LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 IS INCREASED IN PATIENTS WITH IMPAIRED BONE DENSITY

FOSFOLIPAZA A2 UDRUŽENA SA LIPOPROTEINOM JE POVIŠENA KOD PACIJENATA SA SMANJENOM GUSTINOM KOSTIJU

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Summary

Background: Increased levels of lipoprotein-associated phospholipase A2 are associated with atherosclerosis, and may contribute to cardiac disease. The aim of this study was to analyze serum levels of lipoprotein phospholipase A2 (Lp-PLA2) in patients with impaired bone resorption and correlate the findings with markers of bone metabolism (osteocalcin) and other biochemical markers (cholesterol, low density lipoprotein, triacylglycerols).

Methods: Serum Lp-PLA2 was measured by a turbidimetric method in a group of currently treated 85 patients with impaired bone resorption and in a control group of 46 healthy individuals. Serum triacylglycerols was measured by the electrochemiluminescence immunoassay. Cholesterol, low density lipoprotein and triacylglycerols were measured by commercially available enzymatic assays. Bone density was investigated by dual energy X-ray densitometry performed on the lower spine and hips.

Results: Concentrations of LP-PLA2 were significantly elevated in the patients with bone resorption compared to the control group of healthy individuals (225 ng/mL vs. 192 ng/mL, p<0.001) with the highest difference in patients with a T score below −2.5 SD (227 vs. 192 ng/mL). Serum

List of abbreviations: Lp-PLA2, lipoprotein-associated phospholipase A2; PLA2, phospholipase A2; DXA, dual-energy x-ray absorptiometry; PAF-AH, platelet activating factor acetylhydroxylase; LDL, low density lipoprotein; SD, standard deviation; OR, ODDS ratio; PAF, platelet activating factor; ECLIA, electrochemiluminescence immunoassay.
levels of Lp-PLA2 also negatively correlated with decreased levels of serum osteocalcin in patients, and a significant difference in Lp-PLA2 (p=0.02) levels was observed between the control group and group with low levels of osteocalcin. Elevated Lp-PLA2 levels were significantly associated with the therapeutic procedures used, but not with age, gender and concentration of lipids.

**Conclusions:** Lipoprotein-associated phospholipase A2 seems to play an important role also in bone metabolism.

**Keywords:** lipoprotein-associated phospholipase A2, osteocalcin, bone metabolism

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**Introduction**

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a circulating enzyme belonging to the unrelated phospholipase A2 protein families with common enzymatic activity. The two most notable families are secreted phospholipases A2 and cytosolic phospholipases A2. Other families include Ca2+ independent PLA2 (iPLA2) and lipoprotein-associated PLA2, also known as platelet activating factor acetylhydrolase (PAF-AH). Recent evidence has established physiological and pathological roles of PLA2 enzymes in fertility, muscle growth, renal concentration, post-ischemic brain injury, inflammatory and oxidative activities associated with cardiovascular disease and ischemic stroke, inflammatory bone resorption, intestinal polyposis, pulmonary fibrosis, acute respiratory distress syndrome and autoimmune encephalomyelitis (1).

Cytosolic phospholipases preferentially hydrolyze phospholipids containing arachidonic acid and play a key role in the biosynthesis of eicosanoids including prostaglandins and leucotrienes (2). Hydrolysis leads to release of free arachidonic acid which in turn is metabolized to prostaglandins. Prostaglandin (especially prostaglandin E2) is produced by osteoblasts and acts as a potent stimulator of bone resorption (1, 5).

The Lp-PLA2 is platelet-activating factor (PAF) acetylhydrolase (EC 3.1.1.47) catalyzing the degradation of PAF to inactive products by hydrolysis of the acetyl group at the sn-2 position (4). Lp-PLA2 shows mainly proinflammatory and oxidative activities preferably associated with cardiovascular disease (5). The association of Lp-PLA2 with bone resorption is not yet well known, and thus it seems interesting to analyze the serum levels of Lp-PLA2 in patients with impaired bone density.

**Materials and Methods**

**Patients**

Eighty-five patients with various levels of bone density (4 males, mean age 56 years, and 81 post-menopausal women, mean age 70 years) were enrolled in the study. The patients were previously clinically classified for bone mineral density. According to the recent bone density guidelines, the patients were divided into three groups. Group I consists of 45 patients with osteoporosis, group II consists of 20 patients with osteopenia and group III consists of 22 patients with normal bone density. Fifty-seven patients were currently on combined lipid lowering and antiresorption therapy (statins and bisphosphonates), while 11 patients were already on lipid lowering therapy (statins). Seventeen patients were supplemented with vitamin D3.

We compared the patients with a group of 46 healthy individuals (14 males, mean age 55 years, and 32 females, mean age 47 years) without any signs of bone resorption and thus without any therapy. Characteristics of the individuals and patients included in the study are listed in **Table I**.

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**Table I** Characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=46)</th>
<th>All patients (N=85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>55</td>
<td>67</td>
</tr>
<tr>
<td>Males/Females</td>
<td>14/32</td>
<td>4/81</td>
</tr>
<tr>
<td>S-Lp-PLA2 (ng/mL)</td>
<td>192 (159–227)</td>
<td>225 (193–253)</td>
</tr>
<tr>
<td>p &lt;0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>S-Osteocalcin (µg/L)</td>
<td>30.3 (17.6–37)</td>
<td>18.4 (14.2–20.6)</td>
</tr>
<tr>
<td>p &lt;0.05</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>S-LDL cholesterol (mmol/L)</td>
<td>3.1 (1.0)</td>
<td>2.9 (0.7)</td>
</tr>
<tr>
<td>S-Cholesterol (mmol/L)</td>
<td>5.2 (1.0)</td>
<td>5.1 (0.7)</td>
</tr>
<tr>
<td>S-Triacylglycerols (mmol/L)</td>
<td>1.34 (1.02–1.88)</td>
<td>1.13 (0.86–1.5)</td>
</tr>
<tr>
<td>p = 0.03</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations are expressed as mean with (SD), or median and interquartile ranges (25th–50th percentile).
Sample collection

Blood specimens were collected by venipuncture in vacuum collection tubes. After collection, specimens were centrifuged (4 °C at 1500 G for 10 minutes). Serum osteocalcin, total cholesterol, LDL-cholesterol levels, and triglycerides were immediately investigated.

Prior to Lp-PLA2 testing, aliquots of serum were stored at 2–8 °C overnight and assayed on the next day after the blood collection (2nd day after the collection of blood).

Biochemical analysis

Serum levels of Lp-PLA2 were measured under conditions specified in the manufacturer’s instruction, by a commercially available turbidimetric assay for the quantitative determination of Lp-PLA2 (PLAC Test, DiaDexus, San Francisco, USA) on an automatic biochemical analyzer Advia 1800 (Siemens). The reference values for Lp-PLA2 provided by the manufacturer of the diagnostic kit were less than 200 ng/mL.

Expected values for serum levels of osteocalcin were assayed by the commercially available electrochemiluminescence immunoassay (ECLIA Roche, Mannheim, Germany) on an automatic analyzer CO-BAS e411 (Roche). Serum levels of cholesterol, LDL-cholesterol and triglycerides were assayed by the commercially available direct enzymatic assays on an automatic biochemical analyzer Advia 1800 (Siemens).

Bone density investigations

Dual-energy x-ray absorptiometry (DXA) performed on the lower spine and hips was used to evaluate the bone density. Patients were divided into three groups according to their bone density as defined by the T-score. The T-score is a person’s bone mass at a particular site, expressed in standard deviations (SD) away from the mean of a reference population. T-score above –1 SD is considered normal, T-score between –1 and –2.5 SD is classified as osteopenia (low bone mass) and T-score below –2.5 SD is defined as osteoporosis.

Statistical analysis

Shapiro-Wilk normality test was used to determine the distribution of the data. One-way analysis of variance with Newmann-Keuls multiple test or unpaired T-test was used if the distribution of data was normal, and in case of non-parametric data distribution, Kruskal-Wallis test or Mann-Whitney U-test were used to evaluate the Lp-PLA2 levels in selected groups. Fisher’s exact test and odds ratio were used to investigate the association between Lp-PLA2 and lipid parameters (total cholesterol, LDL-cholesterol) or age, sex and therapy.

A value of p<0.05 was considered statistically significant. Statistical software GraphPad Prism, version 6.0 (San Diego, California) was used to perform the statistical analysis.

Results

Serum levels of Lp-PLA2 were measured in the control group of healthy individuals and in the whole group of patients. The median Lp-PLA2 value in patients was significantly elevated in comparison with healthy individuals (225 ng/mL vs. 192 ng/mL, p<0.001 – Mann-Whitney U-test).

Patients were divided into three groups (osteoporosis, osteopenia and normal bone density) according to the T-score estimated from densitometry. T-score is expressed as the standard deviation of bone density from a reference population, as described previously.

Medians of Lp-PLA2 concentrations in patients with osteoporosis, osteopenia and normal bone density according to DXA were significantly elevated, contrary to the control group, with the highest difference in patients with osteoporosis and osteopenia (227 ng/mL and 222 ng/mL vs. 192 ng/mL, p=0.004 and p=0.005 – Mann-Whitney U-test). Concentration of Lp-PLA2 in patients with normal

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Lp-PLA2 (ng/mL)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals</td>
<td>46</td>
<td>192 (159–227)</td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>85</td>
<td>225 (193–253)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>T &gt; –1</td>
<td>20</td>
<td>221 (192–251)</td>
<td>0.024*</td>
</tr>
<tr>
<td>T = –1 to –2.5</td>
<td>22</td>
<td>222 (198–250)</td>
<td>0.005*</td>
</tr>
<tr>
<td>T &lt; –2.5</td>
<td>43</td>
<td>227 (187–263)</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

Results expressed as median and interquartile range (25th and 75th percentile)

*pValue of p<0.05 is considered as statistically significant. Differences between concentrations in patients with osteoporosis, osteopenia and normal bone density according to DXA were considered not significant (p=0.76 Newmann-Keuls multiple comparison test).
Results expressed as median and interquartile range (25th and 75th percentile).

*Value of p<0.05 is considered as statistically significant. Differences between concentrations in patients with osteoporosis, osteopenia and normal bone resorption according to DXA are considered not significant (p=0.11, Newman-Keuls multiple comparison test).

Discussion

Our results support the evidence for a significant role of phospholipase A2 in the metabolic processes of bone metabolism. It is well known that phospholipase A2 is involved in the metabolism of prostaglandins (particularly prostaglandin E2) acting as a stimulator of bone resorption. The lipoprotein phospholipase A2 belongs to a subgroup of the Ca^{2+} independent PLA2 family with a unique substrate preference for lysophospholipids, and thus is mainly involved in atherosclerotic processes. Lp-PLA2 is responsible for generating two proinflammatory mediators following the oxidation of LDL: lysophosphatidylcholine and oxidized fatty acid, and thus is significantly associated with cardiovascular diseases and ischemic stroke (5). The cut off value defined in clinical guidelines for the management of cardiovascular disease is set at 200 ng/mL (6). Our results show that medians of serum Lp-PLA2 in patients with impaired bone remodeling (according to DXA) ranged from 221 to 227 ng/mL, and were therefore elevated above the approved cut off value for cardiovascular disease. This finding could lead to the question of whether these patients with impaired bone metabolism are also at elevated risk of cardiovascular events. Nevertheless, patients were currently clinically investigated and treated by standard therapy (vitamin D3, bisphosphonates, statins). Bisphosphonates are known as powerful inhibitors of bone resorption (7). It is well known that decreased levels of serum osteocalcin correlate with long-term bisphosphonates treatment. This decrease was confirmed in the group of patients with impaired bone resorption, as shown in Table I.

We found a negative correlation of Lp-PLA2 levels with decreased levels of serum osteocalcin (Pearson correlation coefficient r=-0.28). This suggests that Lp-PLA2 might be an additional, promising biochemical marker of bone metabolism, however, this needs to be verified on a larger number of samples.

Recent data show that bisphosphonates may also provide protective effects against cardiovascular events including acute myocardial infarction (8).
Lp-PLA₂ activity correlates with lipid parameters. A strong positive correlation has been demonstrated consistently with LDL and total cholesterol in many epidemiological studies (9–15). We confirmed this correlation in our study (Pearson correlation coefficient r=0.93). We analyzed the association of lipid parameters with Lp-PLA₂ levels and we found this association not significant (p=0.64, Fischer’s exact test, odds ratio – OR=0.76). This suggests that the increased concentration of Lp-PLA₂ in our group of patients with impaired bone density was independent of the levels of LDL-cholesterol and total cholesterol. Although the control group is younger than the patient group (55 vs. 67 years), there was no significant association of Lp-PLA₂ levels with age (OR=1.48, p=0.49). Lp-PLA₂ levels are also independent of gender (OR=1, p=0.67). Serum levels of Lp-PLA₂ were significantly associated with the therapeutic procedures used. We found a significant association of Lp-PLA₂ levels with combined antiresorption and with lipid lowering therapy using bisphosphonates and statins (OR=2.97, p=0.007). Relationship of Lp-PLA₂ concentrations with antiresorption therapy using bisphosphonates was also significant (OR=3.36, p=0.014). On the contrary, the association with statin therapy and vitamin D₃ supplementation was not significant (p=0.09 and p=0.15).

The use of Lp-PLA₂ as a marker of bone resorption in routine laboratory practice implies some analytical specifications including specimen handling, sample storage and the time of analysis. Although Lp-PLA₂ is transported coupling with either LDL, or HDL and lipoprotein (a), it is necessary to perform the assay after sample stabilization during 16 hours at 2–8 °C, and the assay could be performed between 16–72 hours after the blood collection when Lp-PLA₂ is eliminated from coupling with lipoproteins. Alternatively, the assay could be performed on the second day after freezing overnight at less than –20 °C.

**Conclusion**

Lipoprotein-associated phospholipase A₂ seems to play an important role in bone metabolism. However, more analyses need to be performed to confirm its significance.

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**Conflict of interest statement**

The authors stated that they have no conflicts of interest regarding the publication of this article.

**References**


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