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Dietary glycemic index and liver steatosis[1–3]

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ABSTRACT

Background: Insulin resistance (IR) and liver steatosis (LS) are interlinked metabolic derangements whose prevalence is rapidly increasing, but the effect of dietary carbohydrate quality on LS is unknown.

Objective: The objective was to describe the relation of IR and LS to total carbohydrate, total dietary fiber, and the glycemic index (GI) and glycemic load of the diet.

Design: The study was a cross-sectional evaluation of 247 apparently healthy subjects who had no evidence of viral, toxic, or autoimmune hepatitis and who were unselected for alcohol intake. The homeostasis model assessment index was used as a surrogate measure of IR, and a liver echography was used as a proxy for LS grading. Dietary data were collected by using 3-d food records. Total carbohydrate intake, total dietary fiber, GI, and glycemic load were calculated by using a semiquantitative food-frequency questionnaire concerning the dietary sources of carbohydrates.

Results: The prevalence of high-grade LS (HG-LS) increased significantly across quartiles of dietary GI (P for trend < 0.034): HG-LS in the 4th quartile (high GI) was twice that in the first 3 quartiles (low to medium GIs), whereas no relation was observed with total carbohydrates, total dietary fiber, or glycemic load. In insulin-sensitive subjects (first 3 quartiles of homeostasis model assessment index of IR), the prevalence of HG-LS did not differ significantly between GI groups, but, in insulin-resistant subjects (4th quartile of homeostasis model assessment index of IR), it was twice as high in those with high GI as in those with low to medium GIs ($P = 0.005$).

Conclusions: High-GI dietary habits are associated with HG-LS, particularly in insulin-resistant subjects. Dietary advice on the quality of carbohydrate sources therefore may be a complementary tool for preventing or treating LS of metabolic origin. *Am J Clin Nutr* 2006;84:136–42.

KEY WORDS Diet, glycemic index, insulin resistance, liver steatosis, metabolic syndrome

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), a recognized risk factor for nonalcoholic steatohepatitis (NASH) and liver cirrhosis, is the term used to describe an abnormal accumulation of triacylglycerols in the hepatocyte that is commonly observed in obese and insulin-resistant persons (1). Given the increasing worldwide prevalence of insulin resistance (IR) and associated syndromes, the development of dietary strategies to prevent the occurrence of NAFLD and its progression to NASH is of major interest. Weight loss and supplementation with dietary antioxidants have been proposed for potential use in the prevention of NAFLD and NASH, but little or no attention has been paid to the effect of carbohydrate quality on fat accretion in the liver (2-4).

It is well documented that both excess body fat and resistance to the action of insulin impair the suppression of circulating nonesterified fatty acids (NEFAs) in the postprandial state, which favors a greater NEFA influx into the hepatocyte and subsequent synthesis of triacylglycerols. In addition, the metabolic pathway controlling mitochondrial fat oxidation is downregulated in the presence of IR, which further contributes to intracellular fat accretion (5). Because weight loss and insulin-sensitizing medications have shown some efficacy in the treatment of NAFLD, we hypothesize that diets that can modulate either body weight or the metabolic consequences of IR could also have an effect on liver steatosis (LS).

Epidemiologic data indicate that a low dietary glycemic index (GI) is associated with lower food intake and body weight (6, 7). In addition, low dietary GI and glycemic load (GL) seem to be linked to favorable lipid profiles and lower concentrations of C-reactive protein only in overweight and obese persons, which suggests that the metabolic effects of dietary carbohydrates may be particularly important in insulin-resistant persons (8–10). Indeed, randomized controlled intervention trials in insulin-resistant subjects and persons with type 2 diabetes show that low dietary GI can improve the metabolic derangements associated with IR—namely, glucose intolerance, hyperinsulinemia, and the increase in circulating postprandial NEFA—but whether dietary GI or GL has an effect on fat accretion in the liver remains to be established (11–13). The aim of this cross-sectional study was to investigate the relation between dietary carbohydrates,

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[2] Supported by IST-2001-33204, "Healthy Market," from the European Community; COFIN 2001 from the Italian Ministry of University and Research; and CU01.009.23.CT26 from the National Research Council.

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Received September 15, 2005.
Accepted for publication January 31, 2006.

body weight, IR, and LS in a population without diabetes and unselected for alcohol intake.

SUBJECTS AND METHODS

Subjects

The subjects were apparently healthy workers and former workers at a food company in the area of Parma, in Northern Italy, who were surveyed since 1981 for dietary and clinical risk factors for type 2 diabetes and cardiovascular disease. A total of 325 subjects participated in the 2002 call, from which the cross-sectional data presented in this report derive. Exclusion criteria for the study were diagnosis of diabetes, evidence of infection with hepatitis B or C virus (or both), chronic liver disease or nephropathy (or both), autoimmune disease, history of cancer, and the use of statins.

All subjects gave written informed consent at enrollment. The protocol was approved by the Ethics Committee for Human Research of the University of Parma.

Study design

Participants completed both a medical history that provided information on their health status, current medications, alcohol consumption, and history of viral, toxic, or autoimmune hepatitis and a physical exam that included height, weight, waist circumference, and blood pressure. They also underwent a blood draw conducted after a 12-h fast, for biochemical analyses; an oral-glucose-tolerance test (OGTT) that provided 75 g glucose with blood draws up to 120 min for measurement of glucose and insulin to exclude type 2 diabetes (14) and calculate derivative indexes of insulin sensitivity (IS; IS/IR); an ultrasonogram of the liver to assess LS; a 3-d food record to obtain accurate information on short-term diet composition, including alcoholic beverages; and a food-frequency questionnaire (FFQ) that was specifically designed to retrieve detailed data on usual carbohydrate consumption, including GI.

Data collection

We measured height to the nearest 0.1 cm, weight to the nearest 0.01 kg, and waist circumference to the nearest 0.1 cm as described previously (15). On 2 different days, blood pressure was taken twice with the use of a mercury cuff sphygmomanometer as reported in a previous publication (15).

Dietary data

A certified dietitian trained participants to complete a 3-d food record that included all foods, beverages, and supplements consumed during 2 nonconsecutive working days plus a weekend day in the week after the screening visit. The record was checked for completeness and portion sizes by using a book of photographs and standard household measures in the presence of the patient within 48 h after record collection. Nutrient intake was assessed by entering foods into a customized computer program linked to the European Institute of Oncology (IEO) database depicting the macronutrient and micronutrient content of >700 Italian foods (16). During the first screening visit, participants were also asked to complete a semiquantitative FFQ that was specifically designed to gather information about their consumption of carbohydrate-containing foods during the previous 6 mo. The questionnaire was designed to reflect Northern Italian food habits on the basis of 24-h recalls ($n = 2500$) registered by the Varese cohort of the European Prospective Investigation on Cancer and Nutrition (EPIC); it included 131 questions about 62 foods that represent the source of >95% of the carbohydrate intake in Northern Italy. Food intake was assessed semiquantitatively by using portion sizes from the EPIC picture book for the same or similar food items or cooked dishes. For example, for bread, the questions had to do with the amount of the item usually consumed (as shown in the picture book) and the frequency of consumption (number of occasions per day, week, or month). The questionnaire also included qualitative multiple-choice questions related to 12 kinds of bread; the possible answer was never, sometimes, or often to allow some weighing of bread GI value among different items. An in-house program, based on a Microsoft EXCEL spreadsheet, was used to input answers to food questions and to calculate the derived mean daily intakes of food, total carbohydrate, and total dietary fiber (TDF), as well as to calculate directly the average dietary GI and GL during the compilation of the FFQ, which was assisted by a trained dietitian.

For all foods, compositional data on total carbohydrate, starch, simple sugar, and TDF were obtained from the IEO database on food composition, whereas GI values, indexed to a scale in which glucose = 100, were mainly extrapolated from literature data (17) after careful control of the data sources. For 14 foods lacking matching data, direct GI testing was performed according to the standard procedures described by Brouns et al (18). Such data, along with validation of the FFQ, will be published elsewhere (F Brighenti et al, manuscript in preparation).

In addition to mean daily nutrient composition, results were given in terms of daily average food intake. To this purpose, foods were divided into 8 groups according to their composition and the prevalent characteristics of consumption: *1*) breads, pizza, and savory snacks, *2*) pasta, rice, and corn; *3*) pulses and potatoes; *4*) milk and dairy products; *5*) juices and soft drinks; *6*) nuts and fresh and dried fruit; *7*) sugar, sweets, cakes (including cookies), and chocolate; and *8*) fresh vegetables. The median (range) GIs for these food groups were 62 (44) for group 1 [minimum: 51 (rye bread); maximum: 95 (French baguette)]; 50.5 (52) for group 2 [minimum:30 (intact spelleda kernels); maximum: 82 (puffed rice)]; 45 (66) for group 3 [minimum: 19 (dried chickpeas); maximum: 85 (potato purée)]; 34.5 (4) for group 4 [minimum: 32 (partly skimmed milk); maximum: 36 (full-fat fruit yogurt)]; 46 (28) for group 5 [minimum: 40 (unsweetened apple juice); maximum: 68 (orange-flavored soft drink)]; 45.5 (58) for group 6 [minimum: 14 (roasted peanuts); maximum: 72 (watermelon)]; 52 (29) for group 7 [minimum: 42 (chocolate and hazelnut cream); maximum: 71 (hard candies)]; and 54 (59) for group 8 [minimum: 16 (raw carrots); maximum: 71 (yellow pumpkin)]. Besides the 3-d dietary record, we used a food-frequency questionnaire, described elsewhere (19), to estimate the usual alcohol intake.

Ultrasonography

Liver ultrasonography scanning was performed to grade LS on a scale of 0 to 3 (0 = absent; 1 = mild; 2 = moderate; and 3 = severe) on the basis of abnormally bright echoes arising from the hepatic parenchyma, the difference between liver and kidney in echo amplitude, echo penetration into the deep portion of the liver, and clarity of the liver blood vessel structure (20). All ultrasonographic studies were performed by the same operator, who was blinded to laboratory values, using an Hitachi AU 600 echographer (Hitachi Ltd, Tokyo, Japan) equipped with a convex 3.5-MHz transducer.

Biochemical analysis

Serum insulin concentrations were assayed with the use of a microparticle enzyme immunoassay (IMX; Abbott Laboratories, Abbott Park, IL) with intraassay and interassay CVs of 3.0% and 5.3%, respectively. Fasting plasma glucose, total cholesterol, HDL cholesterol, triacylglycerol, uric acid, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations were assessed by a central laboratory that used standard methods.

Statistical analysis

All statistical analyses were performed with SPSS software (version 12.0; SPSS Inc, Chicago, IL). Continuous variables were checked for normality by using the Kolmogorov-Smirnov test. Data are expressed as means ± SDs for variables that are normally distributed and as medians and interquartile ranges (IQRs) for variables with a markedly skewed distribution. Non-normally distributed variables were log-transformed for general linear modeling (GLM) analysis. The GI of the average diet was calculated as the weighed GI value of the diet, according to the FAO/WHO Expert Panel on Carbohydrate in Human Nutrition (21), whereas GL was the sum of available carbohydrate from any given food multiplied by the GI of the same food divided by 100. Fasting insulin and the homeostasis model assessment of IR (HOMA-IR) were used as surrogates of IR, preferentially that of hepatic origin (22–24). The composite insulin sensitivity index (ISI composite) was calculated as 10.000/$\surd$ [(fasting plasma glucose (mg/dL) × fasting plasma insulin (μU/mL)] × [mean OGTT glucose concentration (mg/dL) × mean OGTT insulin concentration (μU/mL)]; it was used as a surrogate for IS at both the hepatic and peripheral levels (25). High-grade LS (HG-LS) was defined as echographically assessed moderate or severe steatosis. The metabolic syndrome (MS) was defined according to the National Cholesterol Education Program Adult Treatment Panel III criteria (26). To remove sex differences observed for dietary GL, TDF, and total carbohydrate intake (data not shown), these variables were adjusted for energy intake obtained by the 3-d questionnaire as described by Willet and Stampfer (27). Sex differences by LS grade were tested by using the chi-square statistic. The main effects of sex and LS grade and of the sex × LS grade interaction for clinical, biochemical, and dietary variables were tested by using univariate GLMs.

The sample population was then divided into quartiles of dietary GI and quartiles of energy-adjusted dietary GL, TDF, and total carbohydrate intake based on the FFQ data. *P* for trend for prevalence of HG-LS across quartiles of GI and energy-adjusted GL, TDF, and total carbohydrate intake was assessed by using the chi-square statistics for linear association. Because the prevalence of HG-LS was significantly higher in the 4th quartile of GI than in the other 3 quartiles and because no significant differences in LS prevalence were observed among the first 3 quartiles of GI (data not shown), the 1st to the 3rd quartiles of GI were grouped for comparisons. Differences between subjects with a low to medium (1st to 3rd quartiles) and a high (4th quartile) dietary GI regarding demographic, clinical, and dietary characteristics were assessed by using the *t* test for independent samples, the Mann-Whitney *U* test, or the Kolmogorov-Smirnov *Z* test, as appropriate. The sample population was further divided into quartiles of HOMA-IR and characterized as insulin-sensitive (1st to 3rd quartiles) or insulin-resistant (4th quartile). Main effects of dietary GI group, insulin resistance status, and their interaction on LS grade were tested by using univariate GLM. Differences on the prevalence of HG-LS between subjects with low to medium and high dietary GI by group of insulin sensitivity were assessed by using the chi-square statistic. Odds ratios (ORs) and the corresponding 95% CIs were estimated by using unconditional logistic regression models including terms for sex, waist circumference ($\leq$88 cm for females and $\leq$102 cm for males compared with $>$88 cm for females and $>$102 cm for males), IR (first 3 quartiles of HOMA-IR compared with 4th quartile of HOMA-IR), and dietary GI (1st to 3rd quartiles compared with 4th quartile). Because additional terms for plasma concentrations of triacylglycerols and HDL cholesterol, alcohol intake, intake of carbohydrates, dietary GL and TDF (1st to 3rd quartiles compared with 4th quartile) did not modify any risk estimate, these were not included in the final model. All tests were two-sided, and a *P* value < 0.05 was considered significant.

RESULTS

Of the 325 volunteers participating in this part of the follow-up, only 241 were eligible for this study and had complete data for all variables considered. Demographic, clinical, and dietary characteristics of eligible volunteers by sex and LS grade are shown in **Table 1**. Eighteen women were taking estrogen replacement therapy, but their distribution into categories of LS (absent, mild, moderate, or severe) did not differ significantly from that of women not taking estrogens, and thus estrogen therapy users were not excluded from the analysis. In addition, no significant differences in sex distribution by LS grade were observed ($P = 0.129$).

Age, blood pressure, HOMA-IR, uric acid concentrations, and total energy and alcohol intakes were significantly higher and HDL-cholesterol concentrations were significantly lower in men than in women. Mean values for the characteristics clustering in the MS, for liver function enzymes, and for dietary GI were significantly higher in subjects with HG-LS than in subjects with absent or mild LS. The sex × LS grade interaction was not significant for any of the variables considered (Table 1). In our sample, 16.6% of subjects ($n = 40$) met the criteria for diagnosis of MS and, as expected, the prevalence of HG-LS was significantly ($P < 0.001$) higher (52.8%) in subjects with MS than in subjects without MS (13.9%).

As shown in **Table 2**, the prevalence of HG-LS increased significantly across quartiles of dietary GI and that in the 4th quartile was approximately double that in the first 3 quartiles, whereas no relation was observed when the sample population was divided into quartiles of carbohydrate intake, dietary GL, or TDF. Therefore, 2 groups of dietary GI defined as high (4th quartile) and low to medium (1st to 3rd quartiles) were considered for comparisons, given that the cutoff for GI in ascertaining an increase in the prevalence of HG-LS appeared to be between the 3rd and 4th quartiles.

Characteristics of subjects by dietary GI group are shown in **Table 3**. High- and low- to medium-GI groups did not differ significantly with respect to most demographic (age and sex), clinical (BMI, waist circumference, lipid profile, uric acid, and AST), or dietary variables (macronutrient assessed by the 3-d record, alcohol intake assessed either by the 3-d or the alcohol-specific FFQ, and total carbohydrate and fiber intakes assessed

TABLE 1

Characteristics of subjects by sex and grade of liver steatosis (LS)[1]

	Absent or mild LS[2] (n = 192)		High-grade LS[3] (*n* = 49)		Main effects		
	Women	Men	Women	Men	Sex	LS grade	Sex × LS
n	90	102	17	32			
Percentage within same sex (%)	84.1	76.1	15.9	23.9			
Percentage within LS group (%)	46.9	53.1	34.7	75.3			
Clinical and biochemical							
Age (y)	58 (7)[4]	62 (9)	57 (7)	62 (9)	0.007	0.517	0.826
BMI (kg/m^2)	26.2 ± 3.4[5]	26.1 ± 2.5	32.0 ± 5.3	30.0 ± 2.3	0.035	<0.001	0.062
Waist girth (cm)	90.9 ± 10.6	93.9 ± 7.8	102.8 ± 11.3)	105.2 ± 7.3	0.076	<0.001	0.856
Systolic blood pressure (mm Hg)	129.5 (15.9)	142.1 (17.0)	133.8 (12.6)	143.8 (19.5)	<0.001	0.297	0.648
Diastolic blood pressure (mm Hg)	82.6 (8.1)	88.8 (9.8)	87.1 (10.0)	93.4 (10.3)	<0.001	0.013	0.763
Fasting glucose (mg/dL)	89 (9)	97 (13)	92 (14)	101 (11)	0.502	0.075	0.831
Fasting insulin (pmol/L)	42.3 (23.7)	46.2 (26.3)	75.0 (56.7)	80.7 (61.0)	0.167	<0.001	0.636
HOMA-IR	1.59 (0.94)	1.84 (1.15)	2.84 (2.20)	3.53 (2.46)	0.028	<0.001	0.710
ISI composite	5.34 (3.84)	4.27 (3.71)	2.89 (2.39)	2.37 (3.12)	0.077	<0.001	0.666
Total cholesterol (mmol/L)	6.12 ± 1.07	5.65 ± 0.99	6.00 ± 0.94	5.83 ± 1.18	0.069	0.849	0.394
HDL cholesterol (mmol/L)	1.71 (0.55)	1.42 (0.44)	1.53 (0.27)	1.30 (0.40)	<0.001	<0.001	0.316
Triacylglycerols (mmol/L)	0.81 (0.63)	0.84 (0.59)	1.25 (0.55)	1.12 (1.18)	0.833	<0.001	0.607
Uric acid (mg/dL)	4.2 ± 0.8	5.3 ± 1.1	5.0 ± 0.8	5.7 ± 1.1	<0.001	<0.001	0.220
AST (U/L)	22 (5)	23 (6)	25 (9)	24 (5)	0.954	0.001	0.254
ALT (U/L)	19 (6)	22 (9)	28 (27)	28 (10)	0.187	<0.001	0.265
Dietary variables							
3-d records							
Energy (kcal/d)	2049 ± 439	2486 ± 505	1979 ± 397	2334 ± 625	<0.001	0.178	0.619
Alcohol (g/d)	12 (20)	26 (25)	12 (18)	31 (35)	<0.001	0.584	0.699
FFQ							
Carbohydrate intake (g/d)[6]	306 ± 81	336 ± 81	337 ± 90	334 ± 89	0.326	0.297	0.238
Glycemic load (g glucose equivalents)[6]	171 ± 47	188 ± 47	191 ± 52	191 ± 51	0.288	0.165	0.277
Glycemic index	55.8 ± 3.1	55.9 ± 3.2	56.8 ± 2.7	57.3 ± 3.9	0.582	0.033	0.734
Total dietary fiber (g/d)[6]	19.3 ± 5.6	19.8 ± 5.6	20.8 ± 6.8	19.8 ± 6.7	0.788	0.827	0.162

[1] HOMA-IR, homeostasis model assessment–insulin resistance; ISI composite, insulin sensitivity index–composite; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Glycemic index and glycemic load were calculated on a scale in which glucose = 100. Main effects for sex and LS and their interaction were tested by using univariate general linear models.

[2] Prevalence was 79.7%.

[3] Prevalence was 20.3%.

[4] Median; interquartile range in parentheses (all such values).

[5] $\bar{x}$ ± SD (all such values).

[6] Energy-adjusted.

by the carbohydrate-specific FFQ). The only significant differences between GI groups were those for dietary GL (as expected, given that total carbohydrate intake was comparable between groups), ALT concentrations, and surrogate markers of IS:IR (fasting insulin, HOMA-IR, and the ISI composite). Therefore, we further classified the study population as insulin-sensitive

TABLE 2

Prevalence of high-grade liver steatosis (HG-LS) by quartile (Q) of glycemic index and of energy-adjusted carbohydrate intake, glycemic load, and total dietary fiber as assessed by using a food-frequency questionnaire[1]

Dietary variable	Q1	Q2	Q3	Q4	*P* for trend
Carbohydrate intake					
QR (g/d)	148–264	264–320	321–378	379–612	
Prevalence of HG-LS (%)	20	18	16	27	0.440
Glycemic index					
QR	49.2–53.6	53.7–55.6	55.7–57.9	58.1–68.5	
Prevalence of HG-LS (%)	17	15	18	32	0.034
Glycemic load					
QR (g glucose equivalents/d)	75–147	147–181	181–211	212–352	
Prevalence of HG-LS (%)	18	20	13	30	0.223
Total dietary fiber					
QR (g/d)	7.3–15.2	15.3–19.5	19.5–23.2	23.3–40.3	
Prevalence of HG-LS (%)	23	17	23	18	0.710

[1] QR, quartile range. From Q1 to Q4, *n* = 60, 60, 61, and 60 for total carbohydrate intake, dietary glycemic load, and total dietary fiber, respectively; and *n* = 60, 61, 61, and 59 for dietary glycemic index, respectively. *P* values are chi-square statistics for linear association. Glycemic index and glycemic load were calculated on a scale in which glucose = 100.

TABLE 3

Demographic, clinical, and dietary characteristics of subjects by dietary glycemic index (GI)[1]

Characteristic	Low to medium GI[2] ($n = 182$)	High GI[3] ($n = 59$)	P
Clinical and biochemical variables			
Age (y)	59 (8)[4]	59 (6)	0.360
Men [n (%)][5]	97 (53)	37 (63)	0.132
BMI (kg/m^2)	26.9 ± 3.4[6]	27.7 ± 4.0	0.135
Waist girth (cm)	94.6 ± 10.4	95.8 ± 10.2	0.450
Fasting glucose (mg/dL)	94 (12)	95 (14)	0.228
Fasting insulin (pmol/L)	46.2 (32.0)	54.0 (33.6)	0.018
HOMA-IR	1.8 (1.4)	2.1 (1.3)	0.020
ISI composite	4.7 (3.8)	3.5 (3.0)	0.005
Total cholesterol (mmol/L)	5.90 ± 1.02	5.80 ± 1.20	0.502
HDL cholesterol (mmol/L)	1.54 (0.44)	1.52 (0.61)	0.568
Triacylglycerols (mmol/L)	0.86 (0.60)	0.94 (0.79)	0.392
Uric acid (mg/dL)	4.86 ± 1.10	5.10 ± 1.13	0.140
AST (U/L)	23 (5)	24 (7)	0.135
ALT (U/L)	21 (9)	24 (12)	0.031
Dietary variables			
3-d records			
Energy (kcal/d)	2287 ± 542	2204 ± 505	0.303
Fat (% of energy)	33.2 ± 5.5	32.7 ± 6.8	0.541
Carbohydrate (% of energy)	48.6 ± 6.2	47.7 ± 8.1	0.489
Protein (% of energy)	14.9 ± 2.4	15.8 ± 2.8	0.094
Alcohol (g/d)	17 (22)	19 (25)	0.880
FFQ alcohol			
Alcohol (g/d)	24 (26)	20 (29)	0.786
FFQ carbohydrates			
Carbohydrate intake (g/d)[7]	322 ± 85	333 ± 77	0.627
GI[7]	54.7 ± 2.0	60.5 ± 2.5	—
Glycemic load (g glucose equivalents)[7]	177 ± 48	200 ± 44	0.007
Total dietary fiber (g/d)	20.1 ± 5.9	18.9 ± 5.8	0.219

[1] HOMA-IR, homeostasis model assessment–insulin resistance; ISI composite, insulin sensitivity index–composite; AST, aspartate aminotransferase; ALT, alanine aminotransferase, Glycemic index and glycemic load were calculated on a scale in which glucose = 100.

[2] The 1st to 3rd quartiles.

[3] The 4th quartile.

[4] Median; interquartile range in parentheses (all such values). P by the Mann-Whitney U test.

[5] Chi-square statistics.

[6] $\bar{x}$ ± SD (all such values). P by the t test for independent samples.

[7] Energy-adjusted.

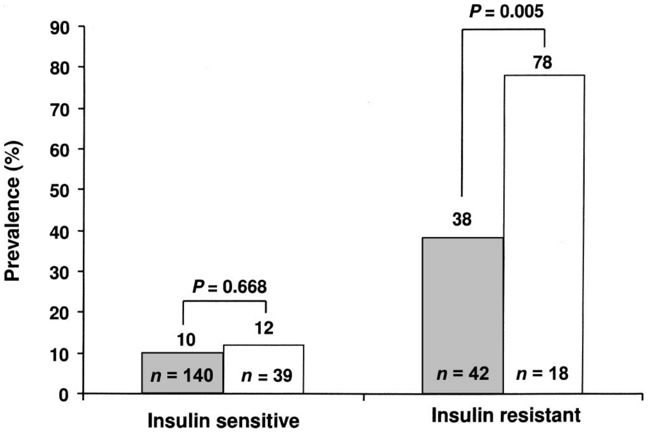

FIGURE 1. The percentage prevalence of high-grade liver steatosis by group of dietary glycemic index (GI) [low GI: first 3 quartiles (▨) compared with high GI: 4th quartile (□)] in insulin-sensitive (first 3 quartiles of homeostasis model assessment–insulin resistance) and insulin-resistant (4th quartile of homeostasis model assessment–insulin resistance) subjects. P values are chi-square statistics. GI subgroup × insulin sensitivity status interaction was significant for liver steatosis grade, $P = 0.002$.

(1st to 3rd quartiles of HOMA-IR) or insulin-resistant (4th quartile of HOMA-IR) to explore the relation between dietary GI, IR status, and LS grade. Because the dietary GI group × IR status interaction was significant for LS grade ($P = 0.002$), the percentage prevalence of HG-LS by dietary GI group in insulin-sensitive and insulin-resistant subjects was calculated. It is interesting that the percentage prevalence of HG-LS in the high-GI group was approximately double that in the low- to medium-GI group when insulin-resistant subjects were considered, but no differences between GI groups were observed when insulin-sensitive subjects were considered (**Figure 1**).

The prevalence of HG-LS by sex and by category of waist circumference, HOMA-IR, and dietary GI and the unadjusted and adjusted ORs associated with the highest category of each risk factor for HG-LS in our population are shown in **Table 4**. In the fully adjusted model, the 4th quartile of dietary GI had ORs for HG-LS that were 3 times those of the 1st to 3rd quartiles, which is approximately half the odds associated with being insulin resistant and approximately one-third the odds associated with having a high waist circumference.

The percentage contribution of different carbohydrate-containing foods to total dietary GL in subjects with low to medium GI compared with high GI is shown in **Table 5**. Differences in dietary pattern may be clearly seen. In particular, the contribution of bread, pizza, and savory snacks to total GL was significantly greater ($P < 0.001$) in subjects with a high dietary GI than in those with low to medium GI, whereas the contribution to total GL of pasta, rice, and corn; milk and dairy products; and fruit was significantly lower ($P < 0.001$, < 0.01, and < 0.001, respectively) as assessed by the Kolmogorov-Smirnov test for independent samples.

DISCUSSION

We have investigated for the first time the relation between dietary carbohydrates and the prevalence of high-grade LS in an adult population without diabetes in whom the prevalence of the MS was comparable to that reported in other Italian cohorts (28). The close relation observed between MS and LS was accounted for in previous publications (1, 29). More attention should be paid to the observation that the prevalence of HG-LS was a function of dietary GI, whereas no relation was observed with either total carbohydrate, dietary GL, or TDF. In addition, dietary GI appears to discriminate individuals primarily on their IR status, and a high GI may be particularly relevant to the risk of HG-LS in insulin-resistant subjects. Low-GI meals, by reducing the rate of glucose absorption, decrease plasma glucose concentrations, reduce the postprandial rise in gut hormones and insulin, and lower serum NEFA concentrations in the late postprandial period more than do high-GI meals (30). Moreover, low-GI meals appear to improve postprandial glycemia of the subsequent meal, possibly by reducing NEFA concentrations, removing NEFA-glucose competition, and ensuring sustained tissue insulinization (31).

It is therefore plausible that, in subjects with low insulin sensitivity and who are known to be at higher risk for LS (1, 5), the

TABLE 4

Percentage prevalence, unadjusted and adjusted odds ratios (ORs), and 95% CIs for high-grade liver steatosis in relation to sex, waist circumference, homeostasis model assessment index for insulin resistance (HOMA-IR), and dietary glycemic index[1]

Risk factors	Prevalence	Unadjusted ORs (95% CIs)	P	Adjusted ORs (95% CIs)[2]	P
	% (n/total)				
Sex					
Females	15.9 (17/107)	1		1	
Males	23.9 (32/134)	1.66 (0.87, 3.19)	0.128[3]	5.76 (2.21, 15.01)	$<$ 0.001
Waist circumference					
Low	9.9 (14/141)	1		1	
High	35.0 (35/100)	4.89 (2.46, 9.72)	$<$ 0.001	9.29 (3.54, 24.43)	$<$ 0.001
HOMA-IR					
Low	10.5 (19/181)	1		1	
High	50.0 (30/60)	7.83 (3.93, 15.6)	$<$ 0.001	6.17 (2.83, 13.44)	$<$ 0.001
Glycemic index					
Low	16.5 (30/182)	1		1	
High	32.2 (19/59)	2.41 (1.23, 4.71)	0.010	3.15 (1.34, 7.39)	0.009

[1] In the logistic regression models, values for waist circumference were $\leq$88 and $\leq$102 cm for females and males, respectively (low), and $>$88 and $>$102 cm for females and males, respectively (high); values for HOMA-IR and glycemic index are first 3 quartiles (low) and 4th quartile (high).

[2] Adjusted for sex, waist circumference, HOMA-IR, and glycemic index. Additional terms for plasma concentrations of triacylglycerols and HDL cholesterol, alcohol intake, intakes of carbohydrates and fiber, and dietary glycemic load (first 3 quartiles compared with the 4th quartile) did not modify any risk estimate. Glycemic index was calculated on a scale in which glucose = 100.

[3] The sex × waist circumference interaction was significant, $P = 0.008$.

effect of high-GI foods may exacerbate liver fat deposition through multiple mechanisms related to the reciprocal control of glucose and fatty acid metabolism in the liver and other tissues. In insulin-resistant persons consuming high-GI foods, the liver is simultaneously exposed to hyperglycemia (deriving from higher glucose availability) and hyperinsulinemia (deriving from both hyperglycemia and IR). This combined exposure may upregulate de novo lipogenesis (DNL) and inhibit NEFA oxidation through the effect of malonyl coenzyme A on carnitine palmitoyl transferase-1–mediated mitochondrial transport of fatty acids (32). In effect, there is evidence that a liquid diet administered as a meal increases hepatic lipogenesis much more than does the same diet administered via continuous feeding, which suggests a role not only of the total amount of glucose consumed but also of the glucose delivery rate (33). However, the availability of fatty acids for conversion into triacylglycerols remains critical to drive fat deposition in the liver and cannot be entirely accounted for by an increase in carbohydrate-stimulated DNL. Donnelly et al (34) recently showed that, in hyperinsulinemic NAFLD patients, DNL contributes up to 26% of liver triacylglycerols, whereas the contribution of peripheral NEFA is $\approx$59%, and that of dietary triacylglycerols is 15%; their findings were discussed in an accompanying commentary (35). Lack of prolonged suppression of circulating NEFA deriving from lack of inhibition of the hormone-sensitive lipase in adipose tissue due to peripheral IR may explain the enhanced availability of NEFA for hepatic triacylglycerol synthesis and fat deposition (35), and this too may be exacerbated by high-GI diets (11, 30).

TABLE 5

Percentage contribution to total dietary glycemic load (GL) from different food sources of carbohydrates according to the average glycemic index (GI) of the diet

Food group	Low to medium GI[1] (n = 182)	High GI[2] (n = 59)	P
Breads, pizza, and savory snacks (% of GL)	45.9 (11.9)[3]	57.8 (11.4)	$<$ 0.001
Pasta, rice, and corn (% of GL)	21.6 (9.7)	16.7 (8.6)	$<$ 0.001
Pulses and potatoes (% of GL)	1.3 (1.5)	1.3 (1.1)	0.610
Milk and dairy products (% of GL)	2.4 (2.9)	1.2 (2.8)	$<$ 0.010
Juices and soft drinks (% of GL)	0.8 (2.3)	0.7 (3.8)	0.760
Fresh and dry fruit and nuts (% of GL)	8.0 (5.9)	3.9 (2.7)	$<$ 0.001
Sugar, sweets, cakes, and chocolate (% of GL)	16.5 (9.8)	13.6 (9.0)	0.077
Fresh vegetables (% of GL)	0.1 (0.2)	0.0 (0.1)	0.408

[1] The 1st to 3rd quartiles.

[2] The 4th quartile.

[3] Median; interquartile range in parentheses (all such values). P by Kolmogorov-Smirnov z test.

Two limitations of this study merit further comment. One is the use of ultrasonography to assess liver fat content. As compared with the gold standard—histologic analysis—which was ethically unacceptable for most of our "healthy" volunteers, the sensitivity (80%–90%) and specificity (85%–95%) of ultrasonography to show increased fat in the liver are very good, and its accuracy approaches 100% for subjects with echographic features of moderate or severe steatosis; thus, we are confident about the stratification of our subjects into subgroups of LS (36, 37). The second limitation is that subjects were unselected for alcohol intake. It is well known that alcohol consumption $>$20–30 g/d has a steatogenic effect, but this variable did not seem to contribute significantly to LS in this population of mild-to-moderate drinkers independently of other known risk factors for LS (ie, sex, waist circumference, and IR). Moreover, alcohol intake did not differ significantly between dietary GI groups, and there is no reason to believe in an undetected association between alcohol intake and GI of the diet.

Finally, it cannot be excluded that other components present in the foods identified as having low GI or some dietary habits associated with a low- or high-GI diet could have played a role in the observed relation between GI and LS. Indeed, the habitual diet of the subjects consuming high-GI foods differs with respect to the type and origin of carbohydrates from the diet of subjects consuming low- to medium-GI foods, even if the total carbohydrate and TDF intakes appear to be comparable. Nevertheless, the fact that GI resulted as a good dietary marker of effect of IR on LS confirms the usefulness of selecting a diet mainly based on low-GI foods as a way to prevent, and possibly manage, IR-induced LS.

In conclusion, high-GI dietary habits are associated with a greater risk of HG-LS, primarily in insulin-resistant subjects, whereas no relation has been observed with other dietary markers of carbohydrate intake, such as carbohydrate quantity or total dietary fiber. These findings seem to indicate that advice on the quality of dietary carbohydrate sources, more than on low-carbohydrate or high-fiber diets, should be primarily given to persons who are at risk of LS of metabolic origin.

We thank Nadia Anelli, of the Department of Public Health, University of Parma, for her skillful collection of dietary data and Valeria Pala, Vittorio Krogh, and Sabina Sieri, of the Milan EPIC group, for their advice in defining the list of carbohydrate foods consumed in Northern Italy.

SV and DA participated in, and IZ was responsible for: requesting approval from the Ethics Committee, recruitment of subjects, collection and interpretation of the clinical and laboratory data, and subject's management. DDR, FS, NP were responsible for the collection and interpretation of dietary data. FN conducted all ultrasonographic studies and interpreted the data. LM supervised the analysis and interpretation of serum insulin. SV and FB performed the statistical analysis and were the primary authors of the manuscript; all of the other authors provided input. FB and IZ were responsible for the study concept and design and for securing the funding for the study. None of the authors had any personal or financial conflict of interest.

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