Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease

Adipokines in NASH: Postprandial Lipid Metabolism as a Link Between Adiponectin and Liver Disease

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Circulating levels of four adipokines (adiponectin, TNF-α, leptin, and resistin) and the postprandial lipid and adiponectin responses to an oral fat load were assessed in 25 nonobese, non-diabetic patients with biopsy-proven nonalcoholic steatohepatitis (NASH) and correlated with metabolic indices and liver histology. Circulating adiponectin was lower in NASH compared with controls (5,476 ± 344 vs. 11,548 ± 836 ng/mL; $P = .00001$) and on multiple regression analysis correlated negatively with liver steatosis, necroinflammation (OR = 5.0; $P = .009$), and fibrosis (OR = 8.0; $P = .003$).The magnitude of postprandial lipemia was significantly higher in NASH than in controls and was related to fasting adiponectin ($\beta = -0.78$; $P = .00003$). Controls showed a significant increase in serum adiponectin in response to the fat load, whereas patients with NASH showed a slight decrease. Postprandial free fatty acids response correlated inversely with adiponectin response in both groups and independently predicted the severity of liver steatosis in NASH ($\beta = 0.51$; $P = .031$). In conclusion, hypoadiponectinemia is present before overt diabetes and obesity appear and correlates with the severity of liver histology in NASH. Impaired postprandial lipid metabolism may be an additional mechanism linking hypoadiponectinemia and NASH and posing a higher cardiovascular risk to these subjects. The mechanism(s) underlying these differences are unknown, but the type of dietary fat seems to play a role. These findings may have important pathogenetic and therapeutic implications in both liver and metabolic disease. (HEPATOLOGY 2005;42:1175-1183.)

Nonalcoholic steatohepatitis (NASH) is a chronic liver disease encountered in individuals without significant alcohol consumption; it is part of a spectrum of liver damage, ranging from simple steatosis to advanced fibrosis and cirrhosis, named nonalcoholic fatty liver disease (NAFLD). NAFLD is the most common chronic liver disease, with a prevalence of 20% in Western populations,[1] and is present from milder stages of the metabolic syndrome until overt hyperglycemia and/or obesity appear.[2]

Adipokines have been implicated in the pathogenesis of type 2 diabetes mellitus and NAFLD, through their metabolic and pro-/anti-inflammatory activity.[3,4] Leptin promotes insulin resistance and hepatic disease in cell cultures and animal models through activation of the transforming growth factor beta axis and stellate cells.[5] Clinical studies yielded controversial results in NAFLD, finding either a link between leptin and steatosis[6] or no direct association between leptin and liver disease.[7,8] Adiponectin and tumor necrosis factor alpha (TNF-α) have been recently related to hepatic steatosis and inflammation, but not to fibrosis, in NASH.[4]

Resistin is another adipose tissue–derived cytokine linking adiposity and insulin resistance in animal models, but data from subjects with NAFLD are lacking.[9,10]

Impaired postprandial triglyceride (Tg) metabolism is a marker of increased cardiovascular risk[11] and may promote hepatic steatosis via an increased uptake of Tg-rich lipoproteins and free fatty acids (FFA) from the blood

Abbreviations: NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; FFA, free fatty acids; TNF-α, tumor necrosis factor alpha; Tg, triglyceride; HDL, high-density lipoprotein; BMI, body mass index; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; VLDL, very low density lipoproteins; AUC, area under the postprandial curve; IAUC, incremental area under the postprandial curve; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids.

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and/or a reduced hepatic secretion of apoB100-containing lipoproteins.[12]

Adiponectin has been recently linked to lipid metabolism, being able to predict fasting Tg and high-density lipoprotein (HDL) cholesterol levels independently of insulin sensitivity and visceral obesity,[13] but data on its relation with Tg and FFA metabolism in the postprandial phase are sparse.

Aims of this study were to assess the association of four adipose tissue–derived cytokines (*i.e.*, adiponectin, TNF-α, leptin, and resistin) with the severity of liver disease and to provide a dynamic assessment of the relations between circulating adiponectin and lipid metabolism in non-obese non-diabetic patients with biopsy-proven NASH.

Patients and Methods

Patient Selection. Twenty-five patients (mean age ± SEM, 37 ± 2 years; body mass index [BMI], 25.3 ± 0.2 kg/m^2) attending our Liver Unit were selected according to the following criteria: persistently (at least 12 months) elevated aminotransferases; ultrasonographic presence of bright liver without any other liver or biliary tract disease; liver histology compatible with a diagnosis of NASH (steatosis involving al least 5% of hepatocytes, presence of lobular inflammation and zone 3 ballooning degeneration[14]). Exclusion criteria were: a history of alcohol consumption > 40 g/wk , as assessed by a detailed interview extended to family members and general practitioners and by a validated questionnaire filled in daily for 1 week by the patients; a BMI ≥30 kg/m^2 for men and ≥28 for women; positive serum markers of viral, autoimmune, or celiac disease; abnormal copper metabolism or thyroid function tests; a diagnosis of overt diabetes mellitus (fasting plasma glucose ≥126 mg/dL or ≥200 mg/dL at +2 hours on a standard oral glucose load, OGTT); serum total cholesterol ≥200 mg/dL; serum triglycerides ≥200 mg/dL; exposure to occupational hepatotoxins or drugs known to be steatogenic or to affect glucose metabolism. Mutations in the hemochromatosis genes HFE and TRF2 were detected in patients and controls using multiplex amplification reaction (Nuclear Laser Medicine, Milan, Italy).

Liver iron concentration was determined on 2 mg dry weight tissue by atomic absorption spectroscopy. The hepatic iron index was obtained dividing liver iron concentration (μmol/g) by age (years; normal range below 0.5).

The controls consisted of 25 healthy subjects matched for age, sex, BMI, percent body fat, waist circumference, and waist-to-hip ratio with normal liver enzymes and abdomen ultrasound scan (Table 1).

Table 1. Baseline Characteristics of Patients With NASH and Controls

	NASH (n = 25)	Controls (n = 25)	P
Age (yr)	37 ± 2	38 ± 2	.725
Sex (M/F)	23/2	23/2	.999
Family history of type 2 diabetes (no. patients)	3	2	.760
Smokers (no. patients)	3	4	.999
BMI (kg/m^2)	25.3 ± 0.2	25.2 ± 0.6	.884
Body fat (%)	23 ± 2	22 ± 4	.824
Waist (cm)	90 ± 2	87 ± 2	.294
Waist/hips ratio	0.92 ± 0.01	0.91 ± 0.05	.865
Abdominal visceral fat area (cm^2)	90 ± 5	84 ± 6	.345
Systolic blood pressure (mm Hg)	129 ± 2	126 ± 3	.784
Diastolic blood pressure (mmHg)	88 ± 1	77 ± 1	.0002
Triglycerides (mg/dL)*	97 ± 9	78 ± 6	.144
Total cholesterol (mg/dL)†	177 ± 10	169 ± 6	.471
HDL cholesterol (mg/dL)†	48 ± 2	58 ± 3	.018
LDL cholesterol (mg/dL)†	110 ± 10	102 ± 5	.454
FFA (mmol/L)	0.66 ± 0.04	0.46 ± 0.03	.006
Uric acid (mg/dL)‡	6.19 ± 0.29	5.19 ± 0.26	.022
Glucose (mg/dL)	97 ± 2	86 ± 2	.019
Insulin (μU/mL)	19.6 ± 2.1	7.3 ± 1.1	.001
Albumin (g/dL)	4.7 ± 0.1	5.0 ± 0.1	.312
AST (U/L)	41 ± 3	26 ± 3	.021
ALT (U/L)	87 ± 7	30 ± 4	.0001
GGT (U/L)	89 ± 18	41 ± 4	.006
ALP (U/L)	86 ± 8	52 ± 7	.011
HFE mutation (H63D) heterozygotes (no. subjects)	4	3	.367
Serum iron (μg/dL)	99 ± 4	91 ± 4	.346
Ferritin (μg/L)	168 ± 21	135 ± 20	.227
Transferrin (% sat)	32 ± 1	29 ± 3	.131

NOTE. Data are presented as mean ± SEM.

Abbreviations: LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; ALP, alkaline phosphatase.

*To convert mg/dL to mmol/L, multiply by 0.01129.

†To convert mg/dL to mmol/L, multiply by 0.02586.

‡To convert mg/dL to μmol/L, multiply by 59.48.

Patients and controls gave their consent to the study, which was conducted according to the Helsinki Declaration.

Alimentary Record. Patients and controls were instructed to fill in a 7-day diet record during a 30-minute individual session with a trained nutritionist; a list of foods was designed, and for each item different portion sizes were specified according to the EPIC study.[15] The recorded period included a complete week, and the record was collected within 1 week of the tolerance tests. The diet record was analyzed using the WinFood database (Medimatica, TETeramo, Italy) according to the table of food consumption of the Italian National Institute of Nutrition[16] and Food Composition Database for Epidemiological Study in Italy.[17]

Anthropometric Measurements. Percent body fat was determined by the bioelectric impedance method

(TBF-202, Tanita, Tokyo, Japan), whose measures closely correlate with those obtained by dual X ray absorption.[18]

Abdominal visceral fat area (cm^2) was estimated using the equations developed by Stanforth et al., validated against computed tomography in Caucasians[19]

Cytokine Measurements. Serum TNF-α, leptin, and adiponectin (both low- and high-molecular-weight forms) were measured by sandwich ELISA (R&D System Europe Ltd, Abingdon, UK). Resistin was measured by a biotin-labeled antibody–based sandwich enzyme immunoassay (Bio Vendor Laboratori Medicine, Inc., Brno, Czech Republic).

Oral Glucose Tolerance Test. After completion of the alimentary record, patients and controls underwent a standard 75-g OGTT; samples for plasma glucose and serum insulin were drawn at +0, +30, +60,+90, and +120 minutes. The whole-body and hepatic insulin sensitivity indices (ISI) were assessed from the OGTT, as previously described.[20]

Oral Fat Load. Patients and controls underwent an oral fat load test, as previously described,[21] within 7 days of the OGTT. Participants were encouraged to avoid strenuous physical efforts and to follow their usual diet during the 24 hours preceding the test. The fat load consisted of a mixture of 200 g dairy cream (35% fat) and 26 g egg yolk for a total energy content of 766 kcal.: 78.3 g fat (55.6% saturated fatty acids, 29.6% monounsaturated fatty acids, 14.8% polyunsaturated fatty acids), 595 mg cholesterol, 8.8 g protein, 7 g carbohydrate. The fat load was consumed during a period of 5 minutes; subjects kept fasting on the test morning, and strenuous activity was forbidden, because exercise can reduce postprandial lipemia. Samples were drawn at 0 (baseline), 2, 4, 6, 8, and 10 hours. Plasma total cholesterol (Chol), Tg, and FFA were measured by automated enzymatic methods. Very-low-density lipoproteins (VLDL) were isolated through preparative ultracentrifugation and assayed for their Tg and total Chol content. Apo E genotype was determined by polymerase chain reaction amplification of genomic DNA using specific oligonucleotide primers.

Statistical Analysis. Data were expressed as mean ± SEM. Differences were considered statistically significant at P less than .05. Differences between groups were analyzed by ANOVA when variables were normally distributed; otherwise, the Mann-Whitney test was used. Normality was evaluated by Shapiro-Wilk test. Chi-square test or Fisher's exact test were used to compare categorical variables.

Data from the oral fat load and from the OGTT were compared by ANOVA and Scheffè *post hoc* test after logarithmic normalization of skewed variables. Differences in mean adiponectin values between the different patient subgroups of the metabolic syndrome were analyzed by ANOVA followed by Student-Neuman-Keuls test.

The area under the curve (AUC) and incremental area under the curve AUC (IAUC; computed on the area exceeding baseline) of plasma total Tg, VLDL-Tg, FFA, and adiponectin during the oral fat load were computed by the trapezoid method. Simple and multiple regression analyses were used to estimate linear relationship between different clinical, dietary, and biochemical continuos variables, after log transformation of skewed data.

Multiple logistic regression analysis was used to identify independent predictors for advanced necroinflammation (grade 3) or fibrosis (stage 3). The covariates for these analyses were age, BMI, waist circumference, waist-to-hip ratio, fasting insulin and cytokine levels, and ISI.

Results

Baseline Parameters. Baseline features of NASH patients and controls are reported in Table 1. Patients had higher mean diastolic pressure, fasting plasma glucose, FFA, and serum insulin and lower HDL-cholesterol than controls.

According to ATP III criteria,[22] eighteen patients had hypertension (systolic/diastolic blood pressure ≥130/85 mm Hg), four were hypertriglyceridemic (fasting plasma triglycerides ≥150 mg/dL), seven had low plasma HDL cholesterol (HDL-C <40 mg/dL in men and <50 mg/dL in women), eight had impaired glucose regulation (two had impaired fasting glycemia, that is, fasting plasma glucose ≥100 mg/dL but <126 mg/dL, and six had impaired glucose tolerance, that is, plasma glucose ≥140 but <200 mg/dL at +2 hours on OGTT), and three had abdominal obesity (waist circumference >102 cm in men and >88 cm in women). Seven patients had the whole picture of the metabolic syndrome (three or more criteria met), eleven met two criteria, and seven met only one criterion.

Cytokines. Serum adiponectin levels were significantly lower in patients with NASH than in controls, whereas no difference was found in levels of the other cytokines (Table 2).

Histopathology. Fatty infiltration was mild (involving 5%-33% of hepatocytes) in eight patients, moderate (33%-66% of hepatocytes) in nine subjects, and severe (>66% of hepatocytes involved) in eight patients. Necroinflammatory activity was grade 1 in nine patients, grade 2 in nine, and grade 3 in seven. Fibrosis was stage 0 in seven patients, 1 in five patients, 2 in five patients, and 3 in eight patients; changes in cirrhosis were absent in our

Table 2. Circulating Cytokine Values in Patients With NASH and Controls

	NASH (n = 25)	Controls (n = 25)	P
TNF-α (pg/mL)	1.18 ± 0.11	0.99 ± 0.04	.111
Adiponectin (ng/mL)	5,476 ± 344	11,548 ± 836	.00001
Leptin (pg/mL)	2,108 ± 789	2,518 ± 678	.695
Resistin (ng/mL)	4.12 ± 0.37	4.28 ± 0.21	.709

NOTE. Data are presented as mean ± SEM.

patients. Liver iron concentration was 17 ± 3 μmol/g dry weight, and hepatic iron index was 0.51 ± 0.04.

Alimentary Record. Daily energy and macronutrient intake did not differ in the 2 groups: 2,582 ± 123 versus 2,498 ± 168 kcal, $P = .888$; carbohydrate, 52% ± 2% vs. 50% ± 2% kcal, $P = .591$; protein, 15% ± 1% versus 16% ± 2 % kcal, $P = .716$; fat, 33% ± 1% versus 34% ± 1% kcal, $P = .482$. Compared with controls, patients consumed a diet richer in saturated and poorer in polyunsaturated fat, expressed both as percentage of total calories and percentage of total fat intake[21]: saturated fatty acids (SFA), 12.1% ± 0.6% versus 10.0% ± 0.6% tot kcal, $P = .009$; polyunsaturated fatty acids (PUFA), 3.9% ± 0.2% versus 5.1% ± 0.2% tot kcal, $P = .002$. Polyunsaturated to saturated fat ratio was also significantly lower in the NASH group (P/S ratio: 0.31 ± 0.03 vs. 0.44 ± 0.04, $P = .0001$),as well as daily intake of vitamin E (5.0 ± 0.4 vs. 7.2 ± 0.5 mg, $P = .0007$)

Oral Glucose Tolerance Test. No patient had diabetes, six had impaired glucose tolerance, two had impaired fasting glycemia, and the others had a normal glucose regulation (fasting plasma glucose <100 mg/dL).

The whole body and hepatic ISI were significantly lower in the NASH group than in controls: whole body ISI: 3.40 ± 1.83 versus 7.81 ± 0.87; $P = .0003$; hepatic ISI: 0.37 ± 0.25 versus 1.31 ± 0.18; $P = .0001$.

Oral Fat Tolerance Test. No significant difference was seen in Apo E allelic frequency: 13 patients with NASH and 11 controls were E3/E3, 8 patients with NASH and nine controls were E4/E3, and four patients with NASH and five controls were E3/E2.

Postprandial plasma total Tg and VLDL-Tg responses are reported in Fig. 1. Plasma total and VLDL Tg were significantly higher in the group with NASH at all times except at 0 hours ($P < .05$ at +2 and +8 hours; $P < .005$ at +4, +6, and +10 hours).

Plasma AUC-Tg and IAUC-Tg were significantly higher in patients with NASH compared with controls (AUC-Tg: 1,281 ± 127 vs. 904 ± 57 mg/dL × hour, $P = .01$; IAUC-Tg: 368 ± 66 vs. 130 ± 53 mg/dL × hour; $P = .004$). VLDL-Tg AUC was higher in patients with NASH than in the controls (1,046 ± 101 vs. 701 ± 94 mg/mL × hour; $P = .01$), as well as VLDL-Tg IAUC (464 ± 65 vs. 237 ± 40 mg/mL × hr; $P = .004$).

Postprandial FFA response is reported in Fig. 1C: plasma FFA levels were higher in NASH than in controls

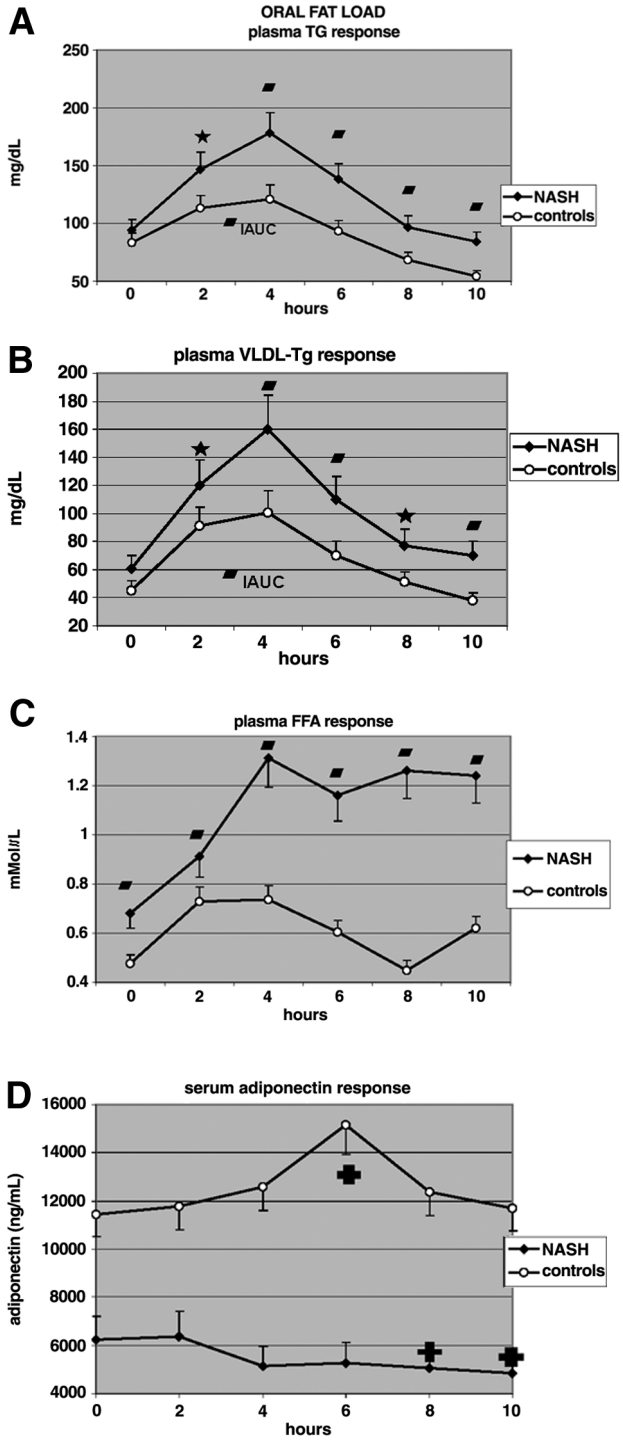

Fig. 1. Oral fat load test. Data are presented as mean ± SEM. Star, $P < .05$ vs. controls. Rhombus, $P < .005$ vs. controls. Plus sign, $P < .05$ vs basal values.

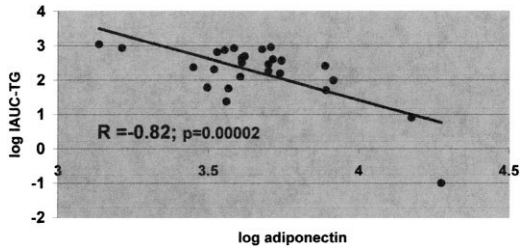

Fig. 2. Correlation between postprandial IAUC-Tg and fasting adiponectin levels (log transformed; r: Pearson correlation coefficient).

at all times ($P < .001$); AUC-FFA was higher in NASH than in controls (11.2 ± 1.5 vs. 6.4 ± 0.3 mmol/L × hour; $P = .003$) as well as IAUC-FFA (4.3 ± 0.9 vs. 1.7 ± 0.4 mmol/L × hour; $P = .006$).

Postprandial adiponectin response is reported in Fig. 1D: in NASH, adiponectin levels were lower than in controls all through the test ($P < .00001$ at all times) and slightly decreased postprandially, reaching statistical significance compared with baseline at +8 hours and +10 hours (4,842 ± 340 vs. 6223 ± 479 ng/mL, $P = .023$), whereas in controls they rose postprandially, reaching statistical significance compared with baseline at +6 hours (15,149 ± 1091 vs. 11,432 ± 948 ng/mL, $P = .014$).

IAUC-adiponectin was also significantly lower in NASH compared with controls: −6,493 ± 2,998 vs. 12,547 ± 5,388 ng/mL × hour; $P = .002$.

Plasma glucose and serum insulin levels did not change through the test (not shown)

Correlations Between Anthropometric, Metabolic, and Histological Parameters. ISI correlated with waist circumference ($r = -0.43$; $P = .010$), serum adiponectin ($r = 0.64$; $P = .00003$), TNF-α ($r = -0.56$; $P = .0005$) and SFA intake, expressed both as percent energy ($r = -0.50$; $P = .003$) and as percent fat intake ($r = -0.49$; $P = .004$) On multiple regression analysis, only SFA intake expressed as percent energy ($F = 10$; $\beta = -0.54$; $P = .0003$) and adiponectin levels ($\beta = 0.43$; $P = .002$) independently predicted ISI.

Hepatic ISI correlated with waist circumference ($r = -0.45$; $P = .007$), serum adiponectin ($r = 0.49$; $P = .003$), serum TNF-α ($r = -0.42$; $P = .011$), plasma triglyceride ($r = -0.44$; $P = .008$), and HDL-cholesterol ($r = 0.40$; $P = .016$). On multiple regression analysis, only waist circumference ($F = 7$; $\beta = -0.53$; $P = .002$) and serum adiponectin levels ($\beta = 0.40$; $P = .01$) predicted hepatic ISI.

IAUC-Tg correlated with basal adiponectin levels ($r = -0.82$; $P = .00002$; Fig 2), with IAUC-adiponectin ($r = -0.62$; $P = .001$), with IAUC-FFA ($r = 0.49$; $P = .003$), and with HDL-cholesterol ($r = -0.49$; $P = .003$). On multiple regression analysis, only basal adiponectin ($F = 20$; $\beta = -0.78$; $P = .00003$) and IAUC-adiponectin ($\beta = -0.40$; $P = .037$) independently predicted IAUC-Tg.

IAUC-VLDL Tg correlated with basal adiponectin ($r = -0.71$; $P = .0008$), with IAUC-adiponectin ($r = -0.60$; $P = .002$) and with HDL-cholesterol ($r = -0.61$; $P = .0018$). On multiple regression analysis, basal adiponectin ($F = 18$; $\beta = -0.68$; $P = .0001$) and IAUC-adiponectin ($\beta = -0.40$; $P = .009$) independently predicted log IAUC-VLDL Tg.

IAUC-FFA correlated with IAUC-adiponectin ($r = -0.58$; $P = .002$), insulin sensitivity index ($r = -0.40$; $P = .020$) and IAUC-Tg ($r = 0.49$; $P = .003$). On multiple regression analysis, only IAUC-adiponectin ($F = 4.43$; $\beta = -0.44$; $P = .041$) and IAUC-Tg ($\beta = 0.33$; $P = .040$) predicted IAUC-FFA.

Fasting adiponectin correlated with whole body and hepatic ISI (see earlier discussion), with IAUC-Tg (see earlier discussion), with PUFA intake expressed as percent energy ($r = 0.60$; $P = .001$) , with P/S ratio ($r = 0.42$; $P = .037$), with HDL-C ($r = 0.42$; $P = .037$), and with fasting insulin ($r = -0.47$; $P = .017$). On multiple regression analysis (adiponectin as the outcome variable), only IAUC-Tg ($F = 10.4$; $\beta = -0.70$; $P = .0007$) and PUFA intake ($\beta = 0.40$; $P = .041$) independently predicted fasting adiponectin levels.

In both groups (only NASH correlation coefficients reported), IAUC-adiponectin correlated with IAUC-Tg ($r = -0.58$; $P = .005$), IAUC-FFA ($r = -0.46$; $P = .030$), ISI ($r = 0.47$; $P = .029$), and hepatic ISI ($r = 0.48$; $P = .026$). On multiple regression analysis, only IAUC-Tg predicted postprandial adiponectin increase in both patients and controls (in NASH, $F = 5$; $\beta = -0.50$; $P = .011$).

Serum leptin levels correlated with BMI ($r = 0.42$; $P = .011$) and with percent body fat ($r = 0.49$; $P = .0029$), but with no other variable.

In patients with NASH, hepatic steatosis correlated with fasting adiponectin ($r = -0.64$; $P = .0006$), IAUC-Tg ($r = 0.51$; $P = .008$), IAUC-FFA ($r = 0.61$; $P = .001$), and waist circumference ($r = 0.43$; $P = .048$). On multiple regression analysis (steatosis as the outcome variable), only fasting adiponectin ($F = 5$; $\beta = -0.55$; $P = .011$) and IAUC-FFA ($\beta = 0.51$; $P = .031$) predicted the severity of steatosis.

On logistic regression analysis, hypoadiponectinemia predicted severe necroinflammation and fibrosis stage 3 even after controlling for the effect of age, BMI, ISI, waist circumference, and the other cytokines (Tables 3, 4).

Table 3. Multiple Ordinal Regression Analysis for Factors Associated With Necroinflammatory Grade 3 in Patients With NASH

Factor	OR	95% CI	P
Age (per 6-year increase)	0.9	0.3-2.1	.69
BMI (per 2 kg/m^2 increase)	1.5	0.4-6.9	.72
Waist (per 3-cm increase)	0.8	0.3-1.9	.59
ISI (per unit increase)	1.9	0.6-5.9	.23
Adiponectin (per 1,000-ng/mL decrease)	5.0	1.1-28.1	.009

Discussion

The main contributions of this work are that it (1) demonstrates the association of low serum adiponectin levels with the severity of liver histology in biopsy-proven NASH; (2) correlates the magnitude of postprandial lipemia to circulating adiponectin and to the severity of hepatic steatosis; and (3) documents a dynamic adiponectin response to an oral fat load, which was strikingly different between healthy subjects and patients with NASH and was related to postprandial FFA response.

The role of circulating adipokines in the pathogenesis of NAFLD remains controversial,[4,6,8] because their levels are related to body fat mass and may simply reflect the association between NALFD and other risk factors for liver disease (*i.e.,* overall or central obesity or diabetes), rather than a true causal relationship. Furthermore, different cytokines may intervene as metabolic syndrome and hepatic inflammation progress: the adipokines altered in diabetes, obesity, and cirrhosis thus may not be the same that switched the whole metabolic and inflammatory cascade. We therefore selected non-obese nondiabetic patients with varying severities of metabolic syndrome and liver histology.

Previous studies correlated hypoadiponectinemia to hepatic fat content, and adiponectin delivery alleviated steatosis and LPS-induced liver injury in animal fatty liver, through modulation of TNF-α and peroxisome proliferator-activated receptor-α activity.[23]

Our study further expands the protective role of adiponectin to liver necroinflammation and fibrosis: significantly lower adiponectin levels were in fact present in advanced fibrosis, despite a similar degree of insulin resistance and visceral fat accumulation. Recently, Hui et al.[4] found that hypoadiponectinemia and insulin resistance independently predicted the severity of steatosis and necroinflammation, but not of fibrosis, in NAFLD with central adiposity distribution.[4] The discrepancy between these two studies deserves comment: unlike steatosis and necroinflammation, which can vary over a short period in relation to lifestyle changes (*i.e.,* diet, physical activity, hormonal status), progression of fibrosis may occur over a much longer period in NASH. Plasma adipokine levels fluctuate over time, depending on the metabolic milieu, as well. Cross-sectional measurements of cytokines therefore may variably correlate with the severity of hepatic fibrosis, depending on the time the measure is made. The finding that changes in severity of steatosis and inflammation run an independent course from those of fibrosis has been previously reported.[24]

Consistently with our data, adiponectin attenuated carbon tetrachloride–induced liver fibrosis in mouse models.[25] The suppressive effect of adiponectin on platelet-derived growth factor– and transforming growth factor-β1–induced proliferation and migration of cultured hepatic stellate cells provides the molecular basis for the anti-fibrotic effect of this adipokine.[25]

These observations point to hypoadiponectinemia as the specific marker of NASH and support the hypothesis that adipokines may underlie both the metabolic milieu that causes liver fat accumulation and precipitate the necroinflammatory and fibrotic responses in NASH.

In keeping with previous studies, most of our patients were males[1,2]; a likely explanation is that the physiologically lower adiponectin levels seen in men may render their liver more susceptible to harmful factors such as visceral fat–released free fatty acids, TNF-α, and possibly dietary factors. Postprandial lipid and adiponectin responses to an oral fat load were also assessed in our study. The absence of carbohydrate in the test meal was designed to prevent the significant postprandial glucose and insulin increase elicited by mixed meals used in other studies.[26-28] The ability of insulin to suppress human adiponectin gene expression[29] and the release of FFA and to enhance triglyceride catabolism may in fact affect postprandial lipid and adiponectin responses.[30]

The higher postprandial lipid response observed in our patients could play a role in liver Tg accumulation, as the magnitude of FFA increase correlated significantly with the severity of hepatic steatosis. In healthy subjects, 36% of lipoprotein lipase–generated FFA escape peripheral storage in adipose tissue and muscle and are available for liver uptake,[31] and this figure may be even higher in insulin-resistant subjects. Donnelly et al.[32] demonstrated in obese NAFLD subjects that the liver usage of different

Table 4. Multiple Ordinal Regression Analysis for Factors Associated With Fibrosis Stage 3 in Patients With NASH

Factor	OR	95% CI	P
Age (per 6-year increase)	0.5	0.1-1.9	.27
BMI (per 2-kg/m^2 increase)	4.1	0.4-40.1	.20
Waist (per 3-cm increase)	0.6	0.2-4.3	.46
ISI (per unit increase)	1.3	0.3-5.7	.75
Adiponectin (per 1,000-ng/mL decrease)	8.0	1.5-81	.003

sources (adipose tissue vs. dietary fat) of plasma FFA varies widely from fasting to feeding conditions, resulting in changes of up to 60% of the FFA used for Tg synthesis. In particular, postprandial dietary FFA spill-over contributed substantially to liver and VLDL Tg synthesis, and this contribution is even larger as dietary fat intake increases.[33]

Interestingly, basal adiponectin levels independently predicted the postprandial triglyceride increase in our patients. This finding may be explained by the ability of this adipokine to enhance Tg-rich lipoprotein catabolism and FFA oxidation. Exogenous adiponectin in fact enhanced fatty acid oxidation by activating adenosine monophosphate–activated protein kinase and to acutely reduce the postprandial FFA increase in mice.[34,35] Tg-rich lipoprotein catabolism also may be affected: hypoadiponectinemia independently predicted postheparin lipoprotein lipase activity in diabetic and nondiabetic patients, accounting for 25% of the variation in lipoprotein lipase activity in these subjects.[36] Furthermore, kinetic studies point to adiponectin as an important regulator of VLDL-apoB catabolism, independently of other adipokines or insulin resistance indices.[37]

An intriguing finding was the striking difference in adiponectin response to the fat challenge between the two groups: controls showed a prompt postprandial increase in circulating adiponectin, which rose up by 35% above basal levels, peaking at 6 hours, whereas NASH patients showed no increase but rather a slight decrease in its levels, reaching statistical significance at +8 and +10 hours (−25% compared with basal values). Consistently, postprandial IAUC-adiponectin was also significantly lower in NASH patients than in controls.

In both groups, postprandial adiponectin response correlated inversely with the magnitude of postprandial FFA increase.

Although further studies are required to elucidate the meaning of this observation, postprandial lipemia physiologically evokes an acute "compensatory" increase in adiponectin secretion aiming at restoring baseline plasma lipid levels by enhancing FFA oxidation and Tg-rich lipoprotein catabolism in target tissues. Consistently with this model, intravenous infusion of lipid emulsions was associated with a small but significant increase in adiponectin levels at 6 hours and, conversely, pharmacological lowering of circulating FFA levels conveyed a significant decrease in adiponectin levels in healthy subjects.[38,39]

The failure of this compensatory mechanism in NASH may contribute substantially to liver steatosis and to hepatic and peripheral insulin resistance.

Alternatively, postprandial lipid metabolism may be primarily impaired in NASH and promote Tg storing and oxidative stress (which is increased in our patients; unpublished data) in hepatocytes and visceral adipocytes, eventually leading to hepatic steatosis and impaired adiponectin secretion.[40,41] If this were the case, the reduction of postprandial lipemia and of oxidative stress would be a primary therapeutic target in patients with NASH, even in the absence of overt obesity or diabetes.

Our findings agree with part, but not all, of the literature. Peake et al.[26] found no difference in adiponectin response to a mixed meal between normal and insulin-resistant subjects over a 6-hour period; however, basal adiponectin values were similar in the two groups, and the mixed meal elicited a significant insulin rise.[26] Vuppalanchi et al.[27] found no difference in adiponectin response to a mixed meal between NASH patients and controls after 3 hours. It is possible that the abundance of adiponectin in blood, as compared with any other adipokines, and its long half-life (2.5-6 hours) may have masked any acute regulatory action of lipid load over the short term in previous studies.

By contrast, English et al.[28] found a 4-fold postprandial increase in adiponectin levels after a mixed meal in obese subjects, with an early steep rise at 1 hour and a plateau lasting 1 hour.

The mechanism(s) responsible for hypoadiponectinemia and for the lack of the physiological increase in NASH are unknown. A genetic background may be involved: in a recent French prospective study, variations at the adiponectin locus affected body weight gain, body fat distribution, and onset of hyperglycemia, as well as adiponectin levels, over a 3-year period.[3]

Among acquired factors, chronic hyperinsulinemia and diet may play a role. In hyperinsulinemic euglycemic clamp studies, in fact, insulin infusion provoked a prolonged inhibition on adiponectin gene expression and circulating levels in lean and obese subjects.[42,43]

Our patients consumed a diet richer in saturated and poorer in polyunsaturated fat compared with controls, and PUFA intake independently predicted fasting adiponectin levels. Available data show that the amount and type of dietary fat regulate adiponectin secretion by acting on nuclear sterol regulatory element-binding protein 1c[44] : a 3-day low-fat diet increased circulating adiponectin without affecting insulin levels or insulin sensitivity in diabetic patients.[45] The addition of different amounts of PUFA increased adipose tissue mRNA and plasma adiponectin and decreased liver lipogenic enzymes and fat content in different mouse models.[46,47]

In healthy humans, dietary saturated fat intake correlated negatively with circulating adiponectin, whereas

omega-3 fatty acids were positively associated with this adipokine.[48]

In conclusion, we confirmed that hypoadiponectinemia is an early finding in NASH, being detectable long before overt diabetes and overall or central obesity appear, and which correlates with the severity of liver histology. We provided evidence of an additional mechanism potentially linking adiponectin and NASH, postprandial lipid metabolism, and found a strikingly different postprandial adiponectin response between our patients and healthy controls, which may promote liver fat accumulation and insulin resistance in NASH.

Our data should prompt future research to focus on lipid metabolism in addition to insulin resistance, given the implications for both liver and cardiovascular disease in these subjects. This study has obvious limitations related to its cross-sectional nature, which prevents any causal inference from the associations we found, and to the prevalence of male sex in our patients, which suggests caution when extending our results to women.

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