Is adiponectin level a predictor of nonalcoholic fatty liver disease in nonobese diabetic male patients?

Haluk Sargin, Mehmet Sargin, Hülya Gozu, Asuman Orcun, Gülcan Baloglu, Murat Ozisik, Mesut Seker, Oya Uygur-Bayramicli

The growing epidemic of obesity has led to many studies on the role of adipose tissue as an endocrine organ that secretes many factors termed as adipokines, which mediate many of the vascular and metabolic complications of adiposity. These products, namely free fatty acids, TNF-α, interleukins, resistin, and leptin reduce insulin sensitivity.[4-3]

Adiponectin is a plasma protein, which is secreted from adipose tissue, and serum levels are markedly reduced in obesity. Adiponectin levels are negatively correlated with body fat percent, central fat distribution, fasting plasma insulin, oral glucose tolerance and positively with glucose disposal during euglycemic insulin clamp[5-7]. It has two receptors; adipor1 is abundantly expressed in skeletal muscle and at moderate levels in other tissues, whereas adipor2 is predominantly expressed in the liver.[8-10]

Adiponectin is a hepatic insulin sensitizer and also an inhibitor of tumor necrosis factor and therefore we studied its levels in nonobese diabetic patients with nonalcoholic fatty liver disease (NAFLD) and compared the results with healthy volunteers and looked for the effect of gender on adiponectin levels.

MATERIALS AND METHODS

Thirty-five patients admitted to the Department of Gastroenterology at Kartal Education and Research Hospital with elevated serum aminotransferase levels for longer than 6 mo and bright liver on ultrasound scan were included in this study. Thirty-four healthy volunteers without liver disease, who were gender and age matched with study group, were enrolled as the control group. Patients were excluded from this cohort if one of the following criteria was present: hepatitis B (hepatitis B surface antigen, antibody to hepatitis surface antigen, antibody to hepatitis B core antigen), hepatitis C (antibody to hepatitis C virus) and Epstein-Barr virus infection, non-organ specific autoantibodies (antimitochondrial antibody, antinuclear antibody, antismooth muscle antibody, antibody to hepatitis C virus), and anti-liver/kidney microsomal antibody, hereditary defects (fasting serum iron, transferrin saturation, ferritin, ceruloplasmin, alpha-1-antitripsin levels), alcohol consumption (ethanol ingestion >20 g/d), use of amiodarone, corticosteroids, tamoxifen, methotrexate, or oral contraceptives, jejunoileal bypass or extensive small bowel resection, total parenteral nutrition, malignancy, hypo-hyperthyroid disease, pregnancy, other known liver diseases like cirrhosis and diabetes. The diagnosis of cirrhosis was based on the clinical and laboratory findings (hyperalbuminemia, prolongation of prothrombin time, hyperbilirubinemia, presence of ascites or other findings of portal hypertension). The absence of
diabetes was confirmed with oral glucose tolerance test (OGTT) in both groups.

The absence of liver disease in the control group was established based on clinical, laboratory and imaging criteria. All controls had normal liver biochemistries and lack of any evidence from physical examination of chronic liver disease. However, an abdominal ultrasound was performed to exclude bright liver in all of the controls. All subjects gave informed consent to take part in the study.

Age, gender, height, weight, and body mass index (BMI) were recorded. Fasting plasma glucose, insulin, proinsulin, lipid profile, uric acid, and serum proteins were measured. Adiponectin levels were measured by Adiponectin Human ELISA Kit. A standard 75 g OGTT with insulin response according to WHO criteria was performed on all patients.

The index of insulin resistance (IR) was calculated on the basis of fasting values of plasma glucose and insulin, according to the homeostasis model assessment (HOMA) method.

Ultrasound liver studies were carried out by the same experienced radiologist who was blinded to laboratory values. The diagnosis of bright liver was based on abnormally intense, high-level echoes arising from the hepatic parenchyma, with amplitude similar to that of echoes arising from the diaphragm.

### Statistical analysis
Statistical analysis was performed with SPSS 11.5 program. Any P value less than 0.05 was considered statistically significant. Results are expressed as mean±SD. Comparison between the two groups was made with Student’s t-test and Mann-Whitney U test. Pearson’s and Spearman’s rank correlations were used to detect the associations between serum adiponectin and various demographic, anthropometric, and metabolic variables. When necessary, multiple regression analysis was performed.

### Results
Demographic, anthropometric, metabolic, and laboratory characteristics of patients with NAFLD and their matched controls are shown in Table 1.

According to the OGTT results, none of the participants had diabetes. However, six patients in NAFLD group and three patients in control group had impaired glucose tolerance (P<0.05). The remaining had normal glucose tolerance.

Insulin (fasting, 60 and 120 min), HOMA, proinsulin, and c-peptide levels were statistically significantly higher in NAFLD group than in control group (Table 2).

Serum adiponectin levels were statistically significantly lower in patients with NAFLD than in the control group (8.14±3.4 μg/mL vs 12.41±9.4 μg/mL, respectively, P<0.01; Table 2). A statistically significant correlation was found between adiponectin and BMI (r: -0.33, P<0.01), HOMA (r: -0.26, P<0.05), proinsulin (r: -0.32, P<0.01), AST (r: -0.25, P<0.05), ALT (r: -0.26, P<0.05) or GGT (r: -0.22, P<0.05). In multiple regressions analysis, gender was found to be a predictor of adiponectin but not the age and BMI (Table 3).

When we subclassified the patient and the control group according to gender, the adiponectin levels were found to be low in males compared to females (P<0.05). But there was no statistically significant difference in age, BMI, HOMA, and proinsulin between the subgroups of gender (P>0.05, Table 4). In multiple regression analysis, adiponectin levels were the only predictor of NAFLD in males (Table 5), whereas in female group it was the BMI (Table 6).

### Table 1 Demographic, anthropometric, and biochemical measurements in NAFLD and control groups

|                       | NAFLD (n: 35) | Controls (n: 34) | P   |
|-----------------------|--------------|-----------------|-----|
| Age (yr)              | 39.1±7.8     | 36.1±6.0        | NS  |
| Gender (female/male)  | 20/15        | 20/14           | NS  |
| BMI (kg/m²)           | 30.5±4.1     | 23.7±3.8        | <0.001 |
| Fasting glucose (mg/dL) | 93±9         | 84±11           | <0.01 |
| Total cholesterol (mg/dL) | 209±35       | 186±36          | <0.01 |
| HDL cholesterol (mg/dL) | 50±12        | 51±12           | NS  |
| LDL cholesterol (mg/dL) | 125±28       | 118±37          | NS  |
| Triglycerides (mg/dL) | 177±100      | 103±57          | <0.01 |
| AST (IU/L)            | 36±10        | 18±5            | <0.001 |
| ALT (IU/L)            | 49±17        | 18±9            | <0.001 |
| GGT (IU/L)            | 43±16        | 16±8            | <0.001 |

NS, nonsignificant.

### Table 2 Comparison of IR parameters in NAFLD and control groups

|                       | NAFLD (n: 35) | Controls (n: 34) | P   |
|-----------------------|--------------|-----------------|-----|
| Fasting insulin (UI/L) | 14.4±6.7     | 9.9±4.5         | <0.01 |
| Insulin 60 min (UI/L)  | 99.6±63.1    | 62.4±45.2       | <0.01 |
| Insulin 120 min (UI/L) | 65.3±89      | 40.2±31.9       | <0.01 |
| HOMA index            | 3.3±1.5      | 2.1±1.1         | <0.001 |
| Proinsulin (pmol/L)   | 10.6±4.6     | 6.2±4.5         | <0.001 |
| C-peptide (ng/mL)     | 2.7±0.7      | 2.0±0.6         | <0.001 |
| Adiponectin (μg/mL)   | 8.14±3.4     | 12.41±9.4       | <0.01 |

### Table 3 Regression model of adiponectin as a dependent variable

| Independent variables | T  | Significance |
|-----------------------|----|--------------|
| Gender (male)         | -2.791 | 0.009 |
| Age (yr)              | 0.774 | NS           |
| BMI (kg/m²)           | -0.523 | NS           |

NS, nonsignificant.

### Table 4 Comparison of male and female patients in both NAFLD and control groups

|                       | Female (n: 40) | Male (n: 29) | P   |
|-----------------------|--------------|-------------|-----|
| Age (yr)              | 37±47.1      | 38±67.1     | NS  |
| BMI (kg/m²)           | 27.5±5.8     | 26.9±4.5    | NS  |
| HOMA                   | 2.6±1.4      | 2.8±1.4     | NS  |
| Adiponectin (μg/mL)   | 11.7±18.9    | 8.2±6.3     | 0.025 |

NS, nonsignificant.

### Table 5 Regression model of NAFLD as a dependent variable in...
For the diagnosis of NAFLD, we used the exclusion of known etiological factors, which are responsible for the liver disease and ultrasound examination. Liver biopsy was not done because the stage and grade of the NAFLD was not of importance in this study and according to Saverymuttu et al., ultrasound examinations can accurately identify steatosis with a sensitivity of 94% and a specificity of 84%.[21]. Ricci et al., also demonstrated that standard ultrasonography may be used for the diagnosis of NAFLD[23].

In NAFLD, most of the liver damage in insulin-resistant and dyslipidemid patients is thought to be caused by accumulation of hepatic triglycerides, and adiponectin might be able to preserve liver function by preventing lipid accumulation in hepatocytes. Adiponectin is also a potent insulin sensitizer and modulates the inflammatory response[23,24-28]. In our study, low adiponectin levels in NAFLD patients are compatible with previous studies.

Adiponectin was found to circulate in inverse proportion to IR syndrome such as BMI, fasting glucose and triglycerides[15,25,27,28]. In our study, we also found an inverse correlation between adiponectin levels with BMI, insulin, HOMA, proinsulin and triglycerides.

A recent study showed that adiponectin levels are correlated in healthy humans with various liver function tests such as ALT and GGT[9]. We also found a statistically significant correlation between adiponectin and liver function tests like AST, ALT, and GGT.

This is the first study looking for adiponectin levels in nondiabetic NAFLD patients. Bajaj et al., demonstrated a relationship between plasma adiponectin levels with hepatic insulin sensitivity and hepatic fat content in patients with type 2 diabetes, for the first time[10]. Yamamoto et al., have reported that adiponectin predicts future IR in a Japanese population in a 2-year follow-up study[11].

In our study, it is remarkable that in males, NAFLD is definitely correlated with low adiponectin levels but the female gender did not show such a correlation. This gender predilection might be due to the correlation of low adiponectin with visceral adiposity in females.

As a summary, adiponectin level is lower in nondiabetic patients with NAFLD in comparison to healthy volunteers. Low adiponectin level might be a predictor of NAFLD especially in male nondiabetics.

REFERENCES

1. Goldstein BJ, Scalia R. Adiponectin: A Novel Adipokine Linking Adipocytes and Vascular Function. JCEM 2004; 89: 2563-2568
2. Bermejo AL, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W, Fernandez-Real JM. Adiponectin, hepatocellular dysfunction and insulin sensitivity. Clinical Endocrinol 2004; 60: 256-263
3. 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
4. Yamamoto Y, Hirose H, Saito I, Nishikai K, Saruta T. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: Two-year follow-up study in Japanese population. JCEM 2004; 89: 87-90
5. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M,
Nonalcoholic fatty liver disease and adiponectin

Sargin H et al. Nonalcoholic fatty liver disease and adiponectin 5877

Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; 20: 1595-1599

Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2004; 24: 29-33

Pittas AG, Joseph NA, Greenberg AS. Adipokines and insulin resistance. *J Clin Endocrinol Metab* 2004; 89: 447-452

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Frogue P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; 423: 762-769

Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang CC, Itani SI, Lodish HF, Ruderman NB. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci USA* 2002; 99: 16309-16313

Song Z, Joshi-Barve S, Barve S, Mc Clain CJ. Advances in alcoholic liver disease. *Curr Gastroenterol Rep* 2004; 6: 71-76

Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; 112: 91-100

Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *New Engl J Med* 2000; 343: 1467-1476

Bajaj M, Suraamornkul S, Piper P, Hardies LJ, Glass L, Cersosimo E, Pratipanawatr T, Miyazaki Y, Defronzo RA. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. *JCEM* 2004; 89: 200-206

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. *J Clin Endocrinol Metab* 2001; 86: 1930-1935

Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency and severity of disease. *Am J Gastroenterol* 2001; 96: 2813-2814

Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; 346: 1221-1231

Dixon JB, Bhathal PS, O’Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; 121: 91-100

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

Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zeneri MB, Monnauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. *Diabetes Care* 2000; 23: 57-63

Wallace TM, Levy JC, Mathews DR. Use and Abuse of HOMA modelling. *Diabetes Care* 2004; 27: 1487-1495

Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *BMJ* 1986; 292: 13-15

Ricci C, Longo R, Gioulis E, Bosco M, Polesello P, Masutti F, Croce LS, Paoletti S, de Bernard B, Tiribelli C, Dalla Palma L. Noninvasive in vivo quantitative assessment of fat content in human liver. *J Hepatol* 1997; 27: 108-113

Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002; 360: 57-58

Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Weyer C, Lindsay RS, Youngren JF, Havel PJ, Pratley RE, Bogardus C, Tataranni PA. Plasma adiponectin concentrations associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. *Diabetes* 2002; 51: 1884-1888

Spranger J, Kroeke A, Möhlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003; 361: 226-228

Tshritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, Staeiger H, Maerker E, Haring H, Stumvoll M. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes* 2003; 52: 239-243

Hotta K, Funahashi T, Bodkin NL, Oermeyer HK, Arita Y, Hansen BC, Matsuzawa Y. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to the type 2 diabetes in rhesus monkeys. *Diabetes* 2001; 50: 1126-1133

Science Editor Wang XL and Guo SY  Language Editor Elsevier HK