Positve correlation between adipocyte fatty acid-binding protein and epicardial fat in patients with a family history of cardiovascular disease

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Background. Adipocyte fatty acid-binding protein (A-FABP) is a promising link between metabolic syndrome and atherosclerosis. Epicardial fat (EPI) is an independent risk factor for cardiovascular disease (CVD).

Objective. The aim of this pilot study was to evaluate the correlation between EPI and A-FABP in asymptomatic patients with a family history of CVD.

Methods. 59 subjects (39 males) (median = 54 years old) were enrolled in the study and their EPI thickness and A-FABP levels were assessed.

Results. EPI was found in 46 patients (77.9%). There were positive correlations between EPI and A-FABP (r=0.336; \( P = 0.010 \)), age (r=0.526; \( P < 0.001 \)), fibrinogen (r=0.304; \( P = 0.023 \)) and systolic blood pressure (r=0.279; \( P = 0.034 \)). A positive correlation was found between EPI and A-FABP in a subgroup of overweight and obese patients (0.389; \( P = 0.041 \), 0.407; \( P = 0.004 \)) and in the subgroup of patients with excluded CVD (r=0.368; \( P = 0.006 \)).

Conclusions. We found a positive correlation between EPI and A-FABP in a group of patients with a family history of CVD and in subgroups of overweight and obese patients.

Key words: Adipocyte fatty acid binding protein, epicardial fat, risk of cardiovascular disease, echocardiography

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death in Europe and USA. There is solid evidence that visceral fat plays an essential role in the development of metabolic and cardiovascular diseases. Ectopic fat deposition has been implicated in the pathophysiology of diabetes, insulin resistance and obesity-related disorders1

Epicardial fat (EPI) is a local deposition of visceral adipose tissue; its occurrence is connected with obesity, diabetes, arterial hypertension and other cardiovascular diseases, and malignant neoplasms2,3. EPI is correlated with various cardiovascular risk factors, independent of abdominal visceral adiposity, body mass index, hypertension and diabetes mellitus4. Two population studies showed that EPI is an independent risk predictor for cardiovascular disease4,5. Adipose tissue is a metabolically active organ secreting adipocytokines and EPI has a considerable secretory activity (more mRNA, protein for IL-1ß, IL-6 and TNFα (ref.6).

Adipocyte fatty acid-binding protein (A-FABP, also known as FABP4 or aP2) is one out of nine members of the family of fatty acid-binding proteins (FABPs) (ref.6). According to Hsu et al., the fasting level of A-FABP is positively correlated with metabolic syndrome diagnostic criteria7. A-FABP may be a promising link between metabolic syndrome and atherosclerosis8 and a new biomarker for predicting the development of type II diabetes mellitus9. In mouse models, a protective effect of A-FABP deficiency on the development of atherosclerosis was demonstrated10. Several studies reported a correlation between serum A-FABP levels and coronary artery disease11,12, with CVD patients having significantly higher A-FABP levels. In a study on A-FABP in atherosclerotic plaques, Peeters et al. found that A-FABP levels correlated with unstable plaque characteristics and symptomatic lesions13. Doi et al. reported that A-FABP levels in males under 65 years of age are significantly associated with coronary artery disease, independent of established risk factors14. Our team has been studying this biomarker for
several years in patients with obstructive sleep apnea as well as in patients with increased cardiovascular risk.\textsuperscript{15-16}

According to the available literature, only one study of correlation between A-FABP and epicardial fat has been published. In that study, Baessler et al.\textsuperscript{17} found a positive correlation between the amount of epicardial fat and A-FABP in a group of morbidly obese subjects.

The aim of our study was to evaluate the correlation between EPI and A-FABP in asymptomatic patients with family history of CVD, stratified according to BMI (normal, overweight, obese).

SUBJECTS AND METHODS

The study comprised 59 subjects (39 males) aged between 32 and 73 years (median = 54 years), all asymptomatic first-degree relatives of patients with CVD – coronary heart disease (CHD) or stroke. Anthropometric and physical measurements – body weight, height, body mass index (BMI), casual blood pressure, and laboratory tests were performed.

Serum uric acid, creatinine, total cholesterol, high density lipoprotein (HDL) cholesterol, LDL cholesterol, triglycerides, insulin, glucose, C-reactive protein (CRP), apolipoprotein A (apo A), apolipoprotein B (apo B), lipoprotein(a) (Lp(a)), fibrinogen and glycated hemoglobin levels were analyzed in fresh serum obtained from venous blood collected after 12 h of fasting according to the manufacturer’s instructions.

Measuring A-FABP levels: Venous blood was collected after 12 h of overnight fasting. Plasma levels were measured by enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine Inc., Brno, Czech Republic) according to the manufacturer’s instructions. To determine variability, intra- and inter-assay variance, human plasma was used with 3 different samples being assessed (CV intra-assay <4.8%, CV inter-assay <10%).

Laboratory analyses were blinded i.e. carried out without access to clinical data.

Echocardiography was performed using VIVID 7 GE Medical. Epicardial fat thickness was measured in the parasternal view (long and short axis) on the right ventricular free wall in diastole, using the mean of three consecutive beats\textsuperscript{2}, as a hypoechoic space between the epicardial surface and the parietal pericardium. If there was a difference between the measurement in long and short axis the result was taken to be the mean value of these two measurements.

Table 1. Basic characteristics and correlation coefficients with epicardial fat.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>53.3±10.8</td>
<td>0.526*</td>
</tr>
<tr>
<td>A-FABP (μg/L)</td>
<td>25.8±12.8</td>
<td>0.336*</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>80.2±3.9</td>
<td>0.046</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>346.4±93.3</td>
<td>-0.073</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.1±4.8</td>
<td>0.120</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.1±0.8</td>
<td>-0.157</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6±0.8</td>
<td>0.183</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4±0.4</td>
<td>-0.105</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.1±0.7</td>
<td>-0.126</td>
</tr>
<tr>
<td>Index.Ch/HDLc</td>
<td>3.9±1.0</td>
<td>0.088</td>
</tr>
<tr>
<td>ApoA lipoprotein (g/L)</td>
<td>1.5±0.3</td>
<td>-0.121</td>
</tr>
<tr>
<td>ApoB lipoprotein (g/L)</td>
<td>0.9±0.2</td>
<td>-0.033</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.4±1.4</td>
<td>0.045</td>
</tr>
<tr>
<td>Glycated Hb (%)</td>
<td>4.0±1.0</td>
<td>0.214</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>11.3±1.4</td>
<td>0.124</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.0±0.9</td>
<td>0.304*</td>
</tr>
<tr>
<td>BP systolic mm Hg</td>
<td>132.3±25.7</td>
<td>0.279*</td>
</tr>
<tr>
<td>BP diastolic mm Hg</td>
<td>82.2±12.0</td>
<td>0.036</td>
</tr>
<tr>
<td>BMI</td>
<td>28.5±3.9</td>
<td>0.085</td>
</tr>
</tbody>
</table>

* P≤0.05

Table 2. Correlations (Spearman’s) between epicardial fat and A-FABP depending on BMI.

<table>
<thead>
<tr>
<th>BMI</th>
<th>n</th>
<th>Correlations</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>-0.100</td>
<td>0.783</td>
</tr>
<tr>
<td>Overweight</td>
<td>29</td>
<td>0.389</td>
<td>0.041</td>
</tr>
<tr>
<td>Overweight plus obesity</td>
<td>20</td>
<td>0.407</td>
<td>0.004</td>
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BP blood pressure, BMI body mass index.
The total CS was calculated using the Agatston method without the need for a contrast agent. To identify calcified lesions, the usual threshold of 130 Hounsfield units was used. Patients were classified into categories with risks corresponding to the CS as follows: normal (0), minimum risk (1-10), mild risk (11-100), moderate risk (101-400) and high risk (above 400). CT scans to determine the CS were performed with the Siemens Biograph-16 16-slice PET/CT scanner; ECG gating was used to obtain images at the same phase of the cardiac cycle (end-diastole) (reconstruction of 3-mm slices).

SPECT was performed with the Siemens e.cam dual-detector scintillation camera equipped with low-energy high-resolution parallel-hole collimators, using 8-frame gating. 180° rotation angle, a total of 64 projections in a 64 x 64 matrix from 45° right anterior oblique projection to 45° left posterior oblique projection.

Coronarography was performed in patients with pathological findings on SPECT with the Phillips Allura Xper FD 10 according to common protocol.

All participants gave informed consent to the study approved by the Ethics Committee of the Faculty of Medicine and Dentistry, Palacky University Olomouc.

Statistical analysis

SPSS software version 15.0 (SPSS Inc., Chicago, USA) was used for statistical analysis. Spearman’s correlation analysis was carried out to evaluate the relationship between EPI and A-FABP and other baseline variables. The normality of distribution was checked by the Shapiro-Wilk test. P<0.05 was considered statistically significant.

RESULTS

Basic characteristics (arithmetic mean ± SD, 1st quartile/median/3rd quartile) of the analyzed data set are shown in Table 1. Of the total 59 participants, 35 patients (59.3%) were pharmacologically treated for arterial hypertension, 5 patients (8.5%) had type 2 diabetes mellitus, 16 patients (27.1%) were smokers, and 1 patient (1.7%) was an ex-smoker. Overweight obese were noted in 29 (49.15%) and 20 (33.89%) patients, respectively. A mild calcium score was present in 13 (22.0%) patients, a moderate calcium score in 6 (10.2%), and a severe calcium score in 2 (3.4%). 6 patients (10.16%) had a pathological finding on SPECT, ischemic heart disease was found in 4 (6.8%) patients (by coronarography).

EPI was present in 46 patients (77.9%) with a mean value of 2.91 mm. Ten patients had EPI >5 mm.

Spearman correlation analysis showed a significant positive correlation between EPI and A-FABP (r=0.336; P=0.010) (see Fig 1), and age (r=0.526; P<0.001), fibrinogen (r=0.304; P=0.023) and systolic blood pressure (r=0.279; P=0.034) (see Table 1).

The correlation between EPI and A-FABP in patients with normal BMI, in overweight patients, and in obese patients is shown in Table 2.
We have found a positive correlation between EPI and A-FABP in group of patients without ischemic heart disease ($r=0.368; P=0.006$).

We found a correlation between EPI and A-FABP ($r=0.385; P=0.004$) in non diabetic patients.

**DISCUSSION**

The growing understanding of the pathogenic role of ectopic fat is a topic of study. The group in the Framingham study$^{19}$ showed that, in 3 086 participants, visceral adipose tissue was associated with cardiovascular disease (hazard ratio 1.44, CI 1.08-1.92; $P=0.01$) and cancer (hazard ratio 1.43, CI 1.12-1.84; $P=0.005$). In a systematic review of 2012 (ref.2) there was a positive correlation in most studies of relationship between EPI and CVD: the presence of EPI increased the risk of CVD. Studies of adipokines and EPI also exist. For example, Ogorodnikova et al showed that at-risk overweight-obese women had significantly elevated epicardial fat and tested the lowest for adiponectin$^{19}$. Other authors found a correlation between leptin levels and subcutaneous and visceral fat volume with controversial results$^{20,21}$.

A-FABP is a promising marker connecting metabolic syndrome, inflammation and atherosclerosis, and recently long term studies$^{22-23}$ have been published showing that persons with higher A-FABP levels have poor cardiovascular prognosis. Huang et al$^{24}$ published a study where persons with higher A-FABP levels have poor cardiovas-

cular prognosis. Huang et al$^{24}$ published a study where persons with higher A-FABP levels have poor cardiovas-
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Thus, there is need to follow up these asymptomatic persons and assess cardiovascular endpoints such as cardiac death and acute myocardial infarction.

The amount of EPI correlated with age, which is in concordance with previously published data by Guglielmi et al$^{26}$. A positive correlation between EPI and fibrinogen was published by Aydin et al$^{27}$.

We evaluated the calcium score. Correlation of this parameter with A-FABP level has been published$^{28}$. The results of direct intervention on risk factors and its influence on A-FABP level have also been published$^{29}$.

Limitations to this pilot study include small sample size, assessment of EPI by echocardiography and not by CT scan, but we judged the greater radiation burden in asymptomatic people would be unethical, and a skilled echocardiographer is able to distinguish between epicardial fat and pericardial fluid. Also, we were not able to perform subanalyses of possible sex differences because of sample size.

**CONCLUSION**

This is the first study to show a positive correlation between EPI and A-FABP in overweight patients with a family history of CVD. Because of possible clinical consequences there is a need for more studies.

**ABBREVIATIONS**

A-FABP, Adipocyte fatty acid binding protein; CI, Confidence interval; CRP, C reactive protein; CV,
Coefficient of variation: CVD. Cardiovascular disease: EPI, Epidemic fat; HDL, High density lipoprotein; FABP, Fatty acid binding protein; IL, Interleukin; LDL, Low density lipoprotein; Lp, Lipoprotein; mRNA, Messenger ribonucleic acid; SD, Standard deviation; TNF, Tumor necrosis factor.

Acknowledgement: Preparation of this manuscript has been supported by grant LF_2017_041.

Author contribution: All authors contributed in equal way in preparation of this manuscript.

Conflict of interest statement: None declared.

REFERENCES


