

Relationship between serum adipocyte fatty acid-binding protein and endothelial/hemostatic markers in dyslipidemic subjects

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Abstract

OBJECTIVES: Some findings support the role of serum adipocyte fatty acid-binding protein (A-FABP) as a key pro-inflammatory mediator that links obesity with cardiovascular diseases. The aim of the study was to evaluate the association of A-FABP with endothelial/hemostatic markers [von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue-plasminogen activator (t-PA), soluble intercellular cell adhesion molecule-1 (s-ICAM-1) and soluble vascular cell adhesion molecule-1 (s-VCAM-1)] in asymptomatic dyslipidemic subjects.

DESIGN: We examined 105 dyslipidemic patients (with apolipoprotein B concentration ≥ 1.2 g/l and/or triglyceride (TG) concentration ≥ 1.5 mmol/l) without clinical manifestation of atherosclerosis and 50 normolipidemic healthy subjects, who served as a control group. Except of endothelial/hemostatic markers, anthropometric and lipid parameters, markers of insulin resistance and inflammation were assessed.

RESULTS: In dyslipidemic patients, A-FABP positively correlated with age ($p < 0.05$), TG ($p < 0.05$), insulin ($p < 0.05$), homeostatic model assessment (HOMA) index ($p < 0.05$), body mass index ($p < 0.001$), waist circumference ($p < 0.05$), high sensitivity C reactive protein ($p < 0.01$), and vWF ($p < 0.05$) and negatively with male gender ($p < 0.05$). There were no correlations between A-FABP and PAI-1, t-PA, s-VCAM-1 or s-ICAM-1. By using linear multivariate regression analysis the positive association between A-FABP and vWF was independent of age, gender, insulin resistance, and visceral obesity.

CONCLUSION: Study displayed an independent positive association of A-FABP with vWF in clinically asymptomatic dyslipidemic subjects. Contribution of A-FABP in the process of endothelial dysfunction could help to explain the role of obesity in cardiovascular damage.

Abbreviations:

Apo	- apolipoprotein
A-FABP	- adipocyte fatty acid-binding protein
BMI	- body mass index
DBP	- diastolic blood pressure
ELISA	- enzyme-linked immunosorbent assay
HDL-C	- high density lipoprotein cholesterol
HOMA	- homeostasis model assessment
hs-CRP	- high sensitivity C-reactive protein
JNK	- C-Jun N-terminal kinases
LDL-C	- low density lipoprotein cholesterol
MCP-1	- monocyte chemo attractant protein-1
NFκB	- nuclear factor kappa B
PAI-1	- plasminogen activator inhibitor-1
SBP	- systolic blood pressure
s-ICAM-1	- soluble intercellular adhesion molecule
s-VCAM-1	- soluble vascular cell adhesion molecule
TC	- total cholesterol
TG	- triglycerides
TNF-α	- tumor necrosis factor-α
t-PA	- tissue-plasminogen activator
vWF	- von Willebrand factor

INTRODUCTION

Adipocyte fatty acid-binding protein (A-FABP) is expressed in adipocytes, macrophages and lymphocytes (Xu & Vanhoute 2012). In adipocytes, A-FABP regulates fatty acid storage and lipolysis. Its putative function is to serve as a lipid-binding chaperone for fatty acids (Hoo *et al.* 2008). In macrophages, A-FABP probably regulates central molecular pathways to coordinate macrophage cholesterol trafficking and inflammatory activity (Makowski *et al.* 2005). It integrates metabolic and immune responses and links the inflammatory and lipid-mediated pathways that are critical in the metabolic syndrome, for which some authors suggest A-FABP as a central mediator of obesity-related cardiovascular diseases (Xu & Vanhoute 2012).

Clinical investigations and animal models identify A-FABP as a factor associated with insulin resistance, adverse lipid profiles – including increased serum triglyceride (TG), LDL-cholesterol (LDL-C) and decreased HDL-cholesterol (HDL-C), hyperglycemia, obesity, metabolic syndrome, type 2 diabetes, hypertension, and also with the development of atherosclerosis (Horakova *et al.* 2011; Hsu *et al.* 2010; Tso *et al.* 2007; Xu *et al.* 2006; Xu *et al.* 2007; Xu & Vanhoute 2012). A-FABP positively correlates with coronary artery disease (Bao *et al.* 2011; Doi *et al.* 2011; Hsu *et al.* 2010; Miyoshi *et al.* 2010; Rhee *et al.* 2009) and ischemic stroke (Tso *et al.* 2011). Serum levels of A-FABP are associated with carotid (Yeung *et al.* 2007) and femoral (Xiao *et al.* 2010) intima-media thickness and with impaired endothelium-dependent vasodilatation (Xiao *et al.* 2010). The pro-atherogenic effect of A-FABP is probably mediated by vascular inflammation. The plasma concentration of A-FABP correlates positively with several pro-inflammatory markers, such as high sensitivity C reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), monocyte chemo attractant protein-1 (MCP-1) and interleukin-6 (Horakova *et al.* 2011; Park *et al.* 2012; Xu *et al.* 2007; Xu

& Vanhoute 2012). These findings also support the role of A-FABP as a key pro-inflammatory mediator that links obesity with cardiovascular damage.

Endothelial dysfunction and atherothrombosis play important roles in atherogenesis. Some serum markers of endothelial dysfunction and/or hemostasis have been shown to predict the development of cardiovascular events in addition to conventional risk factors (Smith *et al.* 2005). There is increasing evidence that endothelial dysfunction is closely associated with insulin resistance accompanying obesity and some types of dyslipidemias (Garanty-Bogacka *et al.* 2005; Karasek *et al.* 2011a and 2011b). Thus, we could suggest that pro-inflammatory effect of A-FABP on artery wall leads to vascular damage detected by serum elevation of the corresponding markers.

The aim of this study was to evaluate the relationship between A-FABP and markers of hemostasis and endothelial dysfunction: von Willebrand factor (vWF), tissue-plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), soluble vascular cell adhesion molecule-1 (s-VCAM-1), and soluble intercellular adhesion molecule-1 (s-ICAM-1) in a high risk population of dyslipidemic subjects without clinical manifestation of atherosclerosis.

MATERIALS AND METHODS

The study was carried out as a cross-sectional study with “lipid-modifying drug naive” asymptomatic patients, their affected relatives, spouses and healthy volunteers. All subjects filled in a questionnaire about their previous medical history, especially the cardiovascular status, medication, and smoking habits. Body mass index (BMI), waist circumference, systolic and diastolic blood pressure (SBP, DBP) were determined. Exclusion criteria were: diabetes mellitus, hypothyroidism, hepatic or renal impairment and nephrotic syndrome (causes of secondary hyperlipidemia); history of clinically manifesting atherosclerosis (coronary artery disease, cerebrovascular ischemic disease and peripheral arterial disease); hypolipidemic therapy in the previous 8 weeks; hormone therapy with estrogens; and clinical presence of acute infectious disease or trauma. The study was reviewed and approved by the institutional Ethics Committee of Medical Faculty and University Hospital Olomouc and informed consent was obtained from all participants.

Individuals who met the above-mentioned criteria (155 subjects, 74 men and 81 women) were divided into two groups: 105 hyperlipidemic patients (males/females: 53/52, mean age: 45.0±13.4 years) and 50 normolipidemic healthy subjects (males/females: 21/29, mean age: 45.2±16.9 years) who served as a control group. Hyperlipidemic subjects were defined as those with serum triglycerides (TG) concentration ≥1.5 mmol/l and/or apolipoprotein B (apoB) concentration ≥1.2 g/l. The value for TG was chosen because small dense LDL

particles become common above this level (Campos *et al.* 1992), the value for apoB was chosen because it is a level from which cardiovascular risk rapidly increases (Demacker *et al.* 2000).

Biochemical analyses

Venous blood samples were drawn in the morning after a 12-h fasting period. Total cholesterol, TG and HDL-C were determined enzymatically on Modular SWA analyzer (Roche, Basel, Switzerland) using commercially available kits (Roche, Basel, Switzerland).

Tab. 1. Basic characteristics.

	NL (n=50)	DL (n=105)
Age (years)	45.2±17.0	45.0±13.5
TC (mmol/l)	5.61±0.88	7.28±1.73***
TG [¶] (mmol/l)	1.05 [0.83-1.21]	2.58 [1.73-4.16]***
HDL-C (mmol/l)	1.68±0.5	1.26±0.38***
LDL-C (mmol/l)	3.47±0.78	4.27±1.53***
non-HDL-C (mmol/l)	3.94±0.81	5.99±1.76***
apoB (g/l)	0.91±0.18	1.30±0.32***
fasting glycemia (mmol)	5.08±0.58	5.48±1.1*
insulin [¶] (mIU/l)	6.9 [4.7-11.3]	9.0 [7.1-12.5]*
HOMA [¶] (mIU*mmol/l ²)	1.57 [0.97-2.6]	2.18 [1.57-3.16]*
SBP (mm Hg)	129.7±14.6	129.6±15.3
DBP (mm Hg)	79.2±9.3	78.9±8.9
BMI (kg/m ²)	25.5±4.7	27.4±4.6*
waist circumference (cm)	85.2±14.7	93.8±13.9*
hs-CRP [¶] (mg/l)	1.0 [0.5-2.2]	1.9 [0.8-3.7]*
vWF (%)	123.5±52.1	134.7±52.3
t-PA [¶] (µg/l)	3.0 [2.0-3.5]	3.0 [2.1-4.2]
PAI-1 [¶] (µg/l)	40.0 [29.5-74.5]	63.5 [34.3-94.1]*
s-ICAM-1 [¶] (µg/l)	327 [240-473]	346 [280-475]
s-VCAM-1 [¶] (µg/l)	697 [488-867]	651 [418-871]
A-FABP [¶] (µg/l)	21.7 [15.0-31.3]	23.3 [16.1-34.3]

NL= normolipidemic controls, DL = dyslipidemic subjects (apoB ≥1.2 g/l and/or TG ≥1.5 mmol/l), TC = total cholesterol, TG = triglycerides, HDL-C = HDL cholesterol, LDL-C = LDL cholesterol, non-HDL-C = non-HDL cholesterol (TC - HDL-C), apoB = apolipoprotein B, HOMA = homeostasis model assessment, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, hs-CRP = high sensitivity C reactive protein, vWF = von Willebrand factor, t-PA = tissue-plasminogen activator, PAI-1 = plasminogen activator inhibitor-1, s-ICAM-1 = soluble intercellular cell adhesion molecule-1, s-VCAM-1 = soluble vascular cell adhesion molecule-1, A-FABP = serum adipocyte fatty acid-binding protein
Values are expressed as mean ± standard deviation, for[¶] (parameters with skewed distribution) as median [25 and 75 percentiles]. Differences in variables between groups were analyzed with ANOVA after adjustment for age and sex. *p<0.05, **p<0.01, ***p<0.001 ... statistical significance level

Determination of HDL-C was made by a direct method without precipitation of apoB containing lipoproteins. LDL-C levels were calculated according to the Friedewald formula (for TG < 4.5 mmol/l). We also calculated non-HDL-cholesterol (non-HDL-C = TC - HDL-C). Concentration of apoB was determined immunoturbidimetrically (TinaQuant Apo B kit, Roche, Basel, Switzerland). A-FABP was determined in serum by ELISA (enzyme-linked immunosorbent assay) according to the manufacturer's instructions (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic). Hs-CRP was assessed by means of an ultra sensitive latex immunoturbidimetric method (CRP latex TinaQuant kit, Roche, Basel, Switzerland). Glycemia was determined by enzymatic-colorimetric method (Glucose GOD-PAP kit, Roche, Basel, Switzerland). Insulin was determined using commercially available kit (Immunotech, Marseille, France) using specific antibodies by immunoradiometric assay method. The result obtained was then used for the calculation of the parameter of insulin resistance HOMA [homeostasis model assessment index (Matthews *et al.* 1985): fasting glycemia (mmol/l) * fasting insulin (mIU/l)/22.5]. Serum levels of the soluble adhesion molecules s-ICAM-1 and s-VCAM-1 were measured by immunoenzymatic assay using standard kits (both Immunotech, Marseille, France). The following endothelial/hemostatic markers were examined: von Willebrand factor by immunoturbidimetric assay (Instrumentation Laboratory Spa, Milan, Italy), plasminogen activator inhibitor-1 and tissue plasminogen activator by ELISA (both Technoclone, Vienna, Austria).

Statistical analysis

All values are expressed as means ± standard deviation or as median with 25 and 75 percentiles for variables with non-normal distribution. The Kolmogorov-Smirnov test was used to test for normal distribution. Variables with skewed distribution (TG, insulin, HOMA, hs-CRP, t-PA, PAI-1, sICAM-1, sVCAM-1, A-FABP) were log transformed to normalize their distribution before statistical testing. Differences in variables between individual groups were analyzed with ANOVA after the adjustment for age and sex. The correlation between individual parameters was tested by a univariate correlation analysis. Multivariate regression analysis was used for testing of an independent association between dependent and independent variables. Statistical analyses were performed by using SPSS for Windows version 12.0 (Chicago, IL, USA). Probability values of p<0.05 were considered as statistically significant.

RESULTS

Basic characteristics of the investigated groups are summarized in Table 1. Compared to normolipidemic controls, dyslipidemic patients had significantly higher insulin levels, fasting glycaemia, HOMA, BMI, waist

circumference, levels of PAI-1 and of hs-CRP. There were no significant differences in vWF, t-PA, s-ICAM-1, s-VCAM-1 and A-FABP levels. Levels of A-FABP correlated positively with age ($r=0.252, p<0.05$), TG ($r=0.368, p<0.001$), insulin ($r=0.229, p<0.05$), HOMA ($r=0.238, p<0.05$), BMI ($r=0.481, p<0.001$), waist circumference ($r=0.233, p<0.05$), hs-CRP ($r=0.279, p<0.01$) and vWF ($r=0.214, p<0.05$), and negatively with male gender ($r=-0.252, p<0.05$) in dyslipidemic subjects. In normolipidemic controls, A-FABP positively correlated with TG ($r=0.311, p<0.05$), insulin ($r=0.561, p<0.001$), HOMA ($r=0.571, p<0.001$), BMI ($r=0.437, p<0.01$) and negatively with HDL-C ($r=-0.324, p<0.05$). By using linear multivariate regression analysis A-FABP was independently associated with gender, BMI, and vWF in dyslipidemic subjects – see table 2, and with BMI (beta=0.43, $t=3.62, p<0.001$) and insulin (beta=0.62, $t=2.99, p<0.001$) in normolipidemic controls.

DISCUSSION

The presented study did not reveal significantly increased levels of A-FABP in asymptomatic dyslipidemic patients compared to normolipidemic controls. A-FABP did not correlate with total cholesterol, LDL-C, HDL-C, non-HDL-C or apoB. But, except of age and gender, A-FABP correlated with TG, markers of insulin resistance, visceral obesity and inflammation.

These findings confirm the key contribution of obesity and insulin resistance in serum increase of A-FABP and some authors suggest A-FABP as a marker for adiposity (Park *et al.* 2012; Xu *et al.* 2006). The positive association of A-FABP with TG levels was not independent in the present study. It might have been caused by adiposity and insulin resistance leading to elevation of TG levels. Nevertheless, some authors suppose the direct role of A-FABP in the increase of TG concentration. A-FABP could serve as a regulator of the liver X receptor ATP-binding cassette transporter A1, which might stimulate the transfer of TG to HDL and catabolism of TG (Park *et al.* 2012).

We found a positive correlation between A-FABP and hs-CRP levels. Recent studies have also reported similar results (Bao *et al.* 2011; Horakova *et al.* 2011; Miyoshi *et al.* 2010; Park *et al.* 2012; Xu *et al.* 2007). Furthermore, A-FABP was also positively associated with other inflammatory cytokines such as MCP-1 and TNF- α , even after adjusting for adiposity (Park *et al.* 2012). This pointed to A-FABP as a pro-inflammatory mediator, connecting insulin resistant states with systemic inflammation. The precise mechanism, whereby A-FABP modulates inflammation, remains unclear. Recently, A-FABP was reported to potentiate vascular inflammation by forming a positive feedback loop with C-Jun N-terminal kinases (JNK) and activator protein-1 to exacerbate lipopolysaccharide induced inflammatory responses in macrophages (Hui *et al.* 2010). Activated JNK increases A-FABP expression by enhancing gene

transcription and conversely, elevated A-FABP potentiates JNK activation leading to augmented production of pro-inflammatory cytokines (Hui *et al.* 2010; Xu & Vanhoute 2012).

From the endothelial/hemostatic markers, only vWF positively correlated with A-FABP in dyslipidemic patients and this relation was independent of other metabolic factors, age, gender, and BMI. As we know, the positive independent association between serum levels of A-FABP and vWF has not been reported yet and the plausible explanation of this finding remains unclear. This factor is produced almost exclusively by vascular endothelial cells activated by pro-inflammatory cytokines. Plasma levels of vWF are elevated in different states of endothelial damage and they have been proposed as a useful marker of endothelial dysfunction (Makin *et al.* 2004). In patients with acute coronary syndrome, vWF correlated significantly with circulating endothelial cells – a marker of endothelial damage (Lee *et al.* 2005). The role of A-FABP as a factor causing endothelial dysfunction was verified by impaired endothelium-dependent vasodilatation in animal and human studies (Lee *et al.* 2011; Xiao *et al.* 2010). A specific inhibitor of A-FABP improved endothelium dependent relaxations by endothelial nitric oxide synthase phosphorylation leading to increased nitric oxide production. Lee *et al.* found the co-localization of A-FABP and vWF in the endothelial layer of apoE^{-/-} mice (Lee *et al.* 2011). In a human study, Agardh *et al.* (2011) identified A-FABP in carotid plaques. Moreover, this expression of A-FABP was increased in unstable plaques and there was a co-localization of A-FABP and macrophage population indicating A-FABP as a key factor connecting vascular inflammation and cellular

Tab. 2. Multivariate regression analysis of the relations between A-FABP as dependent variable and correlated parameters as independent predictors in dyslipidemic group.

	Beta	t	p-value
age	0.07353	1.32655	n.s.
gender	-0.09485	-2.89502	0.006
TG (log)	0.00637	0.16525	n.s.
CRP (log)	0.00398	0.19876	n.s.
insulin (log)	0.30240	1.87779	n.s.
HOMA (log)	-0.08941	-1.24498	n.s.
BMI	0.52517	3.36664	0.002
waist circumference	0.10472	0.46511	n.s.
vWF	0.14483	3.27170	0.002

TG = triglycerides, hs-CRP = high sensitivity C reactive protein, HOMA = homeostasis model assessment, BMI = body mass index, vWF = von Willebrand factor
Parameters with skewed distribution (TG, hs-CRP, insulin, HOMA, A-FABP) were log transformed to normalize their distribution before statistical analysis

lipid accumulation. A-FABP can activate both JNK and nuclear factor kappa B (NFκB) pathways, which regulate the transcriptional activity of inflammatory genes (Makowski *et al.* 2005). This involvement of A-FABP in inflammatory reactions by the activation of the NFκB pathway may result in increased oxidative stress and decreased nitric oxide bioavailability, leading to endothelial dysfunction (Pierce *et al.* 2009). The activation of NFκB pathway could also be a cause of the overexpression of vWF, as was shown in endothelial injury induced by sepsis (Liang *et al.* 2010).

It remains unclear – why A-FABP did not correlate with other endothelial/hemostatic markers investigated in the presented study. PAI-1 is probably less specific for endothelial damage than vWF. PAI-1 originates from several sites, including the endothelium, liver and adipose tissue (Kohler & Grant 2000). Thus, higher levels of PAI-1 detected in dyslipidemic subjects may not reflect only endothelial dysfunction, but they could also be caused by increased production of PAI-1 in the liver or in adipose tissue by a mechanism independent of A-FABP. The production of PAI-1 is connected with t-PA levels, because most of the assessed t-PA antigen in blood is a part of an inactive circulating complex with PAI-1 (Karasek *et al.* 2011a). While VCAM-1 is expressed locally in the endothelium overlying sites of atherosclerotic lesion formation, the expression of ICAM-1 is not restricted only to the endothelium, since it can also be found on macrophages, smooth muscle cells, fibroblasts and hematopoietic cells (Galkina & Ley 2007). In addition, we could expect NFκB pathway activation by A-FABP leading to VCAM- and ICAM-1 expression (Kawakami *et al.* 2006). However, soluble forms of these adhesion molecules may not reflect their initial endothelial overexpression in clinically asymptomatic dyslipidemic patients. Especially the levels of s-VCAM-1 are increased (and predicting a risk for future coronary events) only in patients with manifest atherosclerosis. In contrast, s-ICAM-1 appears to be a marker of a more general inflammatory condition and it may be predictive also in healthy people (Blankenberg *et al.* 2003; Karasek *et al.* 2011b). Moreover, various hemostatic/endothelial markers are probably regulated differently and may represent a different type or severity of endothelial dysfunction. The other reasons and the concurrent limitation of the study may be the small number of patients (105 dyslipidemic patients and 50 normolipidemic controls) and the heterogeneity of dyslipidemic group, a significant increase of A-FABP was detected mostly in subjects with metabolic syndrome (Horakova *et al.* 2011; Hsu *et al.* 2010; Tso *et al.* 2007; Xu *et al.* 2006; Xu *et al.* 2007). Another examinations especially testing microcirculation endothelial function are needed to clarify the importance of A-FABP in an initial step of endothelial dysfunction and during the whole process of atherogenesis.

The presented study found independent positive association of A-FABP with vWF in clinically asymp-

tomatic dyslipidemic subjects. This association could participate in explaining of the role of A-FABP in the process of endothelial dysfunction. Further studies are necessary to elucidate the contribution of A-FABP in atherogenesis.

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