Serum Adipocyte Fatty Acid-Binding Protein Levels Are Associated with Non-Alcoholic Fatty Liver Disease in Type 2 Diabetic Patients

Jang Hyun Koh¹, M.D, Young Goo Shin², M.D, Ph.D, Soo Min Nam², M.D, Mi Young Lee², M.D, Choon Hee Chung², M.D, Ph.D, and Jang Yel Shin², M.D

Health Promotion Center, Samsung Seoul Hospital¹, Department of Internal Medicine, Yonsei University, Wonju College of Medicine², Korea

Corresponding authors:
Jang Yel Shin
E-mail: sjy3290@yonsei.ac.kr

Submitted  25 July 2008 and accepted 18 September 2008.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
Adipocyte fatty acid-binding protein and non-alcoholic fatty liver disease in type 2 diabetes

**Objective** – Adipocyte fatty acid-binding protein (A-FABP) is a major cytoplasmic protein in adipocytes and macrophages and is closely associated with metabolic syndrome (MetS), type 2 diabetes, and atherosclerosis. Here, we investigated whether A-FABP was associated with non-alcoholic 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 ELISA.

**Results** - A-FABP was higher in females, patients with MetS, and patients with overt NAFLD, defined as more than a moderate degree of fatty liver, compared to males, those without MetS, and those without NAFLD. 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 body mass index (BMI), waist-to-hip ratio, waist circumference, triglycerides, γ-glutamyltransferase (GGT), fasting insulin, HOMA-IR, HbA1C, and C-reactive protein (CRP), but showed negative correlation with HDL. The odds ratio (OR) for the risk of overt NAFLD with increasing levels of sex-specific A-FABP was significantly increased [OR (95% CI) = 2.90 (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, HbA1C, HDL, 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, HbA1C, triglycerides, HDL cholesterol, and CRP.
Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of a 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 as a hepatic manifestation of metabolic syndrome (MetS).

Adipocyte fatty acid-binding protein (A-FABP; also known as FABP-4 or aP2) is a major cytoplasmic protein that 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. reported that combined adipocyte-macrophage FABP deficiency improves glucose and lipid metabolism, reduces atherosclerosis, and improves survival in apoE⁻/⁻ mice (12). In cross-sectional studies, A-FABP was closely associated with obesity and MetS (13, 14). In prospective studies, A-FABP levels predicted the development of MetS and type 2 diabetes (15, 16). Furthermore, Yeung et al. reported that A-FABP levels were independently associated with carotid atherosclerosis (17). Tuncman et al. reported that individuals with aP2 variant had lower triglycerides and a reduced risk for coronary heart disease and obesity-induced type 2 diabetes (18). These findings suggested that A-FABP is closely associated with insulin resistance and plays a central role in the development of MetS, type 2 diabetes, and atherosclerosis. Maeda et al. demonstrated protection against fatty liver disease in mice lacking aP2 and mal1 (FABP-5) on high fat diet (19). However, a relationship between A-FABP and NAFLD, a hepatic manifestation of MetS, has not yet been established in a human study.

We hypothesized that patients with NAFLD might have higher A-FABP levels and 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.

RESEARCH DESIGN AND METHODS
We enrolled 181 type 2 diabetic subjects using following inclusion criteria: 1) ages greater than 35 and less than 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 3 times of upper normal, 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. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). All blood samples were obtained after overnight fasting. Fasting plasma glucose, insulin, HbA1c, 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 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; 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, Inc, Korea). A-FABP levels were also assessed by ELISA (Biovendor Laboratory Medicine, Inc, Modrice, Czech Republic). Insulin resistance was measured by the homeostatic model of assessment for insulin resistance (HOMA-IR). The HOMA-IR index was calculated using the formula, fasting plasma glucose (mg/dl) × fasting serum insulin (µU/ml) ÷ 405.

Statistical analyses—Statistical analysis was performed using SPSS (version 13.0; SPSS, Inc, Chicago, IL). Data are presented as means ± 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 a one-way ANOVA. The χ² test was used to compare categorical variables between groups. Correlations of A-FABP with various metabolic parameters were analyzed using Pearson correlation and multiple regression analysis after adjusted 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 to those with the lowest tertile. Two-sided values of P less than 0.05 were considered significant.

RESULTS

Baseline characteristics of the subjects—Duration of diabetes and mean HbA1C levels for all of the subjects were 5.1 years and 8.6%, respectively. Of the 181 type 2 diabetic patients, the users of PPARγ agonists, insulin, statins, and angiotensin converting enzyme inhibitors (ACEi) and/or angiotensin II receptor blockers (ARB) were 7.2%, 4.4%, 17.1%, and 26.5%, respectively. The percentage of patients with MetS and NAFLD were 66.5% and 76.8%, respectively. As shown in Table 1, all subjects were divided into three subgroups: normal, mild degree, and more than moderate degree according to the severity of their fatty liver disease. The proportions of each group were 23.2%, 43.1%, and 33.7%, respectively. Patients with overt NAFLD had a 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 to those without NAFLD. Also, patients with overt NAFLD were more likely to have MetS than those without. Serum A-FABP levels were significantly higher in females than males.
(24.0±16.7 vs. 13.9±9.0 µg/l, p<0.001). Also, A-FABP levels in patients with overt NAFLD and MetS were significantly higher compared to those without NAFLD (24.7±17.9 vs. 15.3±10.2 µg/l, p<0.001) and those without MetS (20.6±14.4 vs. 14.2±12.2 µg/l, p = 0.004). A-FABP levels in users of PPARγ agonists were slightly higher compared to non-users, but this difference was not significant.

**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 (GGT), fasting insulin, HOMA-IR, HbA1C, 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 anti-hypertensive drugs (data not shown).

**Odds ratio of the metabolic parameters for the presence of overt NAFLD**—In multivariate linear regression analysis after adjusted for age and sex, A-FABP was significantly associated with overt NAFLD independent of BMI, waist circumference, HOMA-IR, HbA1C (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 (95% CI) = 2.87 (1.47 - 5.61), p=0.002]. Also, waist circumference, BMI, WHR, HOMA-IR, CRP, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and GGT were significantly associated 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, HbA1C, and serum creatinine, but had lower HDL cholesterol and estimated glomerular filtration rate (GFR) compared to 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 to those in the lowest tertile [OR (95% CI) = 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, HbA1C, HDL, HOMA-IR, CRP, triglycerides, and hepatic enzymes [OR (95% CI) = 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, HbA1C, 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 anti-hypertensive drugs (data not shown).
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. reported that A-FABP in non-diabetic subjects was positively correlated with HOMA-IR, but was negatively correlated with adiponectin (13). On the contrary, Cabre et al. reported that A-FABP in type 2 diabetes was positively correlated with adiponectin, but was not correlated with HOMA-IR (23). 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. reported that high A-FABP plasma concentrations were associated with high plasma creatinine and low GFR in type 2 diabetic patients (24). In our study, patients with estimated GFR <60 ml/min/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 not 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 females compared to males. The sex difference is explained partly by the higher fat percentage and subcutaneous fat in females compared to males, 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 females, A-FABP levels in patients with overt NAFLD were significantly higher than those without overt NAFLD. However, it was not significant in males. These findings suggest that A-FABP is a more specific marker of NAFLD in females than in males.

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 confirm 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% to 94% and 84% to 95%, respectively (25).

In conclusion, we demonstrated that serum A-FABP was closely associated with NAFLD in type 2 diabetic patients. Our data suggests that A-FABP may be an independent marker of NAFLD in type 2 diabetes independent of BMI, waist circumference, HOMA-IR, HbA1C, 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.

ACKNOWLEDGEMENTS:

Previous presentation: No; Source of support: None  Conflicts of interest: None;  Disclaimers: None
REFERENCES
1. Tilg H, Kaser A: Treatment strategies in nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2:148-155, 2005
2. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, Patel N, Madan A, Amarapurkar A, Hafeezunnisa: Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 19:854-858, 2004
3. Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR: Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* in press, 2008.
4. Wanless IR, Lentz JS: Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 12:1106-1110, 1990
5. Angelico F, Del Ben M, Conti R, Francioso S, Feole K, Fiorello S, Cavallo G, Zalunardo B, Liirussi F, Alessandri C, Violi F: Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 90:1578-1582, 2005
6. Kotronen A, Yki-Jarvinen H: Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 28:27-38, 2008
7. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M: Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. *Diabetes Care* 30:2940-2944, 2007
8. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E: Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. *Atheroscler Thromb Vasc Biol* 25:1045-1050, 2005
9. Coe NR, Bernlohr DA: Physiological properties and functions of intracellular fatty acid binding proteins. *Biochim Bio-phys Acta* 1391:287-306, 1998
10. Hertzel AV, Bernlohr DA: The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol Metab* 11:175-180, 2000
11. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS: Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology* 141:3388-3396, 2000
12. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation* 110:1492-1498, 2004
13. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS: Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 25:405-413, 2006
14. Stejskal D, Karpisek M: Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? *Eur J Clin Invest* 36:621-625, 2006
15. Xu A, Tso AW, Cheung BM, Wang Y, Wat NM, Fong CH, Yeung DC, Janus ED, Sham PC, Lam KS: Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation* 115:1537-1543, 2007
16. Tso AW, Xu A, Sham PC, Wat NM, Wang Y, Fong CH, Cheung BM, Janus ED, Lam KS: Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. *Diabetes Care* 30:2667-2672, 2007
17. Yeung DC, Xu A, Cheung CW, Wat NM, Yau MH, Fong CH, Chau MT, Lam KS: Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 27:1796-1802, 2007

18. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, Hotamisligil GS: A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. *Proc Natl Acad Sci U S A* 103:6970-6975, 2006

19. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab* 1:107-119, 2005

20. 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* 274:1377-1379, 1996

21. Cao H, Maeda K, Gorgun CZ, Kim HJ, Park SY, Shulman GI, Kim JK, Hotamisligil GS: Regulation of metabolic responses by adipocyte/macrophage fatty acid-binding proteins in leptin-deficient mice. *Diabetes* 55:1915-1922, 2006

22. Furuhashi M, Tuncman G, Gorgun CZ, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, Sulsky R, Robl JA, Parker RA, Hotamisligil GS: Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature* 447:959-965

23. Cabre A, Lazaro I, Girona J, Manzanares JM, Marimon F, Plana N, Heras M, Masana L: Fatty acid binding protein 4 is increased in metabolic syndrome and with thiazolidinedione treatment in diabetic patients. *Atherosclerosis* 195:e150-e158, 2007

24. Cabre A, Lazaro I, Girona J, Manzanares JM, Marimon F, Plana N, Heras M, Masana L: Plasma fatty acid-binding protein 4 increases with renal dysfunction in type 2 diabetic patients without microalbuminuria. *Clin Chem* 54:181-187, 2008

25. Joy D, Thava VR, Scott BB: Diagnosis of fatty liver disease: is biopsy necessary? *Eur J Gastroenterol Hepatol* 15:539-543, 2003
### Table 1. Characteristics of the subjects according to the severity of fatty liver

|                                | Normal  | Mild          | Moderate       | p    |
|--------------------------------|---------|---------------|----------------|------|
|                                | n=42    | n=78          | n=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  |
| PPARγ agonists use (%)         | 9.5     | 5.1           | 8.2            | 0.6  |
| Insulin use (%)                | 7.1     | 5.1           | 1.6            | 0.4  |
| ARB & 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/m²)                    | 24.0±2.3 | 25.3±3.3‡     | 27.1±3.4‡     | <0.001|
| Waist circumference (cm)       | 84.8±8.0 | 88.1±7.5‡     | 91.2±8.7‡     | <0.001|
| Male                           | 85.8±7.1 | 88.6±6.7      | 92.7±6.7†     | 0.001|
| Female                         | 83.1±9.4 | 87.4±8.5      | 91.8±10.1†    | 0.01 |
| WHR                            | 0.9±0.06 | 0.93±0.05‡    | 0.94±0.05§    | 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±19.2‡    | <0.001|
| ALT (units/l)                  | 23.5±10.2 | 32.6±22.6§    | 42.6±24.7‡    | <0.001|
| GGT (units/l)                  | 27.8±18.5 | 42.0±35.9†    | 52.1±41.9‡    | 0.003|
| A-FABP total (µg/l)            | 13.9±10.1 | 15.9±10.1     | 24.7±17.9‡    | <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±20.9§    | 0.001|
| RBP-4 total (µg/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 (µg/ml)      | 5.0±2.2  | 4.1±2.6§      | 3.8±2.3†      | 0.03 |
| Male                           | 5.1±2.2  | 3.5±2.0†      | 2.7±1.2‡      | <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±4.66*    | 0.04 |
| FPG (mg/dl)                    | 207.9±142.5 | 180.0±68.8   | 174.5±58.5    | 0.2  |
| Fasting Insulin (µg/ml)        | 6.4±6.7  | 7.5±6.1       | 11.1±8.2‡     | 0.001|
| HOMA-IR                        | 3.1±3.5  | 3.1±2.3       | 4.6±3.6*      | 0.01 |
| HbA1C (%)                      | 8.9±2.7  | 8.2±2.2       | 8.8±2.0       | 0.2  |
| 24H 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±0.17‡    | 0.80±0.18     | 0.07 |
| MetS (%)                       | 17 (41.5) | 55 (71.4)     | 47 (77.0)     | <0.001|

Data are means ± SD unless indicated otherwise. p value: the difference among three groups using ANOVA test. *: p value < 0.05 compared with normal. †: p value < 0.01 compared with normal. ‡: p value < 0.001 compared with normal. ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; BP, blood pressure; BMI, body mass index; WHR, waist-to-hip ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; A-FABP, adipocyte fatty acid-binding protein; RBP-4, retinol-binding protein 4; CRP, high sensitivity C-reactive protein; FPG, fasting plasma glucose; MetS, metabolic syndrome.
Table 2. Correlation between A-FABP levels and various metabolic parameters

| A-FABP* | Model 1 | Model 2 |
|---------|---------|---------|
|         | r       | p       | β       | p       |
| Male sex| -0.43   | <0.001  |         |         |
| PPARγ 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   |
| HbA1C   | 0.19    | 0.008   | 0.23    | 0.003   |
| MetS    | 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.

Table 3. Odds ratio of metabolic parameters for the presence of overt fatty liver after adjustment for age and sex

|          | OR      | 95% CI       | p     |
|----------|---------|--------------|-------|
| 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* | 1.16    | 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.
Table 4. Characteristics according to A-FABP tertile levels

| A-FABP tertile | p       |
|----------------|---------|
| Men            |         |
| 1 (< 8.6 µg/L) |         |
| 2 (8.6 – 14.3 µg/L) | 0.5     |
| 3 (> 14.3 µg/L) |         |
| Women          |         |
| 1 (<15.0 µg/L) |         |
| 2 (15.0 – 24.1 µg/L) | 0.02    |
| 3 (> 24.1 µg/L) |         |
| Age (years)    | 53.1±8.1| 55.3±11.4| 54.4±11.4| 0.5 |
| BMI (kg/m²)    | 24.6±3.2| 25.8±3.1| 26.3±3.5†| 0.02|
| Waist circumference (cm) | 85.0±7.0| 89.3±7.7†| 91.6±9.2‡| <0.001|
| WHR            | 0.91±0.05| 0.93±0.05*| 0.95±0.06†| 0.001|
| Triglycerides (mg/dl) | 148.9±94.1| 190.1±124.3*| 187.3±82.1†| 0.05|
| HDL cholesterol (mg/dl) | 52.6±11.6| 45.7±9.3†| 46.7±11.9†| 0.001|
| A-FABP (µg/l)  | 9.0±2.9 | 14.6±4.2†| 31.1±6.9‡| <0.001|
| RBP-4 (µg/ml)  | 67.1±19.6| 78.6±34.4| 73.4±31.5| 0.1 |
| Adiponectin (µg/ml) | 4.3±2.0| 3.9±2.2| 4.4±3.0| 0.5 |
| CRP (mg/l)     | 1.70±3.55| 2.57±4.14| 3.39±4.69*| 0.1 |
| FPG (mg/dl)    | 172.5±69.2| 188.2±119.3| 192.5±70.4| 0.4 |
| Fasting Insulin (µg/ml) | 7.7±7.6| 7.3±6.2| 10.1±7.5| 0.07|
| HOMA-IR        | 3.2±3.3| 3.3±3.2| 4.3±2.8| 0.08|
| HbA1C (%)      | 8.1±2.0| 8.6±2.3| 9.1±2.4*| 0.04|
| 24H albumin (mg/day) | 29.3±38.2| 29.0±26.3| 40.4±52.6| 0.2 |
| Serum Creatinine (mg/dl) | 0.76±0.16| 0.82±0.24| 0.84±0.21†| 0.1 |
| Estimated GFR (ml/min/1.73 m²) | 103.8±19.6| 96.5±26.7| 94.5±23.4*| 0.08|
| MetS (%)       | 25 (43.1) | 46 (76.7)† | 48 (78.7)‡ | <0.001|

Data are means ± SD unless indicated otherwise. p value: the difference among three groups using ANOVA test. *: p value < 0.05 compared with tertile 1. †: p value < 0.01 compared with tertile 1. ‡: p value < 0.001 compared with tertile 1. GFR, glomerular filtration rate.

Table 5. Odds ratio for the presence of overt fatty liver according to the tertile of sex-specific A-FABP levels

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

Data are OR (95% CI) unless otherwise indicated. Model 1: unadjusted. Model 2: model 1 + adjustments for BMI, waist circumference, triglycerides, HDL cholesterol, HbA1C, HOMA-IR, CRP, AST, ALT, GGT.