Adiponectin gene polymorphism modulate acute adiponectin response to dietary fat: possible pathogenetic role in NASH

Adiponectin Gene Polymorphisms Modulate Acute Adiponectin Response to Dietary Fat: Possible Pathogenetic Role in NASH

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Factors underlying the independent association of nonalcoholic steatohepatitis (NASH) with increased cardiovascular risk are unknown. Adiponectin polymorphisms predict cardiometabolic risk in the general population. This association is not always mediated by low fasting adiponectin levels, adipose tissue accumulation, or traditional risk factors. Adiponectin modulates lipid metabolism and liver injury in nonalcoholic fatty liver disease (NAFLD) even in the absence of obesity, dyslipidemia, and diabetes. We hypothesized adiponectin polymorphisms may predispose to NAFLD and may increase cardiovascular risk by modulating circulating lipoprotein and adiponectin response postprandially. The prevalence of adiponectin single-nucleotide polymorphisms (SNPs) 45GT and 276GT was assessed in 70 nonobese, nondiabetic, normolipidemic NAFLD patients and 70 healthy matched controls; the impact of the adiponectin SNPs was subsequently correlated to liver histology and postprandial adiponectin and lipoprotein responses to oral fat load in a subgroup of 30 biopsy-proven patients with NASH and 30 controls. The 45TT and 276GT/TT genotypes were more prevalent in NAFLD patients than in controls and independently predicted the severity of liver disease in NASH. In both patients and controls, these genotypes exhibited a blunted postprandial adiponectin response and higher postprandial triglycerides (Tg), free fatty acids (FFA), oxidized LDL (oxLDL), and VLDL levels than their counterparts, despite comparable fasting adipokines, lipids, dietary habits, adiposity, and insulin resistance. They were also independently associated, together with dietary polyunsaturated fatty acid intake, with postprandial adiponectin response. IAUC adiponectin independently predicted postprandial Tg, FFA, oxLDL, and intestinal and hepatic VLDL subfraction responses in NASH. *Conclusion:* The at-risk adiponectin SNPs 45TT and 276GT are significantly more prevalent in NAFLD than in the general population; they are associated with severity of liver disease, with blunted postprandial adiponectin response, and with an atherogenic postprandial lipoprotein profile in NASH independently of fasting adipokine and lipid levels. (HEPATOLOGY **2008;47:1167-1177.**)

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the Western population, encompassing a spectrum of histological injury ranging from pure benign steatosis to progressive necroinflammation (nonalcoholic steatohepatitis, NASH) and fibrosis. NASH predicts incident cardiovascular disease independently of classical risk factors, insulin resistance, and metabolic syndrome, but mechanisms underlying this association are unclear, nor is it known which subjects with NASH are at greater cardiovascular risk and should therefore be more aggressively treated.[1,2] Altered adipokine action and increased oxidative stress are candidate patogenetic mechanisms in NAFLD. Among the different adipokines, low adiponectin levels predict severity of liver disease in NAFLD, even in the absence of diabetes and obesity.[3,4]

A genetic predisposition is indisputably present in NAFLD, and several candidate genes affecting glucose and lipid metabolism have been proposed.[3,5]

Two common single-nucleotide polymorphisms (SNPs) in exon/intron 2 of the adiponectin gene (45TG

Abbreviations: Apo, apolipoprotein; FFA, free fatty acids; IAUC, incremental area under postprandial curve; ISI, insulin sensitivity index; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; oxLDL, oxidized LDL; PUFAs, polyunsaturated fatty acids; SNP, single-nucleotide polymorphism; Tg, triglycerides; TRLP, triglyceride-rich lipoprotein.

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and 276GT) have been associated with cardiometabolic risk, with haplotypes 45TT and 276GT/TT carrying a higher risk of type 2 diabetes and cardiovascular disease in cross-sectional and prospective studies.[6-10] Furthermore, the same polymorphisms also predicted the thiazolidinedione response independently of basal fasting adiponectin levels.[11] Mechanisms linking adiponectin SNPs to cardiometabolic risk are unclear because these polymorphisms do not always associate with fasting adiponectin level, overall/abdominal obesity, or other traditional risk factors. Adiponectin enhances oxidation of free fatty acid (FFA) and triglyceride-rich lipoprotein (TRLP) metabolism independently of systemic inflammation or insulin action.[12]

Exaggerated postprandial lipemia is an established cardiovascular risk factor in diabetes.[13] The liver takes up circulating FFAs in a dose-dependent fashion and LDL and remnants through the LDL-receptor and the liver-related receptor protein (LRRP). Consistently, postprandial lipid storage contributes substantially to liver triglyceride (Tg) pool in NAFLD[14] and the magnitude of postprandial lipemia correlated with liver steatosis.[4,14,15]

The impact of polymorphisms in the adiponectin gene on liver disease in NASH, as well as on the postprandial adiponectin response and lipoprotein metabolism in health and disease, has not been previously investigated.

Hypothesizing that the association of adiponectin SNPs with cardiometabolic risk is mediated by liver fat accumulation and that adiponectin polymorphisms modulate postprandial adiponectin response and lipoprotein metabolism, we evaluated (1) the association of 2 common polymorphisms in the adiponectin gene with the risk of NAFLD and (2) the impact of adiponectin SNPs on the severity of liver disease and on postprandial adiponectin response and lipoprotein metabolism in nonobese, nondiabetic, normolipidemic patients with NASH.

Patients and Methods

Patient Selection. Because there are no data on NAFLD, we based our study on large cohort studies of Italian subjects.[7,9] Considering a type I error of 0.05 and a type II error of 0.20, at least 65 subjects with NAFLD and 65 healthy controls were needed to detect a significant between-group difference in the prevalence of the 45TG and 276GT adiponectin gene polymorphisms and in circulating adipokines.

NAFLD was defined as persistently (at least 6 months) elevated aminotransferases, the ultrasonographic presence of bright liver without any other liver or biliary tract disease, with a daily alcohol consumption $<$ 20 g/day in men and $<$ 10 g/day in women,[3] as assessed by a detailed inquiry of patients and relatives and a validated questionnaire filled in daily for 1 week by the patients. Exclusion criteria for other causes of fatty liver were: positive markers of viral, autoimmune, or celiac disease; abnormal copper metabolism, serum α_1-antitrypsin level, or thyroid function tests; and exposure to occupational hepatotoxins or to drugs known to be steatogenic or to affect glucose/lipid metabolism. Because we aimed at identifying early mechanisms predisposing to the future development of cardiometabolic disease and different adipokines may intervene at different stages of the development of diabetes and obesity, obese and diabetic subjects were excluded from our study.

Mutations in the hemochromatosis *HFE* and *TRF2* genes were detected in patients and controls using a single multiplex amplification reaction and premade, ready-to-use test strips (Nuclear Laser Medicine, Milan, Italy). The −493 G/T microsomal triglyceride transfer protein (*MTP*) gene polymorphism was assessed by PCR-RFLP using 2-step nested PCR. The adiponectin +45T/G and +276G/T polymorphisms were genotyped by amplification of genomic DNA. The PCR product was digested at 37°C overnight with SmaI or BsmI restriction enzyme, respectively, and the digestion products were resolved by electrophoresis in a 2% agarose gel.

The mean ± SE fibrosis score[16] of the NAFLD group was 0.64 ± 0.21. To ensure biopsying subjects with the progressive form of NAFLD, that is, NASH, a liver biopsy was proposed to patients with a high ($>$0.676) or indeterminate (−1.455-0.676) NAFLD fibrosis score. Thirty-four patients fell within these categories, and 30 accepted undergoing a liver biopsy. All 30 patients had a histological diagnosis of NASH, as proposed by Brunt.[17] Liver iron concentration (LIC) and hepatic iron index (HII) were determined from 2 mg of tissue dry weight by atomic absorption spectroscopy.

To further rule out subclinical liver disease in controls, in addition to a negligible alcohol intake ($<$20 g/day in men and $<$10 g/day in women) and normal abdomen ultrasound, the upper healthy limit for ALT level was lowered to $<$30 U/L for men and $<$20 U/L for women in order to increase the negative predictive value of a normal result.[18,19]

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

Alimentary Record. Patients and controls filled in daily a dietary record for 1 week, according to the EPIC protocol, analyzed using the WinFood database (Medimatica,–TE, Teramo, Italy) as previously described.[4]

Anthropometry. Percent body fat was estimated by

the BIA method (TBF-202, Tanita, Tokyo, Japan), closely correlating with dual X-ray absorption measures.[20]

Cytokines. Serum tumor necrosis factor–α, leptin, and adiponectin were measured by sandwich ELISA (R&D System Europe Ltd., Abingdon, UK). Resistin was measured by an enzyme immunoassay (Bio Vendor laboratory Medicine Inc., Brno, Czech Republic).

Oral Glucose Tolerance Test. After completion of the alimentary record, patients and controls underwent a standard 75-g oral glucose tolerance test. Then whole body insulin sensitivity index (ISI) was calculated.[21]

Oral Fat Load. The 30 biopsied patients and 30 controls comparable in age, body mass index (BMI), abdominal waist circumference, ISI, insulin level, and dietary habits underwent a 10-hour oral fat load test, as described.[4] Plasma total cholesterol (Chol), triglycerides (Tg), and FFAs were measured by automated enzymatic methods. The apolipoprotein E (*ApoE*) genotype was determined by PCR amplification of genomic DNA using specific oligonucleotide primers.

Measurement of TRLP Subfractions and Oxidized LDL during Oral Fat Load Test. VLDL was isolated through preparative ultracentrifugation and their Tg and total Chol content were subsequently measured. One aliquot of plasma was brought to densities of 1,006 g/L by adding a KBr solution (d = 1330 g/L) and centrifuged at 105,000*g* for 21 hours at 10°C in a Beckman L8-70M ultracentrifuge. Tg and total Chol concentration were then determined in the lipoprotein fraction enzymatically. The first, larger amount of blood (10 mL) was drawn for subfractionating TRLP by ultracentrifugation on a discontinuous density gradient. The sample was ultracentrifuged in a Beckman L8-70M centrifuge at 20°C in stages, allowing the separation of 2 VLDL fractions with decreasing Sf values: VLDL 1, Sf > 100; VLDL 2, Sf = 20-100. Chol and Tg content were measured in the 2 fractions.

ApoB48 and ApoB100 content of TRLP subfractions were quantified by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis using 3.9% gel.[22] Nondelipidated samples were reduced in SDS buffer for 4 minutes at 96°C. Samples were applied to the gel and run at 40 mA in 0.025 M Tris, 0.192 M glycine, and 0.1% SDS. Gel was stained with Silver Stain (Bio-Rad Laboratories). Because the chromogenicity of ApoB48 is similar to that of ApoB100, a protein standard was prepared from LDL isolated by sequential ultracentrifugation and used to quantify ApoB100 and ApoB48. The bands were quantified by densitometry using Gel Doc equipment (Bio-Rad Laboratories). Density values were assigned to the ApoB100 bands of the standard LDL, and a standard curve was constructed. The values were recalculated by linear regression.

LDL-conjugated dienes, as markers of LDL lipoperoxidation, were determined by capillary electrophoresis, as previously described.[5]

Statistical Analysis. Data are expressed as means ± SEMs. Differences were considered statistically significant at $P < 0.05$.

Differences between groups were analyzed by ANOVA when variables were normally distributed; otherwise, the Mann-Whitney test was used. The Bonferroni correction was applied when significant between-group differences were detected. Normality was evaluated by the Shapiro-Wilk test. The chi-square test or Fisher's exact test was used to compare categorical variables.

Data from the oral fat load test were compared by ANOVA and the Scheffè post hoc test after log normalization of skewed variables.

The area under the curve and the incremental area under the postprandial curve (IAUC) of plasma Tg, FFAs, and conjugated dienes during the oral fat load test were computed by the trapezoid method.

Simple and multiple regression analyses were used to estimate linear relationships between different variables after log transformation of skewed data.

Logistic regression analysis was used to identify independent predictors of IAUC adiponectin. The covariates were age, sex, BMI, waist, ISI, fasting insulin, adiponectin, SNPs 45GT and 276GT, and dietary polyunsaturated fatty acid (PUFA) intake. Discrete variables were divided into classes for analysis.

A logistic regression model was used to identify independent predictors of severe (>66% hepatocytes) steatosis, necroinflammatory grade 3, or fibrosis stage 3. The covariates were age, BMI, waist, ISI, adiponectin SNP polymorphism, fasting and postprandial adiponectin, IAUC triglyceride/VLDL1 apoB48/apoB100, IAUC LDL-conjugated dienes.

Results

Patient Characteristics. The prevalence of the 45TT haplotype was 59% in the controls versus 83% in the NASH patients ($P = 0.004$), 36% of the controls were heterozygous 45GT carriers versus 15% of the NASH patients ($P = 0.008$), and 5% of the controls were homozygous GG carriers versus 2% of the NASH patients ($P = 0.676$). The prevalence of the 276GG haplotype was 54% in the controls versus 24% in NASH patients ($P = 0.001$), 42% of the controls were heterozygous 276GT carriers versus 73% of the NASH patients ($P = 0.0002$), and 4% of the controls were

Table 1. Baseline Characteristics of Controls and Patients with NAFLD Grouped According to Adiponectin SNP 45G/T

Characteristics	Controls GT/GG (n=29)	Controls TT (n=41)	*P* Controls	NASH GT/GG (n=12)	NASH TT (n=58)	*P* NAFLD
Age (years)	37 ± 2	40 ± 2	0.307	43 ± 2	38 ± 3	0.201
Sex (%M)	67	68	0.890	68	72	0.815
Smokers (%)	34	31	0.618	36	33	0.715
% body fat	20 ± 3	22 ± 3	0.606	23.5 ± 2.1	22.5 ± 0.6	0.489
B.M.I. (kg/m^2)	24.9 ± 0.8	25.0 ± 0.7	0.926	25.6 ± 0.9	25.1 ± 0.7	0.496
Waist (cm)	88 ± 3	90 ± 3	0.649	90 ± 2	91 ± 2	0.936
WHR	0.90 ± 0.02	0.91 ± 0.03	0.801	0.94 ± 0.01	0.92 ± 0.01	0.364
Systolic BP (mmHg)	122 ± 3	124 ± 2	0.711	128 ± 2	127 ± 2	0.818
Diastolic BP (mmHg)	78 ± 3	77 ± 2	0.774	88 ± 1‡§	86 ± 1‡§	0.475
Triglycerides (mg/dL)*	79 ± 6	77 ± 6	0.820	90 ± 11	101 ± 8*†	0.462
Total C (mg/dL) †	169 ± 8	174 ± 9	0.683	183 ± 13	180 ± 5	0.807
HDL-C (mg/dL) †	62 ± 1	59 ± 1	0.043	51 ± 2‡§	46 ± 1†§	0.014
LDL-C (mg/dL) †	99 ± 8	102 ± 8	0.792	113 ± 9	115 ± 6	0.867
Glucose(mg/dL)	88 ± 3	90 ± 3	0.649	94 ± 1	97 ± 1	0.334
Insulin ($\mu\mu$U/mL)	4.8 ± 1.3	4.5 ± 1.8	0.901	9.9 ± 1.6‡§	12.9 ± 1.2‡§	0.263
Whole body ISI	8.59 ± 1.03	7.92 ± 0.97	0.644	4.98 ± 0.91‡§	4.18 ± 0.46‡§	0.406
AST (U/L)	18 ± 3	22 ± 3	0.593	51 ± 5‡§	63 ± 2‡§	0.205
ALT (U/L)	19 ± 4	20 ± 3	0.891	72 ± 9‡§	94 ± 4‡§	0.023
GGT (U/L)	38 ± 6	35 ± 8	0.672	68 ± 13‡§	109 ± 7‡§	0.010
TNF-α (pg/mL)	1.28 ± 0.11	1.12 ± 0.09	0.262	1.34 ± 0.36	1.09 ± 0.11	0.256
Adiponectin (ng/mL)	12843 ± 1106	10914 ± 983	0.316	6469 ± 691‡§	5228 ± 514‡§	0.299
Leptin (pg/mL)	1819 ± 612	1783 ± 749	0.962	1680 ± 148	1594 ± 146	0.895
Resistin (ng/mL)	3.97 ± 0.83	4.29 ± 0.62	0.754	3.92 ± 0.77	4.15 ± 0.31	0.752
ApoE genotype(%)						
2-3	8	7	0.918	12	10	0.931
3-3	83	85	0.984	70	60	0.638
3-4	9	8	0.879	18	20	0.719
−493 GT MTP genotype(%) GG	48	51	0.899	51	58	0.873
GT/TT	52	49	0.911	50	42	0.694
abdominal obesity (% subjects)	7	16	0.452	20	24	0.976
IGR (% subjects)	8	18	0.285	34*†	39*†	0.855
Hypertension (% subjects)	31	39	0.444	68†	65*†	0.786
Low HDL-C (%)	16	9	0.437	16*†	31†	0.603
High Tg (% subjects)	12	8	0.989	15	18*†	0.689
subjects with MS (%)	17	21	0.891	37*†	41*†	0.946
Steatosis (% hepatocyte)§§	–	–	–	21 ± 4	43 ± 5	0.008
Necroinflammatory grade§§	–	–	–	1.3 ± 0.1	2.7 ± 0.3	0.009
Fibrosis stage§§	–	–	–	1.1 ± 0.3	2.9 ± 0.4	0.006

Data are presented as mean ± SEM. Abbreviations: BP, blood pressure; ISI, insulin sensitivity index; total C, total cholesterol; HDL–C, HDL cholesterol; LDL–C, LDL cholesterol; WHR, waist–on–hip ratio; Tg, triglyceride; MTP, microsomal triglyceride transfer protein; IGR, impaired glucose regulation; MS, metabolic syndrome.

*$P < 0.05$ vs. controls TT

†$P < 0.05$ vs. controls GT/GG

‡$P < 0.001$ vs. controls TT

§$P < 0.001$ vs. controls GT/GG

§§ in biopsied patients.

homozygous 276 TT carriers versus 3% of the NASH patients ($P = 0.999$). The genotype frequencies for both adiponectin polymorphisms were in Hardy-Weinberg equilibrium, and the allele frequencies were consistent with published reports in a white population.[7,9] Because of the low prevalence of the 45GG and 276TT genotypes and of the overlapping clinical characteristics of these subjects and heterozygous carriers, they were combined with the GT genotype in all statistical analyses.

The main features of NASH patients and controls grouped according to adiponectin SNP45TG and 276GT status are reported in Tables 1 and 2.

There were no significant differences in *ApoE* allelic frequency or −493GT *MTP* polymorphism among the 4 groups.

ISI, fasting insulin, and adiponectin were significantly lower in patients with NASH than in controls, but they did not differ by adiponectin genotype in NAFLD patients and controls (Tables 1 and 2).

Table 2. Baseline Characteristics of Controls and Patients with NAFLD Grouped According to Adiponectin SNP 276G/T

Characteristic	Controls GG (n=38)	Controls GT/TT (n=32)	*P* Controls	NASH GG (n=17)	NASH GT/TT (n=53)	*P* NAFLD
Age (years)	38 ± 2	39 ± 3	0.726	39 ± 2	41 ± 2	00.590
Sex (%M)	68	65	0.915	70	71	00.970
Smokers (%)	32	36	0.683	44	30	00.478
% body fat	21 ± 3	22 ± 2	0.792	23.4 ± 2.0	22.3 ± 2	00.767
B.M.I. (kg/m^2)	25.0 ± 0.9	24.9 ± 0.7	0.538	25.2 ± 0.4	24.6 ± 0.4	00.936
Waist (cm)	88 ± 2	89 ± 3	0.719	88 ± 2	91 ± 3	00.420
WHR	0.90 ± 0.02	0.91 ± 0.03	0.771	0.91 ± 0.02	0.93 ± 0.02	00.480
Systolic BP (mmHg)	125 ± 3	122 ± 3	0.534	125 ± 4	128 ± 2	00.475
Diastolic BP (mmHg)	76 ± 3	79 ± 3	0.485	87 ± 2‡§	87 ± 1‡§	00.999
Triglycerides (mg/dL)*	76 ± 5	80 ± 6	0.617	83 ± 10	102 ± 8*†	00.215
Total C (mg/dL)†	167 ± 9	174 ± 8	0.639	180 ± 7	181 ± 6	00.930
HDL–C (mg/dL)†	62 ± 1	58 ± 1	0.010	55 ± 2‡§	49 ± 1†§	00.006
LDL-C (mg/dL)†	104 ± 8	106 ± 6	0.712	119 ± 5	114 ± 6	00.649
Glucose(mg/dL)	86 ± 3	88 ± 3	0.699	96 ± 3	97 ± 3	00.857
Insulin (μU/mL)	4.3 ± 1.6	4.0 ± 1.3	0.598	12.2 ± 2.1‡§	12.8 ± 1.2‡§	00.805
Whole body ISI	8.19 ± 1.62	8.03 ± 0.94	0.791	4.56 ± 1.14‡§	3.89 ± 0.26‡§	00.398
AST (U/L)	16 ± 2	18 ± 3	0.568	46 ± 4‡§	59 ± 2‡§	00.342
ALT (U/L)	20 ± 4	19 ± 4	0.735	70 ± 5‡§	88 ± 4‡§	00.021
GGT (U/L)	37 ± 12	40 ± 11	0.813	68 ± 16‡§	110 ± 15‡§	00.137
TNF-α (pg/mL)	1.13 ± 0.11	1.06 ± 0.08	0.690	1.33 ± 0.23	1.07 ± 0.16	00.386
Adiponectin (ng/mL)	11850 ± 710	10591 ± 670	0.498	5905 ± 869‡§	5469 ± 552‡§	00.445
Leptin (pg/mL)	1617 ± 734	1749 ± 812	0.904	1834 ± 192	1626 ± 146	00.455
Resistin (ng/mL)	3.78 ± 0.68	4.01 ± 0.70	0.691	3.86 ± 0.33	4.68 ± 0.39	00.200
ApoE genotype(%)						
2-3	9	7	0.947	12	8	0.612
3-3	80	90	0.319	63	76	0.579
3-4						
−493 GT MTP genotype (%)	11	3	0.363	25	16	0.361
GG	40	53	0.325	64	69	0.839
GT/TT	60	47	0.386	36	31	0.996
abdominal obesity(% subjects)	9	17	0.455	25	22	00.895
IGR (% subjects)	11	17	0.987	38*†	39*†	00.838
Hypertension(% subjects)	34	37	0.976	62†	65*†	00.817
Low HDL-C (%)	6	17	0.234	13*†	35†	00.168
High Tg(% subjects)	9	10	0.898	19	18*†	00.736
subjects with MS (%)	17	23	0.553	43*†	39*†	0.953
Steatosis(% hepatocyte)§§	–	–	–	18 ± 4	45 ± 6	0.004
Necroinflammatorygrade§§	–	–	–	1.3 ± 0.1	2.8 ± 0.4	0.009
Fibrosis stage§§	–	–	–	1.2 ± 0.1	2.9 ± 0.4	0.006

Data are presented as mean ± SEM. Abbreviations: BP, blood pressure; ISI, insulin sensitivity index; total C, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; WHR, waist-on-hip ratio; Tg, triglyceride; MTP, microsomal triglyceride transfer protein; IGR, impaired glucose regulation; MS, metabolic syndrome.

*$P < 0.05$ vs. controls TT

†$P < 0.05$ vs. controls GT/GG

‡$P < 0.001$ vs. controls TT

§$P < 0.001$ vs. controls GT/GG

§§ in biopsied patients.

Plasma high-density lipoprotein–cholesterol (HDL-C) was lower in NASH patients than in controls; NASH patients with SNP 45TT and 276GT/TT had lower HDL-C levels than did those with the other genotypes (Table 1).

The prevalence of metabolic syndrome (ATP III criteria) was higher in NASH patients than in controls but did not differ by adiponectin genotype in the 2 groups (Tables 1 and 2).

To further assess the combined effects of the 2 adiponectin SNPs on baseline characteristics, carriers of the haplotype 45TT+276GT/TT were grouped together and compared with the other haplotypes. The prevalence of haplotype 45TT+276GT/TT was significantly higher in NASH patients than in controls: 64% versus 28% ($P = 0.0002$). Apart from HDL-C, there were no significant between-haplotype differences in the main anthropometric, metabolic, and dietary parameters between 45TT+276GT/TT carriers and their counterpart haplotypes in both the NASH and control groups (not shown).

Histopathology. Fatty infiltration was mild (involving 5%-33% of hepatocytes) in 33% of patients with NASH, moderate (33%-66% of hepatocytes) in 37%

of patients, and severe (>66% of hepatocytes involved) in 30% of subjects. Necroinflammatory activity was grade 1 in 30% of patients, grade 2 in 33%, and grade 3 in 37%.

Fibrosis was stage 0 in 17% of patients, stage 1 in 27% of patients, stage 2 in 27%, and stage 3 in 29%; cirrhotic changes were absent (Table 1). Liver iron concentration was 18 ± 5 μmol/g dry weight, and hepatic iron index was 0.52 ± 0.06.

The severity of liver fatty infiltration, median necroinflammatory grade, and median fibrosis stage were higher in SNP 45TT and SNP 276 GT/TT carriers than in the other genotypes (Tables 1 and 2).

Similarly, 45 TT+276GT/TT carriers with NASH had a significantly higher severity of liver fat infiltration, necroinflammation, and fibrosis than the other haplotypes (not shown). Liver iron concentration and hepatic iron index did not differ by adiponectin SNP.

Alimentary Record. Daily total energy and macronutrient intake of patients with NASH and controls were similar: total calories, 2,466 ± 99 versus 2,503 ± 118 kcal, $P = 0.785$; carbohydrate, 51% ± 2% versus 48% ± 2% kcal, $P = 0.291$; protein, 16% ± 3% versus 19% ± 3% kcal, $P = 0.481$; fat, 32% ± 3% versus 33% ± 3% kcal, $P = 0.814$.

The NASH group consumed more saturated fat and less polyunsaturated fat than did controls, when expressed as both percentage of total calories and percentage of total fat intake, as previously reported: saturated fatty acids (SFAs), 12.2% ± 0.5% versus 10.2% ± 0.4% total kcal, $P = 0.0002$; PUFA, 3.6% ± 0.2% versus 5.2% ± 0.2% total kcal, $P = 0.0001$. Polyunsaturated to saturated fat (P/S) ratio was also lower in NASH patients than in controls: 0.30 ± 0.02 versus 0.50 ± 0.03; $P = 0.0001$.

NASH patients also had a significantly lower daily intake of antioxidant vitamin A (638 ± 71 versus 1,104 ± 107 mg, $P = 0.008$), vitamin C (116 ± 14 versus 152 ± 10 mg, $P = 0.038$), and vitamin E (5.9 ± 0.6 versus 9.6 ± 0.8 mg, $P = 0.0009$).

Daily alcohol intake was similar in the 2 groups: 11 ± 2 versus 12 ± 2 g; $P = 0.782$.

There was no difference in daily antioxidant intake or in any other macro/micronutrient intake by adiponectin genotype in the NASH and control groups (not shown).

Oral Fat Tolerance Test. Plasma adiponectin significantly increased in controls, whereas it decreased in NASH patients (Fig. 1A–C).

In controls, postprandial adiponectin increase was significantly lower in those with the SNP 45TT and SNP 276 GT/TT genotypes than the other genotypes; in NASH patients, the drop in postprandial adiponectin was deeper in SNP 45TT and SNP 276GT/TT carriers than in those with the other genotypes (Fig. 1A–C).

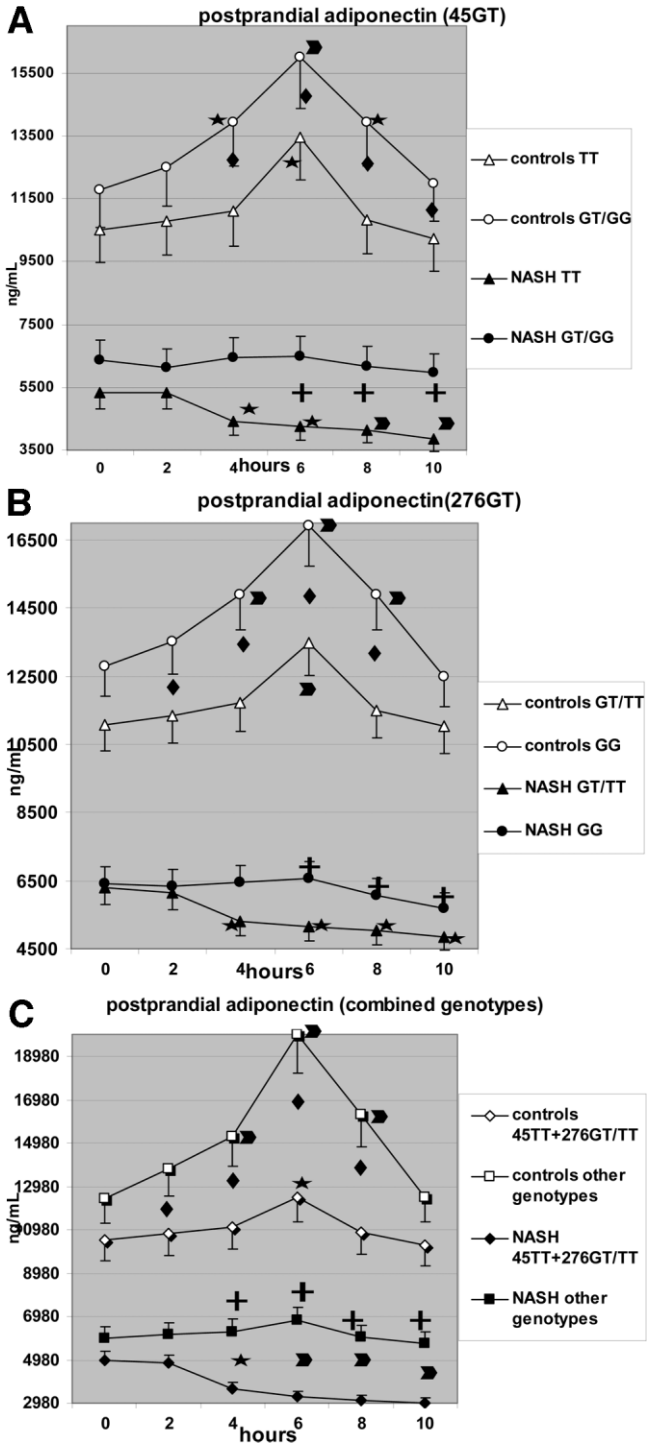

Fig. 1. Oral fat load test. Postprandial adiponectin response in patients and controls grouped according to SNP 45GT (A), SNP 276GT (B), and combined 45GT/276GT (C) genotypes. Data are presented as mean ± SEM; *$P < 0.05$ versus basal; $P < 0.01$ versus basal; ▲$P < 0.05$ versus controls GT/GG; ♦$P < 0.05$ controls GT/GG versus controls TT; $P < 0.05$ NASH GT/GG versus NASH TT.

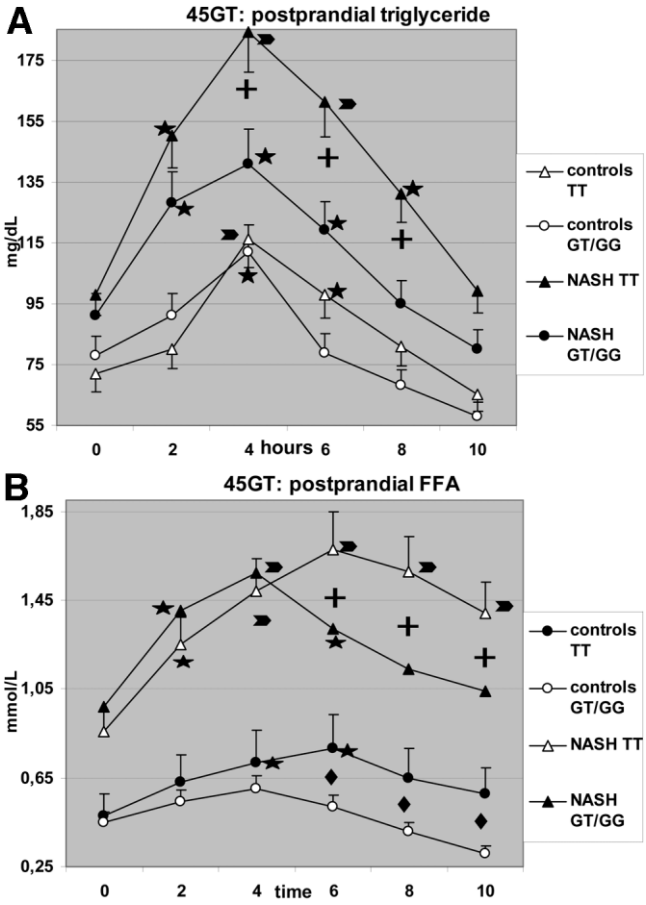

Fig. 2. Oral fat load test. Postprandial plasma Tg and FFA responses, and LDL-conjugated diene responses in patients and controls grouped according to SNP 45GT. Data are presented as mean ± SEM; $^*P < 0.05$ versus basal; $P < 0.01$ versus basal; ▲ $P < 0.05$ versus controls GT/GG; ♦ $P < 0.05$ controls GT/GG versus controls TT; $P < 0.05$ NASH GT/GG versus NASH TT.

Postprandial plasma Tg, FFAs, and LDL-conjugated diene responses were significantly higher in the NASH group than in controls. In the NASH and control groups, SNP 45TT and SNP 276GT/TT carriers displayed significantly higher postprandial Tg, FFAs, and LDL-conjugated diene responses than did those with the other genotypes (Fig. 2A-B; Table 3).

Patients with NASH had higher postprandial intestinal and hepatic VLDL1 and VLDL2 responses than did controls. Postprandial VLDL1 and VLDL2 responses of both intestinal and hepatic origin were higher in SNP 45TT and SNP 276GT/TT carriers than in their counterpart genotypes in both patient and control groups (Fig. 3A–D). The simultaneous presence of SNP 45TT and SNP 276GT/TT had an additive effect on postprandial adiponectin, lipid, and lipoprotein responses in both patients and controls (Fig. 1C; Table 3).

Correlative Analysis. The main correlations between anthropometric, metabolic, and dietary parameters in patients with NASH are shown in Online Supplemental Material Table 1.

On multiple regression analysis, only fasting ($\beta = -0.39$, $P = 0.033$), IAUC adiponectin ($\beta = -0.48$, $P = 0.008$), and IAUC VLDL1 apoB48 ($\beta = 0.51$, $P = 0.002$) predicted IAUC Tg.

On multiple regression analysis, IAUC FFA was predicted by IAUC VLDL1 apoB48 ($\beta = 0.47$, $P = 0.009$), and IAUC adiponectin ($\beta = -0.53$, $P = 0.001$) predicted IAUC FFA. IAUC LDL-conjugated diene was predicted by IAUC VLDL1 apoB48 ($\beta = 0.44$, $P = 0.02$) and IAUC adiponectin ($\beta = -0.51$, $P = 0.002$). IAUC VLDL1 apoB48 was independently predicted by fasting ($\beta = -0.44$, $P = 0.02$) and postprandial adiponectin ($\beta = -0.51$, $P = 0.002$). IAUC VLDL1 apoB100 was independently predicted by fasting ($\beta = -0.42$, $P = 0.03$) and IAUC adiponectin ($\beta = -0.48$, $P = 0.008$). IAUC VLDL2 apoB48 was independently predicted only by IAUC adiponectin ($\beta = -0.49$, $P = 0.007$). IAUC VLDL2 apoB100 was independently predicted by fasting ($\beta = -0.45$, $P = 0.01$) and postprandial adiponectin ($\beta = -0.49$, $P = 0.007$).

On logistic regression analysis, SNP 45TG (OR = 3.8, CI 2.3-6.8, $P = 0.009$), SNP 276GT (OR = 3.6, CI 2.2-5.7, $P = 0.004$), and dietary PUFA intake (expressed as % fat: OR = 2.0, CI 1.5-3.1, $P = 0.02$) independently predicted postprandial adiponectin response in the lowest quartile.

Severe hepatic steatosis was independently predicted by SNP 276GT/TT (OR = 3.5, CI 2.1-6.9, $P = 0.009$), SNP 45TT (OR = 2.0, CI 1.6-4.5, $P = 0.02$), and IAUC VLDL1 apoB48 (OR = 2.4, CI 1.9-5.3, $P = 0.011$). The presence of haplotype 45TT/276TT+GT carried a 4.2-fold increased risk (CI 2.9-7.0, $P = 0.006$) of severe steatosis.

Necroinflammatory grade 3 was predicted by SNP 276 GT/TT (OR = 2.2, CI 1.5-5.3, $P = 0.01$) and IAUC LDL-coniugated dienes (OR = 1.9, CI 1.3-4.9, $P = 0.02$). Haplotype 45TT/276TT+GT carried a 3.2-fold increased risk (CI 1.7-5.2, $P = 0.008$) of necroinflammatory grade 3.

Stage 3 fibrosis was predicted by SNP 45TT (OR = 2.1, CI 1.8-5.7, $P = 0.01$), SNP 276GT/TT (OR = 1.9, CI 1.5-4.6, $P = 0.015$), and IAUC LDL-conjugated dienes (OR = 2.5, CI 1.8-6.0, $P = 0.009$). Haplotype 45TT/276TT+GT carried a 4.0-fold increased risk (CI 2.1-6.7, $P = 0.004$) of fibrosis stage 3.

Discussion

The main findings of our study are:

1. Two common SNPs in adiponectin gene are significantly associated with the presence of NAFLD in nono-

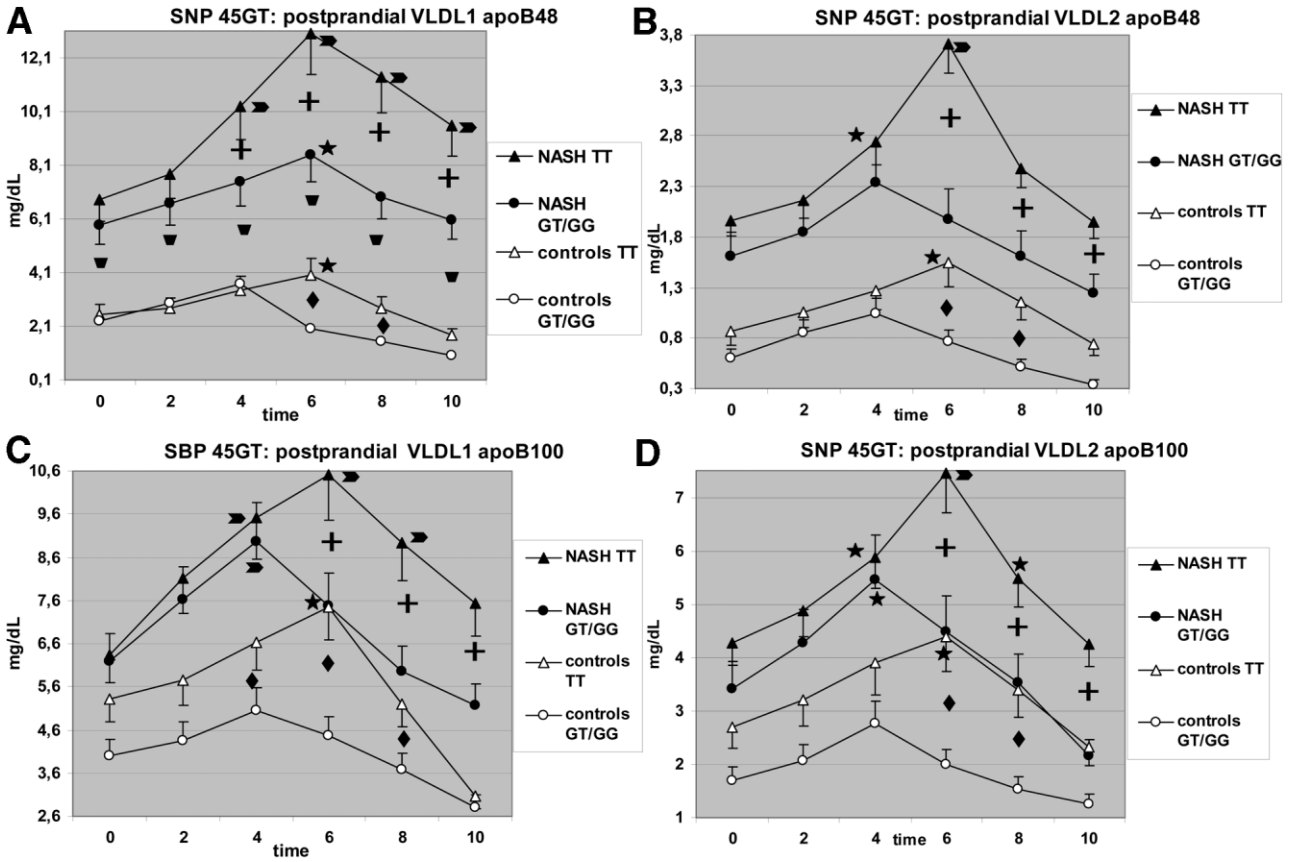

Fig. 3. Oral fat load test. Postprandial VLDL apoB48 and apoB100 subfraction responses in patients with NASH and controls grouped according to SNP 45GT. Data are presented as mean ± SEM; *$P < 0.05$ versus basal; →$P < 0.01$ versus basal; ▲ $P < 0.05$ versus controls GT/GG; ♦ $P < 0.05$ controls GT/GG versus controls TT; $P < 0.05$ NASH GT/GG versus NASH TT.

bese, nondiabetic, normolipidemic subjects.

2. These 2 adiponectin SNPs also predict the severity of liver disease and postprandial serum adiponectin and lipoprotein responses in biopsy-proven NASH.

3. Adiponectin SNPs modulate postprandial adiponectin response and lipoprotein metabolism in healthy controls as well; in fact, 45TT and 276GT/TT genotypes displayed a blunted postprandial adiponectin increase and a more atherogenic lipid profile, with possible implications for primary prevention of hepatometabolic disease.

We report for the first time the association of 2 common SNPs of the adiponectin gene with the presence and severity of NAFLD. Adiponectin SNPs 45TG and 276GT previously have been associated with an increased cardiometabolic risk in different population-based studies, an effect often but not always associated with abdominal/overall adiposity and lower adiponectin levels.[7,9-11,23] In our nonobese, nondiabetic, normolipidemic population, SNP 45TT and 276GT/TT carriers had a significantly increased prevalence and severity of NAFLD than the other genotypes, despite comparable fasting adipokines, adiposity, insulin resistance, and dietary habits. Although the cross-sectional nature of our study prevents causal inference, it is intriguing to speculate that the association between these polymorphisms and the metabolic complications in large population-based cohort studies may be initiated by liver fat accumulation.

Our data provide a novel mechanism linking adiponectin SNPs to fatty liver, that is, the acute modulation of postprandial adiponectin response to fat ingestion. Plasma adiponectin promptly increased postprandially in controls, whereas NASH subjects showed a much smaller increase or even a decrease in plasma adiponectin (Fig. 1A–C). In both patients and controls adiponectin polymorphisms were the main predictors of postprandial adiponectin responses, together with dietary PUFA intake. Mechanisms underlying the differences in adiponectin responses between NASH patients and controls and among different SNPs are unclear. The finding of reduced adiponectin mRNA expression in omental adipocytes from 45T allele carriers compared to G-allele carriers[24] suggests reduced mRNA transcription or stability may underlie

Table 3. Oral Fat Load Parameters of Controls and Patients with NASH Grouped According to Carrying Status of Adiponectin 45-276 GT Haplotype

Parameter	Haplotype 45+276 G/T			Haplotype 45+276 G/T		
	Controls Other haplotypes (n=20)	Controls 45 TT+276 GT/TT (n=10)	*P* controls	NASH Other haplotypes (n=17)	NASH 45 TT+276 GT/TT (n=13)	*P* NASH
Fasting adiponectin (ng/mL)	12390 ± 2128	10513 ± 1582	0.510	6174 ± 1292‡§	4971 ± 1349‡§	0.364
IAUC adiponectin (ng/mL x hr)	29564 ± 4109	5953 ± 1138	0.0002	972 ± 691‡§‡§	−12107 ± 2183‡§	0.0003
Fasting Tg (mg/dL)*	68 ± 6	75 ± 8	0.498	81 ± 11	105 ± 13†	0.201
IAUC Tg (mg/dL x hr)*	182 ± 32	68 ± 23	0.021	558 ± 61‡§	264 ± 43*†	0.0001
Fasting FFA (mMol/L)	0.42 ± 0.11	0.51 ± 0.13	0.162	1.04 ± 0.20‡§	1.18 ± 0.24‡§	0.566
IAUC FFA(mMol/L x hr)	0.40 ± 0.16	1.28 ± 0.21	0.003	3.01 ± 0.86‡§	6.28 ± 1.12‡§	0.009
Fasting VLDL1 ApoB48 (mg/dL)	2.01 ± 0.46	2.31 ± 0.50	0.689	6.32 ± 1.13‡§	6.09 ± 1.25‡§	0.893
IAUC VLDL1 ApoB48 (mg/dL x hr)	1.39 ± 0.33	6.02 ± 0.97	0.00007	11.52 ± 1.98‡§	32.13 ± 2.96‡§	0.00002
Fasting VLDL1 ApoB100 (mg/dL)	4.79 ± 1.39	5.04 ± 1.67	0.714	5.74 ± 1.58	6.02 ± 1.89	0.811
IAUC VLDL1 ApoB100 (mg/dL x hr)	1.78 ± 0.29	6.08 ± 1.17	0.0002	9.03 ± 1.66‡*	28.94 ± 4.39*§	0.0001
Fasting VLDL2 ApoB48 (mg/dL)	0.77 ± 0.36	0.84 ± 0.31	0.563	1.89 ± 0.56*†	1.67 ± 0.64*†	0.698
IAUC VLDL2 ApoB48 (mg/dL x hr)	2.04 ± 0.31	5.79 ± 0.61	0.0009	2.38 ± 1.03*	7.84 ± 1.73*†	0.008
Fasting VLDL2 ApoB100 (mg/dL)	1.91 ± 0.58	2.49 ± 0.74	0.749	3.51 ± 0.63*†	4.77 ± 0.56*†	0.162
IAUC VLDL2ApoB100 (mg/dL x hr)	2.39 ± 0.54	9.11 ± 2.14	0.0001	5.87 ± 1.56*†	16.39 ± 2.13‡§	0.0003
Fasting LDL C.D.(uA 234 nm/uA 200 nm x 100)	6.34 ± 1.29	6.38 ± 1.51	0.881	7.14 ± 1.51	7.59 ± 1.30	0.808
IAUC LDL C.D.(uA 234 nm/uA 200 nm x 100 x hr)	0.20 ± 0.21	2.39 ± 0.45	0.001	4.02 ± 1.53‡§	11.37 ± 2.02‡§	0.001

Data are presented as mean ± SEM. Abbreviations: IAUC: incremental area under the curve; FFA: free fatty acids; Tg: triglyceride; C.D.: conjugated dienes.
*$P < 0.05$ vs. controls 45TT+276 GT/TT
†$P < 0.05$ vs. controls other genotypes
§$P < 0.001$ vs. controls 45TT+276 GT/TT
‡$P < 0.001$ vs. controls other genotypes

the genetic associations described. Alternatively, these SNPs may be in linkage disequilibrium with another functional locus affecting lipoprotein metabolism and lipid peroxidation. Because adipocytes take up FFAs and oxLDL by CD36 receptor and oxidative stress adversely affects adiponectin secretion,[25] a reduced adiponectin response with high-risk polymorphisms would be a consequence of exaggerated postprandial lipemia, thus perpetuating the vicious circle.

Patients with NASH consumed fewer PUFAs and more SFAs than did controls, and PUFA intake was associated with fasting and postprandial adiponectin. The type and amount of dietary fat modulates adipocyte function, with PUFAs exerting a beneficial effect on adiponectin secretion and hepatic steatosis.[26-30] Animal models suggest NASH is characterized by an adaptive failure to a high-fat (mainly saturated) diet, a condition accompanied by higher oxidative stress and lower adiponectin levels than pure steatosis.[31-33] The ability of adiponectin levels to increase following a fat meal can be seen as an acute adaptive mechanism enhancing FFA disposal and TRLP catabolism. This compensatory mechanism is modulated by genetic factors and is compromised to a higher extent when wrong dietary habits are superimposed on an unfavorable genetic background. When a wrong diet is superimposed on an unfavorable genetic background, adipocytes lose their "compensatory" ability to acutely increase adiponectin secretion in response to a fat load. The loss of this "metabolic flexibility" would be an early sign of adipocyte dysfunction and would lead to exaggerated postprandial lipemia, enhanced FFA, and lipid uptake by the liver and adipose tissue,[14,15] eventually resulting in NAFLD, visceral obesity, and lower fasting adiponectin levels.

Despite comparable fasting plasma lipids, adiposity, and insulin resistance, 45TT and 276GT/TT genotypes displayed higher postprandial TRLP and oxLDL levels than the other genotypes in both patients and controls. This highly atherogenic postprandial lipoprotein profile may partially account for the increased atherogenic risk in NASH, independent of insulin resistance, metabolic syndrome, or other traditional risk factors.[2] Interestingly, adiponectin SNPs had similar fasting plasma adiponectin levels, thus suggesting that adiponectin genotyping adds more information on individual cardiometabolic status than fasting adiponectin measurement.[11,24]

The comparison of NASH subjects with healthy controls also suggests that when other factors (i.e., insulin resistance, hypoadiponectinemia, or wrong dietary habits) are superimposed on a high-risk genotype, the higher fluxes of Tg and FFA and the defective TRLP clearance of the insulin resistant state result in more pronounced lipoprotein accumulation and lipoperoxidation than with the other genotypes, thus

accounting for the increased cardiometabolic risk of these adiponectin SNPs.

The postprandial increase in intestinal VLDL1 and oxLDL independently predicted the severity of liver histology in our patients, thus providing a biological mechanism for the epidemiological association between liver disease and atherosclerosis in NASH.[1,2] Consistently, postprandial hepatic FFA uptake contributes substantially to liver Tg pool in NAFLD and diabetes in vivo.[14,15] In cell cultures VLDL-Tg and oxidized LDL activated Kupffer and stellate cells, triggering an inflammatory cascade and extracellular matrix deposition[34]; the amount of oxidized LDL paralleled the severity of the fibrogenic process in NASH.[35] Finally, increased intracellular oxidative stress experimentally impaired VLDL secretion and led to Tg accumulation in hepatocytes, an effect totally reversed by antioxidant treatment.[36]

If the increased cardiovascular risk of adiponectin SNP 45TG and 276GT with NAFLD is prospectively confirmed by larger studies, assessing this polymorphisms may help in the selection of those subjects amenable to more aggressive interventions targeting postprandial lipid metabolism (i.e., statins, PPAR-alpha agonists). Attention should also be paid to the type of dietary fat in addition to total calorie intake. High-risk adiponectin genotypes might benefit from an approach combining PUFA supplementation with insulin sensitizers.[37] Furthermore, the impact of adiponectin SNPs on the response to thiazolidinedione treatment in NASH needs to be addressed in future research.[11]

Limitations of this study were its cross-sectional nature, preventing any causal inference, and the small number of subjects. Furthermore, our measurements were made in patients with the progressive form of NAFLD, that is, NASH, and need to be confirmed in subjects with steatosis alone.

References

1. Schindhelm RK, Dekker JM, Nijpels G, Bouter LM, Stehouwer CDA, Heine RJ, et al. Alanine aminotransferase predicts coronary heart disease events: a 10-year follow-up of the Hoorn Study. Atherosclerosis 2007; 91:391-396.
2. Targher G. Non-alcoholic fatty liver disease, the metabolic syndrome and the risk of cardiovascular disease: the plot thickens. Diabet Med 2007;24: 1-6.
3. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99-S112.
4. Musso G, Gambino R, Durazzo M, Biroli G, Carello M, Fagà E, et al. Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease HEPATOLOGY 2005;42:1175-1183.
5. Gambino R, Cassader M, Pagano G, Durazzo M, Musso G. Polymorphism in microsomal triglyceride transfer protein: a link between liver disease and atherogenic postprandial lipid profile in NASH? HEPATOLOGY 2007;45:1097-1107.
6. Qi L, Doria A, Manson JE, Meigs JB, Hunter D, Mantzoros CS, et al. Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. Diabetes 2006;55:1512-1516.
7. Menzaghi C, Ercolino T, Di Paola R, Berg AH, Warran JH, Scherer PE, et al. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance sindrome. Diabetes 2002; 51:2306-2312.
8. Zacharova J, Chiasson JL, Laakso M, for the STOP-NIDDM Study Group. The common polymorphisms (single nucleotide polymorphism SNP +45 and SNP +276) of the adiponectin gene predict the conversion from impaired glucose tolerance to type 2 diabetes. The STOP-NIDDM Trial. Diabetes 2005;54:893-899.
9. Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, et al. The adiponectin gene SNP +276GT associates with early-onset coronary artery disease and lower levels of adiponectin in younger coronary artery disease (age ≤ 50 years). J Mol Med 2005;83:711-719.
10. Lacquemant C, Froguel P, Lobbens S, Izzo P, Dina C, Ruiz J. The adiponectin gene SNP +45 is associated with coronary artery disease in type 2 (non insulin dependent) diabetes mellitus. Diabet Med 2004;21:776-781.
11. Kang ES, Park SY, Kim HJ, Ahn CW, Nam M, Cha BS, et al. The influence of adiponectin gene polymorphism on the rosiglitazone response in patients with type 2 diabetes. Diabetes Care 2005;28:1139-1144.
12. Von Eynatten M, Schneider JG, Humpert PM, Rudofsky G, Schmidt N, Barosch P, et al. Decreased plasma lipoprotein lipase in hypoadiponectinemia: an association independent of systemic inflammation and insulin resistance. Diabetes Care 2004;27:2925-2929.
13. Adiels M, Olofsson SO, Taskinen MR, Boren J. Diabetic dyslipidaemia. Curr Opin Lipidol 2006;17:238-246.
14. Ravikumar B, Carey PE, Snaar JEM, Deelchand DK, Cook DB, Neely RDG, et al. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. Am J Physiol Endocrinol Metab 2005;288:E789-E797.
15. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005;115:1343-1351.
16. Angulo P,. Hui JM, Marchesini G, Bugianesi E, George J,. Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. HEPATOLOGY 2007;45:846-854.
17. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis 2001;21:3-16.
18. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 2002;137:1-10.
19. Chang Y, Ryu S, Sung E, Jang Y. Higher concentrations of alanine aminotransferase within the reference interval predict non-alcoholic fatty liver disease. Clin Chem 2007;53:686-692.
20. Nunez C, Gallagher D, Visser M, Pi-Sunyer FX, Wang Z, Heynsfield SB. Bioimpedance analysis: evaluation of leg-to-leg system based on pressare contact footpad electrodes. Med Sci Sports Exerc 1997;29: 524-531.
21. Matsuda M, DeFronzo R. Insulin sensitivity indices obtained from oral glucose tolerance testing. Comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-1470.
22. Cassader M., Gambino R., Musso G, Depetris N, Mecca F, Cavallo-Perin P, et al. Postprandial triglyceride-rich lipoprotein metabolism and insulin sensitivity in nonalcoholic steatohepatitis patients. Lipids 2002;36:1117-1124.
23. Loos RJF, Ruchat S, Rankinen T, Tremblay A, Perusse L, Bouchard C. Adiponectin and adiponectin receptor gene variants in relation to resting metabolic rate, respiratory quotient, and adiposity-related phenotypes in the Quebec Family Study. Am J Clin Nutr 2007;85:26-34.
24. Yang WS, Tsou PL, Lee WJ, Tseng DL, Chen CL, Peng CC, et al. Allele-specific differential expression of a common adiponectin gene polymorphism related to obesity. J Mol Med 2003;81:428-434.

25. Soares A, Guichardant M, Cozzone D, Bernoud-Hubac N, Bouzaidi-Tiali N, Lagarde M, et al. Effects of oxidative stress on adiponectin secretion and lactate production in 3T3–L1 adipocytes. Free Radic Biol Med 2005;38: 882-889.
26. Paniagua JA, Gallego de la Sacristana A, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, et al. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care 2007;30:1717-1723.
27. Seo JB, Moon HM, Noh MJ, Lee YS, Jeong HW, Yoo EJ, et al. Adipocyte determination- and differentiation-dependent factor 1/sterol regulatory element-binding protein 1c regulates mouse adiponectin expression. J Biol Chem 2004;279:22108-22117.
28. Nagano K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in diabetic (fa/fa) rats. Biochem Biophys Res Common 2003; 310:562-566.
29. Ide T. Interaction of fish oil and conjugated linoleic acid in affecting hepatic activity of lipogenic enzymes and gene expression in liver and adipose tissue. Diabetes 2005;54:412-423.
30. Mostad IL, Qvistad E, Bjerve KS, Grill VE. Effects of a 3-day low fat diet on metabolic control, insulin sensitivity, lipids and lipoprotein hormones in Norwegian subjects with hypertriacylglycerolemia and type 2 diabetes. Scand J Clin Lab Invest 2004;64:656-574.
31. Arsov T, Carter CZ, Nolan CJ, Petrovsky N, Goodnow CC, Teoh NC, et al. Adaptive failure to high-fat diet characterizes steatohepatitis in Alms1 mutant mice. Biochem Biophys Res Comm 2006;342:1152-1159.
32. Carmiel-Haggai M, Cederbaun, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. FASEB J 2005;19:136-138.
33. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. Endocrinology 2006;147:943-951.
34. Lu L, Zeng M, Li J, Hua J, Fan J, Fan Z, et al. Effects of Kupffer cells stimulated by triglyceride and very low-density lipoprotein on proliferation of rat hepatic stellate cells. Chin Med 1999;112:325-329.
35. Ikura Y, Ohsaka M, Fukushima M, Ryo Y, Muraguchi T. Increased expression of the scavenger receptor CD36 in activated stellate cells in steatotic livers; its relation to fibrotic process in non-alcoholic fatty liver disease. [Abstract] HEPATOLOGY 2005;42(S1):620A.
36. Pan M, Cederhaum AI, Zhang YL, Ginsberg HW, Williams KJ, Fisher FA. Lipid peroxidation and oxidant stress regulate hepatic apolipoprotein B degradation and VLDL production. J Clin Invest 2004;113:1277-1287.
37. Liu LF, Purushotham A, Wendel AA, Belury MA. Combined effects of rosiglitazone and conjugated linoleic acid on adiposity, insulin sensitivity, and hepatic steatosis in high-fat-fed mice. Am J Physiol Gastrointest Liver Physiol 2007;292:G1671-G1682.