Epidermal fatty-acid-binding protein: a new circulating biomarker associated with cardio-metabolic risk factors and carotid atherosclerosis

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Aims

Epidermal fatty-acid-binding protein (E-FABP) is highly homologous to adipocyte FABP (A-FABP), which mediates obesity-related metabolic syndrome (MetS), diabetes and atherosclerosis in animals. Combined deficiency of E-FABP and A-FABP protects against the MetS and atherosclerosis in mice. This study investigated the association of serum E-FABP with cardio-metabolic risk factors and carotid atherosclerosis in humans.

Methods and results

The presence of E-FABP in human plasma was detected by tandem mass spectrometry. Serum E-FABP levels, determined by an enzyme-linked immunosorbent assay in 479 Chinese subjects (age: 55.4 ± 13.5 years; M/F: 232/247), correlated positively (P < 0.05 to <0.001, age-adjusted) with parameters of adiposity, adverse lipid profiles, serum insulin, A-FABP, and C-reactive protein levels and were higher in subjects with the MetS (P < 0.001 vs. no MetS). The association of E-FABP with the MetS was independent of A-FABP. Furthermore, serum E-FABP correlated with carotid intima-media thickness (IMT; P < 0.001) and was independently associated with carotid IMT in men (adjusted P = 0.03).

Conclusion

E-FABP is a new circulating biomarker associated with increased cardio-metabolic risk. It may contribute to the development of the MetS and carotid atherosclerosis in humans, independent of the effect of A-FABP.

Keywords

Fatty-acid-binding proteins • Atherosclerosis • Obesity • Metabolic syndrome • Risk factors

Introduction

The fatty-acid-binding proteins (FABPs) are a family of low-molecular-weight intracellular lipid-binding proteins involved in the regulation of lipid metabolism and inflammation.1 Of these, adipocyte FABP (A-FABP, also known as aP2 or FABP4) and epidermal FABP (E-FABP, also known as mal1 or FABP5) are expressed in adipocytes, with A-FABP accounting for ~6% of the adipocyte cellular proteins.2 A-FABP-deficient mice show partial protection from hyperglycaemia and insulin resistance in both dietary and genetic obesity,3,4 and display resistance to athero-sclerosis when crossed with apolipoprotein E (apoE)-deficient mice.5,6 A more recent study suggests that a selective inhibitor of A-FABP can effectively alleviate diabetes and atherosclerosis in mice.7

We have demonstrated that A-FABP circulates in human blood-stream,8 with levels being increased in obese subjects and correlated closely with adverse lipid profiles, insulin resistance, hyperglycaemia, and hypertension.9 In our recent longitudinal studies, serum A-FABP levels predicted the development of the metabolic syndrome (MetS)10 and type 2 diabetes mellitus (DM).11 Moreover, we observed an independent association between serum A-FABP and carotid atherosclerosis in Chinese women.12 Our clinical data, together with findings from animal
studies, suggest the key role of A-FABP as an etiological mediator of obesity-related metabolic and cardiovascular diseases. E-FABP has a high degree of homology to A-FABP. In addition to the adipocytes and macrophages, E-FABP is present in the skin, brain and mammary glands. Growing evidence from animal studies suggests that, despite being only a minor form of FABP in adipocytes, E-FABP is also an important player in obesity-related disorders. E-FABP knockout mice exhibited enhanced insulin-stimulated glucose uptake and increased systemic insulin sensitivity, while transgenic overexpression of E-FABP aggravated insulin resistance and hyperglycaemia. A marked compensatory upregulation of adipocyte E-FABP expression was observed in A-FABP-deficient mice. Furthermore, studies on mice deficient in both FABPs suggested their additive effects on various components of the MetS and atherosclerosis. Nevertheless, the clinical relevance of these findings remains to be clarified.

In this study, we employed a proteomics-based approach to detect the presence of E-FABP in the human bloodstream. Subsequently, we investigated the relationship of serum levels of E-FABP with obesity-related cardio-metabolic parameters and markers of carotid atherosclerosis, in humans.

**Methods**

**Coupling of the cyanogen bromide (CNBr)-activated Sepharose beads with the anti-human epidermal fatty-acid-binding protein antibody**

The affinity-purified anti-human E-FABP (Biovendor Laboratory Medicine, Inc., Czech Republic) was coupled to Sepharose beads according to manufacturer's instructions (GE Healthcare, Little Chalfont, UK). Briefly, 250 μg of the antibody solution (1 μg/μL) in 0.1 M NaHCO₃ was mixed with 500 μL of the CNBr-activated Sepharose beads media for overnight at 4°C with gentle shaking. After washing and blocking with 0.1 M Tris–HCl (pH 8.0), the beads were stored in 20% ethanol at 4°C.

**Purification and characterization of epidermal fatty-acid-binding protein from human serum**

Albumin and immunoglobulins were depleted from serum as previously described. The remaining supernatant was incubated with Sepharose beads coupled with rabbit non-immune IgG to remove non-specific bindings, followed by incubation with Sepharose beads coupled with anti-human E-FABP IgG at 4°C overnight. The bound protein complexes were extensively washed, eluted, concentrated and then digested with trypsin. Tryptic peptide mixtures were desalted by ZipTip (Millipore) and then subjected to tandem mass spectrometry (MS/MS) analysis using 4800 TOF/TOF system (Applied Biosystems). The instrument settings were as follows: for MS analysis, laser intensity of 2500 was used and eight sub-spectra with 50 shots each were acquired; for MS/MS analysis, laser intensity of 3100 was used and 25 sub-spectra with a total of 2500 shots were acquired with metastable suppressor on. Calmix 1 and 2 (Applied Biosystems) were used for external calibration with mass tolerance of 100 p.p.m. Peptide mass lists and peptide fragment sequences were generated using 4000 series Explorer™ software V 3.5. An in-house MASCOT v2.1 (Matrix Science) searching engine was used for identifying the candidate proteins against NCBInr_200705 FASTA database. For MS/MS protein identifications, the significant probability scores with a P-value of <0.05 were accepted.

**Human participants**

Four hundred and seventy-nine subjects, who participated in previously reported study, were included. They underwent carotid intima-media thickness (IMT) measurement at the Department of Radiology, Queen Mary Hospital. Two hundred and ninety-six subjects were from the Hong Kong Cardiovascular Risk Factor Prevalence (HKCRISP) Study who returned in 2001 for reassessment of their cardiovascular risk. They were classified as having normal glucose tolerance, impaired glucose tolerance (IGT), impaired fasting glucose (IFG) or DM according to WHO 1998 diagnostic criteria. Another 183 subjects were treated type 2 diabetic patients from the Diabetes Clinic of the Queen Mary Hospital, who had the assessment of carotid IMT performed by the same radiologist (S.C.W.C.) in 2001. Altogether there were 232 men (age: 55.7 ± 13.0 years) and 247 women (age: 55.2 ± 13.9 years) with detailed clinical parameters described previously. All subjects gave informed consent and the protocol was approved by the Ethics Committee of the University of Hong Kong.

**Anthropometric and biochemical measurements**

All subjects were assessed after overnight fasting for at least 10 h. The details of anthropometric measurements (height, weight, BMI, waist circumference, and blood pressure) and the measurements of biochemical variables, including lipids, insulin, and high-sensitivity C-reactive protein, were reported previously. Body fat, expressed as a percentage of the total body weight, was quantified with TBF-410 Tanita Body Composition Analyzer (Japan). Insulin resistance was estimated using homeostasis model assessment index (HOMA-IR). The MetS was defined according to the US National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines, modified as recommended in the latest American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement, by adopting the Asian criteria for waist circumference and a lower cut-off of 5.6 mmol/L for elevated fasting glucose, proposed in the International Diabetes Federation (IDF) guidelines. The MetS was defined as having three or more of the following: (i) central obesity (waist circumference ≥80 cm in female and ≥90 cm in male); (ii) hypertriglyceridaemia (fasting triglyceride ≥1.69 mmol/L); (iii) low HDL-cholesterol (fasting HDL <1.29 mmol/L in female and <1.04 mmol/L in male); (iv) elevated fasting glucose (fasting glucose ≥5.6 mmol/L or already on oral hypoglycaemic agents for treatment of type 2 diabetes); and (v) hypertension (sitting BP ≥130/85 mmHg or on regular anti-hypertensive medications).

Human E-FABP ELISA kits were purchased from BioVendor Laboratory Medicine, Inc., Czech Republic. The assay was conducted according to manufacturer’s protocol, using 96-well microtiter plates coated with an affinity-purified sheep polyclonal anti-human E-FABP antibody. The intra- and inter-assay coefficients of variations (CVs) were evaluated by measuring two quality control samples, with high or low concentration of E-FABP, in eight replicates in a single assay or in duplicate in four consecutive assays, respectively. Serum A-FABP levels were measured using the kits from BioVendor Laboratory Medicine, Inc. as previously described. Serum adiponectin levels were determined with our in-house sandwich ELISA.
Measurement of carotid intima-media thickness and definition of carotid plaques

IMT of the common carotids was assessed using high resolution B-mode ultrasound (ATL HDI 3000 and 5000 ultrasound system; Advanced Technology Laboratories, Bothell, WA, USA) and linear transducers with frequency of 10–12 MHz, as previously described.11 Plaques were defined as IMT ≥ 0.13 mm or a focal protrusion into the lumen with a thickness at least 50% greater than the adjacent intima-media complex.

Statistical analysis

All analyses were performed with Statistical Package for Social Sciences version 15.0 (SPSS, Chicago, IL, USA). Data are expressed as mean ± SD or median with inter-quartile range unless otherwise specified. Data not normally distributed, as determined using Kolmogorov–Smirnov test, were logarithmically transformed before analysis. A-FABP and adiponectin levels were adjusted for sex because of their higher levels in women.8 – 11,26 One-way ANOVA and χ² test were used for comparisons between groups for continuous variables and for categorical variables, respectively, and multiple testing was corrected using Bonferroni correction by multiplying the univariate P-value by the number of comparisons. Pearson correlations were used to establish the associations between E-FABP concentrations and various anthropometric and biochemical parameters. Logistic regression analysis was used to calculate the likelihood ratios for the association with the MetS of serum E-FABP, A-FABP, the interaction between serum E-FABP and A-FABP (represented by the cross-product term E-FABP*A-FABP), and each component of the MetS. Multiple linear regression analysis including all relevant factors was performed to determine the variables with independent significant association with carotid IMT and included all variables with significant relationship with carotid IMT in univariate analyses (P < 0.05 after correction for multiple comparisons) as well as variables with biological relevance. Serum E-FABP and A-FABP levels were dichotomized according to their median values. Model assumptions including constant variance, independence, normality, and multicollinearity have been validated. Two-sided P-values less than 0.05 were considered statistically significant. A sample size of 479 subjects was able to detect a significant correlation with α = 0.05 with a detection power >90%.

Results

Epidermal fatty-acid-binding protein was detected in the blood stream

We used affinity chromatography followed by ultra-sensitive mass spectrometry to confirm that E-FABP was indeed present in the circulation. The tandem MS analysis showed that the peptide sequences derived from the affinity purification matched exclusively to E-FABP (Figure 1). Notably, none of the other forms of FabP was detected by this method, suggesting that the anti-human E-FABP antibody used in this study was highly specific.

Serum epidermal fatty-acid-binding protein levels were closely associated with cardio-metabolic parameters

The antibodies in human E-FABP ELISA kits were highly specific to human E-FABP with no cross-reactivities to human A-FABP, liver-FABP, intestinal-FABP, leptin, leptin receptor, adiponectin, resistin, resistin-like molecule-β, interleukine-6, agouti-related protein, and acylation-stimulating protein. The intra-assay CVs for the high- and low-quality control samples were 4.5 and 4.9%, respectively, whereas the corresponding inter-assay CVs were 5.7 and 5.9%, respectively. Serum concentrations of E-FABP were measured in 479 subjects (aged 55.4 ± 13.5) whose demographic and biochemical characteristics had been previously reported.11 Serum E-FABP concentrations ranged 1.19–8.01 μg/L. Unlike A-FABP and adiponectin, serum E-FABP levels (Table 1) were not significantly different between the two sexes.

A significant positive correlation was observed between serum E-FABP levels and age (Table 2). Besides, serum concentrations of E-FABP correlated positively with BMI, waist circumference, fat percentage, systolic blood pressure, triglycerides, serum A-FABP, and hs-C-reactive protein levels, as well as carotid IMT. On the other hand, serum E-FABP concentrations correlated negatively with HDL cholesterol and serum adiponectin levels.

Figure 1 Identification of E-FABP from human serum: the purified protein solutions were digested with trypsin before analysis by tandem mass spectrometry. The three peptides with masses of 927.4, 1813.6, and 3121.0 matched exclusively to the amino acid residues from 25 to 33, 34 to 50, and 83 to 109 of human E-FABP.
Hypertensive or lipid-lowering drugs, the relationship of serum To include also the significant proportions of the subjects on anti-

components of the metabolic syndrome

Serum epidermal fatty-acid-binding protein (E-FABP) was closely related to

Moreover, a significant positive correlation between age-adjusted serum E-FABP level and fasting insulin was found.

Table 1 Serum adipokine concentrations in study subjects

Table 2 Correlations of serum epidermal fatty-acid-binding protein levels with cardio-metabolic risk factors

Moreover, a significant positive correlation between age-adjusted serum E-FABP level and fasting insulin was found.

Serum epidermal fatty-acid-binding protein levels were closely related to components of the metabolic syndrome

To include also the significant proportions of the subjects on anti-hypertensive or lipid-lowering drugs, the relationship of serum E-FABP with hypertension and dyslipidaemia (Table 2) was further examined as categorical variables. In 469 subjects with complete set of data for all the five components of the MetS, serum E-FABP levels were significantly higher in subjects with the MetS vs. subjects without the MetS (P < 0.001) (Figure 2). Serum concentrations of E-FABP were also significantly higher in each of the five components of the MetS (P = 0.006 to P < 0.001) (Figure 2). In addition, serum E-FABP levels increased progressively with an increasing number of components of the MetS. Serum E-FABP levels [median (inter-quartile range)] in subjects with zero, one, two, three, and four or more components of the MetS were 2.18 (1.65–2.94) μg/L, 2.41 (1.62–3.44) μg/L, 2.62 (1.86–3.89) μg/L, 2.92 (1.87–4.46) μg/L, and 3.98 (2.90–6.69) μg/L, respectively (corrected P-value for trend < 0.001). The correlation of serum E-FABP with each MetS component was adjusted against all other four using univariate general linear model: its correlation with hypertension and low HDL cholesterol remained significant (P < 0.001 and P = 0.005, respectively).

Furthermore, the introduction of serum E-FABP into the logistic regression model significantly increased the likelihood of the MetS associated with each component of the MetS (P < 0.001) (Table 3). More importantly, the addition of serum E-FABP into the model that included serum A-FABP together with each component of the MetS (Model B) also significantly increased the likelihood of their association with the MetS (P = 0.007 to P < 0.001). The introduction of the interaction between serum E-FABP and A-FABP into model B did not lead to further significant change in the association with the MetS. These data suggest that serum E-FABP and A-FABP levels had independent effects but no interaction, in a logistic regression model, on the risk of having the MetS.

Serum epidermal fatty-acid-binding protein levels were independently associated with carotid atherosclerosis

In view of the strong positive correlation between serum E-FABP and carotid IMT, even after adjustment for age (Table 2), the relationship between serum E-FABP levels and carotid atherosclerosis was further examined. The correlations between serum E-FABP and carotid IMT remained statistically significant when the data were analysed separately in the two genders (men: r = 0.124, P = 0.010; women: r = 0.155, P = 0.015, age-adjusted). A significantly higher serum E-FABP concentration was observed in subjects with carotid plaques (n = 108): 3.42 (2.28–5.53) vs. 2.52
(1.76–3.74) μg/L] in subjects without carotid plaques (n = 370, P < 0.001). Other parameters which correlated with carotid IMT included age (r = 0.641, P < 0.001), waist circumference (r = 0.248, P < 0.001), serum A-FABP (r = 0.272, P < 0.001), serum hs-C-reactive protein (r = 0.207, P < 0.001), systolic blood pressure (r = 0.486, P < 0.001, in the 307 subjects not receiving anti-hypertensive drugs), fasting triglycerides (r = 0.124, P = 0.015), and HDL-cholesterol (r = −0.121, P = 0.016) (in the 393 subjects not receiving lipid-lowering drug treatment). Increased carotid IMT was found in the presence of hyperglycaemia (IFG/IGT/DM), hypertension, dyslipidaemia (in women only), and smoking (in men only) (all P < 0.05 after Bonferroni correction).

Multiple linear regression analysis was performed, including the above risk factors that had significant associations (P < 0.05 after Bonferroni correction) with carotid IMT, as well as serum A-FABP, which was shown to have independent association with carotid IMT in Chinese women.11 It was found that serum E-FABP, but not A-FABP, was a significant independent risk factor (P = 0.03) of carotid IMT in 225 men with complete set of data (seven men with missing data in smoking status were excluded), together with age (P < 0.001), hyperglycaemia (P = 0.019) and smoking (P = 0.043) (Table 4). On the other hand, serum E-FABP was not independently associated with carotid IMT in 245 women, in whom the final model (adjusted $R^2 =$

$$Table 3$$ Logistic regression analysis of serum epidermal fatty-acid-binding protein and adipocyte fatty-acid-binding protein in the association with the metabolic syndrome as the dependent variable

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Model A</th>
<th>Model B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference (WC)</td>
<td>WC + E-FABPa</td>
<td>15.86b</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>TGa + E-FABPa</td>
<td>27.03b</td>
</tr>
<tr>
<td>HDL cholesterol (HDL)</td>
<td>HDL + E-FABPa</td>
<td>24.27b</td>
</tr>
<tr>
<td>Elevated fasting glucose</td>
<td>Elevated fasting glucose + E-FABPa</td>
<td>25.57b</td>
</tr>
<tr>
<td>Hypertension (HT)</td>
<td>HT + E-FABPa</td>
<td>17.94b</td>
</tr>
<tr>
<td>Waist circumference + A-FABPa</td>
<td>WC + A-FABPa + E-FABPa</td>
<td>7.29b</td>
</tr>
<tr>
<td>Triglycerides + A-FABPa</td>
<td>TGa + A-FABPa + E-FABPa</td>
<td>15.28b</td>
</tr>
<tr>
<td>HDL cholesterol + A-FABPa</td>
<td>HDL + A-FABPa + E-FABPa</td>
<td>10.46b</td>
</tr>
<tr>
<td>Elevated fasting glucose + A-FABPa</td>
<td>Elevated fasting glucose + A-FABPa + E-FABPa</td>
<td>10.28b</td>
</tr>
<tr>
<td>Hypertension + A-FABPa</td>
<td>HT + A-FABPa + E-FABPa</td>
<td>8.79b</td>
</tr>
</tbody>
</table>

E-FABP, epidermal fatty-acid-binding protein; A-FABP, adipocyte fatty-acid-binding protein; n = 469.

aLog-transformed before analysis.

b$\chi^2$ (df = 1).
In the settings of dietary and genetic obesity, whereas played almost complete protection from obesity-related disorders that mice with combined deficiency of E-FABP and A-FABP disappeared. FABP in the MetS, as the possibility of a bystander effect cannot be excluded. In apoE-deficient mice, absence of both these FABPs resulted in an even greater protection against atherosclerosis and increased survival rate. Nevertheless, the correlation of serum E-FABP with triglycerides was rather weak, suggesting that E-FABP may play a more minor role in lipid metabolism, compared with that of A-FABP.

Discussion

Recent studies in mice have addressed the roles of E-FABP in obesity-related metabolic disorders and atherosclerosis. However, the clinical relevance of these animal-based findings has not been confirmed. In the present study, we provided novel evidence demonstrating that E-FABP is present in the human bloodstream. We have also demonstrated several pieces of clinical evidence suggesting that E-FABP is associated with cardiometabolic risks and the MetS in humans, analogous to its proposed role in rodents. First, age-adjusted serum E-FABP levels correlated positively with the parameters of adiposity (BMI, fat percentage and waist circumference), adverse lipid profiles (high triglycerides and low HDL-cholesterol), and hyperinsulinemia. Secondly, serum E-FABP levels were significantly higher in subjects with the MetS and in each of the five components of the MetS. Thirdly, serum E-FABP correlated negatively with adiponectin, but positively with hs-C-reactive protein, a well-known risk factor of the MetS. Lastly, the addition of E-FABP significantly increased the likelihood of the MetS being associated with A-FABP and each individual component of the MetS. Nevertheless, the association of E-FABP with the MetS in this cross-sectional study does not allow us to propose a pathogenic role of this FABP in the MetS, as the possibility of a bystander effect cannot be excluded.

In line with our clinical data, previous animal studies revealed that mice with combined deficiency of E-FABP and A-FABP displayed almost complete protection from obesity-related disorders in the settings of dietary and genetic obesity, whereas deficiency of either FABPs only resulted in a modest protective effect. In apoE-deficient mice, absence of both these FABPs resulted in an even greater protection against atherosclerosis and increased survival rate. Nevertheless, the correlation of serum E-FABP with triglycerides was rather weak, suggesting that E-FABP may play a more minor role in lipid metabolism, compared with that of A-FABP.

Our results also suggest that these two FABPs might have distinct profiles in the two genders. Whereas serum A-FABP exhibited a sexual dimorphism, with higher circulating levels in women, the present study showed comparable levels of E-FABP in men and women. This may be explained by the distinct tissue expression patterns of these FABPs. It is well known that women have a significantly higher body fat composition and more subcutaneous fat than men, whereas men have more visceral fat. The adipose tissue is the major source of A-FABP in the bloodstream, with a higher expression of A-FABP being found in subcutaneous fat. On the other hand, visceral fat expresses a higher level of E-FABP protein. In addition, E-FABP, a minor form of FABP in adipocytes, is expressed in many other tissues, which may also contribute to circulating E-FABP levels.

In this study, we also provided the first clinical evidence demonstrating the association between serum E-FABP and carotid atherosclerosis in humans. Serum E-FABP levels correlated positively with carotid IMT and were significantly higher in subjects with carotid plaques. Furthermore, our multiple regression analysis demonstrated an independent association between serum E-FABP, but not A-FABP, with carotid atherosclerosis. From this and our previous report using the same cohort, serum A-FABP was independently associated with carotid IMT in women, but not in men. Taken together, these results further support the conclusion that E-FABP has a greater impact on men, whereas A-FABP has a more profound effect in women. Further investigations on the potential mechanisms underlying the sexual dimorphism of E-FABP and A-FABP with respect to their serum concentrations and effects on atherosclerosis should allow us to discriminate the specific biological functions of these two FABPs. On the other hand, these independent associations of E-FABP and A-FABP with atherosclerosis in women are in accordance with findings in animals showing that the two FABPs act directly on macrophages to induce atherosclerosis, independent of effects on glycaemia and insulin.

| Table 4 | Multiple regression analysis showing the parameters with associations with carotid intima-media thickness in men |
|---|---|---|---|---|
| Coefficient B | 95% Cls | SE (B) | P-value |
| Serum E-FABPa | 0.026 | 0.003–0.050 | 0.012 | 0.030 |
| Age | 0.005 | 0.004–0.006 | 0.000 | <0.001 |
| Hyperglycaemia | 0.029 | 0.005–0.054 | 0.012 | 0.019 |
| Smoking | 0.024 | 0.001–0.048 | 0.012 | 0.043 |
| Hypertension | 0.016 | –0.009–0.042 | 0.013 | 0.200 |
| Waist circumference | 0.000 | –0.001–0.002 | 0.001 | 0.506 |
| Serum hs-C-reactive proteinb | –0.008 | –0.033–0.017 | 0.013 | 0.534 |
| Serum A-FABPc | –0.002 | –0.026–0.023 | 0.012 | 0.892 |

\(^{a}\)In median. \(^{b}\)Log-transformed. \(^{c}\)In sex-specific median. 

Women, the present study showed comparable levels of E-FABP to that of A-FABP. Two women with missing data on lipid-lowering therapy were excluded. 

0.497) included age ( \(P < 0.001\) ) and serum A-FABP ( \(P = 0.04\) ). Two women with missing data on lipid-lowering therapy were excluded.
sensitivity. Though less abundant in adipocytes, the levels of E-FABP are comparable to A-FABP in macrophages. Macrophage-specific disruption of both these FABPs in apoE-deficient mice resulted in a profound protection against atherosclerosis and a marked increase in survival rate, whereas lipid metabolism and insulin sensitivity were little affected. In summary, we have demonstrated the first clinical evidence that E-FABP is associated with cardio-metabolic risks, as well as carotid atherosclerosis, in humans. In addition, our findings suggest that E-FABP and A-FABP are independent of each other in their association with the MetS and that the two FABPs may have a gender-specific influence on atherosclerosis. The use of serum E-FABP as a single surrogate biomarker for predicting cardiovascular endpoints is probably limited. Whether it may be helpful as part of a multi-marker panel for predicting the risk of future cardiovascular events remains to be investigated. It should be noted, however, that the use of multi-marker panels has been questioned because of the limited effect when analysed with C statistic. Since the current study is only cross-sectional in nature and includes an over-representation of subjects with hyperglycaemia, long-term prospective studies based on cohorts representative of the general population will be carried out to evaluate whether E-FABP, individually or interactively with A-FABP, can be usefully employed as a biomarker for predicting the risk of atherosclerosis and cardiovascular events. The recent development of small molecule A-FABP inhibitors, with one of them successfully shown to protect against diabetes and atherosclerosis in rodent models, suggests that similar therapeutic strategies can also be explored for E-FABP. The measurement of serum E-FABP may serve as a useful biomarker for identifying individuals who are more likely to benefit from the therapeutic effect of such specific E-FABP inhibitors.

Conflict of interest: none declared.

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Sixty-four multislice computed tomography implantation of a Cribier-Edwards bioprosthesis in the aortic position

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A 84-year-old male with severe symptomatic aortic stenosis (valve area 0.66 cm²; transvalvular gradient 48 mmHg) declined for surgical aortic valve replacement due to severe chronic obstructive pulmonary disease underwent transcatheter implantation of a 26 mm Cribier-Edwards aortic bioprosthesis (Edwards Lifesciences Inc, Irvine, CA, USA). The procedure was performed using the retrograde approach through the right femoral artery. The prosthetic heart valve made of three leaflets of bovine pericardium sutured within a balloon expandable stainless steel stent was implanted under rapid pacing. After implantation, the echocardiographic transvalvular gradient decreased to 9 mmHg and the valve area increased to 1.9 cm². The hospital course was uneventful and the patient was discharged home 1 week after implantation.

A 64-multislice computed tomography (MSCT) was performed 6 days after implantation (VCT General Electric HealthCare, Milwaukee, USA). The accurate positioning of the bioprosthesis within the calcific native valve just below the coronary ostia and above the mitral valve could be clearly observed (Figure).

This case illustrates the potential interest of 64-MSCT for evaluating the correct positioning of the new Cribier-Edwards transcatheter heart valve. This technology might be particularly helpful in patients with technically limited approach by echocardiography.

Panel A. Contrast-enhanced ECG-gated 64-MSCT three chambers view reconstruction. The stent (in red) can be seen within the native valve and is clearly above the mitral valve. The cusps of the bioprosthesis (large arrow) are clearly delineated. LV, left ventricle; Ao, aorta; MV, mitral valve; LA, left atrium.

Panel B. MSCT reconstruction in coronal left ventricle outflow tract plane The stent is well below the ostium of the left main coronary artery (LMCA). The large arrow indicates the cusp of the bioprosthesis. The cusps of the bioprosthesis (large arrow) are clearly delineated. LV, left ventricle; Ao, aorta; MV, mitral valve; LA, left atrium.

Panel C. The three cusps are well visualized inside the stent which is surrounded by the calcifications of the native aortic valve.

Panel D. Left ventricle outflow tract plane MSCT reconstruction demonstrating the correct positioning of the stent (in red) below the ostia of the coronary arteries. RCA, right coronary artery; RV, right ventricle.