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Acute Effects of Dietary Glycemic Index on Antioxidant Capacity in a Nutrient-controlled Feeding Study

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Abstract

Oxidative stress, caused by an imbalance between antioxidant capacity and reactive oxygen species, may be an early event in a metabolic cascade elicited by a high glycemic index (GI) diet, ultimately increasing the risk for cardiovascular disease and diabetes. We conducted a feeding study to evaluate the acute effects of low-GI compared with high-GI diets on oxidative stress and cardiovascular disease risk factors. The crossover study comprised two 10-day in-patient admissions to a clinical research center. For the admissions, 12 overweight or obese (BMI: 27–45 kg/m²) male subjects aged 18–35 years consumed low-GI or high-GI diets controlled for potentially confounding nutrients. On day 7, after an overnight fast and then during a 5-h postprandial period, we assessed total antioxidant capacity (total and perchloric acid (PCA) protein-precipitated plasma oxygen radical absorbance capacity (ORAC) assay) and oxidative stress status (urinary F₂ α -isoprostanes (F₂IP)). On day 10, we measured cardiovascular disease risk factors. Under fasting conditions, total antioxidant capacity was significantly higher during the low-GI vs. high-GI diet based on total ORAC (11,736 $\pm$ 668 vs. 10,381 $\pm$ 612 μ mol Trolox equivalents/l, P = 0.002) and PCA-ORAC (1,276 $\pm$ 96 vs. 1,210 $\pm$ 96 μ mol Trolox equivalents/l, P = 0.02). Area under the postprandial response curve also differed significantly between the two diets for total ORAC and PCA-ORAC. No diet effects were observed for the other variables. Enhancement in plasma total antioxidant capacity occurs within 1 week on a low-GI diet, before changes in other risk factors, raising the possibility that this phenomenon may mediate, at least in part, the previously reported effects of GI on health.

INTRODUCTION

The rise of blood glucose in the postprandial period, as quantified by the glycemic index (GI), has been linked to risk for cardiovascular disease and diabetes in prospective observational studies (1,2). Interventional studies have suggested that high-GI compared with low-GI diets may cause insulin resistance, elevated triglyceride concentrations, lower high-density lipoprotein-cholesterol concentration, higher C-reactive protein concentration, and higher plasminogen activator inhibitor-1 (PAI-1) concentration (3–6). One pathophysiological event that may mediate these adverse effects is oxidative stress (7).

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Hyperglycemia causes overproduction of superoxide anions by the mitochondrial electron transport chain that, in turn, generates reactive oxygen species by numerous metabolic pathways (8). Among individuals with or without diabetes, acute elevation in blood glucose during an oral glucose-tolerance test or hyperglycemic clamp increases measures of oxidative stress and lowers antioxidant concentrations in serum (9–11). Over the long-term, oxidative stress has been demonstrated to produce insulin resistance (12), pancreatic β -cell dysfunction (13,14), abnormal serum lipids (15), coagulopathy (16), systemic inflammation (17), and endothelial dysfunction (18).

The aim of this study was to examine the effects of dietary GI on antioxidant capacity, oxidative stress, and risk factors for cardiovascular disease and diabetes in overweight and obese, healthy individuals. Specifically, we hypothesized that compromised antioxidant capacity in response to postprandial hyperglycemia would occur within 1 week of beginning a high-GI diet. In designing this study, we recognized the controversial nature of the GI (19,20) and the variability of published reports on the topic (21–24). Previous positive studies have been criticized for not adequately considering the possibility of confounding by other dietary factors, whereas negative studies have been criticized for potentially lacking treatment fidelity. Thus, we employed a tightly controlled, crossover feeding protocol involving continuous in-hospital observation, with careful attention to process measures.

METHODS AND PROCEDURES

Overview

The study comprised two 10-day in-patient admissions to a clinical research center and was implemented using a crossover design. For the duration of each admission, subjects consumed a low- or high-GI diet. The dietary interventions were presented in random order, with a 2- to 12-week washout period between admissions. Study outcomes were assessed on days 7 (total antioxidant capacity, oxidative stress, endothelial function, and nitric oxide production) and 10 (insulin sensitivity, β -cell function, blood lipids, PAI-1, fibrinogen, and C-reactive protein) of each admission. Blood pressure was measured daily. The institutional review boards at Brigham and Women's Hospital and Children's Hospital Boston approved the protocol. Each subject provided written informed consent prior to enrollment. Data were collected between March 2003 and June 2006.

Subjects

Subjects were recruited using fliers and posters, newspaper advertisements, and Internet listings. Inclusion and exclusion criteria were evaluated during an in-person interview and physician-conducted physical examination. Height was measured using a stadiometer (Harpenden; Seritex, East Rutherford, NJ), and baseline weight was measured using a balance beam scale (Seca, Hanover, MD). Inclusion criteria included male gender, age between 18 and 35 years, BMI between 27 and 45 kg/m², and willingness to abstain from consuming alcohol and caffeinated beverages for the duration of the study. Exclusion criteria included current smoking, adherence to a special diet, allergies or aversions to foods on the study menus, use of medications that could affect body weight or study outcomes, diabetes mellitus (fasting plasma glucose $\geq$ 126 mg/dl (7 mmol/l)), and any other indication of a major medical illness based on a physical examination and laboratory screening tests (complete blood cell count, thyroid hormones, glycosylated hemoglobin, and liver function tests). Of 22 individuals who were screened for participation, 16 met these criteria, anticipated that they could complete the two 10-day hospital admissions, and were enrolled in the study. To characterize the diversity of the subject population, race/ethnicity was assessed by subject self-report according to investigator-defined categories. Subjects were paid \$1,500 for completing the study.

Randomization

The order of the two diets for each subject was determined after enrollment by opening the next in a series of sealed envelopes prepared by the study statistician. The sequence of assignments was computer-generated and balanced equally with respect to order of diets. To ensure that the order of diets for an upcoming subject was not predictable by study staff, the assignments were randomly permuted within blocks of two, four, and six, and the blocks themselves were randomly permuted.

Dietary interventions

The energy requirement of each subject was estimated using the Harris—Benedict equation with an activity factor of 1.4. The estimated requirement was adjusted for body frame size, based on elbow breadth measured using an intercondylar caliper (Holtain, Croswell, UK). Body weight was monitored daily using an electronic scale (Model 5002; Scale-Tronix, Wheaton, IL). The energy content of the diet was adjusted if body weight deviated from baseline weight by >2%.

Each intervention had a single daily menu throughout the 10-day study period (Table 1), calculated using the Food Processor software (v8.3, ESHA Research, Salem, OR). Energy distribution across meals was 25% for breakfast, 30% for lunch, 30% for dinner, and 15% for snack. Macronutrient composition was controlled between diets and across meals, with each containing 60% of energy from carbohydrate, 25% from fat, and 15% from protein. GI was contrasted between diets by selecting carbohydrate-containing foods based on food form (uncooked cornstarch vs. corn syrup), food processing (pasta vs. instant potatoes), and type of cereal (Guardian vs. Bran Flakes, Kellogg's Australia). Uncooked cornstarch has been shown to prevent hypoglycemia in patients with type 1 glycogen storage disease at doses equal to calculated requirements, indicating that the carbohydrate in this product is functionally available (25). Seventy percent of the carbohydrate in each meal came from these contrasting sources. Fiber intake per 2,000 kcal was 28.4 g for the low-GI diet and 26.4 g for the high-GI diet. Intakes of β -carotene (552 vs. 521 μ g), vitamin E (7.2 vs. 7.0 IU), and vitamin C (70 vs. 50 mg) per 2000 kcal were similar, considering the limits of accuracy of our analytic methods. The breakfasts used for the postprandial studies involving oxidative stress were especially closely matched in nutrient composition and food sources, with the difference in GI arising entirely from the difference in solubility and digestion rate between uncooked cornstarch and corn syrup.

Results of a preliminary study indicated that measured GI, based on a white bread standard (26), varied between diets when contrasting sources of carbohydrate were incorporated into mixed meals (data not shown). For example, the breakfast meal with uncooked cornstarch had a GI of 47, and the meal with corn syrup had a GI of 104. The dinner meal with pasta had a GI of 30, and the meal with instant potatoes had a GI of 63. The Guardian and Bran Flakes snacks had GI values of 58 and 70, respectively.

To ensure consistency in delivering the dietary interventions, recipes were standardized and each menu item was weighed to within 0.1 g for portions <10 g or within 1 g for portions $\geq$ 10 g. Subjects were instructed to consume all foods and beverages provided and not to eat or drink anything else, except water and diet ginger ale. Fluid intake was $\geq$ 2,500 ml/day. Sodium intake was 150 mEq/day to mimic a typical Western diet (27) and thereby avoid fluid shifts that could cause fluctuations in body weight during hospital admissions. A spatula was provided with each meal to facilitate consumption of all served food. Research staff monitored dietary compliance. Subjects had two daily 20-min walks in the clinical research center, supervised by nurses.

Body composition

Body composition was measured by dual-energy X-ray absorptiometry using Hologic instrumentation (Models QDR-4500 and Discovery A; Hologic, Waltham, MA) during day 2 of the first admission. Body fat percentage was calculated as the proportion of fat mass to total mass.

Study outcomes

Study outcomes were assessed following a 10-h overnight fast. In addition, antioxidant capacity, oxidative stress, endothelial function, and nitric oxide production were measured during a 5-h postprandial period following standard breakfast meals (Table 1). Plasma glucose and serum insulin concentrations, assessed at 30-min intervals during the postprandial studies, were collected as process data. Blood samples were drawn by indwelling intravenous catheter and centrifuged within 30 min of each draw. All specimens were stored in cryovials at -80°C until analysis. Nurses and technicians who collected or analyzed the specimens were masked to the order of the dietary interventions.

Total antioxidant capacity—The oxygen radical absorbance capacity (ORAC) assay of plasma measures resistance against oxidative damage, reflecting the combined effects of all circulating antioxidants (28). To determine ORAC, heparinized plasma was obtained prior to the meal and at 2.5 and 5 h after the meal. Each sample was divided in aliquots for analysis of total plasma and protein-precipitated plasma obtained by treatment with perchloric acid (PCA, 1:1 vol:vol) and then centrifuging at 100,000 g for 10 min at 4°C and using the supernatant in the assay. The assay was conducted with an automated plate reader, using a modification of the protocol described by Huang *et al.* (29). Fluorescein (3',6'-dihydrospiro[isobenzofuran-1 [3H], 9'[9H]-xanthen-3-one], a synthetic probe sensitive to oxidative damage, was added to heparinized plasma samples and then oxidized at 37°C using 2,2'-azobis (2-amidinopropane) dihydrochloride, a peroxy radical generator. Oxidation of fluorescein was monitored spectrofluorometrically (485 nm excitation, 520 nm emission) at 2-min intervals for 70 min. A water-soluble vitamin E analogue, Trolox, was used to establish a standard curve; assay results are expressed as μmol Trolox equivalents/l. The interassay coefficient of variation is 8.7% for total ORAC and 8.3% for PCA-ORAC.

Oxidative stress status— $\text{F}_{2\alpha}$ -isoprostanes (F_2IP), a family of arachidonic acid a peroxidation products formed in phospholipids, released into the circulation, and excreted in urine, serve as a bio-marker of oxidative stress status. To assess postprandial excretion of F_2IP , urine was collected prior to each breakfast test meal and at the end of the 5-h postprandial period. According to the method of Walter *et al.* (30), an internal standard (prostaglandin $\text{F}_{1\beta}$) was added to samples that were acidified to pH 3.0 with HCl. F_2IP were then separated by extraction with ethyl acetate, and the combined ethyl acetate layers were dried under nitrogen. Pentafluorobenzyl bromide and diisopropylethylamine were added to the residue to prepare pentafluorobenzyl esters of the F_2IP that were then separated by high-performance liquid chromatography, silylated, and analyzed by gas chromatography/mass spectroscopy. The F_2IP were separated and detected with a mass selective detector run in negative chemical ionization mode.

Urine creatinine was measured with the Olympus creatinine reagent OSR6178 and an Olympus AU400e analyzer (Olympus America, Melville, NY) using a kinetic modification of the Jaffe procedure in which creatinine reacts with picric acid and alkaline pH to form a yellow—orange complex. The rate of change in absorbance at 520/800 nm is proportional to the creatinine concentration of the sample at 37°C and expressed at mg/dl. Results for F_2IP are expressed relative to creatinine excretion (ng/mg creatinine).

Endothelial function—Flow-mediated, endothelium-dependent vasodilation of the brachial artery was measured to assess endothelial function before and at 1, 3, and 5 h following consumption of the standard breakfast during respective diets. Subjects were studied in a quiet, dimly lit room after resting supine for at least 5 min. At each time point, brachial artery diameter was measured using ultrasonography. An ultrasound scanner (Toshiba Powervision 8000; Toshiba America Medical Systems, Tustin, CA) equipped with a high-resolution linear array transducer (7.5 MHz) was used to obtain longitudinal images of the brachial artery just proximal to the antecubital fossa. Transducer position was adjusted to obtain optimal images of the near and far wall of the intima. The “R” wave on an electrocardiogram was used as an indicator for acquiring data at end diastole. After baseline image acquisition, a sphygmomanometric cuff placed on the upper arm was inflated to suprasystolic pressure (200 mm Hg) for 5 min. On cuff release, reactive hyperemia causes flow to increase six- to tenfold through the brachial artery. Flow-mediated, endothelium-dependent vasodilation of the brachial artery was determined by calculating the percent change in the brachial artery diameter between the baseline image and the image obtained 1 min after cuff deflation.

Nitric oxide production—Nitric oxide (NO) was measured in serum samples obtained prior to and at 2.5 and 5 h after the meal. To reduce background absorbance due to hemoglobin and improve color formation with the Griess reagents, samples were ultrafiltered through a 10 kDa molecular weight cut-off filter (Microcon YM-10; Millipore, Billerica, MA) prerinsed with ultrapure water, by centrifuging at 16,000 g in an Eppendorf 5415C microcentrifuge (Eppendorf, Westbury, NY). The final products of NO metabolism *in vivo* are nitrate and nitrite, so their sum concentration (NO_x) provides an index of NO production. Samples were analyzed using the Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI) in a 2-step process: conversion of nitrate to nitrite by nitrate reductase and addition of Griess reagent to convert nitrite to an azo compound that can be measured photometrically at 540 nm with a plate reader. Results are expressed as $\mu\text{mol/l}$.

Insulin sensitivity and β -cell function—A frequently sampled intravenous glucose-tolerance test was conducted to evaluate insulin sensitivity and β -cell function (i.e., acute insulin response). Subjects received an infusion of 0.3 g/kg body weight of 25% dextrose solution over a 2-min period. A bolus of insulin (0.03 IU/kg) was given 20 min after the administration of glucose. Blood samples for analysis of plasma glucose and serum insulin concentrations were drawn at the following times relative to the start of the dextrose infusion: -10, -5, -1, 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25, 28, 30, 32, 36, 40, 44, 48, 50, 52, 56, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 min. Glucose concentration was measured using an enzymatic reference method with hexokinase (COBAS Integra 400; Roche Diagnostics, Indianapolis, IN). Insulin concentration was measured by an ultrasensitive assay in a paramagnetic particle, chemiluminescence immunoassay using the Access Immunoassay System (Beckman Coulter, Chaska, MN). Specialized mathematical modeling software (MinMod Millennium, v 6.02; Los Angeles, CA) was used to calculate parameters of interest (insulin sensitivity and acute insulin response).

Other cardiovascular disease risk factors—Fasting blood samples were drawn for analysis of lipids, PAI-1, fibrinogen, and C-reactive protein. Blood lipids were measured on the Hitachi 917 analyzer (Roche Diagnostics). PAI-1 was measured by an enzyme-linked immunosorbent assay (American Diagnostica, Greenwich, CT). Fibrinogen was determined using an immunoturbidimetric assay on the Hitachi 917 analyzer, with reagents and calibrators from Kamiya Biomedical (Seattle, WA). C-reactive protein was measured by particle-enhanced turbidimetric assay using a COBAS Integra 700 analyzer (Roche Diagnostics).

Blood pressure was measured every morning during the 10-day admission. Two readings were obtained from the right arm using an automated system (Dinamap; Criticon, Tampa, FL) after

the subject sat quietly for 5 min with feet flat on the floor. Results are based on the average of all readings from the final 7 days of each admission.

Statistical analysis

Area under the postprandial response curves was calculated by the trapezoidal rule. For glucose and insulin, we calculated the area above baseline over 2 h. For other variables (urinary F₂IP, endothelial function, and serum NO_x), we obtained the full area under the curve over 5 h. We compared outcomes between the low- and high-GI diets by analysis-of-variance techniques appropriate to the crossover design (31). The difference between diets was calculated for each subject and the mean difference compared to zero with adjustment for the sequence of diets. The absence of carryover effects from one diet to the next was verified by testing for association between the order of diets and the sum of the two measures. In a corroborative analysis of postprandial endothelial function, the diet differences were calculated at each time point and analyzed by repeated measures analysis of variance, adjusted for sequence, with a random effect accounting for intrasubject correlation. Computations were performed with SAS statistical software (v.9.1; SAS Institute, Cary, NC).

RESULTS

Of the 16 subjects, 12 completed the study for an overall retention rate of 75%. The characteristics (mean $\pm$ s.d.) of these subjects were as follows: age, 29.4 ± 4.4 years; weight 106.3 ± 15.6 kg; height, 177.1 ± 7.3 cm; and body fat, $29.1 \pm 4.3\%$. Regarding race and ethnicity, six subjects were white (three Hispanic and three non-Hispanic), and six subjects were black (one Hispanic and five non-Hispanic). Of the four subjects who withdrew from the study, one changed his residence and was not able to travel to the study site, two did not complete the first 10-day hospital admission because they did not want to consume the study meals, and one completed the first admission (high-GI diet) but decided not to complete the second admission.

Postprandial glycemia and insulinemia differed as intended between the low- and high-GI breakfasts (Figure 1). Area under the glucose curve above baseline was 2.2-fold greater ($P < 0.001$), and area under the insulin curve above baseline was 1.8-fold greater ($P = 0.001$) during the 2 h after the high-GI breakfast in comparison to the low-GI breakfast. Moreover, blood glucose nadir was lower after the high- vs. low-GI breakfast (-15 ± 2 mg/dl vs. -6 ± 2 mg/dl, mean $\pm$ s.e. compared to baseline, $P = 0.004$).

Effects of the dietary interventions on variables measured under fasting conditions and during the postprandial period on day 7 of respective admissions are presented in Table 2. Under fasting conditions, antioxidant capacity was significantly higher during the low- vs. high-GI diet based on plasma total ORAC ($P = 0.002$) and PCA-ORAC ($P = 0.02$). Area under the postprandial response curve also differed significantly between the two diets for plasma total ORAC ($P = 0.02$) and PCA-ORAC ($P = 0.03$). No diet effects were observed for urinary F₂IP, endothelial function, or NO_x under fasting conditions or during the postprandial period. Repeated-measures analysis of postprandial endothelial function showed no diet difference in the pattern of response ($P = 0.38$) or at any particular time point ($P \geq 0.18$).

Variables calculated from the frequently sampled intravenous glucose tolerance test and other cardiovascular disease risk factors assessed on day 10 of respective admissions are presented in Table 3. The diet differences for insulin sensitivity and PAI-1 were both on the order of 10%, which would be considered clinically important, but were not statistically significant. Post hoc power calculations indicated that effects of the observed magnitude were detectable with 15 and 25% power respectively; to achieve 80% power, the effects would have had to be

twice the magnitude or the sample size four times greater. No differential effect of the diets was observed for any other variables.

DISCUSSION

The average participant in our study consumed 100 ± 3 g of carbohydrate (mean $\pm$ s.e.m., 60% of 669 kcal at 4 kcal/g) for breakfast on each diet, resulting in blood glucose concentrations of 116 mg/dl vs. 147 mg/dl at 1 h after the low- vs. high-GI meals, respectively. Consistent with our findings, Ceriello *et al.* (9) observed an acute decrease in total radical-trapping antioxidant parameter assay after a standard 75-g oral glucose load that elicited a 1-h blood glucose concentration approximating 135 mg/dl in adults without diabetes. Other investigators also have observed increased oxidative stress (10), compromised endothelial function (32), and decreased NO_x concentration (33) in response to a standard oral glucose load. However, we did not observe significant postprandial changes for these variables, suggesting that components in the high-GI mixed meal (e.g., olive oil) (34) or in the overall diet quality during the period of controlled feeding prior to the postprandial study may have provided partial protection against the adverse effects of postprandial hyperglycemia. We speculate that high dietary glycemic load (i.e., the arithmetic product of GI and carbohydrate amount) may interact with poor overall diet quality (i.e., a diet high in refined carbohydrate but low in vegetables, fruits, legumes, nuts, healthful oils, whole grains, or dairy) to increase oxidative stress.

In another feeding study, Jenkins *et al.* (35) found greater protein thiol concentration (indicating less oxidative protein damage), but no difference in total oxidative capacity, after a low-GI meal compared to other meals among 15 healthy adults. Of note, each meal provided 50 g carbohydrate, as compared to an average of 100 g carbohydrate in the present study, and differences in peak blood glucose concentration among meals were only on the order of 15 mg/dl. Our results are consistent with a cross-sectional analysis by Hu *et al.* (36) who identified associations between dietary GI and markers of oxidative stress among 292 healthy adults.

The development of oxidative stress over time has been examined in clinical trials of the Dietary Approaches to Stop Hypertension (DASH) eating plan. After 3 weeks on the DASH diet, antioxidant capacity increased, but oxidative stress (assessed by plasma F₂IP) and insulin resistance (by homeostatic model assessment) did not change in obese, hypertensive participants. By 12 weeks, plasma ORAC tended to increase and urinary F₂IP decreased (37, 38), changes that may underlie, at least in part, the well-documented beneficial effects of the DASH diet on blood pressure, serum cholesterol concentrations, and insulin sensitivity (39–41). The apparently low GI of the DASH diet—with its emphasis on fruits, vegetables, whole grains, and low-fat dairy—may contribute to these observed effects. In any event, these findings suggest that the 10-day duration of our study, with its nutrient-controlled, mixed meal design, would be too short to assess the full impact of GI on oxidative stress, and that the adverse consequences of repeated postprandial hyperglycemia may accumulate over several months.

Strengths of this study include control for potentially confounding dietary factors; continuous observation of participants throughout the feeding protocol, minimizing the possibility of noncompliance; use of a crossover design allowing for within individual comparisons; and state-of-the-art measures of study end points. The main limitations of the study include the relatively short duration of the intervention for assessing change in some outcomes and the possibility of residual confounding from measured or unmeasured dietary components.

In conclusion, this study suggests that changes in total antioxidant capacity comprise an early event in the cascade of metabolic events linking dietary GI to risk for cardiovascular disease and diabetes. In addition, a high glycemic load diet with poor overall nutrient quality (specifically one that is low in antioxidant nutrients) may be deleterious to health.

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Figure 1. Postprandial glycemia and insulinemia after a standard breakfast. Time is measured relative to the start of the meal. Symbols represent the mean of 12 subjects; error bars indicate s.e.m.

Table 1
Menus for the dietary interventions (2,000 kcal)

Low glycemic index		High glycemic index	
Food	Weight, g	Food	Weight, g
Breakfast			
Beverage		Beverage	
Cornstarch ^a	58.0	Corn syrup, light ^a	74.0
Vanilla extract	6.7	Vanilla extract	7.0
Milk, 1% fat	333.0	Milk, 1% fat (lactase added)	351.0
Egg white, dried	9.2	Egg white, dried	9.6
Sugar substitute (Equal)	3.3	Sugar substitute (Equal)	3.5
Olive oil	10.0	Olive oil	10.5
Lunch			
Mexican pasta casserole		Mexican potato casserole	
Pasta (measured dry) ^a	90.0	Mashed potato flakes ^a	78.0
		Water (to reconstitute potato flakes)	350
Black beans	22.0	Black beans	21.0
Chicken breast	14.0	Chicken breast	22.0
Salsa, tomato	50.0	Salsa, tomato	50.0
Cheese, cheddar	12.0	Cheese, cheddar	12.0
Sweet corn	95.0	Sweet corn	95.0
Salt	0.5	Salt	0.4
Margarine (Olivio)	17.5	Margarine (Olivio)	20.0
Dinner			
Pasta (measured dry) ^a	90.0	Mashed potato flakes ^a	77.0
		Water (to reconstitute potato flakes)	350.0
Milk, 1% fat	90.0	Milk, 1% fat (lactase added)	110
Margarine (Olivio)	14.0	Margarine (Olivio)	14.0
Turkey breast	18.0	Turkey breast	24.0
Green peas	35.0	Green peas	35.0
Cheese, parmesan	3.0	Cheese, parmesan	5.0
Salt	0.5	Salt	0.4
Olive oil	5.0	Olive oil	6.0
Peaches, canned in light syrup	120.0	Peaches, canned in light syrup	110.0
Snack			
Guardian cereal (Kellogg's) ^a	49.0	Bran flakes (Kellogg's) ^a	51.0
Milk, 2% fat	77.0	Milk, 2% fat (lactase added)	75.0

Low glycemic index		High glycemic index	
Food	Weight, g	Food	Weight, g
Egg, whole	20.0	Egg, whole	17.0
Orange sections	62.0	Orange sections	54.0
Olive oil	3.7	Olive oil	3.5
		Wheat bran	2.4

^aPrimary sources of carbohydrate for the respective dietary interventions, accounting for 70% of the total carbohydrate.

Table 2
Variables assessed under fasting conditions and during the postprandial period (day 7)

Variable	Fasting state		<i>p</i> ^b	Postprandial AUC ^d		P
	Low-GI diet (<i>n</i> = 12)	High-GI diet (<i>n</i> = 12)		Low-GI diet (<i>n</i> = 12)	High-GI diet (<i>n</i> = 12)	
Plasma total ORAC, μmol TE/l	11,736 ± 668 ^c	10,381 ± 612	0.002	56,472 ± 2,425	51,497 ± 3,273	0.02
Plasma PCA ORAC, μmol TE/l	1,276 ± 96	1,210 ± 96	0.02	6,031 ± 378	5,536 ± 473	0.03
Urinary F ₂ IP, ng/mg creatinine	9.2 ± 1.1	10.1 ± 0.7	0.43	56.0 ± 6.4	50.8 ± 3.1	0.80
Endothelial function, % ΔVD ^d	10.3 ± 1.3	10.1 ± 1.3	0.74	52.6 ± 6.7	56.3 ± 7.8	0.49
Serum NO _x , μmol/l	23.6 ± 3.8	33.6 ± 4.0	0.06	109.5 ± 14.5	145.7 ± 16.8	0.06

F₂IP, F₂*α*-isoprostanes; GI, glycemic index; NO_x, sum concentration of nitrate and nitrite; ORAC, oxygen radical absorbance capacity; PCA, perchloric acid; TE, Trolox equivalents.

^aArea under the curve (AUC) from 5-h postprandial measurement, in measurement unit × h.

^bTesting equality of the two diets. From analysis of variance comparing mean difference of paired measures to zero, controlled for order of diets. Absence of carryover (diet × period interaction) confirmed in all cases, *P* > 0.10, by assessing association between order of diets and sum of paired measures (30).

^cMean ± s.e.m. (all such values).

^dEndothelial function was measured as percent change in vasodilation (ΔVD) at the brachial artery following an ischemic stimulus.

Table 3

Cardiovascular disease risk factors assessed under fasting conditions (day 10)

	Low-GI diet (<i>n</i> = 12)	High-GI diet (<i>n</i> = 12)	<i>p</i> ^a
FS-IVGTT			
Insulin sensitivity, l/mU/min	2.4 ± 0.4 ^b	2.1 ± 0.5	0.13
Acute insulin response, mU/l-min	1,056 ± 200	1,189 ± 262	0.17
Blood pressure, mm Hg			
Systolic	125 ± 3	125 ± 3	0.83
Diastolic	76 ± 2	76 ± 1	0.94
Cholesterol, mg/dl			
Total	165 ± 12	168 ± 11	0.33
High-density lipoprotein	34 ± 3	35 ± 2	0.35
Triglycerides, mg/dl	148 ± 20	147 ± 20	0.86
PAI-1, ng/ml	38 ± 7	43 ± 7	0.12
Fibrinogen, mg/dl	351 ± 14	344 ± 13	0.59
CRP, mg/l	0.24 ± 0.06	0.26 ± 0.10	0.77

CRP, C-reactive protein; FS-IVGTT, frequently sampled intravenous glucose tolerance test; PAI-1, plasminogen activator inhibitor-1.

^aTesting equality under the two diets. From analysis of variance comparing mean difference of paired measures to zero, controlled for order of diets. Possible carryover effect (diet × period interaction) detected for total cholesterol (*P* = 0.03); confirmed absent in all other cases, *P* > 0.10, by assessing association between order of diets and sum of paired measures (30).

^bMean ± s.e.m. (all such values).