

Role of secretory phospholipase A₂ in women with metabolic syndrome

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Background & objectives: Secretory phospholipase A₂ (sPLA₂), a member of the phospholipase A₂ superfamily of enzymes that hydrolyses phospholipids, is a potentially useful plasma biomarker for atherosclerotic cardiovascular disease. Cardiovascular diseases are the leading cause of mortality in women. The purpose of this study was to investigate the correlation between cardiovascular risk factors and the sPLA₂ levels in women with metabolic syndrome as compared to women without metabolic syndrome and men with metabolic syndrome.

Methods: Patients (n=100) with various cardiovascular risk factors consecutively evaluated at the Rehabilitation Hospital-Cardiology Department, Cluj-Napoca, Romania were enrolled during 2011, of whom 10 were excluded. The patients were divided in three groups: group 1 (37 women with metabolic syndrome), group 2 (27 men with metabolic syndrome), and group 3 (26 women without metabolic syndrome). Body weight, smoking habits, glycaemia, hypertension, and serum lipids fractions were analysed as cardiovascular factors. Serum sPLA₂ activity was measured using the chromogenic method.

Results: There were no statistically significant correlations between sPLA₂ levels and the investigated risk factors, irrespective of patient groups. However, there were significant positive correlations between sPLA₂ and hsCRP in all three groups ($P<0.05$). In women with no metabolic syndrome a negative correlation was found between sPLA₂ levels and HDL-C- $r=-0.419$, $P=0.03$. In men with metabolic syndrome there was a direct correlation between sPLA₂ levels and HOMA, $r=0.43$, $P<0.05$, 95% CI (-0.098; 1.15).

Interpretation & conclusions: Women with metabolic syndrome did not display different sPLA₂ levels as compared to men with metabolic syndrome and women without metabolic syndrome. However, women with metabolic syndrome demonstrated a low but positive correlation between sPLA₂ and hsCRP levels.

Key words Cardiovascular risk factors - metabolic syndrome - sPLA₂

Cardiovascular diseases are the leading cause of death in women¹; in Europe almost 55 per cent of mortality in women is caused by cardiovascular diseases, chiefly coronary disease and stroke². In this context, the present research takes into special consideration the novel markers of cardiovascular risk³, under the circumstances in which cardiovascular risk factors and charts downplay cardiovascular risk in women. Moreover, endothelial dysfunction and inflammation play a major role in the pathogenesis of microvascular angina, common among women⁴. The respective markers may be divided into three categories: inflammation, haemostasis, and other factors. It is well known that lipoprotein (a) is highly atherothrombotic, and its elevated levels increase the risk of cardiovascular disease⁵ and represent an independent risk factor for coronary disease⁶. The lipoprotein (a) is shown to be correlated to the progression, extension, and severity of the coronary disease, as well as to a negative prognostic after myocardial infarction⁶. There is evidence that its levels increase with age in women⁶. Elevated levels in women indicate an independent atherogenic risk factor both in pre- and postmenopausal women⁷. It has been shown that high lipoprotein levels in women are associated with an increasing risk of cerebrovascular accident (CVA)⁸. There are studies which show that secretory phospholipase A₂ (sPLA₂) levels are more elevated in women than in men, along with an increase in C-reactive protein (CRP) levels⁹.

There is evidence that the sPLA₂ hydrolyses phospholipids, generating free fatty acids and lysophospholipides, which lead to an increase of proinflammatory factors in the arterial wall¹⁰. These have a chemotactic and oxidative effect on the smooth muscular arterial cells and on the monocytes/macrophages. sPLA₂ also determines the generation of platelet-activating factor (PAF), with proatherogenic and procoagulant role¹⁰.

The present study was undertaken to find out the relationship between sPLA₂ levels and cardiovascular risk factors in women with metabolic syndrome (MS) and compare with women without MS and men with MS.

Material & Methods

A total of 100 patients with various cardiovascular risk factors consecutively evaluated at the Rehabilitation Hospital-Cardiology Department, Cluj-Napoca, Romania, were retrospectively included in the study, of whom 10 were excluded because some of the laboratory

parameters were not determined accurately. Patients with ischaemic heart disease were also excluded. They were divided in three groups: group 1 (37 women with metabolic syndrome), group 2 (27 men with metabolic syndrome), and group 3 (26 women without metabolic syndrome). The study was carried out in 2011. As cardiovascular risk factors body weight, smoking habits, glycaemia, hypertension, serum lipids fractions and high sensitivity C-reactive protein (hsCRP) were analysed. Blood pressure was measured according to standard protocol as the mean of two readings after the participant was at rest for 5 min in a sitting position. The metabolic syndrome was defined according to the criteria of the International Diabetes Federation¹¹. Body mass index (BMI) (kg/m²) was derived from height and weight measured in the clinic with participants wearing light clothing and no shoes. Blood samples (10 ml) were obtained by venipuncture according to the standard Lipid Research Clinics Protocol¹². Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula¹³. The homeostasis model assessment (HOMA-IR) was used to estimate insulin resistance [(HOMA-IR (Insulin resistance) (mmol/LxμU/ml) = fasting glucose (mmol/l) X fasting insulin (μU/ml)/405)]. Plasma glucose levels were measured by the glucose oxidase method¹⁴. The hsCRP was analyzed by chemiluminescent immunometric assay (IMMULITE 2000)¹⁵. sPLA₂ levels were measured by Cayman's secretory PLA₂ (sPLA₂) assay kit (Cayman Chemical Company, Ann Arbor MI, USA). The detection range of the assay was from 0.02 to 0.2 μU/min/ml of sPLA activity, which was equivalent to an absorbance increase of 0.01 to 0.1/min.

Arterial stiffness (pulse wave velocity) was measured using TensioMedTMArteriograph (KFT, Hungary). The study protocol was approved by the ethics committee of University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Statistical analysis: Microsoft Excel 2007 (v.6.0: Microsoft Corporation, Redmond, WA), Epiinfo 2000 (v.6.0, EpiInfo Development team CDC, GA, Atlanta) and SPSS 13 (v.13.0; IBM Corporation, Armonk, NY, USA) for Windows were used for data analysis. The data were presented as mean ± 1 SD or percentages when appropriate. First, the data normality was tested by applying the Kolmogorov-Smirnov test. Then, in agreement with the results, either Levene's test for equality of variances or Bartlett's chi square test was applied. In the former case, the results led to the application of the independent samples t test- equal

variances. In Bartlett's chi square test, Mann-Whitney (Wilcoxon Rank Sum) or the Student t-test was applied. In the case of dichotomous variables, depending on the context, either Fisher or chi-square test was applied.

Results

Male patients with metabolic syndrome displayed a significantly elevated glycaemia than women with MS ($P<0.01$). On the contrary, women without MS had higher levels of total cholesterol and LDL-C ($P=0.01$, $P<0.05$). HDL-C was also more elevated in women with MS, ($P<0.01$) (Table).

In the remaining cardiovascular risk factors, there were no significant differences between the sexes. There were no significant differences in sPLA₂ levels among between the three groups of patients (Table).

To assess whether there were correlations between cardiovascular risk factors, and sPLA₂ the analyses were done. In general, there were no significant correlations between the sPLA₂ levels and either of the studied risk factors, irrespective of the group of patients under examination. However, in women with metabolic syndrome there was a low positive correlation ($r=0.141$, $P<0.05$) between sPLA₂ levels and hsCRP (Fig. 1). But the same correlation appeared

to be present in women with no metabolic syndrome also (Spearman coefficient $\rho=-0.243$, $P<0.05$). A significant correlation between sPLA₂ and hsCRP was also found in men with metabolic syndrome ($-r=0.39$, $P<0.05$) (Fig. 2). At the same time, the female patients without metabolic syndrome displayed a significantly inverse correlation between sPLA₂ and HDL-C- $r=-0.419$, $P<0.05$ (Fig. 3). In men with metabolic syndrome there was a direct relationship between sPLA₂ and HOMA, $r=0.43$, $P<0.05$) (Fig. 4).

Discussion

Traditional risk factors can predict the risk of cardiovascular disease in many, but not in all, patients. About 10 to 20 per cent patients with coronary heart disease display no identifiable risk factor¹⁶. Many studies have established that lipoprotein associated phospholipase A2 (Lp-PLA2) is a cardiovascular marker independent of but correlated and augmentative to established risk factors¹⁷. Lp-PLA2 is produced by the inflammatory cells involved in atherogenesis and is accumulated in atherosclerotic lesions. It is well known that about 70 - 80 per cent of the Lp-PLA2 circulates in blood bound to LDL, and the rest to Lp(a), HDL cholesterol, and a very small quantity to VLDL¹⁸.

Table. Demographic and clinical features of the study participants

Variable	Females with metabolic syndrome (n=37)	Females without metabolic syndrome (n=26)	Males with metabolic syndrome (n=27)
Age (yr)	57.46 ± 10.73	53 ± 10.40	61.41 ± 9.82
Smokers (%)	10.81*	30.76	22.22 ⁺⁺
BMI (kg/m ²)	29.54 ± 4.02 ^{**}	26.17 ± 4.43	30.57 ± 3.91
Glycaemia (mg/dl)	100.68 ± 16.2 ^{***}	86.15 ± 9.65	113.67 ± 26.70 ⁺
HOMA	2.23 ± 2.01 ^{**}	1.16 ± 0.73	2.30 ± 1.08
Total C (mg/dl)	221.24 ± 41.85	225.88 ± 9.65	192.93 ± 46.0 ⁺⁺
LDL-C (mg/dl)	141.14 ± 34.38	150.42 ± 24.77	115.78 ± 35.30 ⁺⁺
HDL-C (mg/dl)	44.95 ± 6.78 ^{***}	51 ± 7.37	38.89 ± 10.60 ⁺⁺
TG (mg/dl)	175.89 ± 83.21 [*]	118.62 ± 49.17	195.52 ± 82.13 ⁺⁺
Hypertension (%)	72.97 [*]	38.5	85.18
Diabetes (%)	27.02	-	33.33
hsCRP (mg/dl)	3.43 ± 3.52	2.84 ± 4.54	4.29 ± 4.93
PWVAo (m/sec)	9.98 ± 2.01	10.32 ± 2.71	10.57 ± 1.82
sPLA2 (µmol/min/ml)	5.69 ± 1.41	5.44 ± 1.0	5.63 ± 1.33

BMI, Body mass index; Total C, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; hsCRP, high sensitivity C reactive protein; PWVAo, pulse wave velocity on aorta; sPLA₂, secretory phospholipase A2

* $P<0.05$, ** <0.01 , *** <0.001 compared with women without metabolic syndrome

⁺ $P<0.05$, ⁺⁺ <0.01 compared with women with metabolic syndrome

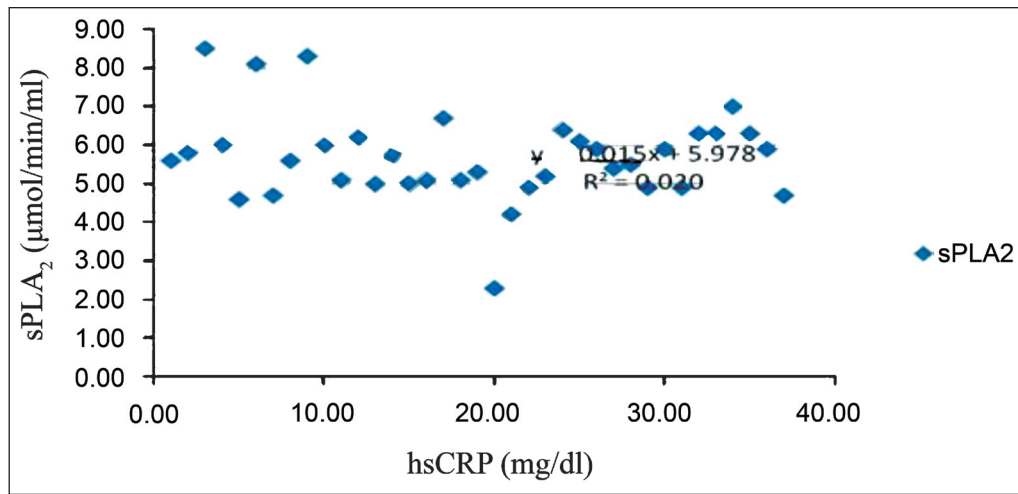


Fig. 1. Correlation between sPLA₂ (µmol/min/ml) and hsCRP (mg/dl) in women with metabolic syndrome.

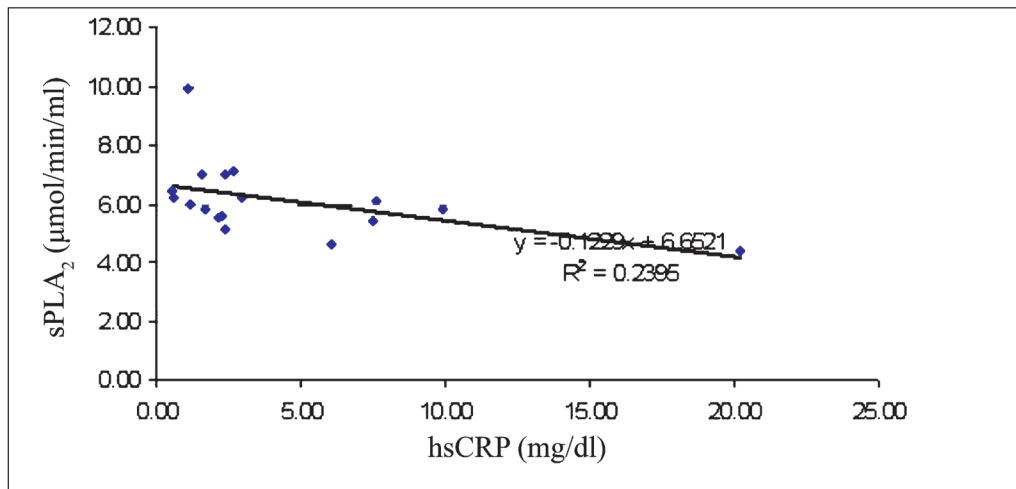


Fig. 2. Correlation between sPLA₂ (µmol/min/ml) and hsCRP (mg/dl) in men with metabolic syndrome.

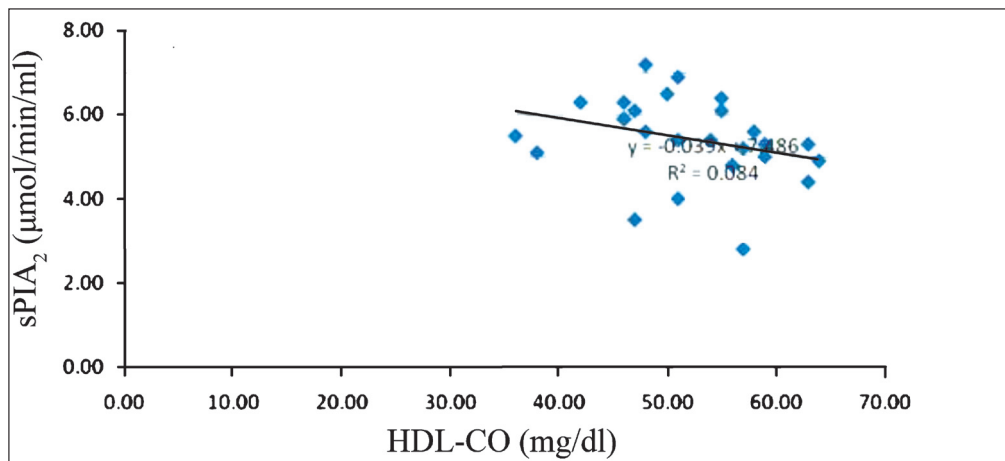


Fig. 3. Correlation between sPLA₂ (µmol/min/ml) and HDL-C (mg/dl) in women without metabolic syndrome.

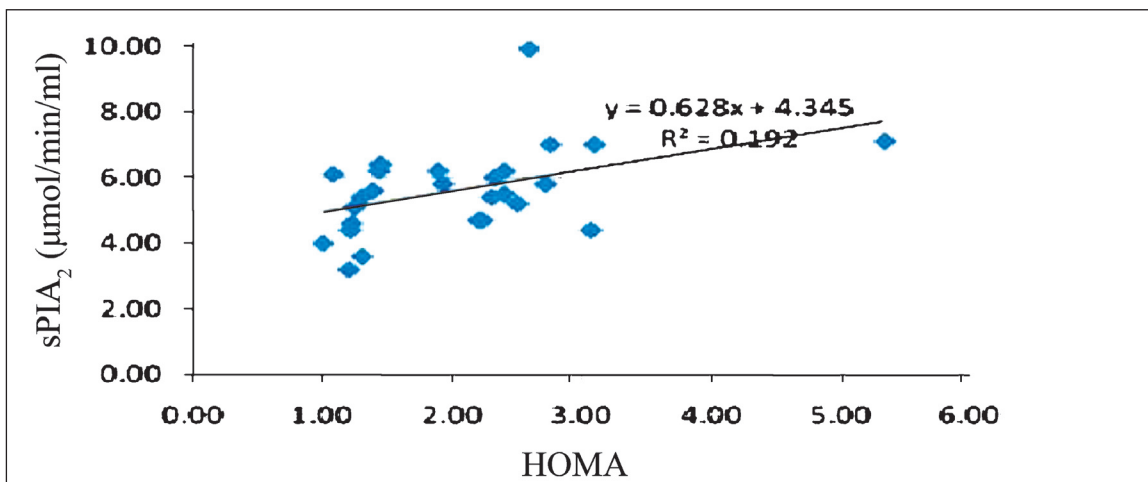


Fig. 4. Correlation between sPLA₂ (µmol/min/ml) and HOMA in men with metabolic syndrome.

Secretory phospholipase A2 (sPLA₂), a member of the phospholipase A2 superfamily of enzymes, is a potentially useful plasma biomarker for atherosclerotic cardiovascular disease. The 2010 ACCF/AHA (American College of Cardiology Foundation / American Heart Association) Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults¹⁹ recommends that Lp-PLA2 might be reasonable for cardiovascular risk assessment in intermediate-risk asymptomatic adults (IIBB). Gong *et al*²⁰ found increased plasma levels of Lp-PLA2 in patients with metabolic syndrome.

There are five major classes of PLA2: the secreted and cytosolic forms, the calcium-independent PLA2s, the lysosomal PLA2s and the platelet-activating factor acetylhydrolases (PAF-AH, which include Lp-PLA2 and are also calcium independent)¹⁰. sPLA2 is also involved in atherogenesis in several ways: it changes the affinity of LDL particles for extracellular matrix proteins²¹⁻²³, with their accumulation in the arterial walls, favours lipid peroxidation²⁴ and mediates the hydrolysis of certain lysophospholipids and free fatty acids²⁵. Circulating levels of sPLA2 are higher in patients with documented CAD than in controls²⁶.

We found an inverse correlation between sPLA2 levels and HDL cholesterol levels in women without metabolic syndrome. An increase in HDL-C levels has been shown to be accompanied by a decline in the levels of inflammatory factors²⁷. An increase in the sPLA2 activity determines the hydrolysis of HDL-C particles both within and without the context of inflammatory processes^{28,29}. sPLA2 may determine changes in the

HDL-C levels that can eventually upset the beneficial mechanisms involved in the macrophage cholesterol efflux²⁹.

An association was seen between sPLA₂ and CRP levels, which represents both acute-phase proteins and markers of vascular inflammation, in patients with and without metabolic syndrome. Koenig *et al*³⁰ also found a significant direct correlation between the levels of sPLA2 type IIA and CRP. Another study conducted in patients with insulin resistance, documented correlations between sPLA2 and CRP, as well as interleukin (IL)-6 and soluble adhesion molecules³¹. The question arises whether sPLA2 also plays a part in the pathogenesis of metabolic syndrome. Ravoux *et al*³² have shown that the ligands of all three peroxisome proliferator activated receptors (PPARs) in the vascular smooth muscle may determine a decrease in the sPLA2 expression. Results from UDACS study demonstrated that in patients with type 2 diabetes mellitus, changes in the *PLA2G2A* genes might alter sPLA₂ levels³³.

Pulse wave velocity on aorta (PWVAo) is deemed to be an investigation that may contribute in assessing individual cardiovascular risk, but is still not used on a large scale. No significant correlation was recorded between sPLA₂ and PWVAo in the present study. Men with metabolic syndrome showed higher values of both sPLA₂ and PWVAo than women with MS.

In conclusion, women with metabolic syndrome did not display different sPLA₂ values as compared to men with metabolic syndrome and women without metabolic syndrome, irrespective of traditional cardiovascular

risk factors taken into account. However, sPLA₂ levels increased with the increase in hsCRP levels in women with MS.

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