Circulating Adipocyte–Fatty Acid Binding Protein Levels Predict the Development of the Metabolic Syndrome: A 5-Year Prospective Study

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Circulating Adipocyte–Fatty Acid Binding Protein Levels Predict the Development of the Metabolic Syndrome

A 5-Year Prospective Study

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Background—Adipocyte–fatty acid binding protein (A-FABP), a major cytoplasmic protein in adipocytes, plays a central role in the development of diabetes and atherosclerotic cardiovascular disease in experimental animals. We have previously shown that A-FABP is present in the bloodstream and that its circulating levels correlate with metabolic risk factors in a cross-sectional study. In the present study, we further evaluated the prospective association of A-FABP with the metabolic syndrome (MetS) as defined by the updated National Cholesterol Education Program criteria.

Methods and Results—In the present study, 495 nondiabetic adults from the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study were prospectively followed up for 5 years. The relationship of serum A-FABP with the MetS and its components was investigated. At baseline, high A-FABP levels were associated with the MetS (odds ratio, 4.0; 95% CI, 1.5 to 10.4; highest versus lowest sex-specific tertile, adjusted for age, body mass index, the homeostasis model assessment index for insulin resistance, C-reactive protein, and adiponectin, $P=0.005$). On long-term follow-up, subjects with higher baseline A-FABP levels had progressively worse cardiometabolic risk profile and increasing risk of the MetS. Among 376 subjects without the MetS at baseline, 50 had developed it at 5 years. Apart from the homeostasis model assessment index for insulin resistance ($P=0.001$), baseline A-FABP was the only independent predictor of the development of the MetS during the 5-year follow-up (odds ratio, 4.7; 95% CI, 1.8 to 11.9; highest versus lowest sex-specific tertile, $P=0.001$, adjusted for the homeostasis model assessment index for insulin resistance and body mass index). A-FABP was predictive of the MetS even after adjustment for each of its individual components.

Conclusions—Circulating A-FABP predicts the development of the MetS independently of adiposity and insulin resistance. (***Circulation*. 2007;115:1537-1543.**)

Key Words: inflammation ■ insulin ■ lipids ■ metabolism ■ obesity

The metabolic syndrome (MetS) encompasses a cluster of interrelated risk factors that are closely associated with the development of atherosclerotic cardiovascular diseases.[1] Obesity, especially excessive accumulation of abdominal fat, is the principal etiological factor that predisposes to insulin resistance and the MetS. Obesity triggers metabolic stress and inflammatory responses in adipose tissue, both of which are now thought to be the important mediators of obesity-related metabolic and cardiovascular pathologies.[2]

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Adipocyte–fatty acid binding protein (A-FABP; also designated aP2 or FABP4) is an ≈15-kDa, small, lipid-binding protein that is expressed predominantly in adipose tissue.[3,4] This protein is the major cytosolic protein of mature adipocytes, accounting for ≈6% of the total cellular protein. In addition, A-FABP is present in macrophages,[5] which have striking similarities to adipocytes in biology and functions. Mounting evidence suggests that this protein plays a key role in linking obesity with various features of the MetS. Mice with A-FABP deficiency are protected from the development of dyslipidemia, hyperglycemia, insulin resistance, and fatty liver disease in the context of both genetic and dietary obesity.[6–8] Furthermore, in apolipoprotein E–deficient mice, ablation of the A-FABP gene causes a remarkable reduction (≈90%) in atherosclerosis[9] and a striking increase in survival rate when challenged with a high-fat atherogenic Western diet.[5] More recently, a reduced risk for hypertriglyceridemia,

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type 2 diabetes, and coronary artery disease has been found in subjects from the Nurses' Health Study and the Health Professionals Follow-Up Study who carry a functional genetic variant of the A-FABP gene that results in reduced adipose tissue A-FABP gene expression.[10]

Although A-FABP was traditionally thought to be a cytoplasmic protein, our recent study showed that a significant portion of this protein also is released from adipocytes into the bloodstream.[11] In a cross-sectional study involving 229 subjects, we found that circulating concentrations of A-FABP correlate significantly with features of the MetS, including central adiposity, dyslipidemia (increased serum triglycerides and decreased high-density lipoprotein [HDL] cholesterol), fasting glucose, insulin resistance, and blood pressure. Furthermore, circulating A-FABP levels increase correspondingly with the increasing number of components of the MetS. These clinical data, together with recent findings from A-FABP–null mice[6] and a genetic study in humans,[10] have provided support for the role of A-FABP as a key player in the development of obesity-related pathologies in humans.

To further evaluate whether A-FABP is an independent risk factor for the cluster of metabolic risk factors that predispose to atherosclerotic cardiovascular disease, we investigated prospectively the 5-year development of the MetS in relation to baseline A-FABP levels in a population-based cohort comprising 495 Chinese subjects recruited from the Hong Kong Cardiovascular Risk Factor Prevalence Study.[12–14]

Methods

Participants

Subjects were recruited from the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study, in which unrelated Chinese subjects were invited to undergo a comprehensive assessment of cardiovascular risks to establish the prevalence of risk factors in southern Chinese subjects.[15,16] Of the 2843 subjects who participated in the cross-sectional study in 1995 to 1996, 644 nondiabetic subjects (as defined by 1998 World Health Organization diagnostic criteria[17]) were subsequently invited to participate in a long-term prospective study to assess the progression of diabetes and other cardiovascular risk factors as reported previously.[12–14] Subjects taking regular lipid-lowering agents without an available pretreatment lipid profile were excluded from analysis. This report includes data from 495 subjects with available stored serum samples for measurement of baseline A-FABP who were not on lipid-lowering drugs at baseline and had returned for the 5-year follow-up assessment. There was no significant difference in baseline characteristics between these subjects and those who did not fulfill the inclusion criteria (n=149; data not shown).

Measurements

The MetS

The MetS and metabolic risks are defined according to the US National Cholesterol Education Program Adult Treatment Panel III guidelines[18] and modified as recommended in the latest American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement[1] by adopting the Asian criteria for waist circumference and a lower cutoff for fasting glucose (≥5.6 mmol/L). The MetS was defined as having ≥3 of the following metabolic risk factors: (1) central obesity (waist circumference ≥80 cm in women and ≥90 cm in men), (2) hypertriglyceridemia (fasting triglycerides ≥1.69 mmol/L), (3) low HDL cholesterol (fasting HDL <1.29 mmol/L in women and <1.04 mmol/L in men), (4) glucose intolerance (fasting glucose ≥5.6 mmol/L or already on oral hypoglycemic agents for treatment of type 2 diabetes), and (5) hypertension (sitting blood pressure ≥130/85 mm Hg obtained as a mean of 2 readings taken after resting for at least 10 minutes or on regular antihypertensive medications).

Clinical and Biochemical Assessments

Subjects returned at years 2 and 5 for reassessment but were under the care of their own primary care physicians between visits. Subjects attended each visit after an overnight fast of at least 10 hours. Details of anthropometric measurements (height, weight, body mass index [BMI], waist circumference, and blood pressure) and the methods for measuring biochemical variables (fasting and 2-hour post–oral glucose tolerance test glucose, insulin, total cholesterol, triglycerides, and low-density lipoprotein and HDL cholesterol) were reported previously.[12–14] Insulin resistance was estimated with the homeostasis model assessment index (HOMA-IR), calculated as fasting glucose (in mmol/L) times fasting insulin (in mIU/L) divided by 22.5.[19] High-sensitivity C-reactive protein (CRP) was measured with a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany) using anti-CRP mouse monoclonal antibodies coupled to latex microparticles.[14] Adiponectin was measured with an in-house sandwich ELISA established in our laboratory (intra-assay and interassay coefficients of variation of 6.2% to 8.3% and 5.1% to 6.4%, respectively).[20] A-FABP was measured with an ELISA (BioVendor Laboratory Medicine, Inc, Modrice, Czech Republic), with calibration and quality control performed as previously reported.[11] All subjects gave informed consent, and the present study was approved by the Ethics Committee of the Faculty of Medicine, University of Hong Kong.

Animal Studies

Serum samples were collected from 14 male C57BL/6J mice (age, 15 to 17 weeks; Laboratory Animal Unit, University of Hong Kong, Hong Kong, China) for measurement of circulating A-FABP concentrations with an ELISA kit specific to mouse A-FABP (BioVendor Laboratory Medicine, Inc). Adipose tissues from 4 different fat depots (visceral, subcutaneous, epididymal, and interscapular) were carefully dissected, rinsed with PBS, and lysed in a buffer containing 1% triton/X-100, 0.1% sodium deoxycholate, and 20 mmol/L Tris (pH 7.4). Tissue lysates were clarified by centrifugation. A-FABP protein levels and total protein concentrations were measured with the mouse A-FABP ELISA and bicinchoninic acid assay (Pierce, Rockford, Ill), respectively. The study protocol was approved by the Animal Ethics Committee of the Medical Faculty, University of Hong Kong.

Statistical Analysis

Statistical analysis was performed with SPSS version 12.0 (SPSS, Inc, Chicago, Ill). Results are presented as mean±SD or median with interquartile range as appropriate. Data that were not normally distributed, as determined with the Kolmogorov-Smirnov test, were logarithmically transformed to obtain near normality before analysis. A-FABP and adiponectin levels were adjusted for sex in all analyses because of the higher levels of these hormones in women.[11,20] Baseline A-FABP levels of the entire cohort with 5-year follow-up data (n=495) were grouped into tertiles in a sex-specific manner so that each subject was classified as being in the low, middle, or high tertile of A-FABP level among individuals of the same sex. The choice of measuring A-FABP levels in tertiles was made a priori to simplify the interpretation of the results of subsequent regression analyses. The χ^2 test was used to compare categorical variables between groups. For continuous variables, 1-way ANOVA was used to compare between 2 groups, and multiple testing was corrected for with Bonferroni correction. Duncan multiple range test was used for comparison of multiple groups. Correlations between A-FABP and anthropometric and biochemical variables were analyzed with Pearson correlation. Logistic regression analysis was used to calculate the odds ratios (ORs) for the development of the MetS at year 5 in subjects with raised baseline A-FABP (second and third tertiles) compared with those with low baseline A-FABP (lowest tertile or

TABLE 1. Baseline Characteristics of Subjects According to the Presence or Absence of the MetS at Baseline

	Yes (n=119)	No (n=376)	*P*
Sex, M/F	60/59	150/226	0.043
Age, y	54.7±11.1	49.0±12.0	<0.001
BMI, kg/m^2	27.8±3.3	23.9±3.4	<0.001
WC, cm			
Men	93.8±7.2	81.7±7.8	<0.001†
Women	86.2±6.6	75.4±7.9	
HT, yes/no	97/22	88/288	<0.001
FG, mmol/L	5.6±0.6	5.1±0.5	<0.001
HDL, mmol/L	1.0±0.2	1.3±0.3	<0.001
TG, mmol/L*	1.8 (1.4–2.3)	0.9 (0.7–1.3)	<0.001
LDL, mmol/L	3.6±0.9	3.3±0.9	0.001
HOMA-IR*	2.0 (1.3–2.8)	1.1 (0.7–1.6)	<0.001
CRP, mg/L*	1.7 (0.8–2.9)	0.8 (0.3–1.6)	<0.001
Adiponectin, μg/mL			
Men	4.7 (2.8–5.8)	5.7 (3.7–7.6)	<0.001†
Women	5.2 (3.8–7.5)	6.7 (5.0–9.2)	
A-FABP, μg/L*			
Men	23.2 (18.2–29.5)	13.3 (9.2–19.0)	<0.001†
Women	26.0 (21.1–33.8)	18.3 (12.8–26.0)	

WC indicates waist circumference; HT, hypertension; FG, fasting glucose; TG, triglycerides; and LDL, low-density lipoprotein. Data are mean±SD or median (interquartile range) unless otherwise indicated. n=495 except for CRP (n=486) and adiponectin (n=480).

*Log transformed before analysis.

†Sex-adjusted.

reference group), with additional adjustment for each component of the MetS. Stepwise logistic regression analyses were used to examine the association of baseline A-FABP and other parameters with the development of the MetS at year 5. The variables selected to enter into stepwise regression were variables that were significantly different (after Bonferroni correction for multiple testing) between subjects with and without the MetS at baseline and at year 5. MetS-defining parameters were not included. Two-sided values of $P<0.05$ were considered significant.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

Results

Among the 495 subjects who returned for the 5-year follow-up assessment, 376 subjects did not have the MetS at baseline. The baseline clinical characteristics of the cohort are summarized in Table 1. More male subjects had the MetS compared with female ($P<0.05$). As expected, subjects with the MetS at baseline had significantly more adverse risk factors than those without the MetS, including higher BMI, age, low-density lipoprotein cholesterol, and insulin resistance index (as assessed by HOMA-IR), as well as MetS-defining parameters (waist circumference, fasting glucose, triglycerides, hypertension, and low HDL cholesterol) ($P<0.001$ for all parameters). They also had higher high-sensitivity CRP and lower serum adiponectin levels (both $P<0.001$). In addition, the A-FABP concentration was significantly higher in subjects with the MetS (Table 1; $P<0.001$, sex-adjusted). A-FABP correlated positively with CRP ($r=0.14$, $P=0.002$) and negatively with adiponectin ($r=-0.12$, $P=0.008$) after adjustment for sex, age, and BMI.

TABLE 2. Multiple Stepwise Logistic Regression Analysis Showing Factors Independently Associated With the MetS at Baseline

Parameters	OR	95% CI	*P*
Age	1.06	1.03–1.09	<0.001
BMI	1.25	1.14–1.37	<0.001
HOMA-IR*	4.85	2.78–8.46	<0.001
A-FABP tertile 2 vs 1†	3.09	1.21–7.93	0.019
A-FABP tertile 3 vs 1†	3.99	1.53–10.39	0.005
A-FABP tertile 3 vs 2‡	1.29	0.71–2.35	0.402

Variables included in the original model are age, BMI, HOMA-IR, low-density lipoprotein, CRP, adiponectin, and A-FABP in sex-specific tertiles.

*Log transformed before analysis.

†Tertile 1 as reference with OR=1.

‡Tertile 2 as reference with OR=1.

On multiple stepwise logistic regression analysis, A-FABP in sex-specific tertiles ($P=0.018$), together with age ($P<0.001$), BMI ($P<0.001$), and HOMA-IR ($P<0.001$), was independently associated with the presence of the MetS at baseline. Subjects with the highest tertile of A-FABP (A-FABP ≥19.9 μg/L in men and ≥25.6 μg/L in women) had a much increased likelihood of having the MetS compared with those in the lowest tertile (A-FABP ≤12.5 μg/L in men and ≤16.4 μg/L in women) (OR, 4.0; 95% CI, 1.5 to 10.4; highest versus lowest tertile, $P=0.005$) (Table 2).

At the 5-year follow up, the baseline age- and sex-adjusted A-FABP was found to have significant positive correlations with multiple adverse metabolic parameters at year 5, including high BMI, waist circumference, systolic and diastolic blood pressures, fasting glucose, triglycerides, total and low-density lipoprotein cholesterol, and HOMA-IR. On the other hand, a significant negative correlation was found between baseline age- and sex-adjusted A-FABP and HDL cholesterol level at year 5 (Table 3). As shown in Table 4, subjects with rising tertiles of baseline A-FABP had progressively worse cardiometabolic risk profile. Rising tertiles of baseline A-FABP also were associated with increasing prevalence of having ≥3 MetS components ($P<0.001$), and thus qualified for the diagnosis of MetS, and a decreasing prevalence of having none of the National Cholesterol Education Program MetS components ($P<0.001$).

Among the 376 subjects who did not have the MetS at baseline, 50 had developed it at year 5. Three subjects were on lipid-lowering drugs at year 5 but did not have pretreatment lipid profiles for accurate classification of their MetS status and hence were excluded from the year 5 analysis. The baseline A-FABP concentration was significantly higher in subjects who had progressed to the MetS at year 5: 19.9 μg/L (interquartile range, 16.7 to 26.2 μg/L) versus 15.0 μg/L (interquartile range, 10.3 to 22.2 μg/L) in subjects without the MetS (sex-adjusted $P=0.0002$). There was a progressive increase in serum A-FABP concentrations so that a significant interval change ($P<0.001$ in both groups, paired *t* test) was seen at year 5. However, there was no significant

TABLE 3. Pearson Correlations of Baseline A-FABP With Cardiometabolic Characteristics at Year 5

	A-FABP*	
	r	*P*
BMI	0.51	<0.001
WC	0.48	<0.001
SBP†	0.15	0.003
DBP†	0.14	0.005
FG	0.20	<0.001
HDL‡	−0.33	<0.001
LDL‡	0.23	<0.001
TG*‡	0.39	<0.001
HOMA-IR*	0.32	<0.001

WC indicates waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting glucose; TG, triglycerides; and LDL, low-density lipoprotein. Model was adjusted for sex and age.

*Log transformed before analysis.

†Includes only subjects not on antihypertensive treatment (n=399).

‡Includes only subjects not on anti–lipid-lowering treatment (n=471).

difference in the interval change in A-FABP concentrations between the 2 groups (the MetS at year 5: median, 9.2 μg/L [interquartile range, −0.1 to 15.0 μg/L]; non-MetS: median, 4.1 μg/L [interquartile range, −2.0 to 9.7 μg/L]; $P=0.246$, independent *t* test).

In addition to higher baseline A-FABP, subjects who had developed the MetS at year 5 had significantly higher baseline BMI, waist circumference, triglycerides, HOMA-IR ($P<0.001$ for all), and low-density lipoprotein cholesterol ($P=0.005$) and lower HDL cholesterol ($P<0.001$). Their higher baseline CRP ($P=0.018$, sex and age adjusted) and apparently lower baseline adiponectin ($P=0.059$, sex and age adjusted) levels were statistically not significant after adjustment for BMI or waist circumference.

TABLE 5. Multiple Logistic Regression Analysis Showing Factors Predicting the Development of the MetS at Year 5

Parameters	OR	95% CI	*P*
HOMA-IR*	2.88	1.58–5.22	0.001
A-FABP tertile 2 vs 1†	4.39	1.79–10.79	0.001
A-FABP tertile 3 vs 1†	4.65	1.82–11.88	0.001
A-FABP tertile 3 vs 2‡	1.06	0.52–2.17	0.878

Variables included in the original model are BMI, HOMA-IR, and A-FABP in sex-specific tertiles. n=373.

*Log transformed before analysis.

†Tertile 1 as reference with OR=1.

‡Tertile 2 as reference with OR=1.

In multiple stepwise logistic regression analysis, baseline A-FABP ($P=0.002$) and HOMA-IR ($P=0.001$) were the only independent predictors for the development of the MetS at year 5 (Table 5). Subjects with the highest sex-specific tertile of baseline A-FABP had an increased risk of developing the MetS at year 5 compared with those with A-FABP in the lowest tertile (OR, 4.7; 95% CI, 1.8 to 11.9; highest versus lowest tertile, $P=0.001$) after adjustment for BMI and HOMA-IR. Adjustment for alcohol intake and smoking status did not alter any of the above results.

Table 6 shows that, compared with subjects in the lowest sex-specific tertile of baseline A-FABP, subjects with baseline A-FABP in the second tertile had an OR of 4.4 (95% CI, 1.9 to 10.2; $P=0.001$) of developing the MetS at year 5. For those with baseline A-FABP in the highest tertile, the OR was 4.5 (95% CI, 1.9 to 10.8; $P=0.001$). These ORs did not

TABLE 4. Association of Baseline A-FABP Concentrations With Cardiometabolic Risk Factors at Year 5

	A-FABP Tertile			
	1 (Men, <12.5 μg/L; Women, <16.4 μg/L)	2 (Men, 12.5–19.9 μg/L; Women, 16.4–25.6 μg/L)	3 (Men, >19.9 μg/L; Women, >25.6 μg/L)	*P*
Age, y	50.8±11.8	55.7±10.6¶	60.4±11.8¶#	<0.001
BMI, kg/m^2	22.9±3.1	25.1±3.2¶	26.6±4.0¶#	<0.001
WC, cm	75.5±7.9	81.5±8.5¶	85.1±11.0¶#	<0.001
HT, yes/no	41/124	92/73¶	92/73¶	<0.001
FG, mmol/L	5.1±0.6	5.3±0.7‖	5.4±0.8¶	0.007
HDL, mmol/L†	1.5±0.4	1.3±0.3¶	1.3±0.4¶	<0.001
TG, mmol/L*†	0.9 (0.7–1.3)	1.10 (0.9–1.6)‖	1.5 (1.0–2.1)¶**	<0.001
LDL, mmol/L†	3.1±0.8	3.4±0.8§	3.6±0.8¶	<0.001
HOMA-IR*	1.5 (1.1–2.1)	1.9 (1.3-2.7)‖	2.2 (1.4–3.3)¶#	0.062
≥3 MetS components, %‡	8 (13/165)	31.1 (51/164)¶	42.9 (70/163)¶#	<0.001
No MetS components, %‡	50.3 (83/165)	17.7 (29/164)¶	9.8 (16/163)¶#	<0.001

WC indicates waist circumference; HT, hypertension; FG, fasting glucose; TG, triglycerides; and LDL, low-density lipoprotein. Data are mean±SD or median (interquartile range) unless otherwise indicated.

*Log transformed before analysis.

†Includes only subjects not on anti–lipid-lowering treatment.

‡Excluded 3 patients on lipid-lowering drugs without available pretreatment lipid profile.

§$P<0.05$, ‖$P<0.01$, ¶$P<0.001$ vs tertile 1.

#$P<0.05$, **$P<0.001$ between tertiles 2 and 3.

TABLE 6. Logistic Regression Analysis of Baseline A-FABP in the Prediction of the Development of MetS: ORs

	A-FABP			
	Tertile 2		Tertile 3	
Variable(s) in Model	OR	95% CI	OR	95% CI
A-FABP*	4.40†	1.90–10.18	4.50†	1.87–10.83
WC+A-FABP*	3.56†	1.51–8.40	3.04‡	1.21–7.64
HDL+A-FABP*	4.19†	1.81–9.75	4.21†	1.74–10.20
TG+A-FABP*	4.31†	1.86–9.99	4.36†	1.81–10.53
FG+A-FABP*	4.42†	1.91–10.23	4.52†	1.88–10.88
HT+A-FABP*	4.35†	1.87–10.08	4.43†	1.84–10.71

Abbreviations as in Table 1.
*A-FABP in sex-specific tertiles.
†$P<0.005$, ‡$P<0.02$, tertile 1 as reference with OR=1.

change substantially after adjustment for individual components of the MetS. On the other hand, the introduction of baseline A-FABP in sex-specific tertiles into the model significantly increased the likelihood of developing the MetS associated with each component of the MetS, as suggested by the highly significant likelihood ratios ($P<0.001$; Table 7). There was no significant interaction between sex and A-FABP in the prediction of the MetS on logistic regression analysis ($P=0.505$ and 0.491 for baseline and year 5, respectively).

We also investigated the relationship between serum levels of A-FABP and its expression in adipose tissues from 4 different anatomic locations (visceral, subcutaneous, epididymal, and interscapular) in 14 male C57BL/6J mice. Mean±SD body weight was 25.9±1.4 g, and mean blood glucose was 7.1±1.0 mmol/L. Mean serum A-FABP level was 25.40±7.67 μg/L, comparable to its concentrations in human subjects. A-FABP protein concentrations in visceral, subcutaneous, epididymal, and interscapular locations were 14.50±6.37, 13.44±6.37, 12.51±5.62, and 11.68±4.69 mg/g total protein, respectively. Correlation analysis (Table 8) demonstrated a strong positive association of serum A-FABP with its expression levels in all the 4 fat depots (visceral, $r=0.71$, $P=0.004$; subcutaneous, $r=0.68$, $P=0.008$; epididymal, $r=0.65$, $P=0.012$; interscapular, $r=0.68$, $P=0.007$). These correlations remained significant after adjustment for body weight, except for interscapular fat ($P=0.08$).

TABLE 7. Logistic Regression Analysis of Baseline A-FABP in the Prediction of the Development of the MetS: Likelihood Ratios

Variable(s) in Model			
A	B	Likelihood Ratio*	*P*
WC	WC+A-FABP†	10.15	<0.001
HDL	HDL+A-FABP†	15.82	<0.001
TG	TG+A-FABP†	16.80	<0.001
FG	FG+A-FABP†	17.64	<0.001
HT	HT+A-FABP†	17.03	<0.001

Abbreviations as in Table 1.
*By χ^2 ($df=2$); tertile 1 as reference with OR=1.
†A-FABP in sex-specific tertiles.

TABLE 8. Correlation Coefficient Between Serum and Tissue Concentrations of A-FABP in Male Mice

	Serum A-FABP	
	Unadjusted	Adjusted*
Visceral fat A-FABP	0.71†	0.61‡
Subcutaneous fat A-FABP	0.68†	0.58‡
Epididymal fat A-FABP	0.65‡	0.58‡
Interscapular fat A-FABP	0.68†	0.50§

*Adjusted for body weight. n=14.
†$P<0.01$; ‡$P<0.05$; §$P<0.1$.

Discussion

Adipose tissue, traditionally thought to be an inert organ for the passive storage of triglycerides, is now recognized to be the critical player in the integration of endocrine, metabolic, and inflammatory signals for the control of energy homeostasis and vascular tone.[21,22] Although the mechanisms by which obesity elicits cardiometabolic risk are not fully understood, dysregulation of lipid metabolism (lipotoxicity) and aberrant inflammatory responses in obese adipose tissue have been proposed to be the 2 major pathways leading to the MetS, diabetes, and cardiovascular disease.[2,23] Recent data obtained from animal models suggest that A-FABP, one of the most abundant cytoplasmic proteins in adipocytes, acts at the interface of metabolic and inflammatory pathways and is central to the development of key pathologies associated with the MetS.[3,5,6] Whether A-FABP plays a similar role in humans and experimental mice remains to be further confirmed, however.

In the present study, we provide the first clinical evidence demonstrating that A-FABP is a significant risk factor for the development of the MetS, independent of adiposity and insulin resistance. Both our cross-sectional and prospective studies have demonstrated an independent association of circulating A-FABP levels with all the metabolic risk factors, including abdominal obesity, insulin resistance, atherogenic dyslipidemia, hyperglycemia, and hypertension. A-FABP levels at baseline predicted the development of the MetS during the 5-year follow-up, even after adjustment for age, sex, BMI, and other confounding factors. Notably, our multiple logistic regression analysis demonstrated that only A-FABP levels and HOMA index were independent predictors of the MetS in this cohort, suggesting that excessive production of A-FABP from obese adipose tissue might be an important contributor to this disease in humans. Furthermore, the increased risk for developing the MetS associated with a high baseline A-FABP was independent of the baseline status of the MetS components. The biological relevance of these clinical findings also is supported by our animal study, which shows a close positive correlation between serum A-FABP levels and its expression in adipose tissues from different depots, suggesting that adipose tissue is the major contributor of circulating A-FABP.

The proatherogenic activities of A-FABP appear to be mediated by its direct actions in macrophages, through modification of cholesterol trafficking, and through activation of several key inflammatory pathways, independently of its effects on lipid metabolism and insulin sensitivity.[24] A-FABP can suppress peroxisome proliferator–activated receptor-γ activity and cholesterol efflux in macrophages, thus leading to foam cell formation.[25] Macrophages derived from A-FABP–deficient mice display reduced IκB kinase and nuclear factor-κB activity, resulting in suppression of inflammatory function, including reduced cyclooxygenase-2 and inducible nitric oxide synthase expression and impaired production of inflammatory cytokines.[24,25] In this study, a positive correlation was found between A-FABP and high-sensitivity CRP, even after adjustment for adiposity, suggesting a contribution of A-FABP to systemic inflammation in humans, as in the murine model. Although low-grade systemic inflammation has been reported to predict the development of the MetS in other population-based studies,[26] the findings from this study suggest that, in addition to its proinflammatory property, A-FABP also can contribute to the pathogenesis of the MetS through other mechanisms.

The unfavorable effects of A-FABP on metabolic and cardiovascular risk factors form a striking contrast to adiponectin, a major adipocyte-derived adipokine with antidiabetic, antiatherogenic, and antiinflammatory activities.[21,27] Unlike A-FABP, circulating concentrations of adiponectin are inversely associated with adiposity and various parameters of the MetS.[28] Low levels of adiponectin independently predict the increased risk of diabetes and cardiovascular disease.[28] Ablation of A-FABP and mal1 (another minor form of FABP in adipocytes) in mice has been shown to enhance the beneficial actions of adiponectin.[6] Furthermore, adipose tissue expression of adiponectin and its circulating concentrations in A-FABP–null mice are increased compared with wild-type mice.[29] In this study and our previous work,[11] we have demonstrated an inverse relationship between serum levels of A-FABP and adiponectin in humans, which persisted after adjustment for adiposity. Although these data would imply that suppression of adiponectin production and/or desensitization of adiponectin actions might account for the adverse effects of A-FABP on the MetS and its associated pathologies, our data from both cross-sectional and prospective studies suggest that A-FABP also contributes independently to the pathogenesis of the MetS. The molecular pathways by which A-FABP elicits or worsens the MetS and its associated pathologies remain to be established. The adverse effects of A-FABP on lipid metabolism and insulin sensitivity might be attributed to its ability to modulate intracellular and systemic fatty acid transport and composition. In mice deficient of A-FABP and mal1, there is an increase in the ratio of shorter-chain (C14) to longer-chain (C18) fatty acids in the muscle and adipose tissues, which favors enhanced insulin receptor signaling, AMP-activated kinase activity, fatty acid oxidation, and insulin-stimulated glucose uptake.[6] On the other hand, ablation of A-FABP and mal1 causes increased accumulation of longer-chain fatty acids in liver tissue, thus leading to decreased expression of SREBP1c and its several downstream lipogenic enzymes.

The major limitation of the present study is the relatively small number of subjects who developed the MetS during the 5-year follow-up. Whether serum A-FABP levels can be useful for predicting the risk of the MetS has to be confirmed in studies involving larger study populations. Our conclusions also need to be confirmed in other ethnic populations with different genetic and environmental backgrounds. Another limitation of the present study is that the statistical analysis was not hypothesis driven but exploratory in nature to find significant factors as a result of application of stepwise methods. Furthermore, although a higher baseline A-FABP level predicted a greater risk of developing the MetS, we did not have sufficient data on cardiovascular end points to investigate whether this would translate into a greater risk of cardiovascular mortality or morbidity. Nevertheless, our data have provided support to the recently published study demonstrating that a genetically determined reduction in the A-FABP mRNA level in human adipose tissue is associated with a reduced risk of cardiovascular disease[10] and suggest a pathogenetic role of A-FABP in cardiovascular risk in humans, as seen in experimental animals. Although our data might suggest a causal relationship between circulating A-FABP levels and the development of the MetS, further studies are required to explain how an increase in circulating A-FABP can lead to a progression in cardiometabolic risk. Studies are ongoing in our laboratory to determine whether A-FABP has a direct action on peripheral insulin sensitivity, as suggested by the positive correlation of serum A-FABP with HOMA-IR, demonstrated in the present study and our previous work.[11]

In conclusion, in this population-based southern Chinese cohort, we have shown that serum A-FABP levels could predict the development of the MetS on long-term follow-up. Our findings suggest that it plays a significant role in the pathogenesis of the MetS that is independent of its relationship with adiposity, insulin resistance as reflected by HOMA-IR, systemic inflammation as reflected by CRP, and hypoadiponectinemia. The significance of these exciting data on a new biomarker for the MetS remains to be confirmed by larger long-term follow-up studies involving other ethnic populations and correlations with cardiovascular end points.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The metabolic syndrome (MetS) encompasses a cluster of interrelated cardiometabolic risk factors that are closely associated with development of atherosclerotic cardiovascular diseases. We have previously found that obesity, the principal causal factor predisposing to insulin resistance and the MetS, is associated with increased circulating levels of adipocyte–fatty acid binding protein (A-FABP), a small protein expressed predominantly in adipose tissue and macrophages. Mice with A-FABP deficiency are protected from the development of dyslipidemia, hyperglycemia, insulin resistance, and atherosclerosis. A reduced risk for hypertriglyceridemia, type 2 diabetes, and coronary artery disease also has been found in humans with a genetic predisposition to reduced A-FABP gene expression. In the present study, we have demonstrated, in both cross-sectional and prospective studies, an association between serum A-FABP levels and the MetS that was independent of indexes of adiposity, insulin resistance, and inflammation. In 495 nondiabetic adults from the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study who were prospectively followed up for 5 years, a high baseline A-FABP level was predictive of the MetS even after adjustment for each of its individual components. Subjects with a high baseline A-FABP ($>$19.9 μg/L in men or 25.6 μg/L in women) had 4-fold the 5-year risk of developing the MetS compared with those with low baseline A-FABP levels ($<$12.5 μg/L in men or 16.4 μg/L in women). The clinical significance of this new biomarker for identifying subjects at high risk of the MetS should be further investigated in larger, long-term follow-up studies involving other ethnic populations and correlations with cardiovascular end points.