Association of Lipoprotein-associated Phospholipase A_2 Activity with Components of the Metabolic Syndrome in Apparently Healthy Boys

Running Title: Lp-PLA_2 in adolescent boys.

Type of manuscript: Original Article

Word count: 2191, 1 table, 1 figure

Valeria Hirschler*, Tomas Meroño**, Gustavo Maccallini*, Leonardo Gomez Rosso**, Claudio Aranda*, Fernando Brites**.

*Durand Hospital, Buenos Aires, Argentina.

**Laboratory of Lipids and Lipoproteins, Department of Clinical Biochemistry, School of Pharmacy and Biochemistry, INFIBIOC, UBA. CONICET. Buenos Aires, Argentina.

Corresponding author:

Valeria Hirschler

Durand Hospital

Maipu 812 5 M. Buenos Aires, 1006 Argentina

Phone: 54-11-4809-0363; fax: 54-11-4893-1647

e-mail: vhirschler@intramed.net

Conflict of interests: None

Word count:2191

List of Abbreviations

Lp-PLA$_2$= Lipoprotein-associated phospholipase A$_2$

CVD= Cardiovascular disease

MS= Metabolic syndrome

BP= Blood pressure

BMI= Body mass index

LDL= Low density lipoprotein

HDL= High density lipoprotein

HOMA-IR= Homeostasis model assessment

CETP= Cholesteryl ester transfer protein

IDF= International Diabetes Federation

Apo= Apolipoprotein

Key Words

Lp-PLA$_2$, children, adolescents, CETP, obesity, metabolic syndrome, lipoprotein profile, insulin resistance, apo B, atherosclerosis, cardiovascular disease.

Abstract

Background: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has been proposed as a biomarker of risk of cardiovascular disease (CVD). Objective: To determine the association between Lp-PLA_2 activity and BMI, insulin-resistance, components of the metabolic syndrome (MS), and lifestyle behaviors in healthy adolescent boys.

Methods: Data were collected cross-sectionally from 164 adolescents from an amateur rugby club. BMI, blood pressure (BP), Tanner stages, glucose, insulin, lipids, and Lp-PLA_2 activity were measured. Questionnaires for lifestyle behaviors were completed. Results: Approximately 26% of the adolescents were obese and 23% overweight. There was a univariate association between Lp-PLA_2 and BMI ($r=0.16;p=0.042$), triglycerides ($r=0.26;p=0.001$), LDL-C ($r=0.46;p<0.001$), apo B ($r=0.55;p<0.001$), whereas waist circumference , BP, glucose, HOMA-IR, and HDL-C were not correlated. None of the lifestyle behaviors were significantly correlated with Lp-PLA_2. In order to analyze Lp-PLA_2 association with known CVD risk conditions, adolescents were categorized according to overweight/obesity and to the presence of metabolic syndrome. Conversely, as it was for LDL-C and apo B concentration, Lp-PLA_2 activity was not higher in adolescents with obesity. Multiple regression analysis showed that apo B was significantly associated with Lp-PLA_2 adjusted for age, BMI, triglycerides and LDL-C ($R2=0.32$).

Conclusion: Lp-PLA_2 activity was only associated with apo B adjusted for several confounding variables, suggesting that its clinical utility to identify individuals at risk for CVD does not surpass LDL-C and apo B in healthy adolescents. As plaque morphology may change with age, associations of Lp-PLA_2 with CVD may likewise vary with age.

Introduction

In the last years, a growing body of evidence supports the conclusion that inflammation plays a significant role in atherosclerosis [1]. Among the serum markers of inflammation, one which has been studied in its relationship with atherosclerosis is the lipoprotein-associated phospholipase A_2 (Lp-PLA_2) [2,3]. Recently, the analysis of data from 32 prospective studies has concluded that Lp-PLA_2 mass and activity were directly associated with the risk of cardiovascular disease (CVD) [2]. In fact, Lp-PLA_2 has been recommended as an inflammatory marker for CVD risk assessment in patients with moderate or high risk based on Framingham criteria [4]. Lp-PLA_2 is an enzyme expressed in atherosclerotic plaques by inflammatory cells and circulates in plasma mainly bound to low density lipoprotein (LDL); thus, its measurement contributes to the identification of patients with rupture-prone plaques [5,6].

Regarding the factors which affect Lp-PLA_2 activity, some studies showed that lipid and non-lipid related parameters, as well as dietary, lifestyle and clinical characteristics could be associated with Lp-PLA_2 activity in adult populations, though the topic is still under debate [2,7,8]. Nonetheless, whether these associations are valid in children and adolescents remains unknown. On the other hand, Lp-PLA_2 activity has also been postulated as a marker of the levels of the highly atherogenic small and dense LDL particles as Lp-PLA_2 is preferably associated with this LDL subclass [9]. To our knowledge, in this context, the relationship between Lp-PLA_2 and factors known to influence the appearance of small and dense LDL particles like the cholesteryl ester transfer protein (CETP) activity has been poorly studied.

Regarding CETP activity in adolescent boys, the lipoprotein profile, milk intake above three glasses per day and watching television more than two hours daily were

associated to higher CETP activities [10]. Moreover, obese adolescents exhibited higher CETP activity than non obese [10]. As CETP's role in atherogenesis remains controversial [11], we sought to study other CVD biomarkers in adolescents, as childhood risk factors may determine the risk of CVD later in life. As far as we know, there are no large studies in healthy adolescents showing the role that Lp-PLA_2 activity plays in relation to the development of future CVD in South American countries.

Therefore, the aim of the present study was to evaluate the association of Lp-PLA_2 activity with clinical characteristics, lifestyle behaviors, insulin resistance markers, lipoprotein profile and CETP activity in healthy adolescent boys.

Methods

Study design and participants:

Data were collected cross-sectionally from 164 adolescent boys aged 16.7±1.8 years from an amateur rugby club in the north side of Buenos Aires suburbs in April 2009. Exclusion criteria included: missing body mass index (BMI) and blood pressure information; not being in the fasting state; known diabetes or other chronic diseases; the use of medication that could alter blood pressure, glucose or lipid metabolism; and the informed consent not being signed. Of the 189 adolescents recruited, 4 were missing the BMI, 4 the blood pressure and 17 declined to participate. The remaining 164 children were included. All subjects were examined by the same physician. The study was approved by the Human Rights Committee of Durand Hospital in Buenos Aires. Each parent and subject gave written informed consent after an explanation of the study and before its initiation.

Socio-demographic characteristics included age and level of parental education and the presence or absence of a refrigerator or a dirt floor. Questionnaires for socio-economic status have been described in detail elsewhere [12].

Anthropometric measures, blood pressure, metabolic syndrome and stage of puberty:

The adolescents' height, weight and waist circumference were measured as previously described [10]. Adolescents were classified as normal weight (< 85th

percentile), overweight (85th to < 95th percentile), or obese (≥ 95th percentile) according to CDC norms [13]. When participants were older than 18 years, they were classified as normal weight (BMI < 25 kg/m^2), overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2), or obese (BMI ≥30 kg/m^2) according to the adult definition [14]. Central obesity was defined as waist circumference ≥ 94 cm per International Diabetes Federation (IDF) criteria [15].

Three separate blood pressure measurements were recorded by a trained technician using a random-zero sphygmomanometer after the participant was seated at rest for five minutes. The averages of the last two measurements of systolic and diastolic blood pressures were used [16]. Hypertension was defined per IDF criteria [15].

The IDF definition was used for diagnosis of metabolic syndrome [15].

The physical examination also included determination of the stage of puberty according to Tanner criteria [17].

Lifestyle behaviors:

Validated questionnaires for lifestyle behaviors were completed by the same pediatrician as previously described. Food frequency questionnaires are a correct measure of patterns of intake [18]. Standard serving sizes and food models were provided as a reference for intake estimation.

Analytical determinations:

Blood samples were obtained from subjects after a 12-hour overnight fast for measurement of glucose, insulin and lipid levels and CETP activity. Plasma glucose and lipid levels were assayed by standardized techniques (Roche Diagnostics, Mannheim, Germany) in a Hitachi 917 analyzer (Hitachi High Technologies Corp., Tokyo, Japan). Plasma insulin levels were measured by radioimmunoassay (Linco Laboratories, St. Charles, MO, USA). The following equation for HOMA-IR index was used: fasting insulin (μU/ml) x fasting glucose (mmol/l)/22.5) [19]. CETP activity was determined employing an isotopic endogenous assay [20]. After incubation of serum with a tracer amount of biosynthetically labeled HDL_3, apo B-containing lipoproteins were separated by the selective precipitation method. Lp-PLA_2 activity was measured following the radiometric assay described by Blank *et al.* [21]. Briefly, The separation of the released radiolabelled acetate from the lipid substrate (1-hexadecyl-2-[^{3}H]acetyl-glicero-3-phosphocholine) was carried out by phase-phase partitioning and measurement of the radioactivity in the aqueous phase.

Data analysis:

Chi squared test was used to compare proportions. When more than 20% of the cells had expected frequencies < 5, Fisher's exact test was used. Data distribution was assessed using the Shapiro-Wilks test. When comparing two groups with normally distributed data, a student t test was performed. When the homogeneity of the variances could not be proven, the non-parametric Kruskal-Wallis test was used with Dunn post-hoc test. To measure the strength of association between two variables, a Pearson correlation coefficient was employed. Multiple linear regression analysis was performed to examine the relationship between Lp-

PLA_2 and other continuous variables, such as age, BMI, triglycerides and LDL-cholesterol (LDL-C). P values <0.05 were considered statistically significant. Data are presented as mean ± SD or as median (Q1-Q3). Analyses were done using the SPSS statistical software package SPSS 10.0 and InfoStat®.

Results

Clinical, lifestyle and metabolic characteristics of the adolescent boys

All subjects were at pubertal or postpubertal stage. The prevalence of Tanner stages 5, 4 and 3 was 51%, 35% and 14%, respectively. Eighty four (51.2%) adolescents had normal weight, 37 (22.6%) were overweight and 43 (26.2%) obese. Furthermore, metabolic syndrome was diagnosed in 11 subjects (6.7%).

Adolescent boys belonged to a middle-low socio-economic class, reflected in the educational backgrounds of the parents, with 49.4% of the mothers and 52.7% of the fathers having only an elementary school education or less. All the families had a refrigerator and none had a dirt floor. Approximately, 58% (94/164) of the adolescents watched television more than two hours per day and 40% (66/164) had television sets in their bedrooms. Nearly, 95% (156/164) of the children drank one or more glasses of sweet beverages, 11% (18/164) drank more than three glasses of milk, 30% (49/163) did not eat breakfast, and 93% (152/164) ate less than five servings of fruit and vegetables per day. Of interest was that only 19.5% of the adolescents drank low-fat or skim milk, as recommended for children who are older than 2 years (30)

General clinical and metabolic characteristics of the total studied population and according to Lp-PLA_2 activity dichotomized at the median are exposed in table 1. Subjects with Lp-PLA_2 activity above the median were older and presented higher waist circumference, systolic blood pressure, plasma triglycerides, total cholesterol, LDL-C, apo B and CETP activity; whereas HDL-C was lower than in the other group (Table 1).

LDL-C, Apo B and Lp-PLA$_2$ activity according to different metabolic situations

Figure 1, shows LDL-C, apo B and Lp-PLA$_2$ activity in the studied group of adolescents classified according to the presence of overweight and obesity and to the presence or absence of metabolic syndrome. LDL-C and apo B plasma concentrations were higher in obese than in overweight and normal weight adolescents, while no significant difference was observed in Lp-PLA$_2$ activity. When adolescents were classified according to the presence of metabolic syndrome, LDL-C, apo B levels and Lp-PLA$_2$ activity were significantly increased in those with metabolic syndrome (Fig. 1).

Factors associated to Lp-PLA$_2$ activity

Lp-PLA$_2$ activity correlations were significant with plasma levels of triglycerides ($r=0.26$; $p=0.001$), total cholesterol ($r=0.46$; $p<0.001$), LDL-C ($r=0.46$; $p<0.001$), apo B ($r=0.55$; $p<0.001$), triglyceride/HDL-C ratio ($r=0.21$; $p<0.01$) and CETP activity ($r=0.24$; $p=0.01$). In contrast, correlations between Lp-PLA$_2$ activity and BMI, waist circumference, systolic blood pressure, insulin concentration, HOMA-IR and HDL-C were not statistically significant. None of the lifestyle behaviors evaluated were significantly correlated with Lp-PLA$_2$ activity. Furthermore, multiple regression analysis showed that apo B was significantly associated with Lp-PLA$_2$ adjusted for age, BMI, triglycerides, and LDL-C ($R^2=0.32$).

Discussion

In this cross sectional analysis of healthy adolescent boys, our main finding was that Lp-PLA_2 activity was positively associated with several lipid-related risk factors such as triglycerides, total cholesterol, LDL-C, apo B, triglyceride/HDL-C ratio, and CETP activity. However, after evaluation by multiple regression analysis, the strongest association of Lp-PLA_2 activity was with apo B adjusted for confounding variables. This correlation agrees with the role of apo B-containing lipoproteins, mainly LDL, as Lp-PLA_2 principal carrier [22].

Lp-PLA_2 promotes vascular inflammation through hydrolysis of oxidized phospholipids and generation of lysophosphatidyl choline and oxidized free fatty acids, which attract monocytes and exacerbate the atherogenic process [8]. On the other hand, some authors postulated that Lp-PLA_2 also has a cardioprotective function because it is implicated in the degradation of platelet-activating factor, a potent mediator of inflammation. Then, this dual role raises the question of which one, proatherogenic or antiatherogenic, is more prominent and under which conditions [8]. As plaque morphology may change with age, associations of Lp-PLA_2 with CVD may likewise vary with age [23].

Regardless the solid evidence from clinical studies that supports the measurement of Lp-PLA_2 in adult populations [2,4], it appears that this determination in apparently healthy adolescent boys does not identify individuals at risk of CVD further than LDL-C and apo B. Actually, LDL-C and apo B plasma concentrations were higher in obese than in non obese adolescents while Lp-PLA_2 activity was not different. Moreover, no significant correlations were found between Lp-PLA_2 activity and BMI or waist circumference and not even with lifestyle behaviors. Consistent with our results, in a previous report carried out in 10 year old schoolchildren, Lp-PLA_2 did not show a consistent association with obesity, whereas other inflammatory markers,

such as C reactive protein and interleukin-6, did [24]. Similarly, Lp-PLA_2 was not associated with obesity or lifestyle behaviors in adult populations [8]. Nonetheless, in a group of obese 11 year old children, a significant correlation between some anthropometric measures, indicative of central obesity, and Lp-PLA_2 has been reported [25]. Overall, it is likely that in children, Lp-PLA_2 association with central obesity might be only evidenced in obese children but not in cohorts where normal weight and/or overweight adolescents are included. Further on, Lp-PLA_2 activity was enhanced in adolescents with metabolic syndrome in agreement with previously reported data in adults [26,27].

In the present study, even though no significant correlation was evidenced between Lp-PLA_2 activity and HDL-C levels, the group of adolescents with Lp-PLA_2 activity above the median exhibited diminished HDL-C concentration. This result points out the fact that other factors might be responsible for the reduction in HDL-C concentration in adolescents with higher activities of Lp-PLA_2. Noteworthy, this group of adolescents also presented higher triglycerides; thus, it could be suggested that the decrease in HDL-C observed might be related to an impaired catabolism of triglyceride-rich lipoproteins. Interestingly, it has been reported that dyslipidemic patients treated with fenofibrate (which enhances very low and intermediate density lipoprotein catabolism) exhibited reductions in Lp-PLA_2 mass and/or activity [28,29]. Furthermore, CETP activity and the triglyceride/HDL-C ratio, which has been postulated as a marker of the proportion of small and dense LDL particles [30], were positively associated with Lp-PLA_2 activity. CETP has been implied in the generation of the highly atherogenic small and dense LDL subclass by different studies [31-33]. Therefore, the fact that Lp-PLA_2 activity might reflect small and dense LDL levels in adolescents deserves further investigation.

The cross-sectional nature of the study, did not allow the analysis of the predictive value of Lp-PLA_2 activity, thus the question whether Lp-PLA_2 activity identifies individuals susceptible to CVD earlier in life has not been analysed. For sure, future longitudinal studies will address this question. Also, only apparently healthy adolescent boys were studied; therefore, the extrapolation of the present results to other populations might be limited. The strengths of this study included the large sample of healthy adolescents, which was more likely to represent the general population, the high response rate of the adolescents, the use of Tanner staging for measurement of puberty, the collection of fasting blood samples, and the use of multiple regression models, which allowed investigators to account for the complex interrelationships between these physiologic traits and potential confounder variables.

In conclusion, in healthy adolescents, Lp-PLA_2 activity is associated with several lipid-related risk factors. As plaque morphology may change with age, associations of Lp-PLA_2 with CVD may likewise vary with age. However, in adolescent boys, its clinical utility to identify individuals at risk of CVD seems to be limited, at least in comparison to LDL-C and apo B levels.

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Acknowledgements

This work was supported in part by grants from University of Buenos Aires (UBACYT B403 and 20020090200017), Fiorini's Foundation, and from CONICET (PIP 931). Tomás Meroño and Leonardo Gómez Rosso are research fellows from CONICET. We would like to acknowledge Mirta Noemi Ismael for help examining the adolescents.

TABLE 1: Clinical and metabolic characteristics according to Lp-PLA$_2$ activity.

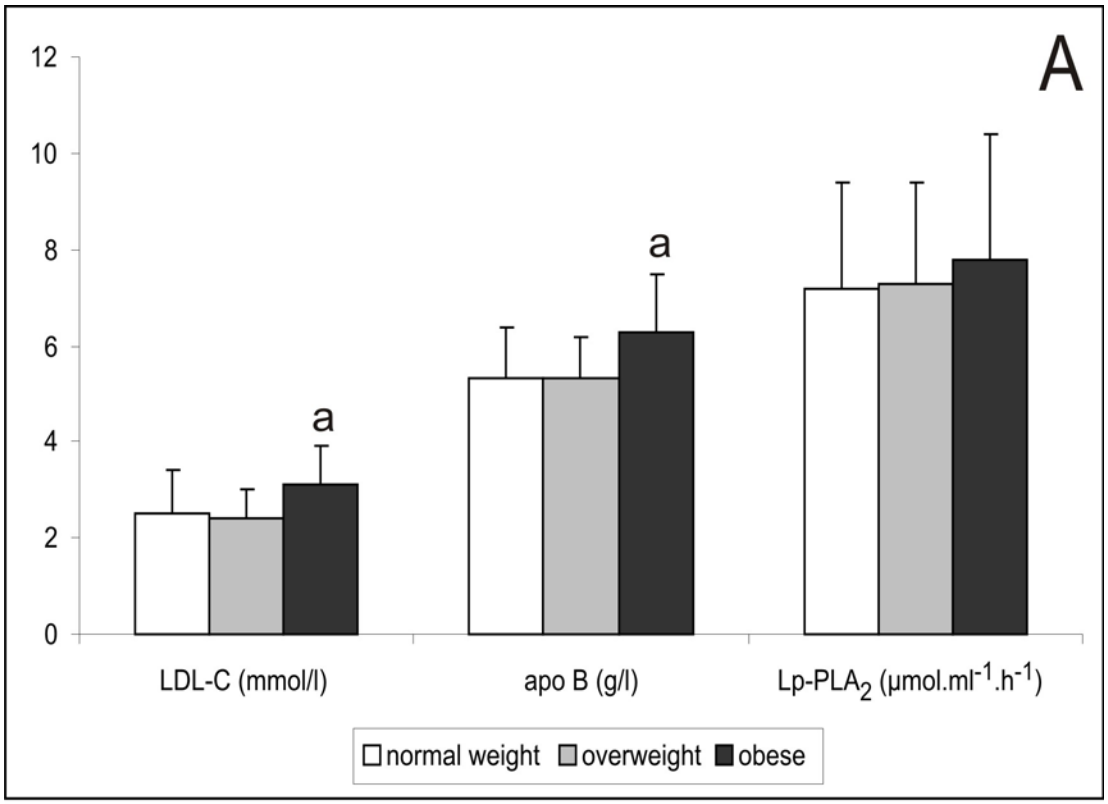
		Lp-PLA$_2$ activity (median=7 µmol/ml.h)		
	Study population	**< median**	**≥ median**	**p<**
N	164	82	82	
Age (years)	17±2	16±2	17±2	0.001
z- BMI	0.97±0.96	0.83±0.89	1.08±1.03	NS
Waist (cm)	84±13	82±12	86±14	0.05
DBP (mmHg)	72±9	71±8	73±9	NS
SBP (mmHg)	116±11	114±11	118±11	0.05

Glucose (mmol/l)	4.56±0.61	4.50±0.55	4.61±0.61	NS
Insulin (mU/l)	6.3 (4.3-8.3)	6.3 (4.3-8.1)	6.4 (4.1-8.9)	NS
HOMA-IR	1.2 (0.8-1.8)	1.2 (0.8-1.6)	1.3 (0.8-1.8)	NS
TG (mmol/l)	0.88 (0.65-1.20)	0.81 (0.61-0.90)	1.02 (0.72-1.34)	0.001
TC (mmol/l)	4.05±0.87	3.69±0.77	4.41±0.85	0.0001
HDL-C (mmol/l)	1.13±0.31	1.15±0.36	1.10±0.23	0,001
LDL-C (mmol/l)	2.64±0.87	2.28±0.77	3.00±0.82	0.0001
TG/HDL-C	2.4±1.9	2.0±1.6	2.6±2.0	0.05
Apo B (g/l)	5.6±1.2	5.0±0.9	6.1±1.2	0.0001
CETP (%/ml.h)	151±36	145±33	158±37	0.05

Lp-PLA_2, lipoprotein-associated phospholipase A_2; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, Homeostasis Model Assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; CETP, cholesteryl ester transfer protein; NS, non significant. Data are expressed as media±S.D. or as median (Q1-Q3).

Figure 1: Panel A, LDL-C, apo B and Lp-PLA_2 activity in adolescent boys classified according to body weight. Obese a $p<0.01$ *vs.* normal weight and overweight adolescents (One way Anova test).

Panel B, LDL-C, apo B and Lp-PLA_2 activity in adolescents classified according to the presence of metabolic syndrome. a $p<0.01$; b $p<0.05$ *vs.* adolescents without metabolic syndrome (Student t test).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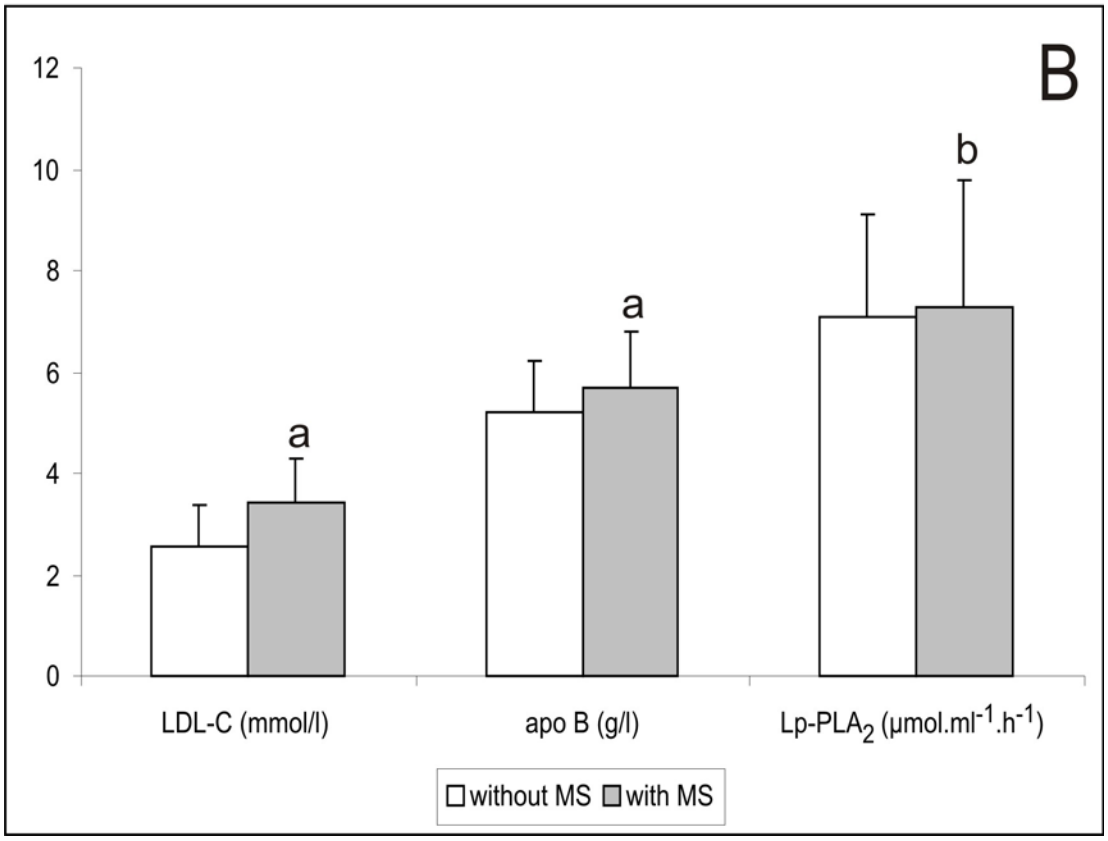
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LDL-C (mmol/l)
apo B (g/l)
Lp-PLA2 (µmol.ml-1.h-1)
without MS
with MS