

Fructose, glycemic load, and quantity and quality of carbohydrate in relation to plasma C-peptide concentrations in US women¹⁻³

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ABSTRACT

Background: Circulating C-peptide concentrations are associated with insulin resistance and the development of type 2 diabetes. However, associations between fructose and the quantity and quality of total carbohydrate intake in relation to C-peptide concentrations have not been adequately examined.

Objective: We assessed the association of dietary fructose, glycemic load, and carbohydrate intake with fasting C-peptide concentrations.

Design: Plasma C-peptide concentrations were measured in a cross-sectional setting in 1999 healthy women from the Nurses' Health Study I and II. Dietary fructose, glycemic load, and carbohydrate intake were assessed with the use of semiquantitative food-frequency questionnaires.

Results: After multivariate adjustment, subjects in the highest quintile of energy-adjusted fructose intake had 13.9% higher C-peptide concentrations (P for trend = 0.01) than did subjects in the lowest quintile. Similarly, in the multivariate model, subjects in the highest quintile of glycemic load had 14.1% (P for trend = 0.09) and 16.1% (P for trend = 0.04) higher C-peptide concentrations than did subjects in the lowest quintile after further adjustment for total fat or carbohydrate intake, respectively. In contrast, subjects with high intakes of cereal fiber had 15.6% lower (P for trend = 0.03) C-peptide concentrations after control for other covariates.

Conclusions: Our results suggest that high intakes of fructose and high glycemic foods are associated with higher C-peptide concentrations, whereas consumption of carbohydrates high in fiber, such as whole-grain foods, is associated with lower C-peptide concentrations. Furthermore, our study suggests that these nutrients play divergent roles in the development of insulin resistance and type 2 diabetes. *Am J Clin Nutr* 2004;80:1043-9.

KEY WORDS Fructose, glycemic load, carbohydrate, C-peptide, dietary questionnaire, insulin resistance

INTRODUCTION

Plasma C-peptide, a marker of insulin secretion, is associated with insulin resistance and the development of many chronic diseases such as diabetes (1, 2), cardiovascular diseases (3), and colon cancer (4). C-peptide is less actively extracted by the liver and has a longer half-life than insulin. In addition, because of the greater linearity of the kinetics of C-peptide relative to that of insulin, plasma concentrations of C-peptide better reflect the true pancreatic secretion of insulin and might be a better marker of insulin demand and pancreatic stress (5).

Fructose is a naturally occurring sugar in fruit and vegetables. In the United States, the intakes of the natural sources of fructose

were relatively stable since the 1970s. However, the consumption of free fructose has dramatically increased over the past 30 y with the increased consumption of soft drinks and other beverages and foods high in fructose. The addition of high-fructose corn syrup sweeteners to foods such as breakfast cereals, baked goods, and prepared desserts account for most of the free fructose (6, 7). Many animal studies showed that intake of fructose increases body weight, plasma glucose, and insulin concentrations and reduces insulin sensitivity and insulin binding activity than other sugars or starch (8-12). However, the long-term effects of fructose intake in relation to insulin action and sensitivity in human studies are less clear. Beck-Nielsen et al (13) and Hallfrisch et al (14) showed that diets with higher proportions of fructose than other carbohydrates lead to reduced insulin action and sensitivity, whereas others found that fructose decreases plasma glucose concentrations (15, 16).

Several studies showed that, when patients with type 2 diabetes are fed isocarbohydrate diets, diets with a low glycemic load (GL) improve glycemic control (17, 18). Some (19, 20) but not all (21, 22) prospective cohort studies showed that a high-GL diet increases risk of type 2 diabetes. However, the use of low-GL diets for the prevention and management of diabetes has still not received conclusive support. The aim of this study was to assess the association of fructose intake, GL, and the quantity and quality of carbohydrate intake in relation to plasma C-peptide concentrations.

SUBJECTS AND METHODS

Study population

The Nurses' Health Study I

The Nurses' Health Study I cohort, established in 1976, consists of 121 700 female registered nurses aged 30-55 y who

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completed a mailed questionnaire and provided detailed medical history and lifestyle information at baseline and on subsequent biennial mailed questionnaires. Diet was assessed approximately every 4 y with the use of a previously validated (23, 24) semi-quantitative food-frequency questionnaire (SFFQ). Between 1989 and 1990, we collected blood samples from 32 826 cohort members who were then aged 43–69 y. As previously published (25), each woman received a kit containing all the supplies needed for blood collection plus a supplemental questionnaire to collect additional information on menopausal status, recent postmenopausal hormone use, time since last meal, and time of day of blood sampling. After blood collection the women mailed the whole-blood sample, cooled with an ice pack, by way of overnight mail. On arrival, samples were centrifuged, and plasma, red blood cells, and buffy coat were stored in the vapor phase of liquid nitrogen freezers. A total of 1534 women were included in this analysis, all of whom were control participants in 3 nested case-control studies of breast cancer, hypertension, and diabetes. None of the women had previously diagnosed cancer, cardiovascular diseases, or diabetes at time of blood drawing. We included only those women who had fasted overnight for ≥ 6 h. The Institutional Review Board of the Brigham and Women's Hospital in Boston approved the study.

The Nurses' Health Study II

The Nurses' Health Study II cohort was established in 1989 and comprises 116 671 female registered nurses from 14 states aged 25–44 y at the start of the study. We used methods similar to those described for the Nurses' Health Study I to obtain questionnaire information and blood samples. Blood was collected in 1997–1999 from 29 604 women. Of those women, 473 healthy women who were free of cancer, cardiovascular diseases, and diabetes were previously sampled to study the effects of alcohol consumption patterns on biologic markers. The selection criteria for that subsample were described elsewhere (26). We selected 465 women from that subsample who had a C-peptide measurement, fasted overnight for 6 h, and filled out an SFFQ in 1999 or 1995. The Institutional Review Board of the Harvard School of Public Health approved the study.

Dietary assessment

The reproducibility and validity of the SFFQs were described in detail (23, 24, 27). Participants were asked to report the average frequency of consumption of 130 food items. Standard portion sizes were listed with each food, and 9 frequency choices from "less than once a month to 6 or more times a day" were given. The specific values of each nutrient were obtained from the Harvard University Food Composition Database, which is derived from the US Department of Agriculture [*Composition of Foods* (28)], information from manufacturers, and published papers. The total nutrient intake was the sum of nutrients derived from each food.

The correlation for energy-adjusted carbohydrate intake between the SFFQ and multiple 7-d diet records was 0.76 (24), and between 2 SFFQs over a 4-y period it was 0.65 (24). The correlation between SFFQ and multiple dietary records for carbohydrate-containing foods was high and included 0.66 for potatoes, 0.71 for white bread, 0.79 for cold cereals, and 0.80 for bananas (29, 30).

Fructose is a monosaccharide, and half of the disaccharide sucrose is fructose, which is split from sucrose in the small

intestine. Therefore, total fructose intake is equal to the intake of free fructose plus half the intake of sucrose. The correlations between the intakes measured by SFFQ and diet records for the 4 top contributors of the monosaccharide fructose intake in our data set were 0.78 for orange juice, 0.84 for soft drink (cola) beverages, 0.59 for raisins, and 0.70 for apples (27).

The use of glycemic index is well documented (31). The glycemic index is a method of ranking foods on the basis of the incremental glucose response and insulin demand they produce for a given amount of carbohydrate (31). We used glycemic index to calculate the average GL during the past year for each participant by multiplying the carbohydrate content (grams per serving) for each food by its glycemic index, multiplying the product by the frequency of consumption (serving of that food per day), and summing values for all food items reported:

$$\text{Individual dietary GL} = \sum(\text{glycemic index} \\ \times \text{carbohydrate content of food} \\ \times \text{servings of food per day}) \quad (1)$$

Each unit of dietary GL represents the metabolic effect of 1 g carbohydrate from a specific food relative to the metabolic effect of 1 g carbohydrate from pure glucose.

Measurement of nondietary factors

Height, current weight, and smoking history were reported at baseline. During follow-up, data on current weight and smoking status were obtained from biennial mailed questionnaires. The correlation coefficient between self-reported weight and weight as measured by trained personnel was 0.96 (32).

Assay of C-peptide

C-peptide was measured with the use of an enzyme-linked immunosorbent assay (Michael Pollak's laboratory at the Lady Davis Research Institute of the Jewish General Hospital, Montreal, and McGill University) and by radioimmunoassay (Linco Research, St Charles, MO) at the laboratories of Robert Cohen (University of Cincinnati Medical Center, Cincinnati) and Nader Rifai (Children's Hospital, Boston). Samples were standardized on the basis of the results obtained from the same quality controls that were previously provided to each laboratory. We obtained a CV < 12%.

Statistical analysis

We used linear regression models with robust variance estimate. This variance estimator allows for valid inference without the normal distribution assumption in the dependent variable (33). For the SAS procedure, we used "proc mixed with empirical" statement, which allows us to use the robust variance model for linear regression analysis. For dietary variables treated as categorical variables (quintiles), data are presented as the adjusted mean difference in C-peptide concentrations associated with difference in each of the dietary predictors for women from quintile 1 to quintile 5. All *P* values are two-sided. We conducted all statistical analyses with SAS software (version 8; SAS Institute Inc, Cary, NC).

In multivariate models, we adjusted for age (5 categories), body mass index (BMI; in kg/m²; continuous), physical activity (quintiles), hypertension, smoking (3 categories), hours since last meal, laboratory batch, menopausal status (premenopausal,

TABLE 1

Age-standardized characteristics by quintile (Q) of percentage of energy from free fructose intake and quintile of energy-adjusted glycemic load in 1999 women who participated in the Nurses' Health Study I and II¹

	Percentage of energy from free fructose			<i>P</i> for trend	Energy-adjusted glycemic load			<i>P</i> for trend
	Q1 (<i>n</i> = 399; median: 2.8%)	Q3 (<i>n</i> = 400; median: 4.9%)	Q5 (<i>n</i> = 400; median: 7.8%)		Q1 (<i>n</i> = 450; median: 84)	Q3 (<i>n</i> = 421; median: 106)	Q5 (<i>n</i> = 346; median: 133)	
Age (y) ²	50	54	56		52	54	55	
C-peptide (ng/mL)	1.96 ± 0.06 ³	1.95 ± 0.06	1.97 ± 0.06	0.7	2.01 ± 0.06	1.92 ± 0.06	1.84 ± 0.06	0.005
BMI (kg/m ²)	26.2 ± 0.2	25.2 ± 0.2	25.4 ± 0.2	0.02	26.0 ± 0.2	25.5 ± 0.2	24.9 ± 0.2	0.0001
Current smoker (%)	16.1 ± 0.02	10.9 ± 0.02	9.4 ± 0.02	0.0005	17.9 ± 0.02	8.6 ± 0.02	6.6 ± 0.02	<0.001
Pre-menopausal women (%)	41.9 ± 0.02	39.9 ± 0.02	34.5 ± 0.02	0.001	41.4 ± 0.02	40.6 ± 0.02	36.3 ± 0.02	0.03
Hypertension (%)	11.6 ± 0.02	10.9 ± 0.02	11.8 ± 0.02	0.6	11.3 ± 0.02	12.7 ± 0.02	11.1 ± 0.02	1.0
Physical activity (METs/wk)	14.3 ± 1.4	16.4 ± 1.4	19.8 ± 1.4	0.002	16.3 ± 1.4	17.9 ± 1.4	17.9 ± 1.5	0.3
Alcohol (g/d)	9.7 ± 0.5	6.6 ± 0.5	4.6 ± 0.5	<0.001	12.9 ± 0.5	5.7 ± 0.5	2.9 ± 0.5	<0.001
Daily dietary intake								
Total energy (kcal)	1754 ± 26	1804 ± 26	1826 ± 26	0.1	1762 ± 24	1847 ± 25	1728 ± 27	0.3
Energy from fat (%)	34.9 ± 0.3	30.8 ± 0.3	26.7 ± 0.3	<0.001	35.5 ± 0.2	31 ± 0.2	24.7 ± 0.3	<0.001
Energy from protein (%)	19.7 ± 0.2	19 ± 0.2	17.4 ± 0.2	<0.001	20.3 ± 0.2	18.7 ± 0.2	16.8 ± 0.2	<0.001
Energy from carbohydrate (%)	43 ± 0.3	50 ± 0.3	57.1 ± 0.3	<0.001	40.4 ± 0.2	50.5 ± 0.2	60.5 ± 0.2	<0.001
Energy from sucrose (%)	7.5 ± 0.2	8.8 ± 0.2	10.4 ± 0.2	<0.001	6.6 ± 0.1	9.1 ± 0.1	11.1 ± 0.1	<0.001
Energy from free fructose (%)	2.7 ± 0.05	4.9 ± 0.05	8.5 ± 0.05	<0.001	3.8 ± 0.1	5.2 ± 0.1	7.2 ± 0.1	<0.001
Energy from total fructose ⁴ (%)	6.4 ± 0.1	9.3 ± 0.1	13.7 ± 0.1	<0.001	7.0 ± 0.1	9.7 ± 0.1	12.7 ± 0.1	<0.001
Glycemic index	52.2 ± 0.2	52.6 ± 0.2	53.4 ± 0.2	<0.001	49.8 ± 0.2	52.7 ± 0.2	55.5 ± 0.2	<0.001
Glycemic load	93 ± 0.99	108 ± 0.98	124 ± 0.98	<0.001	81.6 ± 0.49	109 ± 0.50	139 ± 0.55	<0.001
Cholesterol (mg)	248 ± 3.4	224 ± 3.4	191 ± 3.4	<0.001	265 ± 3	218 ± 3	168 ± 3	<0.001

¹ METs, metabolic equivalent tasks. Glycemic index, glycemic load, and cholesterol intake were energy adjusted by using the residual method.

² Not standardized.

³ $\bar{x} \pm SE$ (all such values).

⁴ Free fructose + sucrose/2.

postmenopausal), and dietary variables, including cholesterol (quintiles), protein (quintiles), total energy (quintiles), and alcohol consumption (5 categories). Because hypertension and alcohol consumption (inversely) are associated with C-peptide concentrations and insulin resistance (21, 34), these 2 variables were included in the multivariate model.

We adjusted all micronutrients and GL for total energy intake with the use of the residual method (35). In addition, when examining the effect of substituting carbohydrate for fat, we used multivariate nutrient-density models (24) that simultaneously included energy intake, the percentage of energy from protein and carbohydrate, and other confounding variables. Similarly, we used the nutrient density model to assess the effect of fructose intake. We used the SFFQs collected at the time closest to the time of blood draw. For the Nurses' Health Study I, blood was taken in 1989–1990, and we used the 1990 SFFQ. For the Nurses' Health Study II, blood was collected in 1997, and we used the average of 1995 and 1999 SFFQs. If a woman's SFFQ was missing, we used the most recent available SFFQ and created an indicator variable set to 1 for these data in multivariate analyses. A total of 232 women were missing the most recent SFFQ.

Because insulin resistance is particularly pronounced in people who are overweight, we repeated several analyses stratified by BMI. To evaluate statistical interaction, we created a new term as the product of dichotomized BMI and dietary predictors (continuous). In the model with main effects and the interaction term, we used the Wald test *P* value for the interaction term to determine the statistical significance for interaction.

RESULTS

Among the 1999 women in this analysis, subjects in the lowest quintile consumed 2.8% of energy from free fructose compared with 7.8% of energy for women in the highest quintile. Women who consumed more free fructose were less likely to smoke and to drink alcohol and more likely to be older, to be postmenopausal, and to be physically active. Free fructose consumption was positively associated with intake of total energy, carbohydrate, sucrose, and GL and inversely associated with intake of total fat, protein, and cholesterol (**Table 1**). BMI was slightly inversely associated with fructose intake. The overall pattern for the associations between GL and other covariates was similar. For example, smoking and alcohol were negatively associated with GL.

The age- and BMI-adjusted (also adjusted for fasting hours and laboratory batch) C-peptide concentrations were 8% higher among women in the highest quintile of free fructose intake than in women in the lowest quintile (**Table 2**). The association was stronger in multivariate analysis (13.9%) and after further adjustment for total carbohydrate intake (20.5%). The associations between total fructose (including the fructose in sucrose) and C-peptide were similar (**Table 2**). The association between sucrose and C-peptide was not significant in multivariate models (data not shown). GL was positively associated with C-peptide in the multivariate model adjusted for total fat or carbohydrate intake, although *P* for trend was only marginally significant after adjustment for total fat in the multivariate analysis. Higher intake

TABLE 2

Changes in fasting plasma C-peptide concentrations from quintile 1 to quintile 5 associated with the change from quintile 1 to quintile 5 in each of the dietary predictors in women¹

Dietary predictor	Change in plasma C-peptide ²	<i>P</i> for trend
	%	
Free fructose ³		
Age and BMI adjusted ⁴	8.0	0.2
Multivariate adjusted ⁵	13.9 (0.002)	0.01
Multivariate plus carbohydrate ⁶ adjusted	20.5 (<0.0001)	0.0008
Total fructose ⁷		
Age and BMI adjusted	3.7	0.8
Multivariate adjusted	13.4 (0.007)	0.08
Multivariate plus carbohydrate adjusted	21.3 (0.0007)	0.01
Glycemic load ⁸		
Age and BMI adjusted	-2.1	0.2
Multivariate adjusted	4.8	0.6
Multivariate plus total fat ⁹ adjusted	14.1	0.09
Multivariate plus carbohydrate adjusted	16.1 (0.02)	0.04
Carbohydrate		
Age and BMI adjusted	-8.8 (0.01)	0.008
Multivariate adjusted	-4.2	0.4
Multivariate plus glycemic load adjusted	-14.2 (0.04)	0.04

¹ For free fructose, total fructose, glycemic load, and carbohydrate, quintiles 5 and 1 were 7.8% and 2.7%, 13.3% and 6.1%, 133.1 and 83.9, and 60.0% and 40.1%, respectively.

² *P* values in parentheses. *P* values are presented only when the *P* for trend was significant.

³ Percentage of energy from free fructose.

⁴ Model included laboratory batches and fasting hours.

⁵ Model included adjustment for dietary factors in quintiles [total energy intake, alcohol intake (0, 0.1–4.9, 5–14.9, 15–29.9, and ≥30 g/d), percentage of energy from protein, and energy-adjusted cholesterol intake], and other confounding factors [age (5 categories), smoking (3 categories: never, past, and current), BMI (continuous), physical activity (quintiles), hypertension, fasting hours, laboratory batches, and menopausal status (premenopausal or postmenopausal)].

⁶ Percentage of energy from carbohydrate.

⁷ Percentage of energy from total fructose (free fructose + sucrose/2).

⁸ Energy adjusted by using residual method.

⁹ Percentage of energy from total fat.

of carbohydrate was inversely associated with C-peptide concentrations after adjustment for GL. Women in the highest quintile of carbohydrate had 14.2% lower C-peptide concentrations

than women in the bottom quintile (*P* for trend = 0.04). We also examined the association between total protein, total fat, and *trans* fatty acids in relation to C-peptide. No association was seen between total protein (animal or vegetable protein) and C-peptide in the multivariate-adjusted model, and no relation was seen between total fat or *trans* fatty acid and C-peptide in the multivariate-adjusted model. BMI was a strong confounder, but the results were similar whether we treated BMI as a continuous variable or as deciles. When BMI was treated as a continuous variable in the multivariate model, we found that every unit increase in BMI was associated with a 0.1 ng/mL increase in C-peptide (*P* < 0.0001). This finding provides an interval validity, because BMI is strongly associated with insulin resistance.

We further examined the association between dietary predictors and plasma C-peptide concentration within strata of BMI (<25 and ≥25). *P* values for interaction were not significant for any of the dietary predictors with BMI strata (<25 and ≥25). The absolute difference for fructose and GL was slightly greater in the overweight group. In the overweight group, the overall range was above normal; thus, even a small increase might have a substantial effect. We also reexamined these associations by using a different cutoff point for BMI (<30 or ≥30) and obtained similar results.

To explore the foods that might contribute to these associations we examined the relation between carbohydrate-contributing foods (Table 3), fructose-contributing foods (Table 4), and different sources of fiber in relation to plasma C-peptide concentrations. Among the top fructose-contributing foods in our SFFQ, a positive association with C-peptide was observed only for the caffeine-containing beverages and fruit punch. In contrast, concentrations of C-peptide were lower among women who consumed ≥1 serving of “ready-to-eat” cereal/d compared with women who consumed <1 serving/wk. Among sources of dietary fiber (cereal, vegetable, fruit), only cereal fiber was significantly associated with C-peptide in the multivariate analysis. Women in the highest quintile of cereal fiber (median concentration: 9 g/d) had 15.6% lower C-peptide concentrations than women in the lowest quintile (median concentration: 2 g/d; *P* for trend = 0.03).

DISCUSSION

In this large cross-sectional study of diet and C-peptide concentrations among premenopausal and postmenopausal women,

TABLE 3

Changes in C-peptide concentrations associated with the change in intake of carbohydrate-contributing foods from 0 servings/wk in women¹

		Servings of carbohydrate-contributing foods ²					<i>P</i> for trend
		0/wk	<2/wk	2–4/wk	≥5–6/wk	≥1/d	
		%					
Mashed potatoes	Ref	3.9	7.4				0.4
Ready-to-eat cereal	Ref	-5.6 (0.2)	-3.0 (0.5)	-6.8 (0.2)	-7.4 (0.09)		0.004
Skim milk	Ref	-2.8	-2.1	-4.3			0.5
Banana	Ref	-5.2	-2.7	-7.3			0.3
Orange juice	Ref	5.3	2.7	3.1			0.3

¹ Data were adjusted for dietary factors in quintiles [total energy intake and alcohol intake (0, 0.1–4.9, 5–14.9, 15–29.9, and ≥30 g/d), and other demographic factors [age (5 categories), smoking (3 categories: never, past, and current), BMI (continuous), physical activity (quintiles), hypertension, fasting hours, laboratory batches, and menopausal status (premenopausal or postmenopausal)].

² *P* values in parentheses. *P* values are presented only when the *P* for trend was significant.

TABLE 4

Changes in C-peptide concentrations associated with the change in intake of fructose-contributing foods from 0 servings/wk in women¹

	Servings of fructose-contributing foods ²				<i>P</i> for trend
	0/wk	<1/wk	≥1/wk	≥2–4/wk	
			%		
Orange juice	Ref	5.3	2.7	3.1	0.3
Apple	Ref	–2	2.6	1.4	0.3
Caffeine-containing beverages	Ref	2.4 (0.5)	11.5 (0.005)		0.05
Raisin	Ref	2.2 (0.5)	2.5 (0.5)	–4.4 (0.2)	0.05
Punch	Ref	3.8 (0.3)	5 (0.2)		0.05

¹ Data were adjusted for dietary factors in quintiles [total energy intake and alcohol intake (0, 0.1–4.9, 5–14.9, 15–29.9, and ≥30 g/d), and other demographic factors [age (5 categories), smoking (3 categories: never, past, and current), BMI (continuous), physical activity (quintiles), hypertension, fasting hours, laboratory batches, and menopausal status (premenopausal or postmenopausal)].

² *P* values in parentheses. *P* values are presented only when the *P* for trend was significant.

dietary fructose intake and GL were positively associated with C-peptide concentrations, whereas cereal fiber was inversely associated with C-peptide. In the early 1960s, fructose was considered beneficial because it was thought to stimulate lower postprandial plasma concentrations of insulin and glucose (7). However, more recently, results from experimental studies in animal models and from short-term feeding trials among humans suggest that higher fructose intake contributes to insulin resistance, impaired glucose tolerance, and hyperinsulinemia (9–12). For example, Thorburn et al (10) fed rats a diet containing 35% of energy as fructose for 4 wk and found reduced insulin sensitivity and whole-body glucose disposal, whereas comparable amounts of starch had no observable effects. Results were similar in rats fed for longer periods (15 mo) with lower amounts of fructose (9). In human studies, Beck-Nielsen et al (13) showed a reduction in insulin binding and insulin activity among healthy subjects fed 1000 extra kcal as fructose for 7 d, whereas intake of 1000 extra kcal glucose had no similar adverse effects. In another study by Hallfrisch et al (14), intake of 15% of total energy as fructose for 5 wk resulted in higher insulin and glucose responses than isocaloric diets with 7.5% of energy from fructose or no fructose. In contrast, among patients with type 2 diabetes, Bantle et al (15) and Osei et al (16) found that fructose reduced blood glucose concentrations in comparison with either isocaloric sucrose or isocaloric diabetic diets in patients with diabetes.

Several mechanisms were proposed to explain the relation between fructose intake and insulin resistance (6). First, an increase in fructose consumption leads to positive energy balance, which might contribute excess body weight (36, 37). Excess adiposity is associated with higher concentration of nonesterified fatty acids (38), which might reduce insulin sensitivity by increasing the intramyocellular lipid content in muscle cells where insulin receptors are located (39). Second, an increased supply of nonesterified fatty acids can lead to an increase in triacylglycerol concentrations, which is associated with reduced insulin sensitivity (40). Furthermore, studies showed that fructose intake increased plasma triacylglycerol concentrations compared with other types of sugar (41–43), and fructose intake resulted in weight gain (36, 37). Taken together, these results suggest high intake of fructose over time might deteriorate insulin sensitivity and promote the development of type 2 diabetes.

We found a positive association between C-peptide concentration with GL and an inverse association with carbohydrates. GL and carbohydrate intake are highly correlated ($r = 0.83$), but

with a large sample size we were able to assess independent associations.

Theoretically, we should observe the effect of GL without controlling for the amount of carbohydrate in the multivariate analysis because GL is a measurement of both the quality and quantity of carbohydrate. However, carbohydrates include components in addition to those contributing to GL. Foods that contribute carbohydrates can be whole grains, refined grains, fruit, and vegetables. If the GL is the same for a woman who consumes mashed potatoes (glycemic index = 102) as for a woman who eats raisin bran cereal (glycemic index = 88) (44), the carbohydrate content of cereal must be 16% higher than that for the mashed potatoes because of the 16% higher glycemic index of mashed potatoes. The food with the additional carbohydrate contains more fiber, vitamins, minerals, and phytochemicals. Therefore, in a model that simultaneously includes GL and carbohydrate, an increase in GL holding carbohydrate constant represents proportionally more carbohydrate from high-GL foods; whereas, carbohydrate holding GL constant represents foods higher in fiber, micronutrients, minerals, and antioxidants. The combination of these components might work synergistically to lower the risk of diabetes (45, 46). The inverse association we observed between intake of cereal fiber and C-peptide concentrations supports our interpretation.

In an additional approach to examine the association between GL and C-peptide, we added total fat (which is not highly correlated with GL) instead of total carbohydrate to the multivariate model. We found a similar positive association between GL and C-peptide. In this particular model, GL represents a true comparison of a high-GL diet with a low-GL diet when holding constant total calories, fat, protein, and implicitly total carbohydrate.

We found that a high consumption of cereals and cereal fiber was inversely associated with C-peptide concentrations, whereas a high consumption of mashed potatoes was positively associated with C-peptide concentrations. Both findings support the possible contribution of a high-GL diet to insulin resistance and of the possible protection by whole-grain foods. Previously, in the Nurses' Health Study and in the Health Professionals Follow-Up Study, we reported a positive association for GL and a negative association for cereal fiber in relation to the incidence of diabetes in both women and men (19, 20). The Iowa Women's Health Study failed to show an association between GL and diabetes (21). The Iowa study might contain more measurement

error in the assessment of dietary intake, because the food-frequency questionnaire was completed only once, and the change in diet during the course of follow-up was not assessed. Several other observational and interventional studies reported the dual benefit of low-GL diets and diets high in whole grains on improved glycemic control and decreased plasma insulin concentrations (1, 10, 47). Kiens et al (22) also were unable to show that high-GL diets increase insulin concentration and increase blood glucose compared with low-GL diets after 30 d of intervention; however, their study included only 7 subjects (young, healthy men) and the high-GL diets might exert their adverse effect later in life.

Some limitations and strengths were found in our study. Because of its cross-sectional design, a causal relation cannot be established. The positive association between total fructose (including free fructose and fructose from sucrose, a disaccharide) and C-peptide was not stronger than the positive association between free fructose and C-peptide. This finding suggests that the fructose in disaccharides (sucrose) might not add greater adverse effect. However, the mechanism for this is not clear. Nevertheless, the large sample size of this study enables us to tease out the independent association of highly correlated covariates such as GL and carbohydrate. The suggested adverse effect of fructose could reflect the harmful effect of the excess of caloric intake from soft drinks. However, we have controlled for total caloric intake in our multivariate model and also especially for the amount of calories from protein and carbohydrate.

In conclusion, we found a significant positive association between high-fructose diets and C-peptide concentrations. The association was independent of total carbohydrate quantity and quality. These results suggest potentially adverse metabolic effects of fructose as sweeteners to soft drinks or other foods, and they indicate a strong need for more research in this area. Furthermore, high glycemic foods and the overall GL of the diet also were associated with higher C-peptide concentrations. These results lend further support to dietary guidelines to replace refined starch with whole grains (28).

TW performed the study design, data collection, and data analysis and wrote the first draft of the manuscript. EG, TP, SEH, JM, NR, and EBR participated in the study design, data collection, and data analysis. The authors had no conflicts of interest.

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