₂	[56]
Case/Control	≈ Lp-PLA ₂ activity	[55]
Case/Control	↑ Lp-PLA ₂ activity	[61]
Cohort	↑ Lp-PLA ₂ activity in CHD mortality	[62]
Case/Control	↑ Lp-PLA ₂ activity	[63]
Cohort	Predictor of cardiovascular outcomes	[4]
Cohort	Lp-PLA ₂ correlated with cardiovascular risk factors	[64]
Cohort	Lp-PLA ₂ activity associated with MS and CVD	[5]
Cohort	Lp-PLA ₂ mass and activity associated with CVD	[65]
Cohort	↑ Lp-PLA ₂ activity associated with CVD	[6]
Meta-analysis	Lp-PLA ₂ mass and activity associated vascular risk	[66]

CVD: Cardiovascular Disease; CHD: Coronary Heart Disease; PAD: Peripheral Arterial Disease; ROS: Reactive Oxygen Species, MS: Metabolic Syndrome

summarizes the antioxidant, inflammatory and neutral links between Lp-PLA₂ and cardiometabolic risk.

6. Modulation of Lp-PLA₂

Studies focused on Lp-PLA₂ modulation are little explored in literature, despite of its possible manipulation. Regarding that Lp-PLA₂ is associated with cholesterol and oxidized lipids in LDL and HDL, it is probable that drugs and environment factors, capable of modulating the lipid metabolism, may change the mass and the activity of this enzyme.

Gerra *et al.* [68] showed that lovastatin was responsible for the simultaneous decrease of LDL-C level and Lp-PLA₂ activity. Similarly, Tsimihodimos *et al.* [56] found reduced Lp-PLA₂ activity in plasma of hypercholesterolemic patients under atorvastatin therapy, with a reduction in LDL-Lp-PLA₂ activity; in contrast, there was no modification in HDL-Lp-PLA₂ activity. The same authors, in an investigation of the effect of fenofibrate on hypercholesterolemic patients, observed a reduction in the LDL-Lp-PLA₂ activity and an increase of the HDL-Lp-PLA₂ activity [69]. Schaefer *et al.* [70], comparing the effect of atorvastatin with placebo in coronary heart disease patients observed a reduction of Lp-PLA₂ under therapy.

Studying the effect of cholesterol feeding and simvastatin treatment on rabbits, Zhang *et al.* [71] found that the LDL-Lp-PLA₂ activity increased with cholesterol feeding and decreased after the treatment. In this way, O'Donoghue *et al.* [72] found that an intensive statin therapy was responsible for 20% of reduction in LDL-Lp-PLA₂ , in average. Likewise, Schaefer *et al.* [70] observed that simvastatin determined a reduction of the Lp-PLA₂ mass in 26%.

In the same way, atorvastatin or fenofibrate therapies can increase the ratio of HDL-Lp-PLA₂ to plasma Lp-PLA₂ (or to LDL-Lp-PLA₂) [57]. Also, the effect of gemfibrozil was monitored in men with low HDL-C, and it was verified that individuals in highest quartile of Lp-PLA₂ showed reduction of cardiovascular events [73]. The use of darapladib (oral Lp-PLA₂ inhibitor) by coronary patients caused a reduction of 59% of the enzyme activity after 12 months of treatment; concomitantly, the placebo group presented a significant increase of necrotic core volume when compared to the therapy group [74]. In a complementary study, the combined effect of atorvastatin and darapladib was evaluated in patients with coronary heart disease in the course of 12 weeks; the individuals under darapladib showed a reduction of approximately 54% in the Lp-PLA₂ activity when compared with controls [75].

Investigating patients under low-fat-diet and orlistat treatment, fenofibrate or both drugs during six months, Filippatos *et al.* [76] observed a significant reduction of

Lp-PLA₂ activity in all groups (14%, 22% and 35%, respectively) when compared to basal time. The results suggested the combination of the two treatments as the optimal therapy.

Hence, the direct influence of lipid metabolism on Lp-PLA₂ was confirmed by the efficiency of hypocholesterolemic drugs. Nonetheless, a similar profile was not observed in patients under anti-hypertension treatment: Spirou *et al.* [77] and Rizos *et al.* [78] verified that anti-hypertensive was not able to change Lp-PLA₂ activity.

Despite the positive effect on Lp-PLA₂ demonstrated by application of drugs, many studies have also investigated the influence of diet and other environment factors on the enzyme. In this context, Pedersen *et al.* [79] compared the effects of high (6.6 g), low (2.0 g) and control doses of n-3 polyunsaturated fatty acids in some metabolic parameters; they did not observe any effect on Lp-PLA₂ activity. Recently, in a sub-sample (n = 150, follow up = 1 y) of PREDIMED study, the authors of the present work, comparing diets enhanced with a mix of nuts (30 g/d), olive oil (50 g/d) or with low concentration of saturated fat (< 7%), observed a reduction in Lp-PLA₂ only in the nuts group [NRTD, personal communication].

The effect of selenium on Lp-PLA₂ was recently evaluated [80] on rats, subject to three different diets (control, high fat and high fat enhanced with selenium). The results showed that the Lp-PLA₂ levels in control group were lower than the other groups, and that the selenium did not affect this enzyme.

The Nurses' Health Study demonstrated that the replacement of energy from carbohydrates for proteins, as well as the alcohol consumption or use of cholesterol-lowering drugs, were associated with a reduction in the Lp-PLA₂ activity. Smoking, overweight, aspirin use, hypercholesterolemia and age were, nevertheless, related to the elevation of Lp-PLA₂ activity [81]. In addition, obese and non-diabetic women submitted to a weight reduction program showed a significant reduction in Lp-PLA₂ activity, directly associated with VLDL-C [82]. The influence of the nutritional status on Lp-PLA₂ activity was also evaluated in adolescents where it was positively associated with body mass index, waist circumference and fat mass percentage [83].

Finally, Chen *et al.* [84] compared vegetarians with omnivores and observed that vegetarians presented lower Lp-PLA₂ activity, with lower total cholesterol and LDL-cholesterol, but with increased chances of higher C-reactive protein.

7. Conclusion

Initially, the discovery of the enzyme Lp-PLA₂ was associated with its ability to hydrolyze PAF and phospholipids, what was seen as a protective function. The enzyme acts as an antioxidant in the presence of

oxidized phospholipids. Thus, Lp-PLA₂, in this sense, represents an important factor, reducing the oxLDL atherogenicity. Nowadays, however, its association with cardiovascular events is the most outstanding characteristic observed.

In addition, associations with several cardiovascular risk markers were also described in the literature. The enzyme hydrolyzes bioactive lipids, reducing their biological activity; the major metabolites generated in the process are the lysophospholipids. Given these results, the enzyme has been associated with a pro-inflammatory action, explained mainly by the production of these compounds that stimulate the inflammatory process in the region of the atherosclerotic plate.

Focusing on the enzyme antiatherogenic function, several studies have been evaluating the distribution of Lp-PLA₂ in the lipid fractions. Surprisingly, the HDL-Lp-PLA₂ enzyme has proven beneficial results to the atherosclerotic process. In the same sense, LDL-Lp-PLA₂ is linked to higher cardiovascular risk. Drugs and diet components that alter the lipid profile, the insulin resistance and the inflammatory markers also affect the enzyme activity and its concentration. Possibly, the effects of these components on the Lp-PLA₂ activity, according to the lipid fraction, represent a new kind of prevention of CVD.

The traditional assessment of cardiovascular risk is based on lipid profile, inflammation and body composition. Since the control of these variables seeks to reduce cardiovascular events and this enzyme is strongly related to them, it is probable that the monitoring of its activity and its distribution on lipoproteins will predict better the cardiovascular risk.

List of abbreviations

Apo AI: Apolipoprotein AI; Apo B: Apolipoprotein B; Apo CII: Apolipoprotein CII; Apo E: Apolipoprotein E; cPLA₂: Cytosolic phospholipase A₂; CVD: Cardiovascular disease; HDL-C: High density lipoprotein cholesterol; HDL-Lp-PLA₂: HDL-Lipoprotein-associated phospholipase A₂; LCAT: Lecithin-cholesterol acyltransferase; LDL(-): Electronegative low-density lipoprotein; LDL-C: Low density lipoprotein cholesterol; LDL-Lp-PLA₂: LDL-Lipoprotein-associated phospholipase A₂; Lp-PLA₂: Lipoprotein-associated phospholipase A₂; NEFAs: Non esterified fat acids; oxLDL: Oxidized low-density lipoprotein; OxNEFAs: Oxidized non esterified fat acids; PAF: Platelet-activating factor; PAF-AH: Platelet-activating factor acetylhydrolase; PAF-like: Platelet-activating factor like; PLA₂: Phospholipases family; PMSF: Phenylmethanesulphonylfluoride; ROS: Reactive oxygen species; VLDL-C: Very low density lipoprotein cholesterol.

Acknowledgements

This study was supported by FAPESP (07/51664-5; 07/52123-8) and CNPQ (474112/07-1). The authors acknowledge Dr Silas Luiz de Carvalho, professor at UNIFESP, whose suggestions contributed to improve the quality of the final version of the manuscript.

Authors' contributions

ITS wrote the manuscript, APQM reviewed the manuscript and NRTD designed, drafted and critically reviewed the manuscript. All authors approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 26 July 2011 Accepted: 28 September 2011

Published: 28 September 2011

References

- Foot DK, Lewis RP, Pearson TA, Beller GA: **Demographics and cardiology, 1950-2050.** *J Am Coll Cardiol* 2000, **35**:1067-1081.
- World Health Organization: **Global health risks: mortality and burden of disease attributable to selected major risks.** [http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf], Accessed January 10,2011.
- O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, Braunwald E: **Lipoprotein-associated phospholipase a2 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**:1745-1752.
- Sabatine MS, Morrow DA, O'Donoghue M, Jablonski KA, Rice MM, Solomon S, Rosenberg Y, Domanski MJ, Hsia J: **Prognostic Utility of Lipoprotein-Associated Phospholipase A2 for Cardiovascular Outcomes in Patients With Stable Coronary Artery Disease.** *Arterioscler Thromb Vasc* 2007, **27**:2463-2469.
- Tsimikas S, Willleit J, Knoflach M, Mayr M, Egger G, Notdurfter M, Witztum JL, Wiedermann CJ, Xu Q, Kiechl S: **Lipoprotein-associated phospholipase A2 activity, ferritin levels, metabolic syndrome, and 10-year cardiovascular and non-cardiovascular mortality: results from the Bruneck study.** *Eur Heart J* 2009, **30**:107-15.
- Li N, Li S, Yu C, Gu S: **Plasma Lp-PLA2 in acute coronary syndrome: association with major adverse cardiac events in a community-based cohort.** *Postgrad Med* 2010, **122**:200-205.
- Iribarren C: **Lipoprotein-Associated Phospholipase A₂ and C-Reactive protein for Measurement of Inflammatory Risk: Independent or Complementary?** *Curr Cardio Risk Rep* 2010, **4**:57-67.
- Farr RS, Cox CP, Wardlow ML, Jorgensen R: **Preliminary studies of an acid-labile factor (ALF) in human sera that inactivates platelet-activating factor (PAF).** *Clinical Immunology and Immunopathology* 1980, **15**:318-30.
- Corson MA, Jones PH, Davidson MH: **Review of the Evidence for the clinical Utility of Lipoprotein-Associated Phospholipase A₂ as a Cardiovascular Risk Marker.** *Am J Cardiol* 2008, **101**(suppl):41F-50F.
- Zalewski A, Macphee C: **Role of Lipoprotein-Associated Phospholipase A2 in Atherosclerosis Biology, Epidemiology, and Possible Therapeutic Target.** *Arterioscler Thromb Vasc Biol* 2005, **25**:923-931.
- Epps KC, Wilensky RL: **Lp-PLA2 - a novel risk factor for high-risk coronary and carotid artery disease.** *J InternMed* 2011, **269**:94-106.
- O'Flaherty JT, Wykle RL: **Biology and biochemistry of platelet-activating factor.** *Clin Rev Allergy* 1983, **1**:353-367.
- Macmanus LM, Pinckard RN: **PAF, aputative mediator of oral inflammation.** *Crit Rev Oral Biol Med* 2000, **11**(2):240-258.
- Chen J, Yang L, Foulks JM, Weyrich AS, Zimmerman GA, Marathe GK, McIntyre TM: **Intracellular PAF catabolism by PAF acetylhydrolase counteracts continual PAF synthesis.** *J Lipid Res* 2007, **48**(11):2365-76.
- Karabina SA, Ninio E: **Plasma PAF-acetylhydrolase: an unfulfilled promise?** *Biochim Biophys Acta* 2006, **1761**:1351-1358.
- Venable ME, Zimmerman GA, McIntyre TM, Prescott SM: **Platelet-activating factor: a phospholipid autacoids with diverse actions.** *J Lipid Res* 1993, **34**(5):691-702.
- Snyder F: **Platelet-activating factor and its analogs: metabolic pathways and related intracellular processes.** *Biochim Biophys Acta* 1995, **1254**(3):231-249.
- Blank ML, Lee T, Fitzgerald V, Snyder F: **A specific acetylhydrolase for 1-alkyl-2- acetyl-sn-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid).** *J Biol Chem* 1981, **256**:175-178.
- Dennis EA: **The growing phospholipase A2 superfamily of signal transduction enzymes.** *Trends Biochem Sci* 1997, **22**:1-2.
- Stremier KE, Stafforini DM, Prescott SM, McIntyre TM: **Human Plasma Platelet-activating factor acetylhydrolase.** *J Biol Chem* 1991, **266**(17):11095-11103.
- Chakraborti S: **Phospholipase A2 isoforms: a perspective.** *Cell Signal* 2003, **15**:637-665.
- Hattori K, Hattori M, Adachi H, Tsujimoto , Arai H, Inoue K: **Purification and characterization of platelet-activating factor acetylhydrolase II from bovie liver cytosol.** *J Biol Chem* 1995, **270**(38):22308-22313.
- Arai H: **Platelet-activating factor acetylhydrolase.** *Prostaglandins Other Lipid Mediat* 2002, **68-69**:83-94.
- Derewenda ZS, Ho YS: **PAF-acetylhydrolases.** *Biochim Biophys Acta* 1999, **1441**:229-236.
- Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM: **Platelet-activating factor and related lipidmediators.** *Annu Rev Biochem* 2000, **69**:419-45.
- Rizos E, Tambaki AP, Gazi I, Tselepis AD, Elisaf M: **Lipoprotein-associated PAF-acetylhydrolase activity in subjects with the metabolic syndrome.** *Prostaglandins Leukot Essent Fatty Acids* 2005, **72**:203-209.
- Tselepis AD, Chapman MJ: **Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet activating factor acetylhydrolase.** *Atherosclerosis* 2002, **3**:57-68.
- Chroni A, Mavri-Vavayanni M: **Characterization of a platelet activating factor acetylhydrolase from rat adipocyte.** *Life Sci* 2000, **67**:2807-2825.
- Manya H, Aoki J, Kato H, Ishii J, Hino S, Arai H, Inoue K: **Biochemical characterization of various catalytic complexes of the brain platelet-activating factor acetylhydrolase.** *J Biol Chem* 1999, **274**(45):31827-32.
- Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM: **Platelet activating factor acetylhydrolases.** *J Biol Chem* 1997, **272**(29):17895-8.
- Okamura K, Miura S, Zhang B, Uehara Y, Matsuo K, Kumagai K, Saku K: **Ratio of LDL- to HDL-Associated Platelet-Activating Factor Acetylhydrolase may be a Marker of Inflammation in Patients With Paroxysmal Atrial Fibrillation.** *Circ J* 2007, **71**:214-219.
- Tjoelker LW, Stafforini DM: **Platelet-activating factor acetylhydrolases in health and disease.** *Biochim Biophys Acta* 2000, **1488**:102-123.
- Matsuzawa A, Hattori K, Aoki J, Arai H, Inoue K: **Protection against oxidative stress-induced cell death by intracellular platelet-activating factor-acetylhydrolase II.** *J Biol Chem* 1997, **272**(51):32315-20.
- Han J, Hajjar DP, Febbraio M, Nicholson AC: **Native and modified low density lipoproteins increase the functional expression of the macrophage class B scavenger receptor, CD36.** *J Biol Chem* 1997, **272**(34):21654-9.
- Glass CK, Witztum JL: **Atherosclerosis: The Road Ahead.** *Cell* 2001, **104**:503-516.
- Sánchez-Quesada JL, Benítez S, Ordóñez-Llanos J: **Electronegative low-density lipoprotein.** *Curr Opin Lipidol* 2004, **15**:329-335.
- Heery JM, Kozak M, Stafforini DM, Jones DA, Zimmerman GA, McIntyre TM, Prescott SM: **Oxidatively modified LDL contains phospholipids with platelet-activating factor-like activity and stimulates the growth of smooth muscle cells.** *J Clin Invest* 1995, **96**(5):2322-30.
- Watson AD, Navab M, Hama SY, Sevanian A, Prescott SM, Stafforini DM, McIntyre TM, Du BN, Fogelman AM, Berliner JA: **Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein.** *J Clin Invest* 1995, **95**(2):774-82.
- Benítez S, Sánchez-Quesada JL, Ribas V, Jorba O, Blanco-Vaca F, González-Sastre F, Ordóñez-Llanos J: **Platelet-activating factor acetylhydrolase is mainly associated with electronegative low-density lipoprotein subfraction.** *Circulation* 2003, **108**(1):92-6.
- Mello AP, da Silva IT, Abdalla DS, Damasceno NR: **Electronegative low-density lipoprotein: origin and impact on health and disease.** *Atherosclerosis* 2011, **215**(2):257-65.
- Lourida ES, Papanthanasou AI, Goudevenos JA, Tselepis AD: **The low-density lipoprotein (LDL)-associated PAF-acetylhydrolase activity and the extent of LDL oxidation are important determinants of the autoantibody titers against oxidized LDL in patients with coronary artery disease.** *Prostaglandins Leukot Essent Fatty Acids* 2006, **75**(2):117-26.
- Noto H, Hara M, Karasawa K, Iso-O N, Satoh H, Togo M, Hashimoto Y, Yamada Y, Kosaka T, Kawamura M, Kimura S, Tsukamoto K: **Human plasma platelet-activating factor acetylhydrolase binds to all the murine lipoproteins, conferring protection against oxidative stress.** *Arterioscler Thromb Vasc Biol* 2003, **23**(5):829-35.
- Bazan NG: **Inflammation. A signal terminator.** *Nature* 1995, **374**(6522):501-2.
- Itabe H: **Oxidized phospholipids as a new landmark in atherosclerosis.** *Prog Lipid Res* 1998, **37**(2-3):181-207.

45. Steinbrecher UP, Pritchard PH: Hydrolysis of phosphatidylcholine during LDL oxidation is mediated by platelet-activating factor acetylhydrolase. *J Lipid Res* 1989, **30**(3):305-15.
46. Frasch SC, Zemski-Berry K, Murphy RC, Borregaard N, Henson PM, Bratton DL: Lysophospholipids of different classes mobilize neutrophil secretory vesicles and induce redundant signaling through G2A. *J Immunol* 2007, **178**(10):6540-8.
47. Schmitz G, Ruebsaamen K: Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis* 2010, **208**(1):10-8.
48. Quinn MT, Parthasarathy S, Steinberg D: Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc Natl Acad Sci USA* 1988, **85**:2995-2998.
49. Lauber K, Bohn E, Kröber SM, Xiao YJ, Blumenthal SG, Lindemann RK, Marini P, Wiedig C, Zobywalski A, Baksh S, Xu Y, Autenrieth IB, Schulze-Osthoff K, Belka C, Stuhler G, Wesselborg S: Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell* 2003, **113**(6):717-30.
50. Müller J, Petković M, Schiller J, Arnold K, Reichl S, Arnhold J: Effects of lysophospholipids on the generation of reactive oxygen species by fMLP- and PMA-stimulated human neutrophils. *Luminescence* 2002, **17**(3):141-9.
51. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A: Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* 2007, **115**(21):2715-21.
52. Herrmann J, Mannheim D, Wohler T, 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, **30**(23):2930-8.
53. Kuniyasu A, Tokunaga M, Yamamoto T, Inoue S, Obama K, Kawahara K, Nakayama H: Oxidized LDL and lysophosphatidylcholine stimulate plasminogen activator inhibitor-1 expression through reactive oxygen species generation and ERK1/2 activation in 3T3-L1 adipocytes. *Biochim Biophys Acta* 2011, **1811**(3):153-62.
54. Vickers KC, Castro-Chavez F, Morrisett JD: Lyso-phosphatidylcholine induces osteogenic gene expression and phenotype in vascular smooth muscle cells. *Atherosclerosis* 2010, **211**(1):122-9.
55. Campo S, Sardo MA, Bitto A, Bonaiuto A, Trimarchi G, Bonaiuto M, Castaldo M, Saitta C, Cristadoro S, Saitta A: Platelet-activating factor acetylhydrolase is not associated with carotid intima-media thickness in hypercholesterolemic Sicilian individuals. *Clin Chem* 2004, **50**(11):2077-82.
56. Tsimihodimos V, Karabina SP, Tambaki AP, Bairaktari E, Goudevenos JA, Chapman MJ, Elisaf M, Tselepis AD: Atorvastatin Preferentially Reduces LDL-Associated Platelet-Activating Factor Acetylhydrolase Activity in Dyslipidemias of Type IIA and Type IIB. *Arterioscler Thromb Vasc Biol* 2002, **22**:306-311.
57. Elisaf M, Tselepis AD: Effect of hypolipidemic drugs on lipoprotein-associated platelet activating factor acetylhydrolase. Implication for atherosclerosis. *Biochem Pharmacol* 2003, **66**(11):2069-73.
58. Theilmeyer G, De Geest B, Van Veldhoven PP, Stengel D, Michiels C, Lox M, Landeloos M, Chapman MJ, Ninio E, Collen D, Himpens B, Holvoet P: HDL-associated PAF-AH reduces endothelial adhesiveness in apoE-/- mice. *FASEB J* 2000, **14**(13):2032-9.
59. Papavasiliou EC, Gouva C, Siamopoulos KC, Tselepis AD: PAF-acetylhydrolase activity in plasma of patients with chronic kidney disease. Effect of long-term therapy with erythropoietin. *Nephrol Dial Transplant* 2006, **21**(5):1270-7.
60. Lagos KG, Filippatos TD, Tsimihodimos V, Gazi IF, Rizos C, Tselepis AD, Mikhailidis DP, Elisaf MS: Alterations in the high density lipoprotein phenotype and HDL-associated enzymes in subjects with metabolic syndrome. *Lipids* 2009, **44**(1):9-16.
61. Noto H, Chitkara P, Raskin P: The role of lipoprotein-associated phospholipase A(2) in the metabolic syndrome and diabetes. *J Diabetes Complications* 2006, **20**(6):343-8.
62. Allison MA, Denenberg JO, Nelson JJ, Natarajan L, Criqui MH: The association between lipoprotein-associated phospholipase A2 and cardiovascular disease and total mortality in vascular medicine patients. *J Vasc Surg* 2007, **46**(3):500-6.
63. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Wiedermann CJ, Oberthaler S, Xu Q, Witztum JL, Tsimikas S: Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. *Arterioscler Thromb Vasc Biol* 2007, **27**(8):1788-95.
64. Persson M, Nilsson J, Nelson J, Hedblad B, Berglund G: The epidemiology of Lp-PLA2: Distribution and correlation with cardiovascular risk factors in a population-based cohort. *Atherosclerosis* 2007, **190**:388-396.
65. 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**(2):528-32.
66. The Lp-PLA2 Studies Collaboration: Lipoprotein-associated phospholipase A2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet* 2010, **375**:1536-44.
67. Tsiionis LD, Katsouras CS, Lourida ES, Mitsios JV, Goudevenos J, Elisaf M, Tselepis AD: Reduced PAF-acetylhydrolase activity associated with Lp(a) in patients with coronary artery disease. *Atherosclerosis* 2004, **177**(1):193-201.
68. Guerra R, Zhao B, Mooser V, Stafforini D, Johnston JM, Cohen JC: Determinants of plasma platelet-activating factor acetylhydrolase: heritability and relationship to plasma lipoproteins. *J Lipid Res* 1997, **38**(11):2281-8.
69. Tsimihodimos V, Kakafika A, Tambaki AP, Bairaktari E, Chapman MJ, Elisaf M, Tselepis AD: Fenofibrate induces HDL-associated PAF-AH but attenuates enzyme activity associated with apoB-containing lipoproteins. *J Lipid Res* 2003, **44**:927-934.
70. Schaefer EJ, McNamara JR, Asztalos BF, Taylor 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 A2 in patients with coronary heart disease versus control subjects. *Am J Cardiol* 2005, **95**(9):1025-32.
71. Zhang B, Fan P, Shimoji E, Itabe H, Miura S, Uehara Y, Matsunaga A, Saku K: Modulating effects of cholesterol feeding and simvastatin treatment on platelet-activating factor acetylhydrolase activity and lysophosphatidylcholine concentration. *Atherosclerosis* 2006, **186**(2):291-301.
72. O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, Braunwald E: Lipoprotein-associated phospholipase A2 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**(14):1745-52.
73. Robins SJ, Collins D, Nelson JJ, Bloomfield HE, Asztalos BF: Cardiovascular events with increased lipoprotein-associated phospholipase A(2) and low high-density lipoprotein-cholesterol: the Veterans Affairs HDL Intervention Trial. *Arterioscler Thromb Vasc Biol* 2008, **28**(6):1172-8.
74. Serruys PW, Garcia-Garcia HM, Buszman P, Erne P, Verheyne S, Aschermann M, Duckers H, Bleie O, Dudek D, Bøtker HE, von Birgelen C, D'Amico D, Hutchinson T, Zambanini A, Mastik F, van Es GA, van der Steen AF, Vince DG, Ganz P, Hamm CW, Wijns W, Zalewski A, Integrated Biomarker and Imaging Study-2 Investigators: Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation* 2008, **118**(11):1172-82.
75. Mohler ER, Ballantyne CM, Davidson MH, Hanefeld M, Ruijlope LM, Johnson JL, Zalewski A, Darapladib Investigators: The effect of darapladib on plasma lipoprotein-associated phospholipase A2 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. *J Am Coll Cardiol* 2008, **51**(17):1632-41.
76. Filippatos TD, Gazi IF, Liberopoulos EN, Athyros VG, Elisaf MS, Tselepis AD, Kiortsis DN: The effect of orlistat and fenofibrate, alone or in combination, on small dense LDL and lipoprotein-associated phospholipase A2 in obese patients with metabolic syndrome. *Atherosclerosis* 2007, **193**(2):428-37.
77. Spirova A, Rizos E, Liberopoulos EN, Kolaitis N, Achimastos A, Tselepis AD, Elisaf M: Effect of barnidipine on blood pressure and serum metabolic parameters in patients with essential hypertension: a pilot study. *J Cardiovasc Pharmacol Ther* 2006, **11**(4):256-61.
78. Rizos EC, Spyrou A, Liberopoulos EN, Papavasiliou EC, Saougos V, Tselepis AD, Elisaf M: Effects of eprosartan on serum metabolic

parameters in patients with essential hypertension. *Open Cardiovasc Med J* 2007, **1**:22-6.

79. Pedersen MW, Koenig W, Christensen JH, Schmidt EB: **The effect of marine n-3 fatty acids in different doses on plasma concentrations of Lp-PLA2 in healthy adults.** *Eur J Nutr* 2009, **48**(1):1-5.
80. Kaur HD, Bansal MP: **Studies on HDL associated enzymes under experimental hypercholesterolemia: possible modulation on selenium supplementation.** *Lipids Health Dis* 2009, **8**:55.
81. Hatoum IJ, Nelson JJ, Cook NR, Hu FB, Rimm EB: **Dietary, lifestyle, and clinical predictors of lipoprotein-associated phospholipase A2 activity in individuals without coronary artery disease.** *Am J Clin Nutr* 2010, **91**:786-93.
82. Tzotzas T, Filippatos TD, Triantos A, Bruckert E, Tselepis AD, Kiortsis DN: **Effects of a low-calorie diet associated with weight loss on lipoprotein-associated phospholipase A2 (Lp-PLA2) activity in healthy obese women.** *Nutr Metab Cardiovasc Dis* 2008, **18**(7):477-82.
83. Silva IT, Timm AS, Damasceno NRT: **Lp-PLA₂ as an important biomarker of cardiovascular risk in obese adolescents.** *Eur J Clin Nutr* , in evaluation.
84. Chen CW, Lin CT, Lin YL, Lin TK, Lin CL: **Taiwanese female vegetarians have lower lipoprotein-associated phospholipase A2 compared with omnivores.** *Yonsei Med J* 2011, **52**(1):13-9.

doi:10.1186/1476-511X-10-170

Cite this article as: Silva et al.: Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A₂ (Lp-PLA₂): a review. *Lipids in Health and Disease* 2011 **10**:170.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

