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Plasma Adiponectin in Nonalcoholic Fatty Liver Is Related to Hepatic Insulin Resistance and Hepatic Fat Content, Not to Liver Disease Severity

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Plasma levels of adiponectin are decreased in patients with nonalcoholic fatty liver disease (NAFLD), but the relationship among plasma adiponectin, insulin sensitivity, and histological features is unclear. In 174 NAFLD patients and 42 controls, we examined plasma adiponectin concentrations in relation to 1) lipid profile, indices of insulin resistance, and features of the metabolic syndrome (n = 174); 2) hepatic insulin resistance (clamp technique with tracer infusion) (10 patients); and 3) histological features at liver biopsy (n = 116).

When the data from all subjects were combined, plasma adiponectin levels were positively associated with increased age, female gender, and plasma high-density lipoprotein levels, and negatively associated with waist circumference, body mass index, triglycerides, indices of insulin resistance, and aminotransferase levels, and also predicted the presence of the metabolic syndrome. In step-wise regression, increased age, female gender, waist circumference, triglyceride levels, and homeostasis model assessment independently associated with adiponectin (adjusted R^2, 0.329). In NAFLD, adiponectin was only associated with increased age, female gender, and triglycerides (adjusted R^2, 0.245). When the measured histological parameters were included in the model, plasma adiponectin levels were also inversely proportional to the percentage of hepatic fat content (adjusted R^2, 0.221), whereas necroinflammation and fibrosis did not fit in the model. Adiponectin was negatively correlated with insulin-suppressed endogenous glucose production during the clamp ($P = 0.011$).

The results demonstrate that decreased levels of circulating adiponectin in NAFLD are related to hepatic insulin sensitivity and to the amount of hepatic fat content. Hypoadiponectinemia in NAFLD is part of a metabolic disturbance characterized by ectopic fat accumulation in the central compartment. **(*J Clin Endocrinol Metab* 90: 3498–3504, 2005)**

NONALCOHOLIC FATTY LIVER (FL) disease (NAFLD) represents the most frequently diagnosed cause of chronic liver disease in Western countries and includes a wide spectrum of hepatic injuries, ranging from FL to steatohepatitis and cirrhosis. An association among NAFLD, the features of the metabolic syndrome (MS), and the parameters of insulin resistance has been extensively reported (1–6). The prevalence of MS increases with the severity of liver disease, from 14% in pure FL to 38% in nonalcoholic steatohepatitis (NASH) (7), and a higher degree of insulin resistance is associated with a higher score of fibrosis. This supports the hypothesis that either insulin resistance plays a role in the pathogenesis and progression of liver damage (8), or the two phenomena have a common pathogenic mechanism.

Adiponectin is an adipocyte-derived plasma protein known to modulate insulin effects (9). Low circulating levels of adiponectin are associated with several components of MS, including visceral adiposity, hyperlipidemia, and insulin resistance/type 2 diabetes (10). A specific role for adiponectin in the liver has also been suggested. Adiponectin levels correlate inversely with hepatic fat and hepatic insulin resistance in diabetic patients (11), whereas in healthy subjects, low adiponectin levels are significantly associated with increased serum concentrations of alanine transaminase (ALT) and γ-glutamyl transpeptidase, suggesting a possible contribution of adiponectin in maintaining liver integrity (12, 13).

In the liver, adiponectin decreases hepatic glucose production (14) and reduces free fatty acid turnover (15). The administration of recombinant adiponectin in *ob/ob* mice reduces hepatomegaly and steatosis and attenuates inflammation in both alcoholic and nonalcoholic FL (16). Recently, reduced adiponectin levels have also been associated with increased fat content and more extensive necroinflammation in NAFLD patients (17), suggesting that the hormone might be at the crossroad between liver fat deposition and progressive hepatic disease.

We examined the relationship among plasma adiponectin

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Abbreviations: ALT, Alanine transaminase; BMI, body mass index; CV, coefficient(s) of variation; EGP, endogenous glucose production; FBG, fasting blood glucose; FL, fatty liver; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NEFA, nonesterified fatty acid; OGIS; oral glucose insulin sensitivity; OGTT, oral glucose tolerance test; OR, odds ratio; QUICKI, quantitative insulin sensitivity check index.

levels, insulin resistance, and biochemical and histological parameters of liver injury in a cohort of NAFLD patients with a low prevalence of type 2 diabetes. To further explore the relationship between plasma adiponectin concentration and hepatic *vs.* peripheral insulin sensitivity, we also performed an euglycemic hyperinsulinemic clamp coupled with tracer infusion in a subgroup of NAFLD patients.

Subjects and Methods

Subjects

One hundred seventy-four consecutive NAFLD patients (156 males) (median age, 41 yr; range, 19–74) and 42 control subjects (34 males) (median age, 43 yr; range, 28–77) were included in the study. In NAFLD, the diagnosis was based on chronically elevated aminotransferase levels (ALT > 1.5 times the upper normal values for 6 months or more), negative hepatitis B and C viral markers, absence of autoimmune hepatitis or celiac disease, and no evidence of genetic, drug-induced, or cholestatic liver disease. Alcohol intake was assessed by interviews extended to family members and general practitioners. Patients with alcohol consumption of 20 g/d more were excluded. All patients had a bright liver at ultrasound scanning (increased liver/kidney echogenicity and posterior attenuation). In 116 cases, the diagnosis was confirmed by a liver biopsy, scored according to the criteria proposed by Brunt *et al.* (18). Steatosis was graded as mild (<33%) in 79 cases (68%), moderate (33–66%) in 26 cases (22%), and severe (>66%) in 11 cases (9%). Necroinflammation was absent in 26 cases (22%), grade I in 36 cases (30%), grade II in 32 cases (28%), and grade III in 23 cases (20%). Finally, fibrosis was absent in 32 cases (28%), perisinusoidal/pericellular in 29 cases (25%), periportal in 34 cases (29%), bridging in 18 cases (15%), and three cases had histological evidence of initial cirrhosis.

Seventy-two cases were classified as NASH on the basis of the presence of fibrosis (stage 1 or over) or necroinflammation (grade 2 or over), whereas 44 were classified as pure FL. Control subjects were free of hepatic diseases, as assessed by normal liver enzymes and normal liver echogenicity. They were selected in a body mass index (BMI) range similar to that of NAFLD cases.

The purpose of the study was explained to all subjects, who gave their informed consent to blood sampling for adiponectin measurement. All the other investigations were carried out during regular follow-up of NAFLD patients, according to specific protocols. Ten NAFLD patients also participated in the euglycemic insulin clamp study after signing an informed consent. The protocol was approved by the senior staff committees of the two University hospitals. These boards regulate noninterventional studies and are comparable with Institutional Review Boards.

Anthropometric and laboratory evaluations

All subjects had a complete clinical, anthropometric, and laboratory investigation. BMI was calculated as weight (in kilograms) divided by height squared (meters squared). Subjects in the BMI range of 25–30 kg/m^2 and 30 kg/m^2 or more were considered overweight and obese, respectively. Waist circumference (at the nearest half centimeter) was measured at the midpoint between the lower border of the rib cage and the iliac crest. The components of MS were classified according to the Adult Treatment Panel III proposal (19), and subjects having three or more positive criteria were labeled as MS.

Laboratory investigations included an oral glucose tolerance test (OGTT) in 129 NAFLD patients and 28 controls. Subjects were classified according to their fasting blood glucose (FBG) as follows: normal fasting glucose (FBG < 110 mg/dl), impaired fasting glucose (FBG, 110–125 mg/dl), and diabetes mellitus (FBG > 125 mg/dl) (20). The 120-min blood glucose after OGTT was used to further classify patients and controls into normal glucose tolerance (<140 mg/dl), impaired glucose tolerance (140–199 mg/dl), and diabetes mellitus (≥200 mg/dl).

All patients were regularly followed up as outpatients and were on a controlled dietary regimen (25 kcal/kg body weight).

Euglycemic insulin clamp

The patients enrolled in this protocol were fed a balanced diet (50% carbohydrate, 35% fat, and 15% protein) for 3 d before the study, when the experiment was performed after an overnight fast. Teflon catheters were placed into an antecubital vein for isotope infusion and into a contralateral dorsal hand vein, heated at 55 C to achieve arterialization of venous blood, for blood sampling. At 0800 h, a primed-continuous infusion of [6,6$^2H_2$]glucose (0.22 μmol/kg·min; prime, 17.6 μmol/kg) was started and continued at a constant rate throughout the study. After a 90-min basal period, a low-dose insulin infusion (insulin infusion rate, 0.25 mU/kg·min; Humulin R, Eli Lilly, Indianapolis, IN) was started and continued for 2 h. During the clamp, a variable rate of a 20% glucose solution, enriched with [6,6 $^2H_2$]glucose, was infused to keep plasma glucose concentration and enrichment constant (21, 22). Arterialized blood samples were collected at 10-min intervals during the last 30 min of the basal period and the last 40 min of the insulin clamp.

Fasting blood samples and clamp samples were drawn into prechilled tubes and immediately cold-centrifuged at +4 C. Serum was stored at −80 C until analyses.

Analytical determinations

Adiponectin concentrations were determined on serum in duplicate, using a specific commercial kit (Human Adiponectin RIA Kit, Linco Research, St. Charles, MO). Intra- and interassay coefficients of variation (CV) for adiponectin were 5.2 and 9.8%, respectively. Plasma glucose levels were measured by the glucose oxidase method (Beckman Instruments, Fullerton CA; interassay CV < 4%). Plasma insulin concentrations were assessed by a double-antibody RIA (Diagnostic Products Corp., Los Angeles, CA; interassay CV < 13%). Nonesterified fatty acids (NEFAs) were measured by an enzymatic colorimetric method (NEFA C, Wako Chemicals GmbH, Neuss, Germany). Fasting serum liver function tests and lipid levels were determined by routine laboratory techniques.

Deuterium enrichment of glucose was measured by a gas chromatography-mass spectrometry system (Hewlett Packard 5972, Hewlett Packard, Palo Alto, CA), selectively monitoring ions at m/z 200, 201, and 202, as previously described (23, 24). Isotopic enrichments were expressed as tracer to tracee ratios (25).

Calculations

In the whole database, insulin resistance was calculated on the basis of fasting glucose and insulin levels, according to the homeostasis model assessment (HOMA-R) method (26). HOMA-R values 3.0 or more were considered to indicate insulin resistance; this cutoff corresponds to the upper quartile of the control population. Insulin sensitivity was assessed by the quantitative insulin sensitivity check index (QUICKI) (27) and by the oral glucose insulin sensitivity (OGIS) (28), a well-established glucose-insulin model derived from OGTT.

In the course of the euglycemic insulin clamp, the rate of glucose disposal was measured during the last 40 min of the insulin infusion (21). The rate of total body glucose appearance was assessed using Steele's equation from tracer data (29). Endogenous glucose production (EGP) during insulin infusion was calculated by subtracting the exogenous glucose infusion rate from the tracer-derived measure of glucose appearance.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Inc., Cary, NC.). Patients were grouped according to categorical variables (gender, presence/absence of MS and its individual components). Adiponectin concentrations were tested for significance using unpaired *t* test (two-tail) or nonparametric analysis (Mann-Whitney *U* test or Kruskal-Wallis test). Contingency test and Fisher's exact test were also used to compare prevalence, whenever appropriate. Linear regression analysis and Spearman rank correlation were used to examine the relationship between plasma adiponectin concentration and different continuous or categorical parameters, both in the whole population and, separately, in controls and in the NAFLD series, as well as in the clamp subgroup. Logistic regression analysis was

used to identify factors independently associated with low adiponectin levels. Results were expressed by odds ratios (ORs) (95% confidence intervals). All data in the text and in the tables are given as means ± SD, when not otherwise indicated. $P < 0.05$ was considered statistically significant.

Results

Anthropometric, clinical, and laboratory data

Clinical and biochemical variables of the patients included in the study are summarized in Table 1. At history, there were no differences in the prevalence of diabetes, hypertension, and dyslipidemia between patients and controls, whereas total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were lower in NAFLD. Despite similar BMI (range, 19.1–35.1 kg/m^2 in controls, 19.8–38.0 kg/m^2 in NAFLD) and similar glucose tolerance, NAFLD patients had a larger waist circumference, indicative of visceral adiposity, and higher insulin levels (both fasting and after the OGTT). As expected, mean HOMA-R was higher in NAFLD and exceeded the cutoff of 3.0, indicative of insulin resistance in 54% of cases, compared with 23% of controls ($P = 0.0006$; Fisher's exact test). Accordingly, both QUICKI and OGIS values were lower in NAFLD. Also, fasting free fatty acid levels were higher in NAFLD.

NAFLD patients had a larger prevalence of metabolic abnormalities corresponding to Adult Treatment Panel III criteria, and the prevalence of MS was more than doubled (26% in NAFLD *vs.* 10% in controls; $P = 0.02$), without differences in relation to gender.

Adiponectin levels were significantly lower in NAFLD patients (Fig. 1). In relation to gender, adiponectin concentrations were systematically lower in males, both in NAFLD and in controls.

Adiponectin levels in relation to clinical, anthropometric, and biochemical parameters

Adiponectin was significantly correlated with several clinical, anthropometric and biochemical variables in the whole group (Table 2). In particular, adiponectin was lower in relation to measures of central adiposity, insulin resistance, and hyperlipidemia. However, when data were analyzed separately in controls and in NAFLD, most correlations were lost in liver patients.

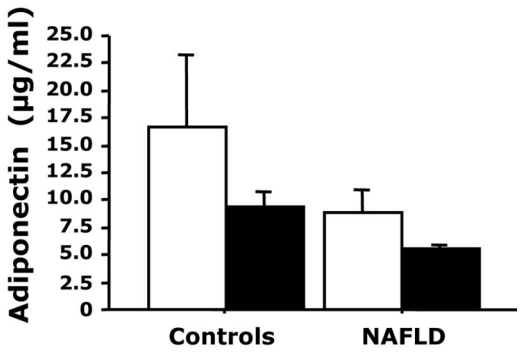

FIG. 1. Adiponectin concentrations in control subjects and in NAFLD patients, according to gender (females, *open bars*; males, *closed bars*). Data are presented as means ± 2 SE.

Adiponectin levels also decreased with the increasing number of positive criteria of MS (P for trend = 0.0046) (Fig. 2), and this relation was maintained in NAFLD patients ($P = 0.0067$). However, there was a large overlap of adiponectin levels among groups, and adiponectin could not identify the individual component of MS. In NAFLD cases, adiponectin levels were lower in relation to low HDL-cholesterol ($P < 0.0001$), high triglycerides ($P = 0.008$), and finally in subjects with MS (5.1 ± 2.0 *vs.* 6.2 ± 2.8 µg/ml; $P = 0.010$).

Hypoadiponectinemia (below the median value of 6.0 µg/ml) significantly predicted the presence of MS in the whole population (OR, 1.39; 95% confidence interval, 1.13–1.70; $P = 0.0017$), after correction for age, gender, waist circumference, and HOMA-R. Similarly, low adiponectin was associated with NAFLD, after correction for gender, waist circumference, and HOMA-R (OR, 1.32, 1.15–1.51; $P < 0.0001$).

TABLE 1. Anthropometric, clinical, and laboratory measurements in NAFLD and controls

	NAFLD (n = 174)	Controls (n = 42)	*P*
Male gender (%)	89	81	0.183
Age (yr)	41 ± 11	43 ± 11	0.297
BMI (kg/m^2)	27.3 ± 3.2	27.8 ± 3.5	0.312
Normal weight/overweight/obesity (%)	27/55/18	24/57/19	0.465
Waist circumference (cm)	99.7 ± 7.4	93.7 ± 9.2	<0.0001
Systolic blood pressure (mm Hg)	128 ± 15	132 ± 13	0.187
Diastolic blood pressure (mm Hg)	80 ± 9	87 ± 5	0.004
FBG − normal/IFG/DM (%)	86/8/6	87/8/5	0.985
OGTT − normal/IGT/DM (%)[a]	71/23/6	89/4/7	0.025
Fasting glucose (mg/dl)	98.2 ± 26.0	93.0 ± 15.9	0.239
Fasting insulin (µU/ml)	15.1 ± 7.9	9.7 ± 3.8	<0.0001
HOMA-R (%)	3.70 ± 2.45	2.30 ± 1.17	0.0006
QUICKI	0.65 ± 0.06	0.69 ± 0.05	0.0002
OGIS (ml/kg·min)[a]	9.24 ± 1.96	10.47 ± 2.00	0.0035
ALT (mU/ml)	79 ± 42	19 ± 6	<0.0001
AST (mU/ml)	39 ± 22	19 ± 5	<0.0001
GGT (mU/ml)	76 ± 74	22 ± 16	0.0032
HDL-cholesterol (mg/dl)	47 ± 11	56 ± 10	<0.0001
Triglycerides (mg/dl)	138 ± 93	130 ± 53	0.621
Fasting NEFAs (mmol/liter)[b]	0.67 ± 0.27	0.37 ± 0.11	<0.0001
Fasting adiponectin (µg/ml)	5.94 ± 2.70	10.7 ± 5.89	<0.0001

IFG, Impaired fasting glucose; DM, diabetes mellitus; GGT, γ-glutamyl transpeptidase.
[a] n = 28 in controls, and n = 129 in NAFLD.
[b] n = 30 in controls, and n = 160 in NAFLD.

TABLE 2. Correlation matrix between adiponectin levels and anthropometric, biochemical, and histological parameters

	All cases (n = 216)	Controls (n = 42)	NAFLD (n = 174)
Age	0.316[c]	0.143	0.395[c]
BMI	−0.092	−0.240	−0.019
Waist	−0.370[c]	−0.417[b]	−0.105
Insulin	−0.252[c]	−0.448[b]	−0.120
HOMA-R	−0.203[b]	−0.426[b]	−0.073
QUICKI	0.263[c]	0.469[b]	0.051
OGIS[d]	0.232[b]	0.288	0.106
Triglycerides	−0.172[a]	−0.303[b]	−0.188[a]
NEFAs[e]	−0.200[b]	0.067	−0.035
HDL-cholesterol	0.178[a]	0.179	0.178[a]
ALT	−0.241[c]	−0.194	−0.060
% Hepatic fat content[f]			−0.219[a]
Necroinflammatory score[f]			0.040
Fibrosis score[f]			0.090

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.
[d] n = 28 in controls, and n = 129 in NAFLD.
[e] n = 30 in controls, and n = 160 in NAFLD.
[f] n = 114.

Euglycemic insulin clamp

The euglycemic hyperinsulinemic clamp was carried out in 10 NAFLD patients, whose anthropometric and biochemical parameters were fully representative of the total NAFLD population. All subjects had a normal fasting glucose and a normal glucose tolerance, but insulin levels were remarkably high, both in the fasting state (14.8 ± 6.4 μU/ml) and during OGTT. ALT levels were on average 106 ± 52 mU/ml. At liver biopsy, six patients were diagnosed as NASH and four as pure FL, with a variable degree of inflammation.

Insulin infusion resulted in steady-state insulin levels of 29.8 ± 9.3 μU/ml in the last 40 min of the clamp, when glucose concentrations varied by less than 5% of basal levels. Fasting plasma adiponectin (5.3 ± 2.2 μg/ml) failed to correlate with glucose disposal (on average, 1.23 ± 0.53 mg/kg·min) (r = 0.430; $P = 0.215$). EGP was suppressed by 66% during insulin infusion (from 1.50 ± 0.15 to 0.51 ± 0.23 mg/kg·min). Fasting plasma adiponectin levels inversely correlated with EGP during the insulin clamp (Fig. 3) (r = −0.761; $P = 0.011$) but not with

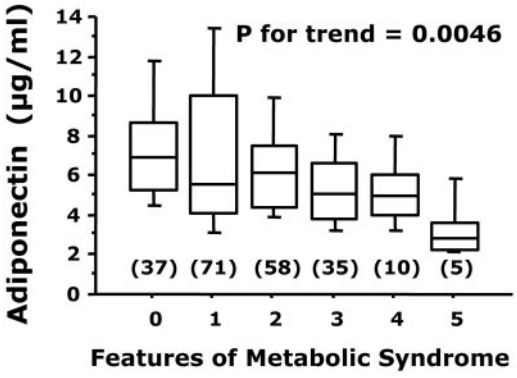

FIG. 2. Adiponectin levels in patients with NAFLD, according to the number of positive criteria for the MS. The values within the *box* range from the 25th to 75th percentiles. The *horizontal bar* corresponds to the median value. The *vertical bars* represent the values between the 10th and 90th percentiles. The relationship is maintained after exclusion of cases with five features of the MS ($P = 0.016$). The number of cases in the various groups is in *parentheses*.

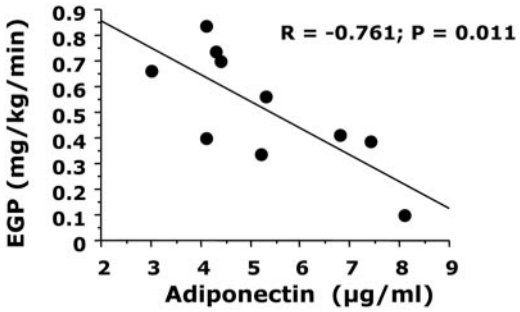

FIG. 3. Relationship between plasma adiponectin concentration and EGP in NAFLD patients participating in the euglycemic hyperinsulinemic clamp study (n = 10).

fasting EGP (r = −0.228; $P = 0.526$). No correlation was observed between postabsorptive or insulin-suppressed EGP and the percentage of hepatic fat (r = −0.258, $P = 0.471$ and r = −0.007, $P = 0.986$, respectively).

NEFAs values decreased from 0.70–0.26 mmol/liter during the clamp (by 63%), but their percent decrease did not correlate with adiponectin.

Adiponectin levels in relation to histology

NAFLD patients submitted to liver biopsy were not different in biochemical and clinical parameters from subjects who did not have a biopsy. No significant differences in laboratory and clinical data were observed when NAFLD subjects were split into NASH and pure FL. Adiponectin concentrations (Fig. 4) and aminotransferases were also similar.

Plasma adiponectin was inversely correlated with the percentage of hepatic fat content (Table 2 and Fig. 5), but not with the score of necroinflammation or fibrosis. Adiponectin was moderately increased in subjects with initial cirrhosis (stage 4, 7.8 ± 3.7 μg/ml), compared with stage 0 (5.8 ± 2.2) and stage 1 (5.1 ± 1.9; $P = 0.023$). A combined score (sum of necroinflammation + fibrosis) was also calculated. The score had a significant positive correlation with HOMA-R (r = 0.228; $P = 0.014$) and a significantly negative correlation with insulin sensitivity, expressed by OGIS (r = −0.416, $P < 0.0001$), but no association with plasma adiponectin (r = 0.041, $P = 0.660$).

Predictive variables of plasma adiponectin levels

Among liver function tests, plasma adiponectin levels were inversely correlated to ALT values in the whole data-

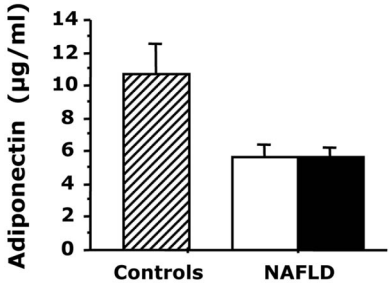

FIG. 4. Plasma adiponectin concentration in NAFLD patients, according to the presence of pure FL (*open bar*) or NASH (*closed bar*). Values of controls are also presented for comparison (mean ± 2SE).

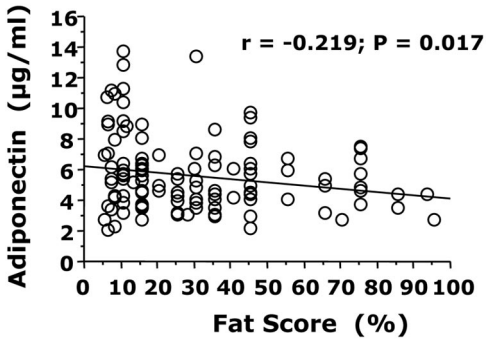

FIG. 5. Relationship between plasma adiponectin concentration and hepatic fat content in the whole database of NAFLD patients (n = 114).

base, after adjustment for age, gender, waist, and HOMA-R ($r = -0.256$, $P < 0.0001$), but the relationship was no longer present in NAFLD patients and in controls when separately analyzed.

Step-wise multiple regression models were constructed to predict adiponectin concentrations. The independent variables tested were the clinical, anthropometric, and biochemical parameters significantly correlated with adiponectin at univariate analysis (see Table 2).

In the general population (Table 3), age, gender, waist circumference, triglycerides, and HOMA-R were associated with adiponectin (adjusted R^2, 0.329). In NAFLD, adiponectin was independently associated only with age, gender, and triglycerides (adjusted R^2, 0.245). When the measured histological parameters were included in the model, plasma adiponectin levels were also inversely proportional to the percentage of hepatic fat content (adjusted R^2, 0.221), whereas necroinflammation and fibrosis did not fit in the model.

A logistic regression analysis, considering adiponectin concentrations below the median value of 6.0 μg/ml as the dependent variable and including histology as an independent variable, identified the percentage of hepatic fat as the sole variable predictive of low plasma adiponectin (OR, 1.14, 1.02–1.31; $P = 0.044$), explaining approximately 5% of the total variance of adiponectin. The histological scores of necroinflammation and fibrosis did not fit in the model.

Discussion

We confirm that average adiponectin levels are low in NAFLD subjects, in agreement with a very recent report (30). Our cohort was well characterized on a clinical, biochemical, and histological basis. In keeping with previous studies (1, 7), most of our patients were males, and results were gender-adjusted to comply with significantly higher values in females (15). Patients with overt type 2 diabetes were excluded, thus allowing an estimate of the significance of adiponectin independently of other severe metabolic disturbances, characterized by hypoadiponectinemia (31, 32).

Low adiponectin levels predicted NAFLD in the whole population, but in NAFLD, hypoadiponectinemia was no longer associated with the anthropometric, clinical, and biochemical parameters of insulin resistance, which characterize MS (13, 33, 34). Adiponectin remained systematically associated only with triglycerides, hepatic insulin sensitivity, and the amount of hepatic fat content, after adjustment for age, gender, and central adiposity.

Adiponectin is a relatively abundant serum hormone, which was reported to influence both lipid and glucose metabolism in the liver and in muscle tissue (35). In the liver, it increases the sensitivity of insulin to inhibit gluconeogenesis (14) and regulates hepatic NEFA metabolism *in vivo*, via suppression of lipogenesis and activation of NEFA oxidation (15). Injection of recombinant adiponectin in mice decreases hepatic gluconeogenesis and peripheral lipid accumulation in nonadipose tissues (9). Other proposed mechanisms of adiponectin activity include an enhanced expression of the PPAR-α gene, leading to increased fat oxidation.

We found lower adiponectin levels in NAFLD compared with controls, despite similar BMI. Available evidence from cross-sectional studies suggests that in humans adiponectin levels are more strictly associated with the amount of centrally located fat (36). In healthy adults, a relationship was also observed between adiponectin deficiency and increased liver fat (37), whereas in mild obesity, hypoadiponectinemia

TABLE 3. Step-wise multiple regression models of independent correlates of fasting serum adiponectin

	Coefficient	SE	Beta	F-to-remove	*P*
All cases (adjusted $R^2 = 0.329$)					
Intercept	20.674	3.021			
Age	0.068	0.021	0.207	10.784	0.0012
HOMA-R	−0.340	0.099	−0.211	11.388	0.0007
Sex	−4.601	0.789	−0.383	33.998	<0.0001
Triglycerides	−0.006	0.003	−0.147	5.949	0.0157
Waist circumference	−0.061	0.030	−0.130	4.158	0.0428
Controls (adjusted $R^2 = 0.484$)					
Intercept	28.291	3.085			
HOMA-R	−1.785	0.553	−0.377	10.409	0.0027
Sex	−7.821	1.585	−0.577	24.350	<0.0001
NAFLD (adjusted $R^2 = 0.245$)[a]					
Intercept	8.565	1.790			
Age	0.068	0.017	0.298	15.491	0.0006
Sex	−2.434	0.705	−0.263	11.900	<0.0001
Triglycerides	−0.006	0.002	−0.208	8.551	0.0018

[a] When the score of hepatic fat (percentage of hepatocytes with fatty droplets) was added as independent variable, also the fat score was fit in the model (adjusted R^2, 0.221; Beta, −0.219; F-to-remove, 6.623; $P = 0.0091$).

predicted the presence of hepatic steatosis at ultrasound (38). Similarly, in our NAFLD cohort, the percentage of hepatic fat was the only variable predicting low plasma adiponectin. However, it explained only 5% of the total variance of adiponectin levels, making it unlikely that decreased adiponectin-mediated NEFA oxidation and lipogenesis might play a major role in fat deposition within the liver. We did not quantitatively measure the amount of visceral and sc fat in our cases; therefore we cannot identify the relative contribution of any of them to adiponectin levels.

Interestingly, the clamp study confirmed that no correlation exists between adiponectin levels and the measure of peripheral insulin resistance in NAFLD, but an inverse correlation was present with hepatic insulin resistance, evaluated by EGP suppression, as previously observed in patients with type 2 diabetes (11). In diabetes mellitus, the impaired suppression of EGP by insulin was found to correlate both with the amount of liver fat and with plasma adiponectin, suggesting that circulating adiponectin may be a crucial link between hepatic fat content and hepatic insulin sensitivity (11). Hepatic fat did not correlate with EGP suppression in our study, but the low number of cases submitted to clamp carries a high risk of both types 2 and 1 error in statistical analysis. The lack of correlation might also stem from the different levels of insulin during the low-dose insulin clamp and the different metabolic profile of NAFLD compared with diabetes. The high levels of triglycerides and NEFAs, coupled with low adiponectin, remain the common soil of hepatic fat deposition and hepatic insulin resistance, but the exact mechanism(s) involved in the onset of NAFLD cannot be ascertained in a cross-sectional analysis.

Adiponectin has antiinflammatory properties in the liver, and its deficiency might account for high aminotransferase and liver disease progression. In KK Ay-obese mice treated by lipopolysaccharide, pretreatment with adiponectin reduces mortality, aminotransferase elevation, and the amount of apoptosis (39). A direct antifibrotic effect of adiponectin has been suggested on the basis of adiponectin receptor gene expression in hepatic stellate cells and the inhibition of stellate cell proliferation and migration after adiponectin treatment (40). The results of our study do not lend support to the previously reported, inverse relationship between adiponectin levels and necroinflammatory activity in NAFLD (17), which also depends on the metabolic milieu and the inflammatory pattern, and might fluctuate over time.

In a recent study (41), decreased plasma adiponectin concentrations were closely related to steatosis but not to severity of fibrosis in hepatitis C virus-infected patients. In patients with advanced fibrosis, TNF-soluble receptor levels (TNFRI and TNRFII) were increased, whereas a nonsignificant increase of adiponectin levels was observed. In NASH, the mRNA expression of adiponectin receptors (AdipoRII) in the liver was negatively correlated with the histological grade of fibrosis but not with serum and hepatic adiponectin (42). Similarly, no correlation was observed between circulating adiponectin levels and liver adiponectin expression. This indicates that liver adiponectin expression is probably regulated by different factors, namely pro-inflammatory cytokines such as TNF-α. Finally, reduced liver function may affect the relationship of adiponectin with fibrosis by increasing adiponectin independently of body composition and the presence of metabolic diseases (43). Our patients with stage 4 fibrosis had adiponectin levels no longer different from control, and the role of reduced liver function and altered hepatic hemodynamics needs extensive investigation.

This study has obvious limitations related to the cross-sectional experimental protocol and to adiponectin measurement. Adiponectin circulates in serum as a hexamer of low molecular weight and a large multimeric structure of high molecular weight (44). Adiponectin concentrations measured in this study reflect the multimeric form of the compound; because assays are improved to identify monomeric adiponectin *vs.* adiponectin complexes, the impact of the different forms in several putative actions of adiponectin and in disease states may be more specifically described. A pharmacologic effect of full-length adiponectin on the liver has been previously demonstrated, suggesting that basal hepatic glucose output can be regulated by adiponectin. The proportion of adiponectin in the multimeric form, rather than its absolute circulating level, may thus explain differences in insulin sensitivity (45).

In summary, the present study demonstrates that low adiponectin levels in NAFLD are mostly associated with hepatic insulin sensitivity and hepatic fat content. The relationship with EGP supports the hypothesis that adiponectin may exert its action mainly on glucose production by the liver. Liver fat accumulation, as well as the features of the insulin resistance syndrome, are nonetheless related to low adiponectin levels, possibly as part of a general metabolic disturbance characterized by ectopic fat accumulation and abnormal secretion of adipokines by a dysfunctional adipose tissue (46).

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