Serum Adipocyte Fatty Acid-Binding Protein Levels Were Independently Associated With Carotid Atherosclerosis


Objective—Adipocyte fatty acid−binding protein (A-FABP) has been shown to be an important player in atherosclerosis in animal models. However, the clinical relevance of these findings is still unknown. This study aims to examine the relationship between serum A-FABP level and carotid intima-media thickness (IMT), an indicator of atherosclerosis in humans.

Methods and Results—The study cohort included 479 Chinese subjects who underwent carotid IMT measurement. Serum A-FABP levels were determined by enzyme-linked immunosorbent assays. Serum A-FABP levels positively correlated with carotid IMT in both men ($r=0.211$, $P=0.001$) and women ($r=0.435$, $P<0.001$). In women, but not in men, the presence of plaques was associated with significantly higher serum A-FABP levels ($P<0.001$ versus women without plaques). Stepwise multiple regression analysis showed that serum A-FABP level was independently associated with carotid IMT in women ($P=0.034$), together with age and hypertension (both $P<0.001$).

Conclusions—A-FABP is an independent determinant of carotid atherosclerosis in Chinese women, but not in men. This gender difference may be attributed to the lower serum A-FABP levels in men, and the effect of other risk factors, such as smoking, among our male participants. Our results have provided clinical evidence supporting the role of A-FABP in the development of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2007;27:1796-1802.)

Key Words: adipocyte fatty acid-binding protein ■ obesity ■ atherosclerosis ■ carotid intima-media thickness ■ Chinese

A dipocyte fatty acid–binding protein (A-FABP, also known as aP2 or FABP4) is highly expressed in mature adipocytes, accounting for approximately 6% of their total soluble protein.1 It belongs to the superfamily of small molecular weight intracellular lipid-binding proteins, and plays a central regulatory role in energy metabolism and inflammation.2 Earlier animal studies showed that A-FABP–null mice were partially protected from developing hyperinsulinemia, hyperglycemia, and insulin resistance when challenged with dietary and genetic obesity.3,4 In apolipoprotein E (apoE)-deficient mice, whether on a low-fat or high-fat diet, ablation of the A-FABP gene provided almost complete protection against atherosclerosis, independent of its effects on glucose and lipid metabolism.5,6 Remarkably, after a high-fat atherogenic Western diet for 1 year, the survival rates of apoE$^{−/−}$ mice null for both A-FABP and mall were 67% higher than those of apoE$^{−/−}$ control mice, primarily because of the increased stability of atherosclerotic plaques.7 These results suggest that A-FABP is a major mediator of atherosclerotic lesion formation in mice. In humans, A-FABP is expressed in monocytes on PPAR$\gamma$ activation,8 and oxidized LDL has been shown to induce A-FABP expression in human THP-1 macrophage cell lines.9 Furthermore, a genetic variant associated with increased A-FABP mRNA expression in adipose tissues predicted coronary artery disease in homozgyous subjects.10 These findings suggest that A-FABP may also play a role in the development of atherosclerotic diseases in humans.

Although A-FABP was traditionally thought to be an intracellular protein, we have demonstrated that a small portion of A-FABP is released from mature adipocytes into the human blood stream,11 with the serum concentrations being ≈8 to 60 ng/mL. In a cross-sectional study we observed a strong positive association between serum A-FABP levels and parameters of adiposity. In addition, serum A-FABP levels correlated closely with several key features of the metabolic syndrome, an aggregate of cardiometabolic risk factors associated with accelerated atherosclerosis,12 including adverse lipid profiles (increased serum triglyceride and LDL-cholesterol, and decreased HDL-cholesterol), insulin resistance, hyperglycemia, and hypertension.13 More importantly, serum levels of A-FABP predicted the development of
the metabolic syndrome, independent of adiposity, in our recently published 5-year prospective study, further supporting the role of A-FABP as a potential mediator of the metabolic syndrome.

To investigate the role of A-FABP in atherosclerosis, we examined in 479 Chinese subjects the relationship between serum A-FABP levels and carotid intima-media thickness (IMT), a well-established indicator of atherosclerosis. High-sensitivity C-reactive protein (hsCRP), a well-known marker of systemic inflammation which predicts cardiovascular mortality in epidemiological studies, and adiponectin, an adipokine with antiatherogenic properties, were also measured to ascertain the role of A-FABP as an independent risk factor for the thickening of carotid IMT.

Methods

Subjects

Our cohort consisted of 479 subjects who underwent carotid IMT measurement at the Department of Radiology, Queen Mary Hospital. They included 296 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study who returned in 2001 for reassessment of their cardiovascular risk. Another 183 were patients with type 2 diabetes recruited from the Diabetes Clinic of the Queen Mary Hospital. All subjects gave informed consent and the protocol was approved by the Ethics Committee of the University of Hong Kong.

Subjects were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG), or diabetes mellitus (DM) according to WHO 1998 diagnostic criteria. Hypertension was defined as a sitting blood pressure of ≥130/85 mm Hg, taken as a mean of 2 readings obtained after at least 10 minutes, or on regular antihypertensive medications. Dyslipidemia was defined as having one or more of the following criteria: (1) fasting triglyceride ≥1.7 mmol/L; (2) HDL-cholesterol <1.29 mmol/L in female and <1.04 mmol/L in male; (3) LDL-cholesterol ≥3.4 mmol/L; (4) already on lipid-lowering drug.

Clinical and Biochemical Assessment

All subjects were assessed after overnight fasting for at least 10 hours. The details of anthropometric measurements (height, weight, BMI, waist circumference, and blood pressure) and the methods for assay of biochemical variables (fasting and 2-hour post-OGTT glucose, insulin, total cholesterol, triglycerides, LDL- and HDL-cholesterol) were reported previously. Insulin resistance was estimated using homeostasis model assessment index (HOMA-IR). Human A-FABP enzyme-linked immunosorbent assay (ELISA) (BioVendor Laboratory Medicine Inc) was performed as we previously described. hsCRP was measured with a particle-enhanced immunoturbidimetric assay (Roche Diagnostics). Serum adiponectin levels were determined with our in-house sandwich ELISA.

Measurement of Carotid IMT

High-resolution B-mode ultrasound (ATL HDI 3000 and 5000 ultrasound system; Advanced Technology Laboratories) was used to measure the IMT of the common carotid arteries. Linear transducers with frequency of 10 to 12 MHz were used. Anterolateral approach was used to longitudinally image the right and left common carotid arteries. Best image was selected to show the far wall intimal–lumen interface as a continuous straight line. Three determinations of IMT were made at 2 cm proximal to the bulb and at the site of greatest thickness. The values at each site were averaged, and the highest value of the averaged IMT used as the representative value for each individual. Plaque was defined as IMT ≥0.13 mm or a focal protrusion into the lumen with a thickness of at least 50% more than adjacent intima-media complex.

Statistical Analysis

All analyses were performed with Statistical Package for Social Sciences Version 14.0 (SPSS). Data are expressed as mean±SD or median with interquartile range as appropriate. Data that were not normally distributed, as determined using Kolmogorov–Smirnov test, were logarithmically transformed before analysis. A-FABP and adiponectin levels were adjusted for sex in all analyses because of higher levels of these hormones in females. Pearson correlations or 1-way ANOVA were used as appropriate for comparisons between 2 groups, and multiple testing was corrected using Bonferroni correction. Stepwise multiple linear regression analysis was done to determine the variables with independent significance 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). Probability values less than 0.05 were considered statistically significant.

Results

The demographic and biochemical characteristics of the subjects are summarized in Table 1. Compared with women, men had significantly higher carotid IMT, triglycerides, diastolic blood pressure, waist circumference, waist-hip ratio, and percentage of former and current smokers, but significantly lower HDL-cholesterol and adiponectin levels. Consistent with our previous reports, women had higher serum A-FABP levels: 26.2 (17.0 to 41.9) μg/L versus 18.9 (12.3 to 29.9) μg/L in men; P<0.001. This sexual dimorphism was seen even when only subjects ≥55 years of age, when the women were likely to be post-menopausal, were considered (Men [n=112; aged 67.5±6.2 years]: 22.9 [13.5 to 36.3] μg/L versus women [n=122; aged 67.4±6.6 years]: 36.8 [24.4 to 51.8] μg/L; P<0.001).

Age-adjusted serum A-FABP levels correlated positively with serum triglycerides, fasting insulin, HOMA-IR, and hsCRP, but inversely with serum HDL-cholesterol and adiponectin levels (Table 2). The positive correlation with LDL-cholesterol was not significant after Bonferroni correction. Serum A-FABP levels were not significantly higher in subjects with IGT/IFG (n=89): 21.1 (12.8 to 31.8) μg/L versus: 20.1 (12.4 to 31.1) μg/L in subjects with NGT (n=174; P=1.0). However, DM patients had significantly higher serum A-FABP levels (n=216; 26.3 [17.2 to 42.8] μg/L) than the NGT and IGT/IFG subjects (both P<0.001).

Serum A-FABP levels showed a significant positive correlation with carotid IMT in both genders (women: r=0.435, P<0.001 [Figure, A]; men: r=0.211, P=0.001 [Figure, B]; Table 3). Furthermore, a significantly higher serum A-FABP concentration was observed in women with plaques (n=48): 41.7 (24.6 to 55.5) μg/L versus 24.2 (16.1 to 37.7) μg/L in women without plaques (n=199; P<0.001). This difference in A-FABP level was not significant in men: 21.5 (12.4 to 35.0) μg/L in men with plaques (n=60) versus 18.5 (12.3 to 28.0) μg/L in men without plaques (n=172; P=0.352).

Subjects with IGT/IFG (n=89) or DM (n=216) had significantly higher carotid IMT (P<0.005 for both), compared those with NGT (n=174; IGT/IFG: 0.65 [0.57 to 0.77] mm; DM: 0.67 [0.55 to 0.80] mm; NGT: 0.58[0.50 to 0.73] mm). There was no significant difference in carotid IMT between IGT/IFG and DM when all subjects were analyzed or when either gender was considered (P=1.0).
Thus, for all subsequent analyses on the relationship between glycemic status and IMT, the IGT/IFG and DM subjects were combined and labeled as the group with hyperglycemia.

The relationships of the various cardiometabolic risk factors with carotid IMT are shown in Table 3 (continuous variables) and Table 4 (categoric variables). When all subjects were considered, serum A-FABP was among the factors positively related to carotid IMT, after correction for multiple testing. These factors also included age, systolic blood pressure/hypertension, waist circumference, waist-hip ratio, hsCRP, hyperglycemia, and smoking. In women, serum triglyceride/dyslipidemia was positively related to IMT, but smoking was not associated with significantly higher carotid IMT levels. In men, the associations of serum A-FABP, waist circumference, and hsCRP with carotid IMT were not statistically significant after Bonferroni correction.

On stepwise multiple regression analysis of the significant risk factors of IMT identified in the above univariate analyses (Table 5), serum A-FABP was independently associated with carotid IMT in women (P=0.034), but not in men. Age was an independent risk factor of increased carotid IMT in both women and men (P<0.001). The other independent risk factors of carotid IMT included hypertension in women only (P<0.001) and, in men only, waist-hip ratio (P=0.024), hyperglycemia (P=0.018), and smoking (P=0.047). hsCRP was not a significant independent risk factor of carotid IMT even when all subjects were included in the analysis. Repeated analysis including the same parameters for both sexes did not alter the results of the stepwise multiple regression models.

**Discussion**

Although recent studies in mouse models have demonstrated A-FABP to be a central player in atherosclerosis,2,5–7 the clinical relevance of these animal-based findings remains to be established. In this study, we provided the first clinical evidence demonstrating the existence of a close association between serum A-FABP levels and carotid atherosclerosis in humans. A positive correlation between serum A-FABP and carotid IMT was observed in both genders and, in women, the
presence of plaques was associated with significantly higher serum A-FABP levels. Furthermore, our multiple regression analysis had identified serum A-FABP, together with age and hypertension, to be independent risk factors in female subjects. Previous studies have revealed the association of increased adipose A-FABP mRNA expression with coronary heart disease, and the correlation between serum A-FABP and its expression in adipose tissues. Our clinical data based on serum A-FABP levels would therefore support a causative role of A-FABP in atherosclerosis in human subjects.

Although serum A-FABP levels significantly correlated with carotid IMT in both genders, our results suggested A-FABP to have a greater impact on atherosclerosis in women. The independent association between serum A-FABP and carotid IMT was not observed in men. Instead, waist-hip ratio, hyperglycemia, and smoking were significantly associated with carotid IMT among our male subjects, whereas women had fewer confounding factors than the men in this study. In particular, these women had a lower prevalence of cigarette smoking, an established risk factor of carotid atherosclerosis. Our female participants had fewer confounding factors than the men in this study. In particular, these women had a lower prevalence of cigarette smoking, as a higher carotid IMT has been found in smokers. Another possible explanation for the gender-specific effect of A-FABP on carotid atherosclerosis is the sexual dimorphism in serum A-FABP concentrations. In this and our earlier studies, serum A-FABP levels in women were significantly higher than those in men. This gender difference could be partly attributed to the higher fat percentage in women, because adipose tissue is the major contributor of circulating A-FABP. A difference in regional fat distribution might also contribute to this sexual dimorphism, as women generally have more subcutaneous fat than men, whereas men have more abdominal (visceral) fat. In a study of obese Whites, A-FABP expression was found to be higher in subcutaneous compared with omental adipose tissue. Notably, the sexual dimorphism of A-FABP resembles that of adiponectin, another major adipokine. We have previously demonstrated that the lower adiponectin levels in men are attributable to the suppressive effects of testosterone on adiponectin secretion. Whether testosterone can modulate A-FABP expression or secretion is currently under investigation in our laboratory.

As in our previous report, serum A-FABP levels correlated positively with serum hsCRP levels in this cohort. hsCRP has been reported to contribute to atherosclerosis in humans. It enhances the expression of adhesion molecules in human endothelial cells, and mediates the uptake of LDL into macrophages. Interestingly, A-FABP also regulates foam cell formation from macrophages, through enhancing intracellular lipid accumulation. Whether hsCRP and A-FABP act in a synergistic manner in promoting atherosclerosis remains to be determined.

In this and our earlier studies, a negative correlation between serum adiponectin and A-FABP is found, although...
both proteins are predominantly produced from adipocytes. Furthermore, adiponectin and A-FABP act in opposite manners in the pathogenesis of atherosclerosis. Firstly, adiponectin has antinflammatory properties, including the suppression of tumor necrosis factor (TNF)-α production in macrophages\(^\text{28}\) and adipocytes.\(^\text{29}\) In contrast, A-FABP is proinflammatory, because the ablation of A-FABP in macrophages leads to reduced IkB kinase and NF-κB activity, diminished cyclooxygenase-2 and inducible nitric-oxide synthase expression, and impaired production of proinflammation cytokines.\(^\text{27,30}\) Secondly, adiponectin has beneficial effects on lipid metabolism\(^\text{31,32}\) and intracellular lipid accumulation in macrophages,\(^\text{33}\) whereas A-FABP is proposed to contribute to dyslipidemia\(^\text{34,35}\) and foam cell formation.\(^\text{27}\) In humans, decreased adipose tissue expression of A-FABP in subjects homozygous for a functional polymorphism is associated with a reduced risk of hypertriglyceridemia.\(^\text{10}\) Thirdly, adiponectin is a potent insulin-sensitizer,\(^\text{36}\) whereas A-FABP induces insulin resistance, as A-FABP null mice are protected from insulin resistance in the context of dietary and genetic obesity.\(^\text{3,4}\)

A-FABP might induce atherosclerotic formation through multiple mechanisms. Studies in both animals\(^\text{2-4}\) and humans\(^\text{10,11,13}\) suggest A-FABP to be an important mediator of insulin resistance and metabolic syndrome, the key risk factors of atherosclerosis. A-FABP may also promote atherosclerosis through direct actions in macrophages. Adenovirus-mediated overexpression of A-FABP in human macrophages directly induces foam cell formation by increasing intracellular cholesterol ester accumulation.\(^\text{27}\) On the contrary, ablation of A-FABP expression in macrophage increases cholesterol efflux and prevents oxidized LDL-induced foam cell formation. Bone-marrow transplantation of A-FABP−/− macrophages into apoE−/− mice reduces atherosclerosis to a level comparable to that in apoE−/− mice with total A-FABP deficiency, indicating an independent role for macrophage A-FABP in atherogenesis.\(^\text{5}\) Nevertheless, the detailed biological pathways whereby A-FABP promotes foam cell formation and atherosclerosis are largely unknown. In agreement with our observation that A-FABP is a serum protein, a recent proteomics-based study on human macrophage cells showed that much A-FABP was released into the conditioned medium.\(^\text{37}\) Whether A-FABP can induce atherosclerosis through its endocrine or autocrine actions on macrophages is being investigated in our laboratory.

**Study Limitations**

The major limitation of this study was its cross-sectional design. Therefore our findings should be investigated in long-term prospective studies before a causal relationship between serum A-FABP and carotid atherosclerosis can be established. Furthermore, this study included an overrepresentation of subjects with hyperglycemia (IGT, IFG, or type 2 diabetes), a well-known risk factor of atherosclerosis. The relationship between serum A-FABP and carotid IMT in the general population, and its role relative to hsCRP\(^\text{38}\) and adiponectin\(^\text{39}\) in this regard, should be further studied in large population-based studies, even though serum A-FABP, but not hsCRP and adiponectin, was found to be independently associated with carotid IMT in our study.

**Conclusions**

In summary, our present study is the first demonstration that high serum A-FABP levels are associated with carotid atherosclerosis in human subjects. Given that A-FABP is 1 of the major adipokines, these findings, together with our previous observations showing the close association of A-FABP with obesity-related insulin resistance and metabolic syn-

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**TABLE 4. Carotid Intima-Media Thickness in Relation to Various Cardio-Metabolic Risk Factors**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Carotid IMT* in All Subjects</th>
<th>Carotid IMT* in Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n)</td>
<td>No (n)</td>
</tr>
<tr>
<td></td>
<td>0.65 (0.55–0.78)</td>
<td>0.58 (0.50–0.70)</td>
</tr>
<tr>
<td></td>
<td>0.70 (0.58–0.83)</td>
<td>0.62 (0.53–0.72)</td>
</tr>
<tr>
<td>Hyperglycemia†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.63 (0.55–0.77)</td>
<td>0.58 (0.50–0.73)</td>
</tr>
<tr>
<td></td>
<td>0.64 (0.56–0.80)</td>
<td>0.69 (0.52–0.78)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.68 (0.60–0.80; n = 282)</td>
<td>0.53 (0.48–0.63; n = 195)</td>
</tr>
<tr>
<td></td>
<td>0.70 (0.58–0.81; n = 156)</td>
<td>0.60 (0.50–0.73; n = 322)</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.68 (0.60–0.82; n = 143)</td>
<td>0.58 (0.52–0.70; n = 88)</td>
</tr>
<tr>
<td>Smoking‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.70 (0.58–0.81; n = 136)</td>
<td>0.62 (0.52–0.72; n = 95)</td>
</tr>
</tbody>
</table>

*Log-transformed before analysis. †Hyperglycemic subjects were those with IGT (impaired glucose tolerance)/IFG (impaired fasting glucose) and type-2 diabetes, whereas nonhyperglycemic was regarded as having NGT (normal glucose tolerance); ‡Smoking included those current smokers and former smokers, whereas nonsmoker referred to those who never smoked.

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**TABLE 5. Stepwise Multiple Regression Analysis Showing the Parameters With Significant Independent Associations With Carotid Intima-Media Thickness**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient B</th>
<th>SE (B)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Women only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum A-FABP*</td>
<td>0.037</td>
<td>0.017</td>
<td>0.034</td>
</tr>
<tr>
<td>Age</td>
<td>0.004</td>
<td>0.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.041</td>
<td>0.011</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B. Men only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.005</td>
<td>0.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.208</td>
<td>0.092</td>
<td>0.024</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>0.029</td>
<td>0.012</td>
<td>0.018</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.023</td>
<td>0.012</td>
<td>0.047</td>
</tr>
</tbody>
</table>

*Log-transformed before analysis. A. The analysis included also waist-hip ratio, serum hsCRP (high-sensitivity C-reactive protein), hyperglycemia, and dyslipidemia, which were excluded from the final model. B. The analysis included also serum A-FABP (adipocyte fatty acid-binding protein) and hypertension, which were excluded in the final model.
Table 4. (Continued)

<table>
<thead>
<tr>
<th>Carotid IMT* in Women</th>
<th>Yes</th>
<th>No</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.65 (0.53–0.75; n=152)</td>
<td>0.55 (0.48–0.67; n=152)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>0.63 (0.53–0.75; n=178)</td>
<td>0.53 (0.47–0.68; n=69)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>0.68 (0.58–0.78; n=139)</td>
<td>0.52 (0.47–0.62; n=107)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>0.67 (0.58–0.79; n=20)</td>
<td>0.60 (0.50–0.73; n=227)</td>
<td>0.058</td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgments

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Disclosures

None.

References


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