Dietary Habits and Their Relations to Insulin Resistance and Postprandial Lipemia in Nonalcoholic Steatohepatitis

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The relations of dietary habits to insulin sensitivity and postprandial triglyceride metabolism were evaluated in 25 patients with nonalcoholic steatohepatitis (NASH) and 25 age-, body mass index (BMI)-, and gender-matched healthy controls. After a 7-day alimentary record, they underwent a standard oral glucose tolerance test (OGTT), and the insulin sensitivity index (ISI) was calculated from the OGTT; an oral fat load test was also performed in 15 patients and 15 controls. The dietary intake of NASH patients was richer in saturated fat (13.7% ± 3.1% vs. 10.0% ± 2.1% total kcal, respectively, $P = .0001$) and in cholesterol (506 ± 108 vs. 405 ± 111 mg/d, respectively, $P = .002$) and was poorer in polyunsaturated fat (10.0% ± 3.5% vs. 14.5% ± 4.0% total fat, respectively, $P = .0001$), fiber (12.9 ± 4.1 vs. 23.2 ± 7.8 g/d, respectively, $P = .000$), and antioxidant vitamins C (84.3 ± 43.1 vs. 144.2 ± 63.1 mg/d, respectively, $P = .0001$) and E (5.4 ± 1.9 vs. 8.7 ± 2.9 mg/d, respectively, $P = .0001$). The ISI was significantly lower in NASH patients than in controls. Postprandial total and very low density lipoproteins triglyceride at +4 hours and +6 hours, triglyceride area under the curve, and incremental triglyceride area under the curve were higher in NASH compared with controls. Saturated fat intake correlated with ISI, with the different features of the metabolic syndrome, and with the postprandial rise of triglyceride. Postprandial apolipoprotein (Apo) B48 and ApoB100 responses in NASH were flat and strikingly dissociated from the triglyceride response, suggesting a defect in ApoB secretion. In conclusion, dietary habits may promote steatohepatitis directly by modulating hepatic triglyceride accumulation and antioxidant activity as well as indirectly by affecting insulin sensitivity and postprandial triglyceride metabolism. Our findings provide further rationale for more specific alimentary interventions, particularly in nonobese, nondiabetic normolipidemic NASH patients. (HEPATOLOGY 2003;37:909-916.)

Nonalcoholic steatohepatitis (NASH) is characterized by liver fatty infiltration with various degrees of inflammation, necrosis, and fibrosis, similar to those of alcoholic liver disease[1]; in the absence of significant alcohol intake, it is part of a spectrum of liver damage, ranging from simple steatosis to advanced fibrosis and cirrhosis, named *nonalcoholic fatty liver disease*. Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver tests in the United States,[2] and NASH has been proposed as a possible cause of cryptogenic cirrhosis[3] and hepatocellular carcinoma.[4]

The prevalence of NAFLD ranges from 10% to 24% of the general population, whereas NASH affects about 3% of the lean population and almost half of morbidly obese people.[5] NASH is associated with several underlying medical disorders, most commonly type 2 diabetes, dyslipidemia, and obesity.[1] Insulin resistance, with the different features of the metabolic syndrome, is regarded as a hallmark and a causal factor of NAFLD, even in the absence of obesity and diabetes mellitus.[6] Several proposed etiologic mechanisms have been proposed, including an increased afflux of free fatty acids to the liver, a reduced

Abbreviations: NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; Tg, triglyceride; BMI, body mass index; OGTT, oral glucose tolerance test; ISI, insulin sensitivity index; VLDL, very low density lipoproteins; Apo, apolipoprotein; ANOVA, analysis of variance; AUC, area under the postprandial curve; IAUC, incremental area under the postprandial curve; SFA, saturated fatty acids; P:S ratio, polyunsaturated to saturated fat ratio.

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free fatty acid β-oxidation, a reduced hepatic secretion of triglyceride (Tg)-rich lipoproteins, and an increased lipid peroxidation. An impaired postprandial Tg response has been recently reported in patients with NASH[7] and may play an etiologic role by favoring Tg accumulation in the liver.

It is increasingly recognized that multiple genetic and acquired factors can influence both insulin action and postprandial lipid metabolism. Among acquired determinants, diet and nutrition, in particular the amount and type of carbohydrate and fat intake, were recently linked to insulin resistance, increased risk of developing type 2 diabetes, and impaired postprandial lipid metabolism[8,9]; furthermore, animal and human models suggest that dietary factors can directly affect hepatic fatty infiltration and oxidative damage in different types of liver disease, including alcoholic fatty liver disease.[10,11]

Little is known about dietary habits and their relations to liver disease, insulin sensitivity, and postprandial Tg metabolism in NASH patients, particularly in the absence of obesity, diabetes, or hyperlipidemia. Although a few studies showed that gradual weight loss may be of limited benefit in diabetic and grossly obese NASH subjects, weight loss is seldom maintained because low-calorie diets are often discontinued; furthermore, caloric restriction and weight loss are hardly feasible in nonobese subjects, who would require a different therapeutic approach.[12] The identification of specific dietary patterns could help to understand the pathogenesis of NASH and would allow a more rationale dietary approach, prior or in addition to pharmacologic interventions. This study investigates the relationship between dietary habits, insulin resistance, the metabolic syndrome, and postprandial lipid metabolism in nonobese, nondiabetic NASH patients with normal fasting plasma lipid levels.

Patients and Methods

Patients. Twenty-five patients (mean age ± SD, 37 ± 9 years; body mass index [BMI] 25.6 ± 2.5 kg/m^2) attending our Liver Unit during the years 2001-2002 were selected according to the following criteria: persistently (at least 12 months) elevated aminotransferases, daily alcohol consumption <20 grams, and ultrasonographic presence of bright liver without any other liver or biliary tract disease. Conditions known to be associated with fatty liver were ruled out by the following exclusion criteria: a BMI ≥30 for men and ≥28 for women; positive serum markers of viral, autoimmune, or celiac disease; abnormal copper metabolism or thyroid function indices; or diagnosis of diabetes mellitus based on plasma glucose ≥126 mg/dL in fasting conditions or ≥200 mg/dL at +2 hours on a standard oral glucose tolerance test, serum total cholesterol ≥240 mg/dL, or serum Tg ≥200 mg/dL. The patients did not take drugs known to be steatogenic or to affect glucose metabolism and were not exposed to occupational hepatotoxins. Mutations in the hemochromatosis genes HFE and TRF2 were detected in patients and controls using a single, multiplex amplification reaction, and premade, ready-to-use teststrips (Nuclear Laser Medicine, Milan, Italy). Liver biopsy specimens could be obtained from 21 patients and were examined blindly by a single pathologist (E.D.). A diagnosis of NASH was made if inflammatory infiltrate and hepatocyte ballooning, with or without fibrosis, were present in addition to steatosis. Fatty infiltration was graded as mild (involving <40% hepatocytes), moderate (involving 40%-70% hepatocytes), and severe (involving >70% hepatocytes). Inflammatory infiltrate and fibrosis were graded subjectively on a scale of 0 to 3, in which 0 = none, 1 = mild, 2 = moderate, 3 = severe, as proposed by Diehl et al.[13] Cirrhosis was considered to be grade 4 fibrosis.

The control group consisted of 25 healthy subjects with normal liver enzymes and abdomen ultrasound scan, matched for age, sex, and BMI (see Table 1). Patients and controls gave their consent to the study, which was conducted in conformance with the Helsinki Declaration.

Alimentary Record. Patients and controls completed a 7-day diet record after a training session with a dietician; a list of foods was designed and, for each item, different portion sizes were specified according to the EPIC study.[14,15] The recorded period included a complete week, and the record was collected within 2 weeks of the metabolic tests. The diet record was analyzed using the WinFood database (Medimatica, 64014 Martinsicuro-TE), 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]

Oral Glucose Tolerance Test and Insulin Sensitivity Assessment. Patients and controls underwent a standard 75-g oral glucose tolerance test (OGTT), with measurement of plasma glucose and serum insulin concentrations at different times. Insulin sensitivity was assessed by using the whole body insulin sensitivity index (ISI), a novel index of whole body insulin sensitivity derived from the OGTT that correlates closely with the euglycemic insulin clamp measurement in nondiabetic people.[18]

Oral Fat Load. A subgroup of 15 patients and 15 controls, representative of the whole groups, underwent an oral fat load 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 dairy cream (35% fat) and egg yolk, for a total energy content of 766 kcal. The total amount of fat was based on the subject's body surface area (78.3 g fat 55.6% saturated fatty acids, 29.6% monounsaturated fatty acids, 4.2% polyunsaturated fatty acids, 0.5 g cholesterol per m^{-2}). The fat load was consumed during a period of 5 minutes; subjects continued fasting on the test morning, and strenuous activity was forbidden because exercise can reduce postprandial lipemia. A catheter (Venflon Viggo AB, Helsingborg, Sweden) was inserted in the antecubital vein and during the test was used to draw samples after 0 (baseline), 2, 4, 6, 8, and 10 hours for biochemical determinations. Plasma total cholesterol and Tg were measured by means of automated enzymatic methods. Very low density lipoproteins (VLDL) were isolated through preparative ultracentrifugation and subsequently assayed for their Tg and total cholesterol content. Apolipoprotein (Apo) E genotypes were determined by polymerase chain reaction amplification of genomic DNA using specific oligonucleotide primers. Tg-rich lipoprotein (defined by a Svedberg flotation rate >20) ApoB48 and ApoB100 content were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis using 3.9% gel as previously described.[7]

Statistical Analysis. Data were expressed as means ± SD. Differences between groups were analyzed by analysis of variance (ANOVA) when variables were normally distributed; otherwise, the Mann-Whitney *U* test was used. Normality was evaluated by the Shapiro-Wilk test. Data from the oral fat load were compared by ANOVA and Scheffè post hoc test after logarithmic normalization of skewed variables. Differences in mean saturated fat intake between the different components of the metabolic syndrome were analyzed by ANOVA followed by Student-Neuman-Keuls test. χ^2 Test or Fisher exact test were used to compare categorical variables as appropriate. Spearman rank test was used to estimate linear relationship between different variables relating to dietary intake, anthropometry, glucose, and lipid metabolism.

Plasma total Tg and VLDL-Tg area under the curve (AUC) and incremental area under the curve AUC (IAUC; computed on the area exceeding baseline) were computed by the trapezoid method to estimate the overall response of plasma Tg and VLDL-Tg during the entire 10-hour postprandial period. Differences were considered statistically significant at $P < .05$.

Results

Baseline Parameters. The main anthropometric, clinical, and biochemical parameters in NASH patients and controls are reported in Table 1. BMI was ≤25 in 17 (68%) patients and <30 in the remaining 8 (32%) patients. Patients had a higher mean diastolic pressure (90 ± 9 vs. 78 ± 6 mm Hg; $P = .0001$) than controls and lower high-density lipoprotein cholesterol (47 ± 9 vs. 59 ± 17 mg/dL; $P = .014$). There was no significant difference in the number of smokers between the 2 groups: 3 patients and 2 controls. A family history of type 2 diabetes mellitus (first degree relatives) was present in 7 patients and 4 controls ($P = .496$). Eight patients and 4 controls were heterozygous carriers for the H63D mutation of the HFE gene ($P = .324$). There was no significant difference in Apo E allelic frequency: 8 NASH patients and 7 controls were E3/E3, 5 NASH subjects and 5 controls were E4/E3, and 2 NASH subjects and 3 controls were E3/E2.

Fatty infiltration was mild in 13 patients (62%), moderate in 4 (21%), and severe in 4 (17%). Inflammatory infiltrate and hepatocyte ballooning, compatible with a diagnosis of NASH, were present in all 21 liver biopsy specimens. Inflammatory activity was grade 1 in 11 (52%) patients, grade 2 in 7 (33%) patients, and grade 3 in 3 (15%) patients. Fibrosis was graded as 0 in 15 (71%) patients, 1 in 1 (5%) patient, 2 in 3 (14%) patients, and 3 in 2 patients. Cirrhotic changes were not seen in any of the biopsy specimens.

Alimentary Record. The main nutritional data are reported in Table 2. Total energy, carbohydrate, protein, and fat intake did not differ in the 2 groups. Saturated fatty acids (SFA) intake, expressed both as percentage total energy and as percentage total fat intake, was higher in NASH patients compared with controls: 13.7% ± 3.1% vs. 10.0% ± 2.1% kcal, respectively ($P = .0001$), and 39.1% ± 4.8% vs. 31.1% ± 5.2% total fat, respectively ($P = .0001$). Daily cholesterol intake was higher in patients than in controls: 506 ± 108 vs. 405 ± 111 mg/d ($P = .002$). Polyunsaturated fat intake was lower in NASH patients when expressed both as percentage kcal (3.5% ± 1.3% vs. 4.7% ± 2.0%, respectively, $P = .015$) and as percentage total fat (10.0% ± 3.5% vs. 14.5% ± 4.0%, respectively, $P = .0001$). The polyunsaturated to saturated fat ratio (P:S) was significantly lower in NASH group (P:S ratio: 0.24 ± 0.10 vs. 0.46 ± 0.12, respectively, $P = .0001$). NASH patients had also a significantly lower daily intake of fiber (12.9 ± 4.1 vs. 23.2 ± 7.8, respectively, $P = .0001$) and antioxidant vitamins C (84.3 ± 43.1 vs. 144.2 ± 63.1 mg, respectively, $P = .0001$) and E (5.4 ± 1.9 vs. 8.7 ± 2.9 mg, respectively, $P = .0001$). Dietary habits of controls were comparable with those of a large sample of healthy Piedmont population, as assessed by a recent diethological survey.[15]

Oral Glucose Tolerance Test and Insulin Sensitivity. No patient was diabetic (fasting plasma glucose ≥126 mg/dL or plasma glucose ≥200 mg/dL at +120

Table 1. Baseline Characteristics of Patients With NASH and Controls

	NASH Patients (n = 25)	Controls (n = 25)	*P* Value
Age (y)	37 ± 9	37 ± 10	.999
Sex (M/F)	24/1	24/1	.999
BMI	25.6 ± 2.5	24.9 ± 2.5	.327
Family history of type 2 diabetes (No. subjects)	7	4	.496
Smokers (No. subjects)	3	2	.921
Waist (cm)	91 ± 6	89 ± 5	.207
Systolic blood pressure (mm Hg)	131 ± 13	128 ± 10	.365
Diastolic blood pressure (mm Hg)	90 ± 8	79 ± 5	.000
Triglycerides (mg/dL)*	100 ± 44	76 ± 34	.036
Total cholesterol (mg/dL)†	176 ± 35	181 ± 24	.559
HDL cholesterol (mg/dL)†	47 ± 8	57 ± 15	.005
LDL cholesterol (mg/dL)†	109 ± 36	108 ± 27	.912
Uric acid (mg/dL)‡	6.18 ± 1.00	5.21 ± 1.31	.005
Glucose (mg/dL)	97 ± 9	85 ± 13	.000
Insulin (μU/mL)	13.5 ± 8.7	6.3 ± 2.6	.000
Albumin (g/dL)	4.9 ± 0.4	5.0 ± 0.3	.322
AST (U/L)	39 ± 12	26 ± 16	.002
ALT (U/L)	86 ± 35	33 ± 21	.000
GGT (U/L)	93 ± 72	45 ± 23	.003
ALP (U/L)	88 ± 43	54 ± 32	.003
HFE mutation (H63D) heterozygotes (No. subjects)	8	4	.324
Serum iron (μg/dL)	99 ± 24	90 ± 22	.173
Ferritin (μg/L)	176 ± 79	136 ± 87	.095
Transferrin (% saturated)	32 ± 7	28 ± 10	.108

NOTE. Data are presented as mean ± SD.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-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.

minutes), 5 patients had impaired glucose tolerance (plasma glucose ≥140 mg/dL but <200 mg/dL at +120 minutes of the OGTT), 3 patients had impaired fasting glycemia (fasting plasma glucose ≥110 mg/dL but <126 mg/dL), and the others had a normal glucose regulation (fasting plasma glucose <110 mg/dL).

The whole body ISI was significantly lower in the NASH group than in controls: 3.38 ± 1.61 vs. 7.07 ± 1.50; $P = .0001$). ISI correlated significantly with total energy intake ($r_s = -0.51$; $P = .013$), with saturated fatty acid intake expressed both as percentage kcal ($r_s = -0.57$; $P = .004$) and as percentage fat ($r_s = -0.62$; $P = .017$), with P:S ratio ($r_s = 0.46$; $P = .028$), cholesterol intake ($r_s = -0.72$; $P = .0001$), and diastolic blood pressure ($r_s = -0,46$; $P = .028$) but not with BMI ($r_s = -0.36$; $P = .086$), waist circumference ($r_s = -0.37$; $P = .074$), or other clinical or dietary parameter.

Metabolic Syndrome. Adopting the recently proposed criteria for clinical definition of the metabolic syndrome,[19] 16 patients were hypertensive (systolic/diastolic blood pressure ≥130/85 mm Hg), 5 had abdominal obesity (waist circumference >102 cm in men and >88 cm in women), 3 were hypertriglyceridemic (fasting plasma Tg ≥150 mg/dL), 5 had low plasma high-density lipoprotein-cholesterol (<40 mg/dL in men and <50 mg/dL in women), and 8 had impaired glucose regulation (defined as impaired fasting glycemia or impaired glucose tolerance on OGTT).

NASH patients were classified into 3 subgroups on the basis of the number of the above-mentioned criteria present in each subject: group A (1 criterium present: 11 subjects), group B (2 criteria met: 8 subjects), and group C (3 or more criteria met: 6 subjects). Group B and group C had significantly higher dietary SFA intake than group A after controlling for age, BMI, total caloric intake, and other dietary parameters: group A, 11.9% ± 1.9% total calories; group B, 13.7% ± 1.7% total calories; group C, 14.8% ± 2.5% total calories) (B vs. A: $P = .048$; C vs. A: $P = .017$). ALT levels were also higher in group C than in group A (109 ± 44 vs. 66 ± 21 U/L; $P = .031$).

Oral Fat Tolerance Test. Postprandial plasma total Tg and VLDL-Tg responses are reported in Fig. 1. Plasma total Tg and VLDL-Tg levels were higher in the NASH group at all time points and peaked at 4 hours, with a significant difference at 4 hours ($P = .029$ for total Tg; $P = .038$ for VLDL-Tg) and at 6 hours ($P = .014$ for total Tg; $P = .043$ for VLDL-Tg).

Table 2. Daily Intake of Main Dietary Constituents in NASH Patients and Controls

	NASH Patients (n = 25)	Controls (n = 25)	*P* Value
Total energy intake (kcal)	2,638 ± 444	2,570 ± 739	.695
Kcal/kg body weight	33 ± 5	32 ± 6	.580
Dietary fat (g)	102.8 ± 31.6	92.1 ± 35.2	.264
Dietary carbohydrate (g)	295.1 ± 53.7	315.2 ± 101.9	.387
Dietary protein (g)	121.2 ± 25.2	107.2 ± 32.7	.096
Alcohol (g)	13.3 ± 7.3	13.5 ± 8.9	.705
Dietary fat (% kcal)	35.1 ± 7.1	32.3 ± 6.7	.158
Dietary carbohydrate (% kcal)	44.7 ± 8.7	48.6 ± 9.1	.128
Simple carbohydrate (% total carbohydrate)	30.3 ± 6.4	32.5 ± 5.1	.185
Fiber (g)	12.9 ± 4.1	23.2 ± 7.8	.000
Dietary protein (% kcal)	20.2 ± 3.7	16.7 ± 4.3	.003
SFA (g)	40.2 ± 12.7	28.7 ± 11.1	.001
MUFA (g)	52.1 ± 17.4	47.8 ± 16.7	.377
PUFA (g)	10.3 ± 4.9	13.4 ± 4.1	.019
Cholesterol (mg)	506 ± 108	405 ± 111	.002
SFA (% total kcal)	13.7 ± 3.1	10.0 ± 2.1	.000
MUFA (% total kcal)	17.7 ± 4.4	16.7 ± 5.1	.462
PUFA (% total kcal)	3.5 ± 1.3	4.7 ± 2.0	.015
SFA (% total fat)	39.1 ± 4.8	31.1 ± 5.2	.000
MUFA (% total fat)	50.9 ± 6.5	51.9 ± 5.9	.572
PUFA (% total fat)	10.0 ± 3.5	14.5 ± 4.0	.000
(P:S ratio)	0.24 ± 0.10	0.46 ± 0.12	.000
Vitamin A (μg)	582.6 ± 383.7	647.1 ± 507.3	.614
Vitamin C (mg)	84.3 ± 43.1	144.2 ± 63.1	.000
Vitamin E (mg)	5.4 ± 1.9	8.7 ± 2.9	.000
Iron (mg)	12.1 ± 2.3	14.5 ± 3.9	.011

NOTE. Data are presented as mean ± SD.

Abbreviations: SFA, saturated fat intake; PUFA, polyunsaturated fat intake; MUFA, monounsaturated fat intake; P:S ratio, polyunsaturated to saturated fat ratio.

Plasma AUC-Tg and IAUC-Tg were significantly higher in NASH patients compared with controls (AUC-Tg: 1,330 ± 537 vs. 918 ± 280 mg/dL × hour^{-1}, P = .014; IAUC-Tg: 405 ± 296 vs. 184 ± 240 mg/dL × hour^{-1}; P = .033). VLDL-Tg AUC was not different between the 2 groups.

Baseline plasma Tg-rich lipoprotein ApoB48 and ApoB100 concentrations were lower in NASH patients than in controls, and the postprandial ApoB curves were almost flat in NASH subjects, whereas they were coupled to total Tg and VLDL-Tg responses in controls (Fig. 2). The difference was statistically significant at all time points for ApoB48 ($P < .001$) and at 4 hours for ApoB100 levels ($P = .028$).

In NASH subjects, fasting total Tg correlated with ISI ($r_s = -0.67$; $P = .042$), total calories ($r_s = 0.61$; $P = .04$), saturated fat intake expressed as percentage calories ($r_s = 0.73$; $P = .01$); total Tg at 4 hours correlated with SFA percentage calories ($r_s = 0.73$; $P = .01$) and cholesterol intake ($r_s = 0.62$; $P = .04$) but not with ISI ($r = -0.28$; $P = .41$), fasting Tg ($r_s = 0.57$; $P = .07$) or any other anthropometric or dietary parameters; total Tg at 6 hours correlated with fasting Tg ($r_s = 0.70$; $P = .01$) and SFA expressed as percentage calories ($r_s = 0.67$; $P = .02$).

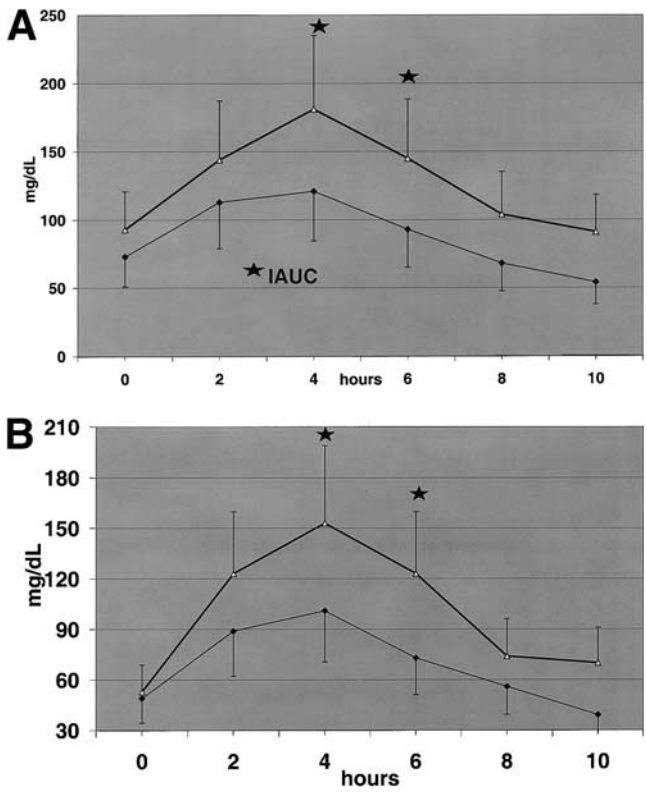

Fig. 1. Oral fat load. Plasma total triglyceride (A) and VLDL-triglyceride (B) responses in patients with NASH (△) and controls (◆). IAUC: incremental area under the curve. *$P < .05$.

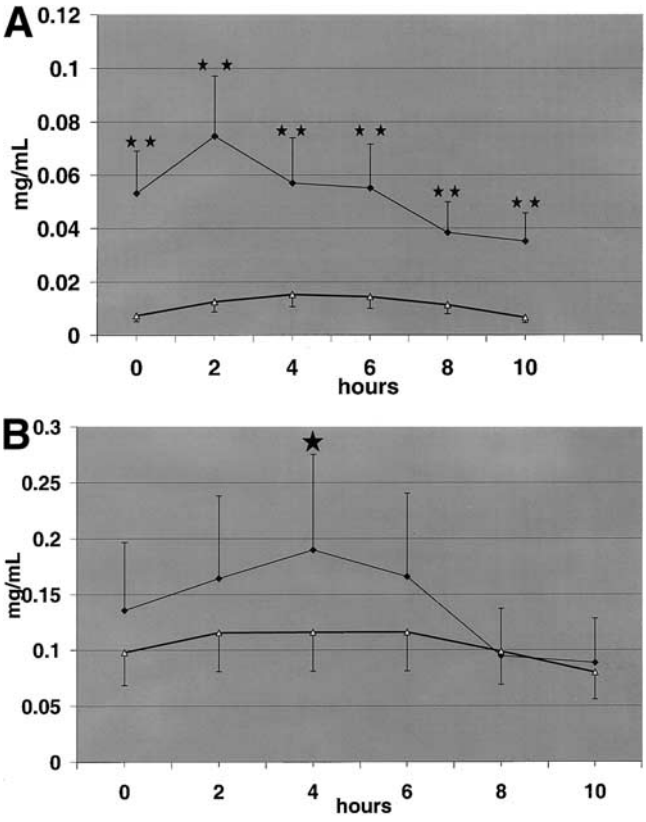

Fig. 2. Oral fat load. Plasma triglyceride-rich lipoprotein ApoB48 (A) and ApoB100 responses (B) in patients with NASH (△) and controls (◆). $^{**}P < .001$, $^{*}P < .05$.

AUC-Tg correlated with fasting Tg ($r_s = 0.84$; $P = .0001$), fat intake ($r_s = 0.58$; $P = .023$), saturated fat intake expressed both as percentage calories ($r_s = 0.74$; $P = .002$) and percentage fat ($r_s = 0.70$; $P = .004$), and cholesterol intake ($r_s = 0.67$; $P = .006$).

Fasting VLDL-Tg correlated with ISI ($r_s = -0.61$; $P = .04$); VLDL-Tg correlated with the P:S ratio at 2 hours ($r_s = -0.68$; $P = .02$), 4 hours ($r_s = -0.61$; $P = .04$), and 6 hours ($r_s = -0.68$; $P = .02$). No other clinical or alimentary parameter correlated with VLDL-Tg at any time of the test. There was no correlation between Tg-rich lipoprotein ApoB48 and ApoB100 responses and any metabolic, anthropometric, or dietary parameter.

Correlation Between Aminotransferases and Metabolic Parameters. Alanine aminotransferase levels, chosen as an index of hepatocellular damage, correlated with ISI ($r_s = -0.49$; $P = .018$), plasma Tg levels ($r_s = 0.69$; $P = .0001$), total energy intake ($r_s = 0.58$; $P = .004$), saturated fat intake expressed both as percentage calories ($r_s = 0.58$; $P = .004$) and as percentage fat ($r_s = 0.44$; $P = .038$), cholesterol intake ($r_s = .49$; $P = .017$), and dietary polyunsaturated to saturated fat ratio (P:S ratio; $r_s = -0.50$; $P = .016$).

Discussion

Our NASH patients were mostly characterized by a mild to moderate degree of inflammation and fibrosis and were free of hyperlipidemia, diabetes and obesity, conditions known to entail abnormal dietary and metabolic profiles and to carry an increased risk of advanced liver disease and cirrhosis.[5] A novel finding of this study is that NASH subjects consumed a diet richer in saturated fatty acids and poorer in polyunsaturated fatty acids, fiber, and antioxidant vitamins C and E compared with controls. These dietary habits may promote the development of NASH by directly affecting liver steatosis and oxidative damage and by modulating postprandial Tg metabolism and insulin sensitivity.

Experimental data suggest that the different types of dietary fat play a pivotal role in modulating hepatic Tg metabolism and accumulation in the liver, independently of insulin or other hormonal signaling.[20] Polyunsaturated fatty acids, in particular, may act as "fuel partitioners," directing fatty acids away from Tg storage and toward oxidation and enhancing glucose flux to glycogen; their site of action lies at the nuclear level, at which they bind to nuclear transcription factors, such as PPAR-α and sterol regulatory element binding protein, up-regulating transcription of genes involved in fatty acid oxidation and simultaneously down-regulating genes involved in lipid synthesis.[21,22]

The significant differences in dietary fiber and vitamin C between the 2 groups, despite a similar total and simple carbohydrate consumption, suggest a low intake of fresh fruit and vegetables in NASH subjects. Daily vitamin E intake of NASH patients was nearly half that of controls as well. A reduced availability of antioxidant vitamins, coupled with the enhanced lipid peroxidation and oxidative stress[23] present in our patients (data not reported), may contribute to hepatocellular injury, inflammation, and fibrosis in patients with NASH; this is supported by the reported efficacy of vitamin E supplementation.[24,25]

Interestingly, postprandial total Tg and VLDL-Tg were significantly higher in NASH group compared with controls (Fig. 1). An impaired postprandial Tg metabolism may promote fatty liver in several ways, such as an increasing hepatic uptake of Tg-rich lipoproteins and their remnants in the postprandial period.[7,26] The amount and type of dietary fat is emerging as an important predictor of postprandial Tg response and appears to exert its effect independently of other variables, such as fasting Tgs, insulin resistance, and central obesity.[27] In our patients, the amount of saturated fat intake, expressed both as percentage total energy or as polyunsaturated to saturated fat ratio, was strongly associated with, respec-

tively, postprandial total Tg and VLDL-Tg concentrations at different times of the fat load test. Whether this effect depends on faster intestinal absorption, delayed Tg-rich lipoprotein lipolysis, or reduced cholesterol ester transfer protein activity is not known at present.[9,28]

Postprandial Tg-rich lipoprotein ApoB48 and ApoB100 responses were coupled to the Tg rise in controls, whereas they were surprisingly flat in NASH subjects (Fig. 2), in striking dissociation from total and VLDL-Tg responses; because plasma Tg and VLDL-Tg responses were higher in patients with NASH, the net effect is a lower number of circulating ApoB-containing lipoproteins (namely, VLDL and chylomicrons) that are larger and richer in Tg than in controls. This finding suggests an impaired secretion of VLDL and chylomicrons in NASH and is consistent with the reduced basal hepatic ApoB100 synthesis reported in a recent study.[29]

A reduced hepatic secretion of VLDL may promote liver steatosis combining with the increased afflux of free fatty acids to hepatocytes commonly seen in insulin resistance. The mechanism(s) underlying the reduced secretion of ApoB-containing lipoproteins in patients with NASH are unknown: ApoB responses are not apparently related to any dietary or metabolic parameter in our patients. An interesting candidate is microsomal Tg transfer protein, which plays a key role in assembly and secretion of chylomicrons and VLDL by the intestine and the liver, respectively.[30] Actually, a reduced activity of microsomal Tg transfer protein, as a consequence of different gene polymorphism, has been recently associated with the development of hepatic steatosis in diabetic subjects[31] and can be part of the genetic susceptibility to steatohepatitis.

The ISI of patients with NASH was nearly half that of controls, and the different features of the metabolic syndrome were evident in many patients. The ISI and the presence of the features of the metabolic syndrome correlated significantly with saturated fat intake but not with BMI or waist circumference; therefore, the effect of the different types of fatty acids on insulin sensitivity would not seem to be mediated by body or abdominal fat accumulation. This finding agrees with recent experimental and epidemiologic data: A high ratio of saturated to polyunsaturated dietary fat has been linked to insulin resistance and the risk of type 2 diabetes, an effect thought to be mediated by the fatty acid composition of cell membranes.[32]

In conclusion, dietary habits of our NASH patients are characterized by striking differences in the quality of fat and vitamins compared with controls, despite similar intakes in calories and macronutrients. This finding provides the rationale for alimentary intervention based on diets equilibrated in the types of fat and in antioxidant vitamins, preferably with the help of professional dietary instruction. If these changes proved to be effective, dietary modification should be considered the first line approach to these patients, before the introduction or in complement to drug therapy, such as insulin-sensitizing agents. This intervention would be particularly valuable in subjects free of diabetes, obesity, or hyperlipidemia. Further studies, addressing the prevalence of NASH in different countries with different dietary habits, are needed to confirm our findings and to assess the feasibility and benefit of alimentary interventions.

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