

# Adipocyte Fatty Acid–Binding Protein Is a Plasma Biomarker Closely Associated with Obesity and Metabolic Syndrome

AIMIN XU,<sup>1,2\*</sup> YU WANG,<sup>3,4</sup> JIAN YU XU,<sup>1,2</sup> DAVID STEJSKAL,<sup>5</sup> SIDNEY TAM,<sup>6</sup>  
JIALIANG ZHANG,<sup>1,2</sup> NELSON M.S. WAT,<sup>1,2</sup> WAI KEUNG WONG,<sup>6</sup> and KAREN S.L. LAM<sup>1,2</sup>

**Background:** Adipocyte fatty acid–binding protein (A-FABP) is traditionally thought to be a cytosolic fatty acid chaperone expressed in adipocytes. Mice with targeted disruption of the A-FABP gene exhibit a striking phenotype with strong protection from insulin resistance, hyperglycemia, and atherosclerosis. The clinical relevance of these findings remains to be confirmed.

**Methods:** We used tandem mass spectrometry–based proteomic analysis to identify proteins secreted from adipocytes and present in human serum. We measured serum A-FABP concentrations in 229 persons (121 men and 108 women; age range, 33–72 years), including 100 lean [body mass index (BMI) <25 kg/m<sup>2</sup>] and 129 overweight/obese individuals (BMI >25 kg/m<sup>2</sup>) selected from a previous cross-sectional study.

**Results:** A-FABP was released from adipocytes and was abundantly present in human serum. Mean (SD) circulating concentrations of A-FABP were significantly higher in overweight/obese than in lean persons [32.3 (14.8) vs 20.0 (9.8) µg/L; *P* <0.001]. Age- and sex-adjusted serum A-FABP concentrations correlated positively (*P* <0.005) with waist circumference, blood pressure, dyslipidemia, fasting insulin, and the homeostasis model assessment insulin resistance index. Moreover, we observed a significant increase in A-FABP concentrations corresponding with increases in the number of components of the metabolic syndrome (*P* <0.05).

**Conclusions:** A-FABP is a circulating biomarker closely associated with obesity and components of the metabolic syndrome, and measurement of serum concentrations of A-FABP might be useful for clinical diagnosis of obesity-related metabolic and cardiovascular disorders. © 2006 American Association for Clinical Chemistry

Obesity, characterized by excess accumulation of adipose tissue, is the most common risk factor for metabolic syndrome, a cluster of abnormalities including dyslipidemia, insulin resistance, type 2 diabetes, hypertension, and atherosclerosis (1). Although the molecular pathways that link obesity with such a wide spectrum of metabolic and cardiovascular defects are not well understood, recent studies have indicated a central role of adipose tissue in the development of this syndrome (2, 3). Accumulating evidence suggests that adipose tissue is not simply an inert energy storage depot but also functions as a major endocrine organ, producing and releasing a variety of bioactive substances (such as adipokines, chemokines, and free fatty acids) into the bloodstream (4, 5). These adipose tissue–derived bioactive molecules, through their local and systemic actions, coordinate to regulate energy metabolism, insulin sensitivity, inflammation, and vascular responses. Discordant production by adipose tissue of these substances, such as decreased adiponectin and increased tumor necrosis factor- $\alpha$ , interleukin-6, plasminogen activator inhibitor 1, and free fatty acids, contributes to the development of metabolic syndrome (6, 7).

Adipocyte-specific fatty acid–binding protein (A-FABP)<sup>7</sup> belongs to the fatty acid-binding protein super-

<sup>1</sup> Department of Medicine; <sup>2</sup> the Research Center of Heart, Brain, Hormone, and Healthy Aging; <sup>3</sup> Department of Biochemistry; and <sup>4</sup> Genome Research Center, University of Hong Kong, Hong Kong, China.

<sup>5</sup> Department of Laboratory Medicine, Sternberk Hospital, Sternberk, The Czech Republic.

<sup>6</sup> Clinical Biochemistry Unit, Queen Mary Hospital, Hong Kong, China.

\* Address correspondence to this author at: Department of Medicine, University of Hong Kong, L8-43, 21 Sassoon Road, Hong Kong, China. Fax 852-28162095; e-mail amxu@hkucc.hku.hk.

Received October 21, 2005; accepted December 20, 2005.

Previously published online at DOI: 10.1373/clinchem.2005.062463

<sup>7</sup> Nonstandard abbreviations: A-, H-, L-, E-, I-, and B-FABP, adipocyte, heart-type, liver-type, keratinocyte, intestine-type, and brain-type fatty acid-binding protein, respectively; apo E, apolipoprotein E; 2-DE, 2-dimensional gel electrophoresis; MS/MS, tandem mass spectrometry; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BMI, body mass index; and HOMA, homeostasis model assessment.

family whose members have relative molecular masses ( $M_r$ ) of  $\sim 15\,000$ , and it is highly expressed in adipose tissue (8, 9). A-FABP is a predominant cytosolic protein of mature adipocytes, accounting for  $\sim 6\%$  of total cellular proteins. This protein may be an important regulator of systemic insulin sensitivity and lipid and glucose metabolism (8). Mice deficient in A-FABP are protected from development of hyperinsulinemia, hyperglycemia, and insulin resistance in the context of both dietary and genetic obesity (10, 11). Adipocytes obtained from A-FABP-null mice had markedly reduced efficiency of lipolysis *in vivo* and *in vitro* (12, 13) and exhibited a 2- to 3-fold decrease in fatty acid release, suggesting that A-FABP mediates efflux of fatty acids in normal physiology (14). Furthermore, the acute insulin secretory response to  $\beta$ -adrenergic stimulation was profoundly suppressed in A-FABP ( $-/-$ ) mice compared with their wild-type littermates (13), suggesting that this protein might modulate systemic insulin sensitivity through its actions on other distal target tissues.

A-FABP is present in macrophages (9, 15), which have striking similarities to adipocytes in biology and function (16). The expression of A-FABP in macrophages can be induced by oxidized LDL (17) and by toll-like receptor agonists (18) and can be suppressed by the cholesterol-lowering statin drugs (19). In these cells, A-FABP modulates inflammatory cytokine production and cholesterol ester accumulation (20). In apolipoprotein (apo) E-deficient mice, ablation of the A-FABP gene conferred remarkable protection against atherosclerosis, which commonly occurs in this mouse strain (21, 22). Taken together, these animal studies demonstrate that A-FABP, by integrating metabolic and inflammatory pathways, provides a key link between various components of metabolic syndrome. Nevertheless, the clinical relevance of these findings remains to be confirmed.

The original purpose of our study was to use a proteomics approach based on 2-dimensional gel electrophoresis (2-DE) to systematically characterize adipokines secreted from adipocytes. Unexpectedly, we found that A-FABP, traditionally thought to be a cytoplasmic protein, was released from adipocytes into the bloodstream. We therefore designed the present study to investigate whether the circulating concentrations of A-FABP correlate with adiposity and components of the metabolic syndrome in humans.

### Materials and Methods

#### SEPARATION OF PROTEINS SECRETED FROM 3T3-L1 ADIPOCYTES BY 2-DE

We maintained 3T3-L1 cells as subconfluent cultures in DMEM supplemented with 100 mL/L fetal calf serum. We induced differentiation of postconfluent cells into mature adipocytes by incubation with 0.25  $\mu\text{mol/L}$  dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine, and 10 mg/L insulin, as described previously (23). More than

80% of cells became lipid-filled adipocytes at day 8 after differentiation induction.

To harvest proteins secreted by adipocytes, we washed cells 3 times with phosphate-buffered saline and then incubated them with serum-free medium for another 4 h. The medium was collected, filtered through a 0.20  $\mu\text{m}$  filter, and then concentrated and desalted by use of a concentrator with a molecular mass cutoff of 5000 (Vivascience AG). We quantified the proteins with bicinchoninic acid (BCA) reagent, then separated 50  $\mu\text{g}$  of the proteins by 2-DE and visualized them with silver staining, as we described previously (24, 25).

#### COUPLING OF CNBr-ACTIVATED SEPHAROSE BEADS WITH ANTI-HUMAN A-FABP4 ANTIBODY

Affinity-purified antihuman A-FABP (Biovendor Laboratory Medicine, Inc.) was coupled to Sepharose beads according to the manufacturer's instructions (GE Healthcare). Briefly, 250  $\mu\text{g}$  of the antibody solution (1 g/L) in 0.1 mol/L  $\text{NaHCO}_3$  was mixed with 500  $\mu\text{L}$  of CNBr-activated Sepharose beads overnight at 4  $^\circ\text{C}$  with gentle shaking. After being washed and blocked with 0.1 mol/L Tris-HCl (pH 8.0), the beads were stored in 200 mL/L ethanol at 4  $^\circ\text{C}$ .

#### PURIFICATION OF A-FABP FROM HUMAN SERUM BY AFFINITY CHROMATOGRAPHY

Albumin and IgG, the 2 major components of human serum, were depleted by the ProteoExtract Albumin/IgG Removal Kit (Calbiochem), and the remaining supernatant was incubated with 100  $\mu\text{L}$  of Sepharose beads coupled with rabbit nonimmune IgG to prevent nonspecific binding. The clarified supernatant was then incubated with 100  $\mu\text{L}$  of Sepharose beads coupled with anti-human A-FABP IgG at 4  $^\circ\text{C}$  overnight. The beads were washed with Tris-buffered saline, and the bound protein complexes were eluted with 0.1 mol/L glycine-HCl (pH 2.5). The eluted fractions were concentrated, separated by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and visualized by silver staining.

#### PROTEIN IDENTIFICATION BY EDMAN DEGRADATION SEQUENCING AND MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT TANDEM MASS SPECTROMETRY

Proteins of interest separated by 1- or 2-DE were excised and digested in gel with trypsin; the tryptic peptide mixtures were then fractionated by reversed-phase HPLC on a Jupiter 5  $\mu\text{C}_{18}$  column (250  $\times$  2.00 mm; Phenomenex Inc.) as we described previously (25). Well-separated fractions were collected and subjected to amino acid sequencing by Edman degradation using a PerkinElmer Life Sciences Procise Model 492 protein sequencer (24).

For matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis, 0.5  $\mu\text{L}$  of the tryptic peptide mixture or peptides separated by reversed-phase

HPLC was mixed with an equal amount of  $\alpha$ -cyano-4-hydroxycinnamic acid matrix (10 g/L in 600 mL/L acetonitrile–3 mL/L trifluoroacetic acid), spotted on the sample plates, and air-dried. Reflectron MS analyses were performed on a Voyager DE PRO Biospectrometry Workstation (Applied Biosystems) equipped with a pulsed laser beam (nitrogen laser,  $\lambda = 337$  nm). All ion spectra were recorded in the positive mode with an acceleration voltage of 20.0 kV. The spectrometer was externally calibrated by use of Cal Mix 2 standard mixture. Tandem MS (MS/MS) experiments were performed with a QSTAR XL quadrupole orthogonal acceleration time-of-flight mass spectrometer equipped with an oMALDI ion source (Applied Biosystems), as described previously (25).

#### HUMAN PARTICIPANTS

We recruited a total of 229 persons from our previous cross-sectional Hong Kong Cardiovascular Risk Factor Prevalence Study (26, 27). The participants were stratified to lean [body mass index (BMI)  $<25$  kg/m<sup>2</sup>] and overweight/obese (BMI  $\geq 25$  kg/m<sup>2</sup>) groups. For some analyses, the overweight/obese participants were further classified into 2 BMI groups according to the guidelines of the World Health Organization: overweight (BMI = 25–29.9 kg/m<sup>2</sup>) and obese (BMI  $\geq 30$  kg/m<sup>2</sup>). The study protocol was approved by the Ethics Committee of the Medical Faculty, University of Hong Kong.

#### ANTHROPOMETRIC AND BIOCHEMICAL MEASUREMENTS

Anthropometric measurements (height, weight, waist circumference, BMI, and systolic and diastolic blood pressures) were performed as reported previously (26). The methods for measurement of biochemical variables, including fasting insulin and glucose concentrations, 2-h postprandial glucose concentrations, fasting lipid profiles (total cholesterol, LDL-cholesterol, HDL-cholesterol, and total serum triglycerides and free fatty acids) were described elsewhere (27, 28). The homeostasis model assessment (HOMA) of insulin resistance, a simple assessment of insulin sensitivity, was calculated with the following formula: fasting plasma glucose (mmol/L)  $\times$  fasting insulin (mIU/L)/22.5. Body composition was determined by bioelectric impedance analysis (Model TBF-410; Tanita). The serum concentrations of adiponectin were quantified by an in-house ELISA, as we described recently (29).

The metabolic syndrome was defined, according to criteria proposed by the National Cholesterol Education Program's Adult Treatment Panel III report (30), as the presence of 3 or more of the following abnormalities: (a) hypertension (systolic blood pressure  $\geq 130$  mmHg, diastolic blood pressure  $\geq 85$  mmHg, and/or receiving blood pressure-lowering drugs); (b) hyperglycemia [fasting serum glucose concentration  $\geq 6.1$  mmol/L (110 mg/dL) and/or receiving glucose-lowering drugs]; (c) hypertriglyceridemia [fasting serum triglyceride concentration  $\geq 1.69$  mmol/L (150 mg/L)]; (d) low HDL-cholesterol

[fasting serum HDL-cholesterol concentration  $<1.04$  mmol/L (40 mg/dL) in males and  $<1.29$  mmol/L (50 mg/dL) in females]; (e) central obesity (waist circumference  $\geq 80$  cm in females and  $\geq 90$  cm in males, according to the classification criteria for Asians) (31).

#### ELISA FOR QUANTITATIVE MEASUREMENT OF HUMAN A-FABP

The human A-FABP ELISA reagent sets were obtained from Biovendor Laboratory Medicine, Inc. Serum samples were diluted 1:10 before assay, then 100- $\mu$ L volumes of diluted sera, calibrators, and quality-control samples were applied to 96-well microtiter plates coated with an affinity-purified goat anti-human FABP antibody. The assay was conducted according to the manufacturer's instructions. A calibration curve was constructed by plotting the absorbance values at 450 nm vs the A-FABP concentrations of the calibrators, and concentrations of unknown samples were determined by use of this calibration curve. The intra- and interassay variations were evaluated by measuring 3 different samples in 10 replicates in a single assay, or in duplicate in 5 consecutive assays, respectively.

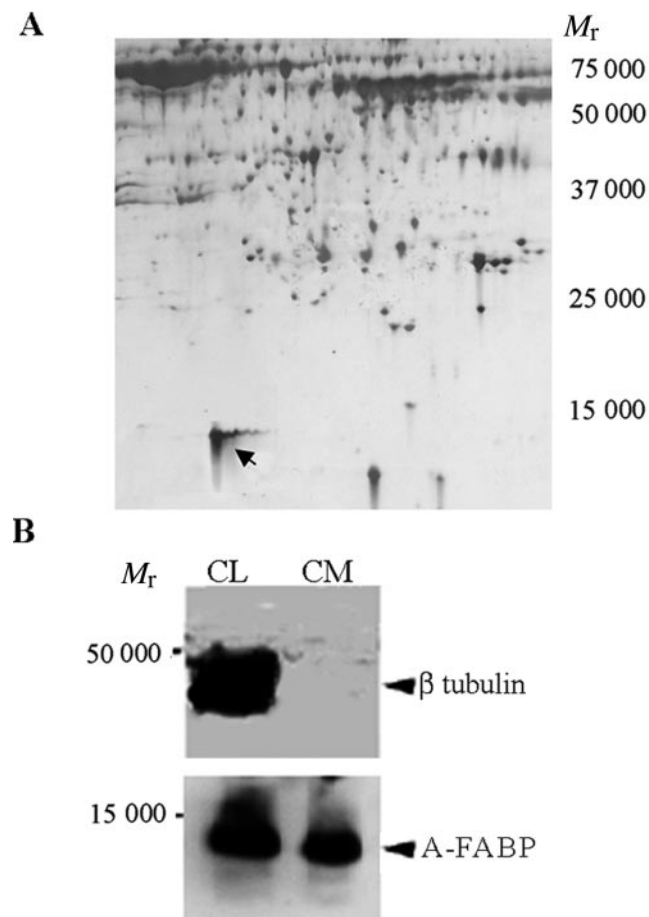
#### DATA ANALYSIS

All statistical calculations were performed with the SPSS 11.5 statistical software package (SPSS Inc.). We used the Pearson correlation coefficient to establish the association between A-FABP concentrations and various anthropometric and biochemical measures. Comparison of values between lean and overweight/obese individuals was made by one-way ANOVA. Participants were also stratified into 5 groups according to the number of components of the metabolic syndrome (0, 1, 2, 3, or  $\geq 4$ ). We used one-way ANOVA with a Scheffé-type multiple comparison test to compare serum A-FABP concentrations among these groups. All data are expressed as the mean (SD), and *P* values  $<0.05$  were considered statistically significant.

## Results

#### A-FABP IS RELEASED FROM ADIPOCYTES INTO EXTRACELLULAR MEDIUM

Adipocytes are now known to secrete a variety of bioactive molecules involved in systemic insulin sensitivity and energy metabolism (4). We therefore used a 2-DE-based proteomic approach to comprehensively identify the proteins secreted from 3T3-L1 adipocytes. This analysis detected  $>130$  proteins in the conditioned medium of fully differentiated adipocytes (Fig. 1A). MS-based peptide fingerprinting identified many of these proteins as previously described adipokines with typical secretory signal peptides such as adiponectin, adipisin, complement factor 3, and others (data not shown). Notably, 1 protein spot, with an apparent  $M_r$  of  $\sim 15$  000 and pI of 4.8, was found to be murine A-FABP. Image analysis with PDQUEST software (Bio-Rad Laboratories) revealed that this protein accounted for  $>1\%$  of the total protein secreted from



**Fig. 1.** A-FABP is an abundant protein secreted from 3T3-L1 adipocytes. The culture supernatants from 3T3-L1 adipocytes at day 8 after differentiation were harvested, concentrated, and separated by 2-DE (A). The protein denoted with the arrow was murine A-FABP, as determined by Edman degradation sequencing. In panel B, 20  $\mu$ g of proteins from cell lysates (CL) or conditioned culture medium (CM) of adipocytes was resolved by 16.5% SDS-PAGE and probed with mouse anti- $\beta$ -tubulin monoclonal antibody or goat anti-mouse A-FABP antibody (R&D System, Inc.).

adipocytes. We further confirmed the identity of the protein by Edman degradation sequencing of 2 tryptic peptides derived from the protein spot. This analysis

yielded the internal sequences EVGVGFAT and LGVEF-DEITA, which exclusively matched amino acid residues 22–29 and 67–76 of murine A-FABP, respectively.

Western blot analysis revealed that A-FABP was abundantly present inside the cells as well as in the extracellular medium (Fig. 1B). On the other hand,  $\beta$ -tubulin, a cytoskeleton protein, was detected only in the cell lysates but not in the extracellular culture medium of adipocytes, suggesting that A-FABP detected in the extracellular medium was not caused by nonspecific cell lysis.

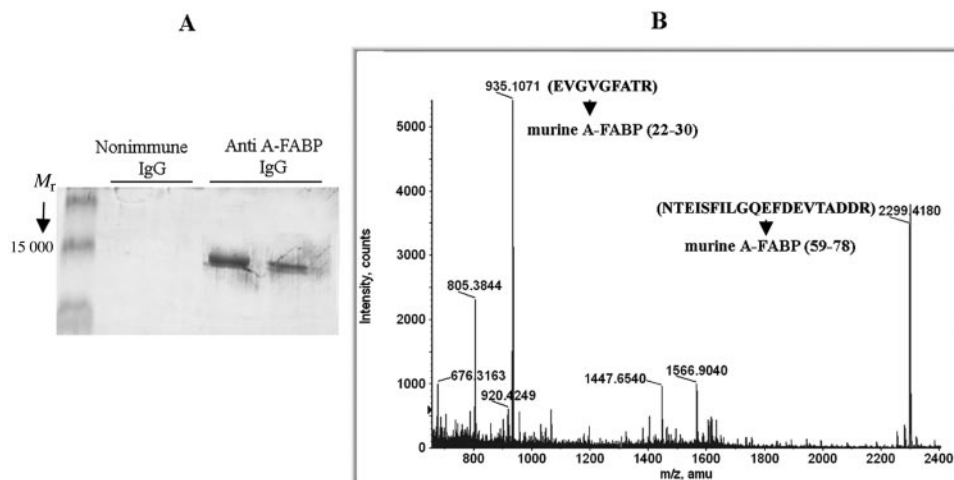
#### A-FABP IS PRESENT IN HUMAN SERUM AND IS INCREASED IN OVERWEIGHT/OBESE INDIVIDUALS

To investigate whether A-FABP is a circulating protein, we subjected human serum to chromatographic purification using the anti-human A-FABP antibody (Biovendor Laboratory Medicine) as an affinity ligand. SDS-PAGE revealed a protein with an  $M_r$   $\sim$ 15 000 that was selectively eluted from the gel matrix coupled with the anti-A-FABP antibody (Fig. 2). MS/MS analysis unequivocally identified the protein as A-FABP, indicating the presence of this protein in human serum. Notably, there were no noise signals for other types of FABPs, suggesting that the anti-A-FABP used in this study was highly specific to this protein.

We next measured the serum concentrations of A-FABP in 100 lean and 129 overweight or obese individuals. The clinical and biochemical characteristics of these individuals are given in Table 1. With a sandwich ELISA method recently developed by Biovendor Laboratory Medicine, Inc., we found that the assay is highly specific for human A-FABP, with no detectable cross-reactivity with other types of FABPs [heart-type FABP (H-FABP), liver-type FABP (L-FABP), or keratinocyte FABP (E-FABP)], leptin, adiponectin, resistin, tumor necrosis factor- $\alpha$ , C-reactive protein, or interleukin-6. The intra- and interassay CVs were 3.7%–6.4% and 2.6%–5.3%, respectively. In lean individuals, mean (SD) serum concentrations of A-FABP were 20.0 (9.8)  $\mu$ g/L (range, 7.2–36.4  $\mu$ g/L). In both men and women, the circulating concen-

**Fig. 2.** Purification and identification of A-FABP from human serum.

Serum samples were precleared with the ProteoExtract Albumin/IgG Removal Kit, and then incubated with Sepharose beads coupled with goat nonimmune IgG or goat IgG against human A-FABP. After extensive washing, proteins selectively bound to the beads were eluted, concentrated, and separated by 15% SDS-PAGE (A). The  $M_r$   $\sim$ 15 000 protein was digested in the gel with trypsin, and the tryptic peptide mixture was analyzed by a QSTAR mass spectrometer (B). Two peptides with masses of 935.10 and 2299.41 were selected for further MS/MS analysis to obtain their amino acid sequences, which were used for database searching.



**Table 1. Clinical and biochemical characteristics of the 229 Chinese individuals recruited in this study.<sup>a</sup>**

Variable	Lean (n = 100)	Overweight/Obese (n = 129) <sup>b</sup>
Age, years	57.6 (12.8)	53.9 (12.7)
Men/Women	54/46	67/62
BMI, kg/m <sup>2</sup>	22.3 (2.0)	29.4 (4.7) <sup>c</sup>
Waist-to-hip ratio	0.86 (0.06)	0.90 (0.08) <sup>c</sup>
Systolic blood pressure, mmHg	126.8 (22.6)	130.4 (19.7)
Diastolic blood pressure, mmHg	75.0 (11.3)	78.4 (10.4) <sup>d</sup>
Triglycerides, mmol/L	1.3 (0.8)	1.7 (1.0) <sup>c</sup>
LDL-cholesterol, mmol/L	3.0 (0.8)	3.5 (0.8) <sup>c</sup>
HDL-cholesterol, mmol/L	1.3 (0.3)	1.1 (0.3) <sup>c</sup>
Free fatty acids, mmol/L	0.4 (0.2)	0.4 (0.1)
Fasting insulin, mIU/L	7.5 (4.7)	13.4 (7.4) <sup>c</sup>
Fasting glucose, mmol/L	6.3 (2.4)	6.1 (1.8)
HOMA for insulin resistance	2.1 (1.4)	3.6 (2.2) <sup>c</sup>
2-h postprandial glucose, mmol/L	8.0 (3.9)	8.9 (4.5)
Serum adiponectin concentration, mg/L	6.2 (3.0)	4.6 (2.3) <sup>d</sup>

<sup>a</sup> All values except for sex are the mean (SD).

<sup>b</sup> The overweight/obese group included 93 overweight and 36 obese individuals.

<sup>c,d</sup> Compared with the lean group: <sup>c</sup>  $P < 0.001$ ; <sup>d</sup>  $P < 0.01$ .

trations of this protein in obese/overweight individuals were significantly higher than those in lean individuals (Table 2). Notably, serum A-FABP concentrations in men were significantly lower than in women among obese/overweight individuals ( $P < 0.01$ ) but not in lean individuals. We observed a strong positive correlation between serum A-FABP concentrations and BMI in both men ( $r = 0.477$ ;  $P < 0.001$ ) and women ( $r = 0.651$ ;  $P < 0.001$ ; Fig. 3).

#### SERUM A-FABP CONCENTRATIONS ARE CLOSELY RELATED TO THE COMPONENTS OF METABOLIC SYNDROME

Several recent studies on animal models have demonstrated that A-FABP plays a central role in linking obesity with metabolic syndrome. We next investigated the relationship between serum A-FABP concentrations and various biochemical and anthropometric markers for metabolic syndrome in humans. Because there was a wide variation in BMI among the 36 obese individuals (ranging

**Table 2. Comparison of serum concentrations of A-FABP ( $\mu\text{g/L}$ ) between lean and overweight/obese individuals.**

	Lean	Overweight/Obese <sup>a</sup>
Men	18.5 (9.2) (n = 54)	28.1 (13.9) <sup>b</sup> (n = 67)
Women	21.7 (10.4) (n = 46)	36.8 (14.6) <sup>b,c</sup> (n = 62)
All persons	20.0 (9.8) (n = 100)	32.3 (14.8) <sup>b</sup> (n = 129)

<sup>a</sup> The overweight/obese group includes 93 overweight and 36 obese persons.

<sup>b</sup>  $P < 0.01$  compared with the lean group.

<sup>c</sup>  $P < 0.01$  compared with the male overweight/obese group.

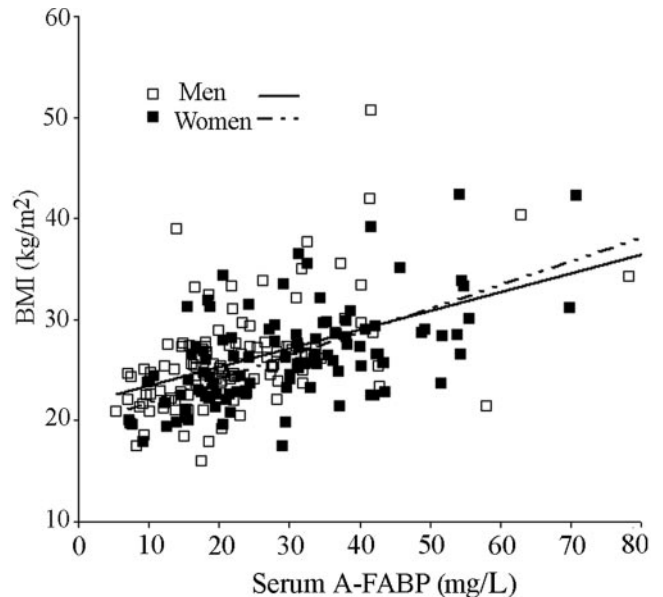


Fig. 3. Correlation between serum concentrations of A-FABP and BMI in men ( $\square$ ;  $n = 121$ ) and women ( $\blacksquare$ ;  $n = 108$ ).

from 30.1 to 50.8 kg/m<sup>2</sup>) and a strong correlation between A-FABP and BMI, we excluded these obese individuals to ensure that the correlation analysis would be examined in a more homogeneous cohort. After adjustment for sex and age, serum concentrations of A-FABP were positively correlated with waist-to-hip ratio; waist circumference; fat percentage; systolic and diastolic blood pressure; fasting serum concentrations of insulin, triglycerides, and LDL-cholesterol; 2-h postprandial glucose; and the HOMA index (Table 3). On the other hand, A-FABP was negatively correlated with fasting serum concentrations of HDL-cholesterol. Notably, the correlations of serum A-FABP with fat percentage and triglyceride, insulin, and 2-h postprandial glucose concentrations were significant even after adjustment for BMI.

Age- and sex-adjusted serum concentrations of A-FABP were inversely associated with circulating concentrations of adiponectin, an important adipokine with antidiabetic and antiatherogenic activities (32). After adjustment for serum adiponectin, the association of serum A-FABP concentrations with triglycerides ( $r^2 = 0.319$ ;  $P < 0.001$ ), LDL ( $r^2 = 0.148$ ;  $P < 0.05$ ), HDL ( $r^2 = -0.213$ ;  $P < 0.001$ ), fasting insulin ( $r^2 = 0.315$ ;  $P < 0.001$ ), and 2-h postprandial glucose ( $r^2 = 0.212$ ;  $P < 0.05$ ) remained significant, whereas its relationship with systolic blood pressure ( $r^2 = 0.002$ ;  $P = 0.984$ ) and diastolic blood pressure ( $r^2 = 0.084$ ;  $P = 0.415$ ) did not persist. This result suggests that the association of A-FABP with blood pressure is largely attributable to its inverse correlation with serum adiponectin.

On the basis of National Cholesterol Education Program criteria, metabolic syndrome was diagnosed in 32.7% of the 229 individuals studied. By stratification of serum concentrations of A-FABP, we found that the

**Table 3. Correlation between serum A-FABP concentrations or BMI and several study variables in 193 Chinese individuals with BMI <30 kg/m<sup>2</sup>.<sup>a</sup>**

Variable	Correlation (r) with A-FABP concentration		
	Adjusted for sex and age	Adjusted for sex, age, and BMI	Correlation (r) with BMI, adjusted for age and sex
Waist circumference, cm	0.446 <sup>b</sup>	0.026	0.833 <sup>b</sup>
Fat percentage, %	0.543 <sup>b</sup>	0.231 <sup>c</sup>	0.837 <sup>b</sup>
Waist-to-hip ratio	0.275 <sup>b</sup>	-0.001	0.529 <sup>b</sup>
Systolic blood pressure, mmHg	0.206 <sup>d</sup>	0.062	0.204 <sup>c</sup>
Diastolic blood pressure, mmHg	0.208 <sup>d</sup>	0.093	0.256 <sup>c</sup>
Triglycerides, mmol/L	0.352 <sup>b</sup>	0.265 <sup>b</sup>	0.260 <sup>b</sup>
LDL-cholesterol, mmol/L	0.155 <sup>c</sup>	0.057	0.190 <sup>d</sup>
HDL-cholesterol, mmol/L	-0.228 <sup>d</sup>	-0.089	-0.288 <sup>b</sup>
Free fatty acids, mmol/L	0.083	0.111	-0.032
Fasting insulin, mIU/L	0.368 <sup>b</sup>	0.194 <sup>d</sup>	0.424 <sup>b</sup>
Fasting glucose, mmol/L	0.100	-0.144	0.043
2-h postprandial glucose, mmol/L	0.224 <sup>c</sup>	0.191 <sup>c</sup>	0.121
HOMA for insulin resistance	0.284 <sup>b</sup>	0.092	0.415 <sup>b</sup>
Serum adiponectin concentration, mg/L	-0.193 <sup>d</sup>	-0.036	-0.311 <sup>b</sup>

<sup>a</sup> All significant correlations remained valid even if the 36 obese persons were included in the analysis.

<sup>b</sup>  $P < 0.001$ .

<sup>c</sup>  $P < 0.05$ .

<sup>d</sup>  $P < 0.01$ .

prevalence of metabolic syndrome in individuals with A-FABP >35  $\mu\text{g/L}$  was 54.5% in women and 71.4% in men. On the other hand, the prevalence rate of this disorder in individuals with A-FABP <15  $\mu\text{g/L}$  was only 2% in women and 6.9% in men. To further explore the relationship between A-FABP and metabolic syndrome, we stratified the mean concentrations of serum A-FABP by the number of components of the metabolic syndrome present in each individual (Fig. 4). Mean A-FABP concentrations for those with 0, 1, 2, 3, or  $\geq 4$  components of metabolic syndrome were 16.63, 17.82, 20.98, 29.34, and 37.67  $\mu\text{g/L}$  in men and 17.81, 20.1, 26.35, 37, 42, and 43.21

$\mu\text{g/L}$  in women, respectively. The serum concentrations of A-FABP increased significantly as the number of components of metabolic syndrome increased. In both sexes, a linear and significant trend was observed ( $P < 0.05$ ).

### Discussion

Accumulating evidence from animal experiments suggests that A-FABP is a central regulator of systemic insulin sensitivity, lipid metabolism, and inflammation (8, 9), although its functional mechanisms remain poorly understood. We demonstrated that A-FABP, traditionally thought to be a major cytoplasmic protein of adipocytes, is released from the cells and is present in the bloodstream. We also found that A-FABP is one of the most abundant proteins secreted from adipocytes and confirmed the presence of circulating A-FABP in humans. In line with our results, data from previous studies have shown that several members of the FABP family, including H-FABP (33), L-FABP (34), intestine-type FABP (I-FABP) (35), and brain-type FABP (B-FABP) (36), are present in the human bloodstream, presumably being released from the cells via direct diffusion. Recent data indicated that H-FABP is a promising plasma biomarker for early and sensitive diagnosis of acute coronary heart syndrome and myocardial infarction in heart failure (33) and that B-FABP may be a plasma biomarker for the detection of brain injury (36).

Our immunoassay results for human A-FABP revealed that in lean persons the circulating concentration of this protein is substantially higher than those of several other major adipokines and/or cytokines secreted from adipose tissue, including resistin, leptin, tumor necrosis factor- $\alpha$ ,

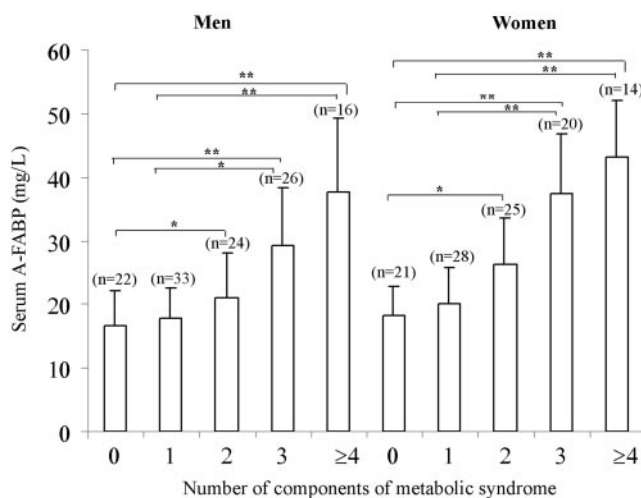


Fig. 4. Mean (SD) A-FABP concentrations stratified by the number of the components of metabolic syndrome.

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

interleukin-6, and C-reactive protein (27, 37, 38). In overweight/obese persons, we found that circulating A-FABP was markedly increased in both men and women. The strong positive association between serum A-FABP concentrations and indicators of adiposity (BMI, waist-to-hip ratio, waist circumference, and fat percentage) further suggests that adipose tissue, which is composed of adipocytes and macrophages, is probably the major contributor of A-FABP secreted into the circulation. For serum A-FABP in obese individuals, we observed a sexual dimorphism similar to those that occur with many adipokines derived from adipose tissue (29, 39). This sex difference may be the result primarily of the relatively higher fat percentage in women than in men. Indeed, we found that the fat percentage is a determinant of circulating A-FABP concentrations. In addition, the sex difference in regional fat distribution might also contribute to the sexual dimorphism of A-FABP in overweight/obese men and women. Women generally have more subcutaneous fat than men, whereas men have more abdominal (visceral) fat (40), and different fat depots have differential gene expression. For example, it has previously been shown that visceral fat produces more angiotensinogen, interleukin-6, and plasminogen activator inhibitor-1, but less leptin and adiponectin than subcutaneous fat (41). Further study is needed to investigate whether fat depots differ in the production of A-FABP in humans.

Several previous studies in A-FABP-null mice have suggested that A-FABP plays a key role in the link between obesity and various features of metabolic syndrome (8, 21). Earlier reports showed that mice with targeted disruption of the A-FABP gene alone exhibited moderate improvement in systemic glucose and lipid metabolism and alleviation of insulin resistance associated with both dietary (11) and genetic (10) obesity. Nevertheless, study results suggest that dramatic compensatory up-regulation of E-FABP (also called mal1), an otherwise minor form of adipocyte FABP, in adipose tissue of A-FABP-null mice might lead to underestimation of phenotypic changes (42). To overcome this limitation, mice with targeted disruption of both A-FABP and E-FABP were developed (43). Mice lacking these 2 FABPs exhibited a striking phenotype with almost complete protection against diet-induced obesity, insulin resistance, dyslipidemia, type 2 diabetes, and fatty liver disease. In addition, compared with controls in the apo E<sup>-/-</sup> model, mice with either A-FABP deficiency alone (21) or combined deficiency of both A-FABP and E-FABP (22) showed as much as a 90% lower rate of arteriosclerosis throughout the aorta. Remarkably, when mice were challenged with a high-fat atherogenic Western diet for 1 year, the survival rates of apo E<sup>-/-</sup> mice null for both FABPs were 67% higher than those of apo E<sup>-/-</sup> controls (22). In addition, adenovirus-mediated overexpression of A-FABP facilitated foam cell formation in human macrophages (44).

Consistent with these animal findings, our study results in humans demonstrated a close positive association

between circulating concentrations of A-FABP and features of the metabolic syndrome, including adverse lipid profiles (increased serum triglycerides and LDL-cholesterol and decreased HDL-cholesterol), fasting insulin, 2-h postprandial glucose, HOMA index, and systolic and diastolic blood pressure. Notably, the correlation between circulating concentrations of A-FABP and serum concentrations of triglycerides, 2-h postprandial glucose, and fasting insulin remained significant even after adjustment for BMI, suggesting that A-FABP might be an independent contributor to hypertriglyceridemia and glucose intolerance in humans. Our clinical data, taken together with the findings from A-FABP-null mice, support the notion that A-FABP might be a central player in the development of key pathologies associated with metabolic syndrome in humans.

The physiologic functions of circulating A-FABP remain to be determined. Like most FABPs, A-FABP can bind with a variety of hydrophobic lipid ligands known to influence systemic metabolism and inflammation (8, 45). Cytoplasmic A-FABP has been proposed to be involved in the intracellular trafficking and targeting of fatty acids inside cells (46). Nevertheless, this local action in adipocytes cannot fully account for the observed metabolic effect of A-FABP on its several distal targets, such as the liver and muscle (14, 43). Circulating A-FABP might participate in transporting free fatty acids or other types of lipid hormones, which in turn modulate systemic insulin sensitivity and energy metabolism, in the bloodstream. Indeed, a recent report has demonstrated that retinol-binding protein 4, another small lipid-binding protein produced in adipose tissue and liver, functions as an endocrine hormone that contributes to obesity-related insulin resistance and diabetes in mice (47). Research is ongoing in our laboratory to define the potential roles of circulating A-FABP in mediating the metabolic and inflammatory effects of this protein in mice.

In summary, our study demonstrates for the first time that A-FABP is a circulating biomarker of adiposity in humans. Our clinical data, taken together with the previous findings in animal models, support the central role of A-FABP in linking obesity with key components of the metabolic syndrome and suggest that this protein might represent a promising diagnostic marker for these disorders. Further prospective studies are needed to evaluate whether measurement of circulating concentrations of A-FABP can be used to predict the risk of diabetes and cardiovascular diseases.

This work was supported by Hong Kong Research Council Grant HKU 7486/04M and by a research and conference grant from the University of Hong Kong.

## References

1. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab* 2004;89:2595–600.
2. Moller DE, Kaufman KD. Metabolic syndrome: a clinical and molecular perspective. *Annu Rev Med* 2005;56:45–62.
3. Ferroni P, Basili S, Falco A, Davi G. Inflammation, insulin resistance, and obesity. *Curr Atheroscler Rep* 2004;6:424–31.
4. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004;89:2548–56.
5. Chaldakov GN, Stankulov IS, Hristova M, Ghenev PI. Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Curr Pharm Des* 2003;9:1023–31.
6. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115:911–9; quiz 920.
7. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* 2004;92:347–55.
8. Makowski L, Hotamisligil GS. Fatty acid binding proteins—the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr* 2004;134:2464S–8S.
9. Boord JB, Fazio S, Linton MF. Cytoplasmic fatty acid-binding proteins: emerging roles in metabolism and atherosclerosis. *Curr Opin Lipidol* 2002;13:141–7.
10. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology* 2000;141:3388–96.
11. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 1996;274:1377–9.
12. Coe NR, Simpson MA, Bernlohr DA. Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res* 1999;40:967–72.
13. Scheja L, Makowski L, Uysal KT, Wiesbrock SM, Shimshek DR, Meyers DS, et al. Altered insulin secretion associated with reduced lipolytic efficiency in aP2<sup>-/-</sup> mice. *Diabetes* 1999;48:1987–94.
14. Baar RA, Dingfelder CS, Smith LA, Bernlohr DA, Wu C, Lange AJ, et al. Investigation of in vivo fatty acid metabolism in AFABP/aP2<sup>(-/-)</sup> mice. *Am J Physiol Endocrinol Metab* 2005;288:E187–93.
15. Pelton PD, Zhou L, Demarest KT, Burris TP. PPAR $\gamma$  activation induces the expression of the adipocyte fatty acid binding protein gene in human monocytes. *Biochem Biophys Res Commun* 1999;261:456–8.
16. Cousin B, Munoz O, Andre M, Fontanilles AM, Dani C, Cousin JL, et al. A role for preadipocytes as macrophage-like cells. *FASEB J* 1999;13:305–12.
17. Fu Y, Luo N, Lopes-Virella MF. Oxidized LDL induces the expression of ALBP/aP2 mRNA and protein in human THP-1 macrophages. *J Lipid Res* 2000;41:2017–23.
18. Kazemi MR, McDonald CM, Shigenaga JK, Grunfeld C, Feingold KR. Adipocyte fatty acid-binding protein expression and lipid accumulation are increased during activation of murine macrophages by toll-like receptor agonists. *Arterioscler Thromb Vasc Biol* 2005;25:1220–4.
19. Llaverias G, Noe V, Penuelas S, Vazquez-Carrera M, Sanchez RM, Laguna JC, et al. Atorvastatin reduces CD68, FABP4, and HBP expression in oxLDL-treated human macrophages. *Biochem Biophys Res Commun* 2004;318:265–74.
20. Makowski L, Brittingham KC, Reynolds JM, Suttles J, Hotamisligil GS. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor  $\gamma$  and I $\kappa$ B kinase activities. *J Biol Chem* 2005;280:12888–95.
21. Makowski L, Boord JB, Maeda K, Babaev VR, Uysal KT, Morgan MA, et al. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nat Med* 2001;7:699–705.
22. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, et al. Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation* 2004;110:1492–8.
23. Xu A, Yin S, Wong L, Chan KW, Lam KS. Adiponectin ameliorates dyslipidemia induced by the HIV protease inhibitor ritonavir in mice. *Endocrinology* 2004;145:487–94.
24. Wang Y, Xu A, Knight C, Xu LY, Cooper GJ. Hydroxylation and glycosylation of the four conserved lysine residues in the collagenous domain of adiponectin. Potential role in the modulation of its insulin-sensitizing activity. *J Biol Chem* 2002;277:19521–9.
25. Wang Y, Lu G, Wong WP, Vliegthart JF, Gerwig GJ, Lam KS, et al. Proteomic and functional characterization of endogenous adiponectin purified from fetal bovine serum. *Proteomics* 2004;4:3933–42.
26. Wat NM, Lam TH, Janus ED, Lam KS. Central obesity predicts the worsening of glycemia in southern Chinese. *Int J Obes Relat Metab Disord* 2001;25:1789–93.
27. Tan KC, Wat NM, Tam SC, Janus ED, Lam TH, Lam KS. C-reactive protein predicts the deterioration of glycemia in Chinese subjects with impaired glucose tolerance. *Diabetes Care* 2003;26:2323–8.
28. Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, et al. Angiotensin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. *Proc Natl Acad Sci U S A* 2005;102:6086–91.
29. Xu A, Chan KW, Hoo RL, Wang Y, Tan KC, Zhang J, et al. Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *J Biol Chem* 2005;280:18073–80.
30. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.
31. Thomas GN, Ho SY, Janus ED, Lam KS, Hedley AJ, Lam TH. The US National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) prevalence of the metabolic syndrome in a Chinese population. *Diabetes Res Clin Pract* 2005;67:251–7.
32. Trujillo ME, Scherer PE. Adiponectin—journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *J Intern Med* 2005;257:167–75.
33. Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. *Clin Chim Acta* 2005;352:15–35.
34. Pelsers MM, Morovat A, Alexander GJ, Hermens WT, Trull AK, Glatz JF. Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients. *Clin Chem* 2002;48:2055–7.
35. Lieberman JM, Sacchettini J, Marks C, Marks WH. Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery* 1997;121:335–42.
36. Wunderlich MT, Hanhoff T, Goertler M, Spener F, Glatz JF, Walleesch CW, et al. Release of brain-type and heart-type fatty acid-binding proteins in serum after acute ischaemic stroke. *J Neurol* 2005;252:718–24.
37. Liu MY, Xydakis AM, Hoogeveen RC, Jones PH, Smith EO, Nelson KW, et al. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. *Clin Chem* 2005;51:1102–9.
38. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, et al. Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab* 2003;88:5452–5.



39. Rosenbaum M, Pirotbelli A, Vasselli JR, Heymsfield SB, Leibel RL. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. *Int J Obes Relat Metab Disord* 2001;25:1365–71.
40. Blaak E. Gender differences in fat metabolism. *Curr Opin Clin Nutr Metab Care* 2001;4:499–502.
41. Wajchenberg BL, Giannella-Neto D, da Silva ME, Santos RF. Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. *Horm Metab Res* 2002;34:616–21.
42. Maeda K, Uysal KT, Makowski L, Gorgun CZ, Atsumi G, Parker RA, et al. Role of the fatty acid binding protein mal1 in obesity and insulin resistance. *Diabetes* 2003;52:300–7.
43. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, et al. Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab* 2005;1:107–19.
44. Fu Y, Luo N, Lopes-Virella MF, Garvey WT. The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. *Atherosclerosis* 2002;165:259–69.
45. Zimmer JS, Dyckes DF, Bernlohr DA, Murphy RC. Fatty acid binding proteins stabilize leukotriene A4: competition with arachidonic acid but not other lipoxygenase products. *J Lipid Res* 2004;45:2138–44.
46. Hertzel AV, Bernlohr DA. The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol Metab* 2000;11:175–80.
47. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005;436:356–62.