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Fatty acid-binding protein 4 is associated with endothelial dysfunction in patients with type 2 diabetes

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ABSTRACT

Objective: Adipocyte fatty acid-binding protein (FABP4) plasma levels are higher in type 2 diabetes (T2D). Endothelial dysfunction is also common in T2D. We have investigated the relationship between circulating FABP4 levels and endothelial function in diabetic patients.

Methods: In 257 patients (105 diabetic and 152 non-diabetic) at increased risk of cardiovascular disease, we measured circulating FABP4, reactive hyperemia index (RHI) by peripheral artery tonometry, intima-media thickness, and biomarkers of inflammation, oxidation and endothelial function.

Results: In T2D subjects, FABP4 was negatively associated with endothelial function, as measured by RHI ($r = -0.226$, $P = 0.05$). In a stepwise multivariate linear regression model, FABP4 was a predictor of RHI in T2D patients ($P = 0.04$).

Conclusion: Circulating levels of FABP4 are inversely associated with endothelial function in T2D patients, as measured by RHI. We suggest a direct effect of plasma FABP4 on the vascular endothelium in those with T2D.

1. Introduction

The adipose fatty acid-binding protein (FABP), also known as FABP4 and aP2, is one of the best characterised intracellular lipid transport proteins. It is expressed in adipocytes and macrophages [1]. A recent study has shown that FABP4 is expressed in microvascular endothelial cells [2]. It appears to function primarily as a cytoplasmic chaperone of long chain fatty acids; it transports them to other sub-cellular compartments for metabolism, secretion [3] or translocation to the nucleus to regulate the function of transcription factors, such as PPARγ [4]. FABP4 knock-out mice are protected against hyper-insulinemia and insulin resistance induced by obesity [1]. FABP4 −/− mice in an apo E −/− background show delayed development of arteriosclerotic plaques [1]. FABP4 is also found circulating in plasma; however, neither its secretion pathways nor its functions are known. Our group and others have shown that FABP4 plasma concentrations are increased in patients with obesity, metabolic syndrome (MS), type 2 diabetes (T2D), familial combined hyperlipidemia or lipodystrophy syndromes [5–9]. FABP4 levels have also been associated with the presence of cardiovascular disease. However, FABP4's causal role in these processes has not been established. There is indirect evidence that circulating FABP4 could promote inflammation and mediate insulin resistance and atherogenic dyslipidemia; circulating FABP4 has also been associated with carotid intima-media thickness and coronary atheroma [10]. It has recently been demonstrated that FABP4 has a direct impact on myocardial muscle cells by decreasing their contractility [11]. This finding suggests that circulating FABP4 could also have a direct effect on peripheral cells and tissues. One hypothesis is that the elevated FABP4 plasma concentrations observed in patients with obesity, MS and T2D could account for the accelerated arterial damage seen in these conditions. Endothelial dysfunction is a common finding in the above mentioned metabolic diseases. Several factors, such as insulin resistance, dyslipidemia, hypertension, hyperglycaemia, abdominal obesity and low-grade inflammation, have been associated with endothelial dysfunction in subjects with T2D [12], but the exact cause of this alteration is not clear. Despite the strong evidence relating endothelial dysfunction and cardiovascular risk, an assessment of endothelial function is not usually performed at the clinical level [13], primarily because of technical difficulties. Reactive hyperemia index (RHI), which is measured by peripheral artery tonometry (PAT), is a non-invasive, reproducible and reliable method used to assess endothelial function in clinical settings. In this work, we have explored the relationship between FABP4 plasma levels and endothelial function assessed by RHI in diabetics and non-diabetics at elevated cardiovascular risk.

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Table 1
Clinical, metabolic, biochemical and vascular function parameters according to diabetes status.

Characteristic	All CVR patients (n = 257)	Non-T2D (n = 152)	T2D (n = 105)	P
Women, %	29.6	28.9	30.5	0.792
Age, years	57.29 ± 10.66	55.38 ± 11.06	60.07 ± 9.44	<0.0001
Systolic blood pressure, mm Hg	137.99 ± 16.88	136.22 ± 14.66	140.55 ± 19.44	0.043
Diastolic blood pressure, mm Hg	83.51 ± 10.72	83.42 ± 11.06	83.63 ± 10.27	0.879
Body mass index, kg/m^2	31.11 ± 4.41	30.22 ± 4.06	32.43 ± 4.60	<0.0001
Waist circumference, cm	103.83 ± 10.62	101.91 ± 9.72	106.70 ± 11.29	<0.0001
Total cholesterol, mmol/l	5.10 ± 1.13	5.33 ± 1.08	4.77 ± 1.12	<0.0001
Triglycerides, mmol/l	1.89 (1.20–2.97)	1.88 (1.22–2.77)	1.94 (1.11–3.23)	0.596
LDL, mmol/l	3.19 ± 0.94	3.44 ± 0.93	2.84 ± 0.85	<0.0001
HDL, mmol/l	1.40 ± 0.30	1.43 ± 0.30	1.36 ± 0.28	0.061
Glucose, mmol/l	6.80 ± 2.10	5.80 ± 1.18	8.24 ± 2.29	<0.0001
HbA1c, %	6.37 ± 1.16	5.35 ± 0.59	6.74 ± 1.09	<0.0001
Creatinine, μmol/l	84.00 (71.00–93.00)	85.00 (75.00–93.00)	82.50 (70.25–91.75)	0.412
FABP4, ng/ml	26.02 (19.39–7.99)	25.30 (17.77–35.81)	29.22 (21.52–39.64)	0.069
ICAM-1, ng/ml	71.11 (28.50–181.04)	70.20 (28.95–182.85)	78.33 (27.94–175.32)	0.908
VCAM-1, ng/ml	833.34 (394.61–1328.40)	791.24 (339.30–1265.50)	984.86 (459.73–1430.0)	0.082
E-selectin, ng/ml	23.88 (10.77–43.03)	21.01 (9.79–37.96)	27.53 (13.73–48.63)	0.036
IL-6, pg/ml	47.66 (24.00–149.67)	44.91 (24.00–143.89)	53.33 (24.00–155.54)	0.754
TNFα, pg/ml	143.77 (24.00–502.08)	129.31 (24.00–476.25)	158.13 (36.17–549.91)	0.395
HsCRP, mg/l	1.44 (0.76–3.87)	1.33 (0.66–3.42)	1.69 (0.84–4.50)	0.259
Ab ox-LDL, U/ml	16.27 (14.33–19.47)	16.22 (14.51–19.56)	16.31 (14.13–19.14)	0.497
ox-LDL/LDL, U/mmol	56.05 (45.62–68.60)	54.87 (44.29–67.94)	58.02 (47.47–69.75)	0.175
RHI	1.58 (1.45–1.88)	1.58 (1.46–1.89)	1.58 (1.45–1.86)	0.884
CCA mean, mm	0.75 (0.66–0.87)	0.75 (0.66–0.88)	0.75 (0.64–0.86)	0.836
IMT mean, mm	0.79 (0.70–0.88)	0.80 (0.70–0.88)	0.79 (0.70–0.90)	0.847
IMT max, mm	1.10 (0.99–1.25)	1.10 (0.99–1.25)	1.10 (0.96–1.26)	0.920
ABI	1.02 (0.93–1.10)	1.03 (0.94–1.11)	1.01 (0.92–1.08)	0.607

Normally distributed data are presented as mean ± SD or percentage of patients. Non-normally distributed data are presented as the median (25th percentile–75th percentile). P-value: the difference among non-T2D and T2D using one-way ANOVA or Mann–Whitney U-test. Abbreviations: CVR, cardiovascular risk; T2D, type 2 diabetes; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FABP4, fatty acid-binding protein 4; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule-1; sE-selectin, soluble E-selectin; IL-6, interleukin 6; TNFα, tumor necrosis factor α; HsCRP, Hs C-reactive protein; ox-LDL/LDL, oxidized low-density lipoprotein/LDL; Ab ox-LDL, oxidized low-density lipoprotein antibodies; RHI, reactive hyperemia index; CCA, carotid common artery; IMT, intima-media thickness; ABI, ankle brachial index.

2. Subjects and methods

2.1. Subjects

Two hundred and fifty-seven men and women, aged 22–80 years, with no evidence of cardiovascular disease but with T2D or intermediate global cardiovascular risk (CVR) were included in the study. Complete physical examination, anthropometry, CVR assessment, vascular function and imaging studies were performed. The study was approved by the Ethical and Clinical Investigation Committee of the hospital, and all participants signed the written consent form.

2.2. Biochemical and biomarker determinations

Plasma levels of FABP4 were assessed using commercial kits (Bio Vendor Laboratory Medicine Inc., Brno, Czech Republic). Results were expressed as ng/ml. The performance characteristics of this assay were <5% CV intra-assay and <8% inter-assay.

Differential expression of inflammatory cytokines (sVCAM-1, sICAM-1, sE-Selectin, TNFα and IL-6) was determined using the Human Cytokine Antibody Array (RayBiotech, Norcross, GA, USA).

Oxidized low-density lipoproteins (ox-LDL) in plasma were measured by the Mercodia Oxidized LDL ELISA, a solid-phase two-site enzyme immunoassay (Mercodia AB, Uppsala, Sweden). The ox-LDL-antibodies (Ab ox-LDL) were determined using the enzyme immunoassay (IMTEC Immunodiagnostika GmbH, Berlin, Germany).

2.3. Vascular function and imaging studies

The endothelial function was assessed using the EndoPAT-2000 (Itamar Medical Ltd., Israel). This method compares the pulse wave amplitude in two fingertips, one on each hand, before and after five minutes of brachial ischemia in one of the arms (test arm). The obtained value is referred to RHI.

The carotid intima-media thickness (IMT) was evaluated by B-mode ultrasonography using an Acuson Sequoia 512 sonograph (Siemens Medical Solutions, Erlangen, Germany).

The lowest value of the ankle/arm SBP ratio measured with an 8 MHz continuous wave Doppler probe (Hadeco Mini Doppler Es 100X) was recorded as the ABI index.

2.4. Statistical analysis

All data were tested for normality using the Kolmogorov–Smirnov test. Continuous variables are presented as the mean ± SD for normally distributed data, as the median (25th percentile–75th percentile) for non-normally distributed data and as frequencies (n) or percentages (%) for categorical variables. Comparisons between groups were analyzed by one-way ANOVA or the Mann–Whitney U-test. Differences in FABP4 tertile groups according to diabetes status were tested using the chi-square test. Univariate association of FABP4 with anthropometric, biochemical and vascular variables was tested by Spearman or Pearson correlation analysis. To account for inflation of the experiment-wise type 1 error resulting from multiple testing, we implemented the Bonferroni test. After this adjustment, a $P \leq 0.05$ was considered statistically significant. The predictors for stepwise regression analysis were based on correlation analysis and selected from the variables known to be associated with the dependent variable. Analyses were performed using SPSS (version 17.0, SPSS Inc., Chicago, IL). A $P \leq 0.05$ was considered as statistically significant.

3. Results

The clinical and biochemical characteristics of the 257 patients distributed according to T2D status is summarised in Table 1.

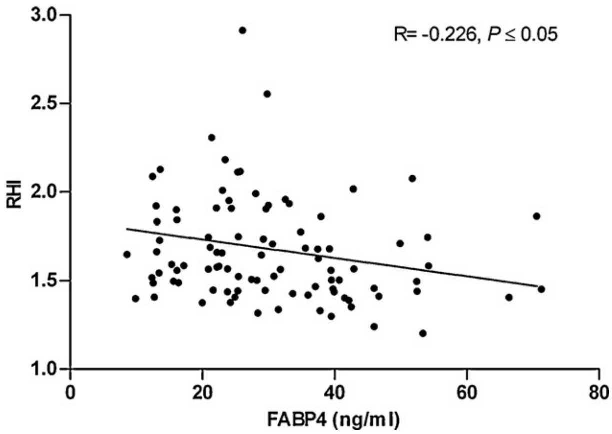

Fig. 1. Relationship between fatty acid-binding protein 4 (FABP4) and RHI in subjects with T2D. Univariate association was tested by Pearson correlation analysis. Results remained statistically significant at $P \leq 0.05$ after we adjusted for α inflation caused by multiple testing.

FABP4 plasma concentrations tended to be higher in T2D patients ($P = 0.069$). When the study population was divided into tertiles by FABP4 levels, the upper tertiles contained a significantly higher percentage of subjects with T2D than without it (24.8%, 36.2%, 39.0% vs. 38.8%, 31.6%, 29.6% respectively; $P = 0.05$).

FABP4 correlations with age, body mass index (BMI), waist circumference, ICAM-1, VCAM-1, E-selectin, IL-6, TNFα, HsCRP, Ab ox-LDL, ox-LDL/LDL, RHI, CCA mean, IMT mean, IMT max and ABI were assessed in patients with and without T2D. In T2D patients, we observed a significant positive association with Ab ox-LDL ($r = 0.251$, $P = 0.02$) and a significant negative association with RHI ($r = -0.226$, $P = 0.05$) (Fig. 1). This correlation remained significant after adjusting for age, gender and BMI ($P = 0.05$).

A linear regression model, which included age, gender, BMI, total cholesterol, triglycerides, glucose, sE-selectin, Ab ox-LDL and Hs C-reactive protein as independent variables, revealed that FABP4 was the only variable associated with RHI levels (inverse) in T2D patients ($R^2 = 0.034$, $P = 0.04$).

4. Discussion

The main finding of our study was that circulating FABP4 levels are inversely associated with peripheral reactive hyperemia, a subrogated marker of endothelial dysfunction, in T2D patients. Multivariate analysis revealed that plasma FABP4 was the only determinant of endothelial dysfunction in T2D. This finding could reflect a direct impact of FABP4 on vascular function in this group of patients.

We also observed a significant correlation between FABP4 and Ab ox-LDL in T2D patients. These results suggest that FABP4 may be related to lipid oxidation, which is one of the main pathophysiological mechanisms leading to endothelial dysfunction.

The role of circulating FABP4 remains unknown. Because some metabolic alterations can be associated with an increase in the plasma concentration of FABP4, it could be considered as a hazard to peripheral tissues. It has recently been shown that FABP4 levels cause direct harm to myocardiocytes, which could contribute to myocardial dysfunction in situations where FABP4 is increased [11]. Our results support the hypothesis that circulating FABP4 is not only a biochemical marker but also an effector of vascular damage in T2D. Other groups have shown that FABP4 is associated with the presence of clinical and subclinical arteriosclerosis [10,14,15]. By connecting FABP4 with endothelial dysfunction, our results provide more information about the relationship between FABP4 and arterial lesions. Although our data cannot establish a causal effect, we speculate that FABP4 may injure the endothelium.

FABP4 was associated with Ab ox-LDL in diabetic patients. Lipid oxidation appears to promote endothelial dysfunction; thus, we cannot exclude an interaction FABP4, oxidation and RHI alteration. On the other hand, it has been argued that FABP4 has a possible antioxidant effect at cellular level. However, Ab ox-LDL was not correlated to RHI in either binary or logistic correlation studies, and the association between FABP4 and RHI remained after adjusting for Ab ox-LDL.

This study has several limitations. Because it was cross-sectional, it allowed us to detect correlations but not to formulate predictions. To assess the clinical relevance of plasma FABP4 levels, prospective studies are needed.

In summary, we have demonstrated for the first time that circulating FABP4 levels are associated with endothelial dysfunction, as assessed by RHI, in T2D. If a causal relationship can be established, FABP4 plasma concentrations could be a therapeutic target for the prevention of vascular damage in T2D patients.

Conflict of interest

None.

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