CLINICAL STUDY

Relationship between serum adiponectin concentration, pulse wave velocity and nonalcoholic fatty liver disease

S G Kim, H Y Kim, J A Seo, K W Lee, J H Oh, N H Kim, K M Choi, S H Baik and D S Choi

Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Korea University, Seoul, South Korea

(Correspondence should be addressed to D S Choi; Email: cdongs@kumc.or.kr)

Abstract

Objectives: We aimed to investigate the relationship between nonalcoholic fatty liver disease (NAFLD), serum adiponectin concentration and brachial-ankle pulse wave velocity (baPWV) as a risk marker for atherosclerosis.

Methods: A total of 213 nonalcoholic subjects (67 males, 146 females) participated in this study. Division of subjects into the NAFLD group or the normal group was based on the existence of fatty liver detected by sonography.

Results: Serum adiponectin levels in the NAFLD group were significantly lower than those in the normal group. After adjusting for age, body-mass index (BMI) and the homeostasis model of assessment (HOMA), there was a significant negative correlation between NAFLD and serum adiponectin level only in females ($r = -0.22$, $P = 0.008$). Multiple logistic regression analysis showed a tendency of inverse correlation between NAFLD and serum adiponectin level in females ($P = 0.055$). After adjustment for age, BMI and HOMA value, serum adiponectin levels were inversely correlated with serum alanine aminotransferase (ALT) and gamma-glutamyltranspeptidase (GGT) levels ($r = -0.199$ ($P = 0.004$) and $r = -0.282$ ($P < 0.001$)). On the other hand, baPWV in the NAFLD group was also significantly higher than that in the normal group in females ($P = 0.005$). Individual levels of serum ALT, aspatate aminotransferase (AST), alkaline phosphatase (ALP) and GGT were positively correlated with baPWV after adjusting for age, sex, BMI, HOMA and systolic blood pressure ($P < 0.05$).

Conclusion: Serum adiponectin level and baPWV were significantly associated with NAFLD and various liver enzymes, especially in females.

European Journal of Endocrinology **152** 225–231

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a major cause of liver-related morbidity with possible progression to cirrhosis (1). There are two histologic patterns of NAFLD: fatty liver alone (simple steatosis) and steatohepatitis (1). Now there is increasing evidence that NAFLD often represents a component of the metabolic syndrome characterized by obesity, hyperinsulinemia, peripheral insulin resistance, diabetes, hypertriglyceridemia and hypertension (2–6). Recently, it was reported that there is a near-universal association of nonalcoholic steatohepatitis (NASH) and insulin resistance, irrespective of obesity (7). Although their clinical association seems to be well established, the pathogenesis of NAFLD has not been fully elucidated, and its clinical association with cardiovascular disease (CVD), which is an important outcome of insulin resistance, is not well known.

Adiponectin is an adipocytokine that is highly specific to adipose tissue (8). In contrast to other adipocytokines, adiponetin levels are decreased in the metabolic syndrome.

Hypoadiponectinemia was documented in subjects with obesity, type 2 diabetes (9), dyslipidemia (10), hypertension (11) and coronary artery disease (12). Adiponectin may have anti-inflammatory and anti-atherogenic properties (13–15), and it is considered an independent risk factor for CVD (16, 17). Adiponectin is also known to act directly on hepatic tissue and to inhibit glucose production (18).

Pulse wave velocity (PWV) offers a noninvasive method of measuring arterial stiffness for assessment of atherosclerosis, and many reports have described the relationship between PWV and the development of atherosclerotic disease (19–21). PWV is thought to be a risk marker (22, 23) for and prognostic predictor of atherosclerosis (24, 25). Recently, a device to measure brachial-ankle pulse wave velocity (baPWV) was developed. baPWV is also considered a marker of atherosclerotic vascular damage and cardiovascular risk (26).

We hypothesized that NAFLD by itself may be associated with atherosclerosis, which is an important outcome of insulin resistance, and adiponetin may play a role in the pathogenesis and outcome of NAFLD.

Accordingly, the purpose of this study was to investigate the relationship between NAFLD, serum adiponectin concentration and baPWV as a risk marker of atherosclerosis.

Subjects and methods

Subjects and measurement

A total of 341 Koreans (146 males, 193 females) who underwent a routine health checkup at Korea University Anam Hospital were enrolled. Subjects were excluded from this study for any of the following criteria: (i) alcohol consumption over 140 g/week; (ii) evidence of viral or toxic hepatitis; (iii) known diabetes or fasting glucose of at least 126 mg/dl; (iv) other endocrine disease (such as thyroid dysfunction); (v) known liver or renal dysfunction. Finally, a total of 213 Koreans (67 males, 146 females) were included in this study. Informed consent was obtained from all subjects before they participated in the study, which was approved by the ethics committee of the institution. Clinical data such as age, sex, height, weight, body-mass index (BMI) and blood pressure were recorded. The systolic and diastolic pressures were measured by Baumanometer (W A Baum, New York, NY, USA) on the arm of the seated subject, who had rested in a sitting position for 5 min before the measurement. Body fat mass, body fat percentage and waist/hip ratio were measured by bioelectrical impedence analysis (Inbody 2.0; Biospace, Seoul, South Korea). Waist to hip ratio (cm/cm) was determined by measurement of the circumference of the waist and hips in a standing position. Waist circumference was measured to the nearest 0.1 cm at the level of the iliac crest by a tape while the subject was at minimal respiration. Hip circumference was measured at the level of the anterior superior iliac spine. Blood samples were drawn after an overnight fast and immediately centrifuged. Serum total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, uric acid and liver enzyme levels were determined by enzymatic methods with a chemistry analyzer (Hitachi 747, Tokyo, Japan). Plasma glucose was measured by the glucose oxidase method. High-sensitivity C-reactive protein (CRP) was determined by the photometric latex agglutination method (LX 2200; Aloka, Tokyo, Japan). Serum insulin levels were measured by immunoradiometric assay (Biosource, Nivelles, Belgium), which had a reactivity of less than 0.2% to human proinsulin. The insulin resistance was estimated using a homeostasis model of assessment (HOMA), calculated from baseline glucose and insulin levels as fasting glucose (mmol/l) $\times$ fasting insulin (μU/ml)/22.5 (27). Serum adiponectin concentration was determined by radioimmunoassay with kits and protocols from Linco Research (St Charles, MO, USA) (human adiponectin sensitivity of 1 ng/ml with a 100 μl sample size; intra-assay coefficient of variation (CV) of 8.7%).

The following variables were measured by the Waveform Analyzer (model BP203RPE II; Colin, Komaki, Japan). Extremity blood pressure was measured by the oscillometric method, and ankle/brachial pressure index (ABI) was automatically calculated. Right, left and mean baPWV was measured.

A single experienced radiologist blinded to the laboratory data performed ultrasonographic liver examinations. Fatty liver was defined as a bright liver on ultrasonography. The diagnosis of bright liver was based on abnormally intense and high level echoes arising from the hepatic parenchyma, with amplitude similar to that of echoes arising from the diaphragm. Subjects were divided into the NAFLD group or the normal group by whether fatty liver was seen by sonography or not.

Statistical analyses

Data are expressed as means±S.D. Parameters that did not fulfill normal distribution (that is, ALT, AST, ALP, GGT, adiponectin, triglyceride and HOMA) were log-transformed for subsequent analysis. Correlation analysis was used to establish the relations between adiponectin, PWV and NAFLD. The correlations between various hepatic enzymes and adiponectin or PWV were also examined. Differences between mean values in the NAFLD or the normal group were analyzed by unpaired *t*-test. Multiple logistic regression analysis using NAFLD as a dependent variable was conducted to determine the relative contributions made by each variable to the outcome variable. Age, weight, BMI, waist/hip ratio, body fat mass, HDL cholesterol, triglyceride, HOMA, uric acid, CRP, systolic blood pressure, adiponectin and PWV were employed as independent variables. Significant independent variables were chosen using the stepwise variable selection method. A value of $P < 0.05$ was used to indicate statistical significance. Data were analyzed by SPSS for Windows (Version 10.0; SPSS, Inc., Chicago, IL, USA).

Results

The characteristics of the study subjects are summarized in Tables 1 and 2 . In males, weight, BMI, waist/hip ratio, body fat mass, body fat percentage (%), systolic blood pressure, diastolic blood pressure, ALT, AST, HOMA, triglyceride, CRP, uric acid, and adiponectin were significantly higher in the NAFLD group than in the normal group. HDL cholesterol was significantly lower in the NAFLD group. However, age and baPWV were not significantly different between the groups (Table 1). In females, most variables, including age and baPWV, were higher in the NAFLD group than in the normal group (Table 2). In particular, serum adiponectin levels in the NAFLD group were significantly lower than those in the normal group in both sexes. Multiple logistic regression analysis was performed

Table 1 Basal characteristics in males.

|	**Normal** ($n = 31$)	**NAFLD** ($n = 36$)	**P**
Age (years)	47.7±13.6	48.1±14.6	0.982
Weight (kg)	66.1±7.8	79.0±11.9	<0.0001
BMI (kg/m^2)	22.7±2.4	27.8±3.4	<0.0001
Waist-to-hip ratio (cm/cm)	0.87±0.04	0.94±0.04	<0.0001
Body fat mass (kg)	12.5±3.4	21.3±6.3	<0.0001
Body fat percentage (%)	18.7±3.7	22.6±4.8	<0.0001
Systolic blood pressure (mmHg)	113.6±13.4	127.9±16.2	<0.0001
Diastolic blood pressure (mmHg)	69.6±11.4	78.8±11.8	0.002
Triglyceride (mg/dl)	123.6±63.9	181.8±92.1	0.004
HDL cholesterol (mg/dl)	47.1±9.1	41.0±7.9	0.005
HOMA	1.49±0.7	3.04±1.6	<0.0001
Uric acid (mg/dl)	5.6±1.2	6.4±1.7	0.039
ALT (IU/l)	20.7±9.3	42.9±29.1	<0.0001
AST (IU/l)	19.7±4.3	28.4±14.2	0.001
CRP (mg/l)	0.89±0.93	1.48±1.12	0.026
Adiponectin (μg/ml)	6.2±3.4	4.5±2.8	0.026
Right ankle-brachial index	1.10±0.09	1.08±0.13	0.48
Left ankle-brachial index	1.13±0.09	1.17±0.09	0.08
Right brachial-ankle PWV (cm/s)	1376.5±236.0	1528.2±338.6	0.035
Left brachial-ankle PWV (cm/s)	1393.5±263.0	1491.8±353.0	0.207
Mean brachial-ankle PWV (cm/s)	1385.0±244.3	1491.8±340.3	0.086

Table 2 Basal characteristics in females.

	Normal ($n = 107$)	**NAFLD** ($n = 39$)	**P**
Age (years)	47.1±11.5	55.6±8.5	<0.0001
Weight (kg)	55.8±6.4	67.2±8.9	<0.0001
BMI (kg/m^2)	22.6±2.5	27.1±2.9	<0.0001
Waist-to-hip ratio (cm/cm)	0.87±0.05	0.94±0.05	<0.0001
Body fat mass (kg)	16.3±4.3	23.7±4.3	<0.0001
Body fat percentage (%)	28.8±5.0	34.9±4.3	<0.0001
Systolic blood pressure (mmHg)	116.8±16.7	124.7±13.6	0.008
Diastolic blood pressure (mmHg)	71.5±11.6	76.7±10.4	0.015
Triglyceride (mg/dl)	98.7±53.7	150.0±73.2	<0.0001
HDL cholesterol (mg/dl)	57.2±11.4	47.8±9.0	<0.0001
HOMA	1.46±0.8	2.56±1.1	<0.0001
Uric acid (mg/dl)	4.04±0.75	4.82±1.36	0.002
ALT (IU/l)	16.6±6.8	28.0±19.7	0.008
AST (IU/l)	20.0±5.9	25.0±10.7	0.001
CRP (mg/l)	0.72±0.87	1.22±0.92	0.004
Adiponectin (μg/ml)	10.6±4.8	7.2±3.5	<0.0001
Right ankle-brachial index	1.09±0.10	1.11±0.09	0.264
Left ankle-brachial index	1.11±0.09	1.13±0.07	0.324
Right brachial-ankle PWV (cm/s)	1387.3±332.1	1564.9±325.1	0.005
Left brachial-ankle PWV (cm/s)	1390.6±320.1	1553.9±285.0	0.006
Mean brachial-ankle PWV (cm/s)	1388.9±323.5	1559.4±302.9	0.005

with NAFLD as a dependent variable. Age, BMI and HOMA were selected as significant variables in women. Although adiponetin was not selected in the final model, there was a tendency of inverse correlation between NAFLD and serum adiponectin level in females ($P = 0.055$). In males, the final model for NAFLD included systolic blood pressure, body fat mass and HOMA. After adjusting for age, BMI and HOMA, there was a significant negative correlation between NAFLD and serum adiponectin levels only in females ($r = -0.22$, $P = 0.008$). In addition, serum adiponectin levels were inversely correlated with serum ALT and GGT levels before ($r = -0.344$ ($P < 0.001$)

and $r = -0.393$ ($P < 0.001$) respectively) and after adjustment for age, BMI and HOMA value ($r = -0.199$ ($P = 0.004$) and $r = -0.282$ ($P < 0.001$) respectively) (Fig. 1) in all subjects. Correlation was not observed between AST, ALP and serum adiponectin levels.

On the other hand, baPWV in the NAFLD group was significantly higher than that in the normal group in females ($P = 0.005$). After adjusting for age, NAFLD showed positive correlation with baPWV ($r = 0.14$, $P = 0.039$), and the positive correlation was stronger in NAFLD with elevated levels of liver enzymes ($r = 0.27$, $P = 0.001$). Interestingly, individual levels
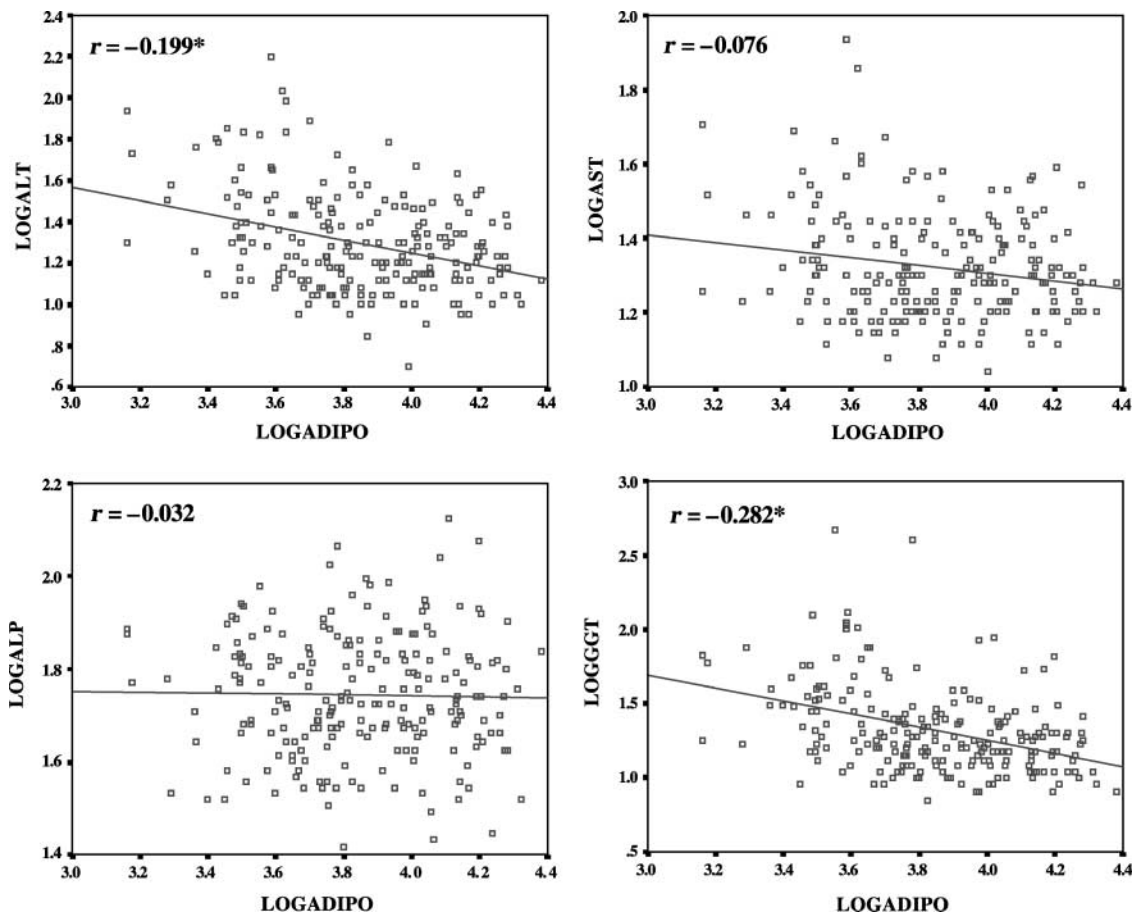
Figure 1 Partial correlation graphs between log-transformed adiponectin and log-transformed ALT, AST, ALP and GGT in controlling for age, BMI and HOMA in all subjects (LOGADIPO: log adiponectin, *$P < 0.05$).

of serum ALT, AST, ALP and GGT were positively correlated with baPWV before ($r = 0.272$ ($P < 0.001$), $r = 0.321$ ($P < 0.001$), $r = 0.370$ ($P < 0.001$) and $r = 0.224$ ($P = 0.001$) respectively) and after adjustment for age, sex, BMI, HOMA and systolic blood pressure ($r = 0.244$ ($P < 0.001$), $r = 0.203$ ($P = 0.003$), $r = 0.216$ ($P = 0.002$) and $r = 0.166$ ($P = 0.016$) respectively) (Fig. 2) in all subjects. However, there was no significant correlation between adiponectin levels and PWV.

When NAFLD subjects were compared with 40 selected controls matched for age, sex, BMI and systolic pressure, which were the factors known to affect adiponectin levels and baPWV, there were still significant differences in adiponectin levels and baPWV between the NAFLD group and the control group (6.1 ± 3.5 vs $8.5 \pm 4.2\,\mu g/ml$ ($P = 0.002$) and 1544.9 ± 291.6 vs $1407.6 \pm 290.0\,cm/s$ ($P = 0.023$) respectively).

Discussion

Adiponectin, a novel adipocytokine, is highly specific to adipose tissue and is secreted predominantly from it.

(8). In contrast to other adipocytokines, circulating adiponetin levels are decreased in the metabolic syndrome. Hypoadiponectinemia was documented in subjects with various components of the metabolic syndrome (9–11). However, the relationship between adiponectin and NAFLD, which is a component of the metabolic syndrome, is not well known. As far as we know, there have been only three reports on that subject (28–30). Xu's group reported that delivery of recombinant adiponectin into nonalcoholic ob/ob mice with fatty liver ameliorated hepatomegaly, steatosis and alanine aminotransferase abnormality (28). Recently, Hui *et al.* reported that hypoadiponectinemia is a feature of NASH independent of insulin resistance (29). According to this report, reduced adiponectin levels are associated with more extensive necroinflammation and may contribute to the development of necroinflammatory forms of NAFLD. In our study, the serum adiponectin level was significantly associated with NAFLD and various liver enzymes, especially in females. After adjustment for age, BMI and HOMA, there was a significant negative correlation between NAFLD and serum adiponectin level only in females. We do not know exactly why this relationship is
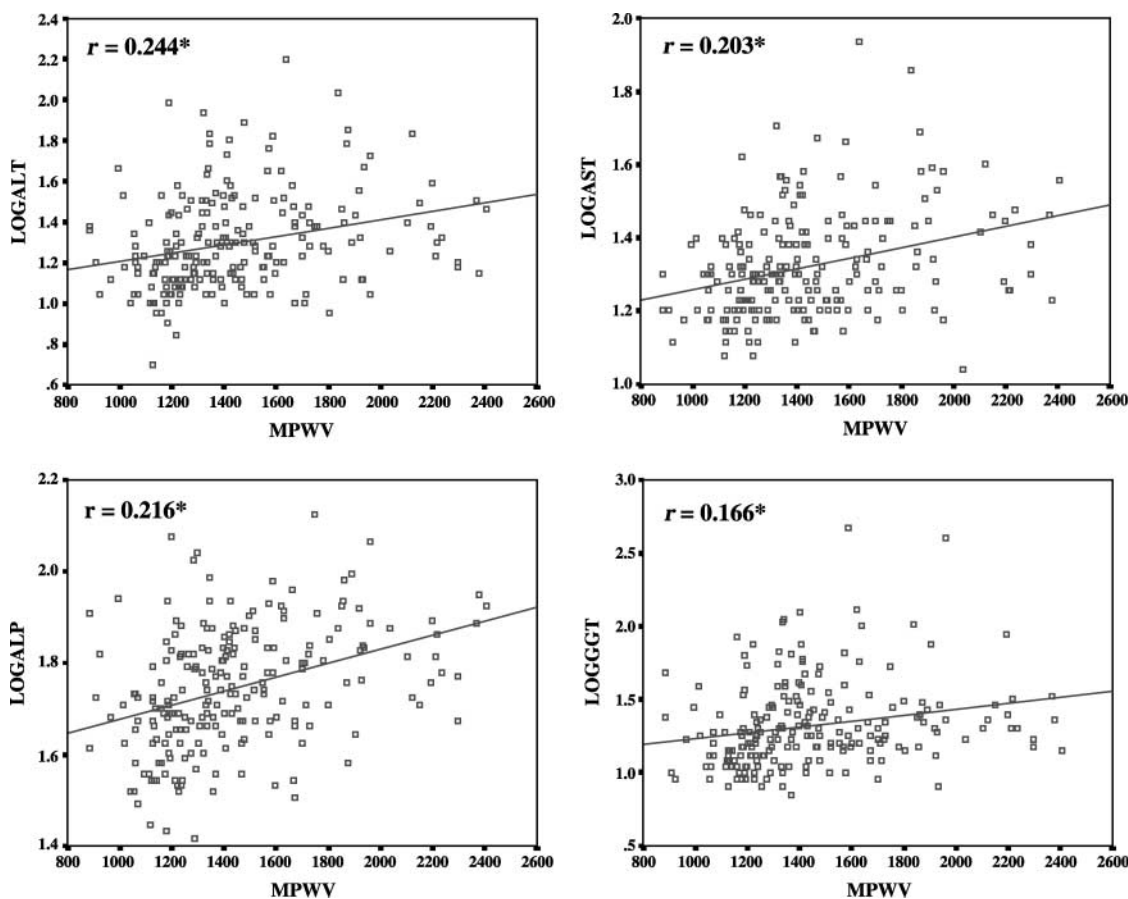
Figure 2 Partial correlation graphs between baPWV and log-transformed ALT, AST, ALP and GTT in controlling for age, BMI, HOMA and systolic blood pressure in all subjects. MPWV, mean brachial-ankle PWV; *P < 0.05.

observed only in females. A possible explanation is the smaller number of male subjects. The power of the study may not be enough to detect differences in men. While Hui *et al.*'s report mainly focused on steatohepatitis, the subjects in our study had normal to mild hepatic dysfunction. Therefore, possibly more simple steatosis than steatohepatitis was included in our study. We found that serum adiponectin levels were inversely correlated with serum ALT and GGT levels after adjusting for age, BMI and HOMA value. This result is compatible with the findings in an apparently healthy Spanish population (31). Recently, several prospective studies have shown that elevated ALT and GGT are predictors of the development of type 2 diabetes (32, 33), and it was reported that low serum adiponectin levels predict the development of type 2 diabetes and metabolic syndrome (34, 35). In combination, these reports and our data suggest that adiponectin plays a role in the pathogenesis of NAFLD or hepatic dysfunction. Adiponectin is known to stimulate PPAR-α, which is thought to accelerate fatty acid oxidation and upregulate the expression of insulin-receptor substrate, increasing insulin utility in various organs expressing adiponectin receptors, including the liver (36). Thus, hypoadiponectinemia may result in fat

accumulation in the liver; that is, the development of fatty liver causing increased transaminase activity (37). In addition, it was reported that adiponectin prevents LPS-induced hepatic injury by inhibiting the synthesis and/or release of tumor necrosis factor-alpha by KK-Ay obese mice (38).

PWV is considered a risk marker (22, 23) and prognostic predictor of atherosclerosis (24, 25). baPWV is a newly developed device using a volume-rendering method. This instrument determines baPWV with simultaneous oscillometric measurement of pulse waves in all four extremities (39). Because of its technical simplicity and short sampling time, baPWV is more appropriate for screening a large population than previous methods.

Ohnishi *et al.* reported that there are significant differences between baPWV values in normal and impaired fasting glucose (IFG) groups (40). Yamashina *et al.* reported that baPWV in patients with coronary artery disease (CAD) is significantly higher than in non-CAD patients (41). Our study found for the first time that baPWV is positively associated with NAFLD and various liver enzymes. NAFLD showed stronger correlation with baPWV when it was combined with hepatic dysfunction. These data suggest that NAFL or hepatic dysfunction by itself may be associated with

atherosclerosis, which is an important outcome of insulin resistance. However, our study is a cross-sectional study. Therefore, further studies need to be performed to determine whether NAFLD by itself results in CVD.

On the other hand, there was no significant correlation between adiponectin levels and PWV. It is possible that a confounding factor such as age causes the lack of association between adiponectin and baPWV. Contrary to our expectation, old age has a higher adiponectin level than young age. Actually, after controlling for age, there was a significant negative correlation ($r = -0.1829$, $P = 0.017$) between baPWV and serum adiponectin level in 171 healthy subjects without confounding diseases.

We defined NAFLD as fatty liver on ultrasonography. Because the reference standard for diagnosing fatty liver is histopathologic findings, there is concern about the diagnostic accuracy of using ultrasonography. Yet, according to a recent review, ultrasound scanning, when positive, can give a high degree of diagnostic certainty, depending on the prevalence of fatty liver in the population being studied (42). Ultrasound is also the cheapest, safest and most patient-friendly imaging technique, and it is a reasonable alternative to liver biopsy in certain circumstances, especially studies conducted on relatively healthy subjects such as ours.

In conclusion, serum adiponectin level and baPWV are significantly associated with NAFLD and various liver enzymes, especially in females. These findings suggest that adiponectin may play a role in the pathogenesis of NAFLD, and NAFLD by itself may be associated with atherosclerosis, which is an important outcome of insulin resistance.

References

1 McCullough AJ. Update on nonalcoholic fatty liver disease. *Journal of Clinical Gastroenterology* 2002 **34** 255–262.

2 Cortez-Pinto H, Camilo ME, Baptista A, De Oliveira AG & De Moura MC. Non-alcoholic fatty liver: another feature of the metabolic syndrome? *Clinical Nutrition* 1999 **18** 353–358.

3 Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G & Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001 **50** 1844–1850.

4 Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P & Rizzetto M. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 2002 **35** 367–372.

5 Chen W, Yu Z & Li Y. [The relationship between nonalcoholic fatty liver and insulin resistance with abnormal glucose metabolism]. *Zhonghua Gan Zang Bing Za Zhi* 2000 **8** 76–77.

6 Sharabi Y & Eldad A. Nonalcoholic fatty liver disease is associated with hyperlipidemia and obesity. *American Journal of Medicine* 2000 **109** 171.

7 Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M & George J. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002 **35** 373–379.

8 Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *Journal of Biological Chemistry* 1995 **270** 26746–26749.

9 Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE & Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1930–1935.

10 Matsubara M, Maruoka S & Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2764–2769.

11 Huang KC, Chen CL, Chuang LM, Ho SR, Tai TY & Yang WS. Plasma adiponectin levels and blood pressure in nondiabetic adolescent females. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4130–4134.

12 Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T & Matsuzawa Y. Association of hypoadiponectinemia with coronary artery disease in men. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2003 **23** 85–89.

13 Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T & Noda T. Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry* 2002 **277** 25863–25866.

14 Yamauchi T, Hara K, Kubota N, Terauchi Y, Tobe K, Froguel P, Nagai R & Kadowaki T. Dual roles of adiponectin/Acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. *Current Drug Targets. Immune, Endocrine and Metabolic Disorders* 2003 **3** 243–254.

15 Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T & Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999 **100** 2473–2476.

16 Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K, Kihara S, Funahashi T, Rakugi H, Matsuzawa Y & Ogihara T. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension* 2004 **43** 1318–1323.

17 Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB & Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *Journal of the American Medical Association* 2004 **291** 1730–1737.

18 Berg AH, Combs TP, Du X, Brownlee M & Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Medicine* 2001 **7** 947–953.

19 Blacher J, Asmar R, Djane S, London GM & Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999 **33** 1111–1117.

20 Safar H, Mourad JJ, Safar M & Blacher J. Aortic pulse wave velocity, an independent marker of cardiovascular risk. *Archives Mal Coeur European Vaiss* 2002 **95** 1215–1218.

21 Amar J, Ruidavets JB, Chamontin B, Drouet L & Ferrieres J. Arterial stiffness and cardiovascular risk factors in a population-based study. *Journal of Hypertension* 2001 **19** 381–387.

22 Cohn JN. Vascular wall function as a risk marker for cardiovascular disease. *Journal of Hypertension* 1999 **17** (Suppl 5) S41–S44.

23 Van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, Van Der Kuip DA, Hofman A & Witteman JC. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. *Stroke* 2001 **32** 454–460.

24 Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, Ducimetiere P & Benetos A. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001 **37** 1236–1241.

25 Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ & London GM. Aortic pulse wave velocity index and mortality in

end-stage renal disease. *Kidney International* 2003 **63** 1852–1860.

26 Yamashina A, Tomiyama H, Arai T, Hirose K, Koji Y, Hirayama Y, Yamamoto Y & Hori S. Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. *Hypertension Research* 2003 **26** 615–622.

27 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419.

28 Xu A, Wang Y, Keshaw H, Xu LY, Lam KS & Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and non-alcoholic fatty liver diseases in mice. *Journal of Clinical Investigation* 2003 **112** 91–100.

29 Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A & George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004 **40** 46–54.

30 Targher G, Bertolini L & Zenari L. Hypoadiponectinemia is closely associated with nonalcoholic hepatic steatosis in obese subjects. *Diabetes Care* 2004 **27** 2085–2086.

31 Lopez-Bermejo A, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W & Fernandez-Real JM. Adiponectin, hepatocellular dysfunction and insulin sensitivity. *Clinical Endocrinology* 2004 **60** 256–263.

32 Perry IJ, Wannamethee SG & Shaper AG. Prospective study of serum gammaglutamyltransferase and risk of NIDDM. *Diabetes Care* 1998 **21** 732–737.

33 Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C & Tataranni PA. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002 **51** 1889–1895.

34 Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, Ohnuma H, Igarashi M, Tominaga M & Kato T. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese population: the Funagata study. *Diabetes Care* 2003 **26** 2015–2020.

35 Choi KM, Lee J, Lee KW, Seo JA, Oh JH, Kim SG, Kim NH, Choi DS & Baik SH. Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly Koreans. *Clinical Endocrinology* 2004 **61** 75–80.

36 Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB & Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine* 2002 **8** 1288–1295.

37 Yokoyama H, Hirose H, Ohgo H & Saito I. Inverse association between serum adiponectin level and transaminase activities in Japanese male workers. *Journal of Hepatology* 2004 **41** 19–24.

38 Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M & Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology* 2004 **40** 177–184.

39 Kubo T, Miyata M, Minagoe S, Setoyama S, Maruyama I & Tei C. A simple oscillometric technique for determining new indices of arterial distensibility. *Hypertension Research* 2002 **25** 351–358.

40 Ohnishi H, Saitoh S, Takagi S, Ohata J, Isobe T, Kikuchi Y, Takeuchi H & Shimamoto K. Pulse wave velocity as an indicator of atherosclerosis in impaired fasting glucose: the Tanno and Sobetsu study. *Diabetes Care* 2003 **26** 437–440.

41 Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, Koji Y, Hori S & Yamamoto Y. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. *Hypertension Research* 2002 **25** 359–364.

42 Joy D, Thava VR & Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? *European Journal of Gastroenterology and Hepatology* 2003 **15** 539–543.