

Serum Adipocyte Fatty Acid-Binding Protein Levels Are Associated With Nonalcoholic Fatty Liver Disease in Type 2 Diabetic Patients

JANG HYUN KOH, MD¹
YOUNG GOO SHIN, MD, PHD²
SOO MIN NAM, MD²

MI YOUNG LEE, MD²
CHOON HEE CHUNG, MD, PHD²
JANG YEL SHIN, MD²

OBJECTIVE — Adipocyte fatty acid-binding protein (A-FABP) is a major cytoplasmic protein in adipocytes and macrophages and is closely associated with metabolic syndrome, type 2 diabetes, and atherosclerosis. Here, we investigated whether A-FABP was associated with nonalcoholic fatty liver disease (NAFLD) in type 2 diabetes.

RESEARCH DESIGN AND METHODS — We enrolled 181 type 2 diabetic patients. Clinical and biochemical metabolic parameters were measured. The severity of NAFLD was measured by ultrasound. A-FABP, adiponectin, and retinol-binding protein-4 (RBP-4) were determined by enzyme-linked immunosorbent assay.

RESULTS — A-FABP levels, defined as more than a moderate degree of fatty liver compared with men, those without metabolic syndrome, and those without NAFLD, were higher in women, patients with metabolic syndrome, and patients with overt NAFLD, respectively. Adiponectin was decreased according to the severity of NAFLD, but RBP-4 showed no difference. Age- and sex-adjusted A-FABP showed positive correlations with BMI, waist-to-hip ratio, waist circumference, triglycerides, γ -glutamyltransferase, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), A1C, and C-reactive protein (CRP) but showed negative correlation with HDL cholesterol. The odds ratio (OR) for the risk of overt NAFLD with increasing levels of sex-specific A-FABP was significantly increased (OR 2.90 [95% CI 1.15–7.29] vs. 7.87 [3.20–19.38]). The OR in the highest tertile of A-FABP remained significant after adjustments for BMI, waist circumference, A1C, HDL cholesterol, triglycerides, HOMA-IR, CRP, and hepatic enzymes.

CONCLUSIONS — Our study demonstrates that serum A-FABP is significantly associated with NAFLD in type 2 diabetes, independent of BMI, waist circumference, HOMA-IR, A1C, triglycerides, HDL cholesterol, and CRP.

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Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic elevation of hepatic enzymes in the general population without known liver disease. NAFLD is observed in 20–30% of the total population (1) and in 75% of type 2 diabetic patients (2,3) in developed countries. NAFLD is characterized by hepatic insulin resistance. In epidemiologic studies,

NAFLD has been reported to be closely associated with obesity, dyslipidemia, and diabetes (4–6). In prospective studies, NAFLD was a risk factor for type 2 diabetes and cardiovascular disease independent of the classic risk factors (7,8). Hence, NAFLD is considered a hepatic manifestation of metabolic syndrome.

Adipocyte fatty acid-binding protein (A-FABP; also known as FABP-4 or aP2)

is a major cytoplasmic protein and is involved in the regulation of lipid metabolism. A-FABP is expressed abundantly in mature adipocytes and activated macrophages. A-FABP binds fatty acid ligands with high affinity and functions in intracellular fatty acid trafficking, regulation of lipid metabolism, and modulation of gene expression (9,10). In obese mice lacking A-FABP, dyslipidemia and peripheral insulin resistance are improved and β -cell function is preserved (11). Boord et al. (12) reported that combined adipocyte-macrophage fatty acid-binding protein deficiency improves glucose and lipid metabolism, reduces atherosclerosis, and improves survival in apoE^{-/-} mice. In cross-sectional studies, A-FABP was closely associated with obesity and metabolic syndrome (13,14). In prospective studies, A-FABP levels predicted the development of metabolic syndrome and type 2 diabetes (15,16). Furthermore, Yeung et al. (17) reported that A-FABP levels were independently associated with carotid atherosclerosis. Tuncman et al. (18) reported that individuals with an aP2 variant had lower triglycerides and a reduced risk of coronary heart disease and obesity-induced type 2 diabetes. These findings suggested that A-FABP is closely associated with insulin resistance and plays a central role in the development of metabolic syndrome, type 2 diabetes, and atherosclerosis. Maeda et al. (19) demonstrated protection against fatty liver disease in mice lacking aP2 and mall on high-fat diet. However, a relationship between A-FABP and NAFLD, a hepatic manifestation of metabolic syndrome, has not yet been established in a human study.

We hypothesized that patients with NAFLD might have higher A-FABP levels and that A-FABP might show a positive correlation with the severity of NAFLD on ultrasound. To test this hypothesis, we investigated the relationship between serum A-FABP levels and NAFLD in type 2 diabetic patients.

From the ¹Health Promotion Center, Samsung Seoul Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; and the ²Department of Internal Medicine, Yonsei University, Wonju College of Medicine, Wonju, Korea.

Corresponding author: Jang Yel Shin, sjy3290@yonsei.ac.kr.

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RESEARCH DESIGN AND

METHODS— We enrolled 181 type 2 diabetic subjects using the following inclusion criteria: 1) ages >35 and <75 years, 2) serum creatinine levels less than 1.4 mg/dl and albumin excretion rate less than 300 mg/day, 3) hepatic enzymes levels less than three times upper normal, and 4) alcohol consumption less than 20 g/day. Patients with known hepatic disease, cardiovascular disease, acute or chronic inflammation, and malignancy were excluded. The mean age of the subjects was 54.3 ± 10.4 years, and 55.2% of the total subjects were male. The protocol was approved by the ethics committee of Yonsei University Wonju College of Medicine. All of the subjects gave written informed consent, and all of the reported investigations were carried out according to the principles of the Declaration of Helsinki (the year 2000 revision).

Alcohol intake, smoking habits, medication history, and medical history were assessed using a standardized questionnaire. Anthropometric data including weight, height, waist and hip circumference, and blood pressure were assessed. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). All blood samples were obtained after overnight fasting. Fasting plasma glucose, insulin, A1C, urine albumin excretion rate, hepatic enzyme levels, high-sensitivity C-reactive protein (CRP), and lipid profiles were measured.

All of the abdominal ultrasounds were performed by the same specialist. The severity of NAFLD on ultrasound was graded as follows: mild (grade 1), defined as a slight diffuse increase in liver echogenicity in the hepatic parenchyma with normal visualization of the diaphragm and the portal veins; moderate (grade 2), defined as a moderately diffuse increase in liver echogenicity with a slightly impaired visualization of the diaphragm and the portal veins; and severe (grade 3), defined as a marked increase in liver echogenicity with poor or no visualization of the diaphragm and the portal veins. We defined overt NAFLD in this study as more than a moderate degree of fatty liver.

Adiponectin and retinol-binding protein-4 (RBP-4) levels were determined by ELISA (Adipogen, Seoul, Korea). A-FABP levels were also assessed by ELISA (Bio vendor Laboratory Medicine, Modrice, Czech Republic). Insulin resistance was measured by the homeostasis model of assessment for insulin resistance (HOMA-IR). The HOMA-IR index was calculated

Table 1—Characteristics of the subjects according to the severity of fatty liver

| | Normal | Mild | Moderate | P |
|---|-------------------|------------------------|--------------------------|--------|
| n | 42 | 78 | 61 | |
| Age (years) | 53.4 ± 10.5 | 54.0 ± 9.3 | 55.2 ± 11.8 | 0.7 |
| Sex (male %) | 64.3 | 57.7 | 45.9 | 0.2 |
| Hypertension (%) | 52.4 | 34.6 | 44.1 | 0.2 |
| Current smoker (%) | 26.2 | 23.1 | 11.5 | 0.1 |
| PPAR γ agonists use (%) | 9.5 | 5.1 | 8.2 | 0.6 |
| Insulin use (%) | 7.1 | 5.1 | 1.6 | 0.4 |
| ARB and ACEi use (%) | 40.5 | 21.8 | 23.0 | 0.06 |
| Statins use (%) | 21.4 | 12.8 | 19.7 | 0.4 |
| Diabetes duration (years) | 6.7 ± 6.0 | 4.6 ± 4.7 | 4.6 ± 6.3 | 0.1 |
| Systolic BP (mmHg) | 129.6 ± 21.1 | 131.5 ± 13.7 | 134.8 ± 17.2 | 0.3 |
| Diastolic BP (mmHg) | 75.4 ± 15.0 | 77.1 ± 8.2 | 78.7 ± 11.6 | 0.4 |
| BMI (kg/m^2) | 24.0 ± 2.3 | $25.3 \pm 3.3^*$ | $27.1 \pm 3.4^\ddagger$ | <0.001 |
| Waist circumference (cm) | 84.8 ± 8.0 | $88.1 \pm 7.5^*$ | $91.2 \pm 8.7^\ddagger$ | <0.001 |
| Male | 85.8 ± 7.1 | 88.6 ± 6.7 | $92.7 \pm 6.7^\ddagger$ | 0.001 |
| Female | 83.1 ± 9.4 | 87.4 ± 8.5 | $91.8 \pm 10.1^\ddagger$ | 0.01 |
| WHR | 0.91 ± 0.06 | $0.93 \pm 0.05^*$ | $0.94 \pm 0.05^\ddagger$ | 0.003 |
| Total cholesterol (mg/dl) | 180.3 ± 34.4 | 193.4 ± 39.5 | 190.3 ± 39.1 | 0.2 |
| Triglycerides (mg/dl) | 149.4 ± 101.8 | 182.0 ± 115.1 | 186.7 ± 83.4 | 0.2 |
| HDL cholesterol (mg/dl) | 50.0 ± 13.1 | 48.0 ± 12.1 | 47.8 ± 8.8 | 0.6 |
| Male | 48.8 ± 9.4 | 46.6 ± 10.2 | 48.1 ± 8.7 | 0.6 |
| Female | 52.1 ± 18.3 | 49.4 ± 14.4 | 47.5 ± 8.9 | 0.5 |
| LDL cholesterol (mg/dl) | 104.9 ± 30.8 | 112.4 ± 36.6 | 111.5 ± 33.7 | 0.5 |
| AST (units/l) | 20.5 ± 6.5 | 24.9 ± 10.2 | $33.8 \pm 19.2^\ddagger$ | <0.001 |
| ALT (units/l) | 23.5 ± 10.2 | $32.6 \pm 22.6^*$ | $42.6 \pm 24.7^\ddagger$ | <0.001 |
| GGT (units/l) | 27.8 ± 18.5 | $42.0 \pm 35.9^*$ | $52.1 \pm 41.9^\ddagger$ | 0.003 |
| A-FABP total ($\mu\text{g}/\text{l}$) | 13.9 ± 10.1 | 15.9 ± 10.1 | $24.7 \pm 17.9^\ddagger$ | <0.001 |
| Male | 12.3 ± 9.0 | 13.6 ± 10.2 | 15.9 ± 6.6 | 0.3 |
| Female | 16.7 ± 11.6 | 19.1 ± 9.3 | $32.2 \pm 20.9^\ddagger$ | 0.001 |
| RBP-4 total ($\mu\text{g}/\text{ml}$) | 72.7 ± 28.7 | 74.1 ± 32.6 | 72.1 ± 26.1 | 0.9 |
| Male | 76.2 ± 23.3 | 83.3 ± 38.1 | 77.8 ± 23.6 | 0.6 |
| Female | 66.3 ± 36.5 | 61.6 ± 17.0 | 67.3 ± 27.5 | 0.7 |
| Adiponectin total ($\mu\text{g}/\text{ml}$) | 5.0 ± 2.2 | $4.1 \pm 2.6^*$ | $3.8 \pm 2.3^\ddagger$ | 0.03 |
| Male | 5.1 ± 2.2 | $3.5 \pm 2.0^\ddagger$ | $2.7 \pm 1.2^\ddagger$ | <0.001 |
| Female | 5.0 ± 2.3 | 4.9 ± 3.2 | 4.7 ± 2.5 | 0.9 |
| CRP (mg/l) | 1.50 ± 2.50 | 2.39 ± 4.46 | $3.60 \pm 4.66^*$ | 0.04 |
| FPG (mg/dl) | 207.9 ± 142.5 | 180.0 ± 68.8 | 174.5 ± 58.5 | 0.2 |
| Fasting insulin ($\mu\text{U}/\text{ml}$) | 6.4 ± 6.7 | 7.5 ± 6.1 | $11.1 \pm 8.2^\ddagger$ | 0.001 |
| HOMA-IR | 3.1 ± 3.5 | 3.1 ± 2.3 | $4.6 \pm 3.6^*$ | 0.01 |
| A1C (%) | 8.9 ± 2.7 | 8.2 ± 2.2 | 8.8 ± 2.0 | 0.2 |
| 24-h albumin (mg/day) | 40.3 ± 45.1 | 31.4 ± 38.6 | 30.3 ± 40.6 | 0.5 |
| Serum creatinine (mg/dl) | 0.87 ± 0.28 | $0.78 \pm 0.17^*$ | 0.80 ± 0.18 | 0.07 |
| Metabolic syndrome (n [%]) | 17 (41.5) | 55 (71.4) | 47 (77.0) | <0.001 |

Data are means \pm SD, unless indicated otherwise. P value: the difference among three groups using ANOVA test. * $P < 0.05$ compared with normal. $^\ddagger P < 0.01$ compared with normal. $^\ddagger P < 0.001$ compared with normal. ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; FPG, fasting plasma glucose; GGT, γ -glutamyltransferase.

using the following formula: fasting plasma glucose (mg/dl) \times fasting insulin /405 ($\mu\text{U}/\text{ml}$).

Statistical analyses

Statistical analysis was performed using SPSS (version 13.0; SPSS, Chicago, IL). Data are presented as means \pm SD and as a number (in percentages) for categorical

measures. Data that were not normally distributed were logarithmically transformed before analysis. For continuous variables, the differences between groups were compared using either an unpaired Student's *t* test or one-way ANOVA. The χ^2 test was used to compare categorical variables between groups. Correlations of A-FABP with various metabolic param-

ters were analyzed using Pearson correlation and multiple regression analysis after adjustments for age and sex. Logistic regression analysis was performed to assess the odds ratio (OR) of the metabolic parameters for the presence of overt NAFLD after adjustments for age and sex. A-FABP levels were grouped into tertiles in a sex-specific manner. Multiple logistic regression analysis was used to assess the OR for the presence of overt NAFLD in subjects with the higher A-FABP tertiles compared with those with the lowest tertile. Two-sided values of $P < 0.05$ were considered significant.

RESULTS

Baseline characteristics of the subjects

Duration of diabetes and mean A1C levels for all of the subjects were 5.1 years and 8.6%, respectively. Of the 181 type 2 diabetic patients, the users of peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists, insulin, statins, and angiotensin-converting enzyme inhibitors and/or angiotensin II receptor blockers were 7.2, 4.4, 17.1, and 26.5%, respectively. The percentage of patients with metabolic syndrome and NAFLD were 66.5 and 76.8%, respectively. As shown in Table 1, all subjects were divided into three subgroups according to the severity of their fatty liver disease: normal, mild degree, and more-than-moderate degree. The proportions of each group were 23.2, 43.1, and 33.7%, respectively. Patients with overt NAFLD had higher BMI, waist circumference, waist-to-hip ratio (WHR), hepatic enzymes, CRP, A-FABP, and HOMA-IR ($P < 0.05$) and had lower adiponectin levels ($P < 0.05$) compared with those without NAFLD. Also, patients with overt NAFLD were more likely to have metabolic syndrome than those without. Serum A-FABP levels were significantly higher in women than men (24.0 ± 16.7 vs. 13.9 ± 9.0 $\mu\text{g/l}$; $P < 0.001$). Also, A-FABP levels in patients with overt NAFLD and metabolic syndrome were significantly higher than in those without NAFLD (24.7 ± 17.9 vs. 15.3 ± 10.2 $\mu\text{g/l}$; $P < 0.001$) and those without metabolic syndrome (20.6 ± 14.4 vs. 14.2 ± 12.2 $\mu\text{g/l}$; $P = 0.004$). A-FABP levels in users of PPAR- γ agonists were slightly higher compared with nonusers, but this difference was not significant.

Table 2—Correlation between A-FABP levels and various metabolic parameters

| | A-FABP* | | | |
|----------------------------|----------|----------|---------|----------|
| | Model 1 | | Model 2 | |
| | <i>r</i> | <i>P</i> | β | <i>P</i> |
| Male sex | −0.43 | <0.001 | — | — |
| PPAR γ agonists use | 0.09 | 0.2 | — | — |
| ACEi or ARB use | 0.05 | 0.3 | — | — |
| BMI | 0.22 | 0.003 | 0.22 | 0.004 |
| WHR | 0.33 | <0.001 | 0.35 | <0.001 |
| Waist circumference | 0.28 | <0.001 | 0.32 | <0.001 |
| Current smoking | −0.22 | 0.003 | −0.03 | 0.7 |
| Triglycerides* | 0.15 | 0.03 | 0.20 | 0.003 |
| HDL cholesterol | −0.18 | 0.01 | −0.17 | 0.03 |
| GGT* | 0.12 | 0.06 | 0.22 | 0.001 |
| Fasting insulin | 0.18 | 0.01 | 0.17 | 0.03 |
| HOMA-IR* | 0.22 | 0.003 | 0.22 | 0.004 |
| A1C | 0.19 | 0.008 | 0.23 | 0.003 |
| Metabolic syndrome | 0.31 | <0.001 | 0.25 | 0.001 |
| CRP* | 0.32 | <0.001 | 0.30 | <0.001 |
| Adiponectin* | 0.09 | 0.1 | — | — |

Model 1: Pearson correlation coefficient. Model 2: Regression coefficient adjusted for age and sex. *Log transformed data before analysis. ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; GGT, γ -glutamyltransferase.

Correlations between serum A-FABP levels and various metabolic parameters

As shown in Table 2, age- and sex-adjusted A-FABP showed significant positive correlations with BMI, WHR, waist circumference, triglycerides, γ -glutamyltransferase, fasting insulin, HOMA-IR, A1C, and CRP. However, A-FABP was negatively correlated with HDL cholesterol ($P < 0.05$). However, there were no significant correlations between age- and sex-adjusted A-FABP and adiponectin, RBP-4, and the use of PPAR- γ agonists, statins, or antihypertensive drugs (data not shown).

OR of the metabolic parameters for the presence of overt NAFLD

In multivariate linear regression analysis after adjustment for age and sex, A-FABP was significantly associated with overt NAFLD independent of BMI, waist circumference, HOMA-IR, and A1C ($P < 0.01$) (data not shown). In multiple logistic regression analysis after adjustment for age and sex, high A-FABP was associated with overt NAFLD (OR 2.87 [95% CI 1.47–5.61]; $P = 0.002$). Also, waist circumference, BMI, WHR, HOMA-IR, CRP, triglycerides, aspartate aminotransferase, alanine aminotransferase, and γ -glutamyltransferase were significantly associ-

Table 3—OR of metabolic parameters for the presence of overt fatty liver after adjustment for age and sex

| | OR | 95% CI | <i>P</i> |
|---------------------|------|------------|----------|
| BMI | 1.24 | 1.11–1.38 | <0.001 |
| Waist circumference | 1.07 | 1.02–1.13 | 0.003 |
| WHR | 2.20 | 1.18–4.11 | 0.01 |
| HOMA-IR* | 1.46 | 1.07–1.98 | 0.02 |
| A-FABP* | 2.87 | 1.47–5.61 | 0.002 |
| CRP* | 2.40 | 1.36–4.23 | 0.002 |
| Triglycerides* | 1.99 | 1.07–3.68 | 0.03 |
| ALT* | 3.97 | 2.10–7.49 | <0.001 |
| AST* | 7.16 | 2.93–17.45 | <0.001 |
| GGT* | 2.55 | 1.53–4.23 | <0.001 |

Data are OR (95% CI) unless otherwise indicated. *Log transformed data before analysis. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyltransferase.

Table 4—Characteristics according to A-FABP tertile levels

| | A-FABP tertile | | | P |
|--|---|--|---|--------|
| | 1 (<8.6 $\mu\text{g/l}$ [men], <150 $\mu\text{g/l}$ [women]) | 2 (8.6–14.3 $\mu\text{g/l}$ [men], 15.0–24.1 $\mu\text{g/l}$ [women]) | 3 (>14.3 $\mu\text{g/l}$ [men], >24.1 $\mu\text{g/l}$ [women]) | |
| Age (years) | 53.1 \pm 8.1 | 55.3 \pm 11.4 | 54.4 \pm 11.4 | 0.5 |
| BMI (kg/m^2) | 24.6 \pm 3.2 | 25.8 \pm 3.1 | 26.3 \pm 3.5† | 0.02 |
| Waist circumference (cm) | 85.0 \pm 7.0 | 89.3 \pm 7.7† | 91.6 \pm 9.2‡ | <0.001 |
| WHR | 0.91 \pm 0.05 | 0.93 \pm 0.05* | 0.95 \pm 0.06‡ | 0.001 |
| Triglycerides (mg/dl) | 148.9 \pm 94.1 | 190.1 \pm 124.3* | 187.3 \pm 82.1* | 0.05 |
| HDL cholesterol (mg/dl) | 52.6 \pm 11.6 | 45.7 \pm 9.3† | 46.7 \pm 11.9† | 0.001 |
| A-FABP ($\mu\text{g/l}$) | 9.0 \pm 2.9 | 14.6 \pm 4.2† | 31.1 \pm 16.9‡ | <0.001 |
| RBP-4 ($\mu\text{g/ml}$) | 67.1 \pm 19.6 | 78.6 \pm 34.4 | 73.4 \pm 31.5 | 0.1 |
| Adiponectin ($\mu\text{g/ml}$) | 4.3 \pm 2.0 | 3.9 \pm 2.2 | 4.4 \pm 3.0 | 0.5 |
| CRP (mg/l) | 1.70 \pm 3.55 | 2.57 \pm 4.14 | 3.39 \pm 4.69* | 0.1 |
| FPG (mg/dl) | 172.5 \pm 69.2 | 188.2 \pm 119.3 | 192.5 \pm 70.4 | 0.4 |
| Fasting insulin ($\mu\text{U/ml}$) | 7.7 \pm 7.6 | 7.3 \pm 6.2 | 10.1 \pm 7.5 | 0.07 |
| HOMA-IR | 3.2 \pm 3.3 | 3.3 \pm 3.2 | 4.3 \pm 2.8 | 0.08 |
| A1C (%) | 8.1 \pm 2.0 | 8.6 \pm 2.3 | 9.1 \pm 2.4* | 0.04 |
| 24-h albumin (mg/day) | 29.3 \pm 38.2 | 29.0 \pm 26.3 | 40.4 \pm 52.6 | 0.2 |
| Serum creatinine (mg/dl) | 0.76 \pm 0.16 | 0.82 \pm 0.24 | 0.84 \pm 0.21* | 0.1 |
| Estimated GFR (ml/min/1.73 m^2) | 103.8 \pm 19.6 | 96.5 \pm 26.7 | 94.5 \pm 23.4* | 0.08 |
| Metabolic syndrome (n [%]) | 25 (43.1) | 46 (76.7)‡ | 48 (78.7)‡ | <0.001 |

Data are means \pm SD unless indicated otherwise. P = the difference among three groups using ANOVA test. * P < 0.05 compared with tertile 1. † P < 0.01 compared with tertile 1. ‡ P < 0.001 compared with tertile 1. FPG, fasting plasma glucose.

ated with the presence of overt NAFLD (Table 3). As shown in Table 4, the patients in the highest tertile of sex-specific A-FABP had significantly higher BMI, waist circumference, WHR, triglycerides, A1C, and serum creatinine but had lower HDL cholesterol and estimated glomerular filtration rate (GFR) compared with those in the lowest tertile ($P < 0.05$). Patients in the higher tertiles of sex-specific A-FABP had higher OR for the presence of overt NAFLD compared with those in the lowest tertile (2.90 [1.15–7.29] vs. 7.87 [3.20–19.38]). The OR in the highest tertile of sex-specific A-FABP remained significant after adjustment for BMI, waist circumference, A1C, HDL cholesterol, HOMA-IR, CRP, triglycerides, and hepatic enzymes (8.53 [2.63–27.65]) (Table 5).

CONCLUSIONS— In the present study, we demonstrate that serum A-

FABP levels in type 2 diabetic patients are closely associated with NAFLD independent of BMI, waist circumference, HOMA-IR, A1C, triglycerides, HDL cholesterol, and CRP levels. Patients in the highest tertile of A-FABP were eight times more likely to have overt NAFLD compared with those in the lowest tertile. In animal studies, there was no protection against fatty liver disease in aP2-deficient mice because of compensation through increased expression of mall (20). However, a profound protection against fatty liver disease was shown in aP2-mall combined-deficient mice on high-fat diet (19). Also, Cao et al. (21) demonstrated that there was striking protection from liver fatty infiltration in *ob/ob*-aP2-mall-deficient mice with strong suppression of liver stearoyl-CoA desaturase-1. Furthermore, Furuhashi et al. (22) reported that fatty infiltration of the liver was attenuated and total liver triglyceride content

was reduced in aP2-inhibitor-treated *ob/ob* mice. However, the relationship between A-FABP and fatty liver disease has not yet been established in a human study. This study is the first to demonstrate an association between A-FABP and NAFLD in type 2 diabetic patients. Taken together, these findings suggest that chemical inhibition of A-FABP might show beneficial effects against fatty liver disease.

Like previous studies, serum A-FABP levels in our type 2 diabetic patients were associated with markers of obesity, dyslipidemia, hyperglycemia, insulin resistance, and inflammation. However, there are discrepancies in the correlation between A-FABP and adiponectin. Xu et al. (13) reported that A-FABP in nondiabetic subjects was positively correlated with HOMA-IR but was negatively correlated with adiponectin. On the contrary, Cabre et al. (23) reported that A-FABP in type 2 diabetes was positively correlated with adiponectin but was not correlated with HOMA-IR. In our type 2 diabetic patients, A-FABP was not correlated with adiponectin but was positively correlated with HOMA-IR. Differences in the adiposity of the populations and sex difference of A-FABP and adiponectin levels might partly explain this discrepancy. Recently, Cabre et al. (24) reported that high A-FABP plasma concentrations were as-

Table 5—OR for the presence of overt fatty liver according to the tertile of sex-specific A-FABP levels

| | OR (95% CI) | | |
|---------|-------------|------------------|-------------------|
| | Tertile 1 | Tertile 2 | Tertile 3 |
| Model 1 | 1 | 2.90 (1.15–7.29) | 7.87 (3.20–19.38) |
| Model 2 | 1 | 3.14 (0.99–9.99) | 8.53 (2.63–27.65) |

Data are OR (95% CI). Model 1: unadjusted. Model 2: model 1 + adjustments for BMI, waist circumference, triglycerides, HDL cholesterol, A1C, HOMA-IR, CRP, alanine aminotransferase, aspartate aminotransferase, and γ -glutamyltransferase.

sociated with high plasma creatinine and low GFR in type 2 diabetic patients. In our study, patients with estimated GFR <60 ml/min per 1.73 m² were only 2.8% of the total subjects. In multiple regression analysis, A-FABP was associated with serum creatinine after adjustments for age, sex, and BMI but was not associated with estimated GFR (data not shown).

Similar to previous studies (15,23), sex difference in A-FABP was observed in our study. A-FABP was significantly higher in women than in men. The sex difference is explained partly by the higher fat and subcutaneous fat percentages in women compared with men because adipose tissue is a major source of circulating A-FABP and A-FABP expression is higher in subcutaneous fat than in visceral fat. In our data, patients with NAFLD had higher A-FABP levels than those without NAFLD. In women, A-FABP levels in patients with overt NAFLD were significantly higher than in those without overt NAFLD. However, it was not significant in men. These findings suggest that A-FABP is a more specific marker of NAFLD in women than in men.

This study has several limitations. One limitation of the present study is that it is cross-sectional. We could not prove a causal link between serum A-FABP levels and the development of NAFLD. Second, we could not analyze our data stratified by sex because of the small sample size. Nevertheless, we assessed the OR for the presence of NAFLD according to the sex-specific tertiles of A-FABP. Third, the severity of NAFLD was assessed by ultrasound in this study but was not confirmed pathologically. Although liver biopsy is the gold standard to assess pathologic grading of NAFLD, it is difficult to perform liver biopsies for the assessment of NAFLD in clinical practice. It has been reported that the sensitivity and specificity of ultrasound in the diagnosis of fatty liver, as assessed on liver biopsy, were 60–94 and 84–95%, respectively (25).

In conclusion, we demonstrated that serum A-FABP was closely associated with NAFLD in type 2 diabetic patients. Our data suggest that A-FABP may be an independent marker of NAFLD in type 2 diabetes, independent of BMI, waist circumference, HOMA-IR, A1C, triglycerides, HDL cholesterol, and CRP levels. Large population-based prospective studies are warranted to confirm whether A-FABP is an independent predictor of NAFLD and whether it plays a causative role in the pathogenesis of NAFLD.

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