

High-glycemic index carbohydrate increases nuclear factor- κ B activation in mononuclear cells of young, lean healthy subjects¹⁻³

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ABSTRACT

Background: High-glycemic index diets have been linked to greater risk of cardiovascular disease and type 2 diabetes. Postprandial glycemia within the normal range may promote oxidative stress and inflammatory processes underlying the development of disease.

Objective: We explored acute differences in the activation of the inflammatory marker nuclear factor- κ B after consumption of 2 carbohydrate meals matched for macronutrient and micronutrient composition but differing in glycemic index.

Design: After an overnight fast, 10 young, lean healthy subjects were fed in random order 3 meals providing 50 g of available carbohydrate as glucose, white bread, or pasta. Venous blood samples were collected at 0, 1, 2, and 3 h, and nuclear proteins were extracted from mononuclear cells. Changes in nuclear factor- κ B-p65 proteins were detected by Western blotting. Acute changes in other markers of oxidative stress (nitrotyrosine and soluble intercellular adhesion molecule-1) were also assessed.

Results: The maximum increase in nuclear factor- κ B activation was similar after the bread meal [mean (\pm SEM) area under the curve: $69 \pm 16\%$ optical density \cdot h] and the glucose challenge ($75 \pm 9\%$ optical density \cdot h), but was 3 times higher than after the pasta meal ($23 \pm 5\%$ optical density \cdot h) ($P < 0.05$). Similarly, changes in nitrotyrosine, but not soluble intercellular adhesion molecule-1, were higher after glucose and bread than after pasta ($P = 0.01$ at 2 h).

Conclusions: The findings suggest that high-normal physiologic increases in blood glucose after meals aggravate inflammatory processes in lean, young adults. This mechanism may help to explain relations between carbohydrates, glycemic index, and the risk of chronic disease. *Am J Clin Nutr* 2008;87:1188-93.

INTRODUCTION

Observational studies have linked high blood glucose concentrations with a higher risk of chronic disease, particularly cardiovascular disease (CVD). Persons with diabetes are 2 to 4 times as likely to have coronary heart disease as are persons without diabetes (1), and poor glycemic control produces a further increase in risk (2). Persons with impaired glucose tolerance share a similar CVD risk profile as do those with newly diagnosed diabetes (3). Among normal glucose-tolerant subjects, the highest quintile of glycated hemoglobin (a long-term measure of average glycemia) is associated with a 1.7 times greater risk of coronary heart disease (4). Because postprandial hyperglycemia is the most important contributor to glycated hemoglobin at this level (5), this raises the possibility that higher levels of postprandial glycemia, even within the normal range, augment the pathological process underlying atherosclerosis (6).

Studies on the glycemic index (GI) and dietary glycemic load (GL) provide further support for the hypothesis that postprandial glycemia increases the risk of CVD. Using data from the 75 000 women in the Nurses' Health Study, Liu et al (7) showed that over 10 y of follow-up, diets with a high GL doubled the risk of coronary heart disease after adjustment for known risk factors, including fiber intake. In the 20-y follow-up, GL was the strongest independent predictor of CVD risk and a better predictor of risk than was fat or protein intake (8). Beulens et al (9) recently found an increased risk of CVD in a population of 15 700 Dutch women when comparing the highest and lowest quartiles of GI and GL. After adjustment for CVD risk factors, the highest and lowest quartiles of GI and GL had hazard ratios of 1.47 and 1.33, respectively. Finally, Hu et al (10) observed a step-wise relation between dietary GI and oxidative stress markers in healthy adults. Concentrations of plasma malondialdehyde and F₂-isoprostane from the lowest to the highest quartile of GI were comparable with the differences found in those passively exposed to cigarette smoke and in active smokers.

Inflammation is now recognized as a central mechanism underlying the atherosclerotic process (11). Elevated postprandial blood glucose concentrations may contribute to a state of chronic low-grade inflammation by increasing oxidative stress (12, 13). An important mediator of proinflammatory gene transcription is the redox-sensitive transcription factor nuclear factor- κ B (NF- κ B). Once activated, NF- κ B translocates to the nucleus, where it acts on genes that regulate proinflammatory cytokines, adhesion molecules, and other mediators of inflammation (14). Because a 75-g oral glucose load has been shown to cause an acute increase in NF- κ B binding activity (15), we hypothesized that high-GI carbohydrates would also induce high NF- κ B binding activity. The aim in the present study was to examine acute differences in postprandial NF- κ B activation in mononuclear cells of healthy subjects following physiologic carbohydrate loads differing in GI. Nitrotyrosine generation and soluble intercellular adhesion

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molecule-1 (sICAM-1) concentrations—alternate measures of oxidative stress—were also measured.

SUBJECTS AND METHODS

Seventeen volunteers (8 men, 9 women) of white European extraction with a mean age of 23.4 ± 2.5 y and body mass index (BMI; in kg/m^2) of 22.4 ± 2.4 participated in 2 studies. All met the inclusion criteria of 18–30 y of age, BMI of 18–25, and no medication known to interfere with glucose tolerance. In study 1, 6 subjects consumed 50-g carbohydrate portions of white bread (TipTop Sunblest, NSW, Australia) and cooked pasta (San Remo vermicelli, SA, Australia) to confirm differences in postprandial glycemia by use of fingerprick capillary testing and standardized GI methods (16). In study 2, on 3 separate mornings, 10 subjects consumed in random order an oral carbohydrate challenge containing 50 g of available carbohydrate as a glucose drink (Glucodin, Boots Healthcare, Auckland, NZ), white bread (TipTop Sunblest), or cooked pasta (San Remo vermicelli). The 2 starchy foods were selected on the basis of their shared origins from wheat and close similarity in macronutrient and micronutrient contents (Table 1) but widely different published GI values (GI: bread = 70, pasta vermicelli = 35) (17). All meals were consumed with water so that the total volume was 600 mL. Approximately 40 mL of venous blood was collected from the median cubital vein by using a standard butterfly needle at time zero into 4×10 mL K_2EDTA evacuated tubes (BD Biosciences, CA) and at 1, 2, and 3 h. For each time point, blood from 3 of the tubes was used for mononuclear cell nuclear extraction. Blood from the fourth tube was centrifuged and the plasma was divided into 1.5-mL Eppendorf tubes for measurement of glucose, insulin, sICAM-1, and nitrotyrosine concentrations. The protocol was approved by the Ethics Review Committee of the University of Sydney, and the subjects gave written, informed consent.

Whole anticoagulated venous blood samples were loaded onto Histopaque-1077 separation media and were centrifuged at $400 \times g$ for 30 min at room temperature. Mononuclear cells were collected and the nuclear proteins extracted as previously described (15). Protein concentrations of nuclear extracts were determined by the BCA protein assay. Western blotting was performed by using the NuPAGE Electrophoresis System (Invitrogen Life Technologies, CA) and the ECL Western Blotting

Detection System (Amersham Biosciences, Buckinghamshire, United Kingdom). Nuclear protein extracts ($500 \mu\text{g}/\text{mL}$) were prepared, loaded onto a precast polyacrylamide gradient gel (NuPAGE Novex 4–12% Bis-Tris Gel), and separated for 60 min in NuPAGE MOPS SDS running buffer (1 mol/L tris base, 1 mol/L MOPS, 20.5 mmol/L EDTA, 69.3 mmol/L SDS, pH 7.7) on an XCell SureLock™ Mini-Cell (180 V). SeeBlue Plus2 standard (Invitrogen Life Technologies) was used to allow easy visualization of the protein molecular weight ranges during electrophoresis. Proteins were transferred to an Invitrolon PVDF membrane (Invitrogen Life Technologies). The membrane was incubated with a mouse monoclonal IgG antibody specific for the p65 subunit of NF- κ B (Santa Cruz Biotechnology, CA) followed by an anti-mouse IgG, HRP-linked polyclonal antibody (Amersham Biosciences). ECL chemiluminescent substrate detection reagent (Amersham Biosciences), and Hyperfilm ECL (Amersham Biosciences) was used to allow visualization of the NF- κ B p65 subunit. Images were digitized and optical densities of the bands were quantified by densitometry using IMAGEQUANT 5.1 software (Molecular Dynamics/GE Healthcare, NJ) and the Object Average background method. Analysis of sICAM-1 was carried out by using an enzyme-linked immunosorbent assay (Bender MedSystems, CA). Nitrotyrosine was analyzed as previously described (18).

Glucose and insulin responses were expressed as the incremental area under the curve (AUC) with the fasting concentration as baseline. The bread and pasta meals were compared by using independent-sample *t* tests for paired data (study 1, in which only bread and pasta were investigated). An analysis of variance model for AUC values was run with *food* as a fixed factor and *subject* as a random factor (study 2). Post hoc tests included a Bonferroni adjustment for multiple comparisons. The ANOVA model used automatically allowed for the fact that not all subjects contributed data for all foods. Statistical significance was indicated by $P < 0.05$. Data are presented as the mean \pm SEM. Statistical analyses were conducted by using SPSS for WINDOWS, version 12 (SPSS Inc, Chicago, IL).

RESULTS

Glucose and insulin responses

Fasting glucose and insulin concentrations were within the normal range at the beginning of each test (all data combined, glucose = 4.6 ± 0.1 mmol/L, insulin = 14 ± 2.2 pmol/L). Using capillary fingerprick blood sampling, study 1 confirmed the expected 1.7 fold difference in postprandial glycemia between the bread and pasta meals (AUC: 189 ± 39 versus 114 ± 38 ; $n = 6$, $P = 0.01$; Figure 1A). Differences in forearm venous blood glucose (Figure 1B) and insulin concentrations (Figure 1C) were also documented in the 10 subjects who participated in study 2. Differences in venous glucose and insulin concentrations were not significant.

Intranuclear NF- κ B binding activity

Changes in the concentrations of NF- κ B in intranuclear mononuclear cell extracts in response to 50 g carbohydrate portions of glucose, bread, and pasta are shown in Figures 2 and 3. Compared with pasta, the bread meal produced a 3-fold greater increase in NF- κ B as determined by the AUC (mean \pm SEM: glucose = 75 ± 9 , bread = 69 ± 16 , pasta = 23 ± 5 , $P = 0.02$)

TABLE 1
Nutritional composition of the test meals¹

	White bread (GI = 70)	Cooked pasta (GI = 35)
Energy (kJ)	1145	1042
Protein (g)	9.4	8.2
Fat (g)	2.9	0.6
Available carbohydrate (g)	50.0	50.0
Starch (g)	47.2	50.0
Fiber (g)	3.3	3.7
Thiamine (mg)	0.46 ²	0.04
Riboflavin (mg)	0.05	0.04
Niacin (mg)	1.23	1.02
Iron (mg)	1.44	0.82
Vitamin C (mg)	0.0	0.0

¹ Composition data were determined by using FOODWORKS (Xyris Software, Brisbane, Australia, 2007).

² White bread is fortified with thiamine in Australia.

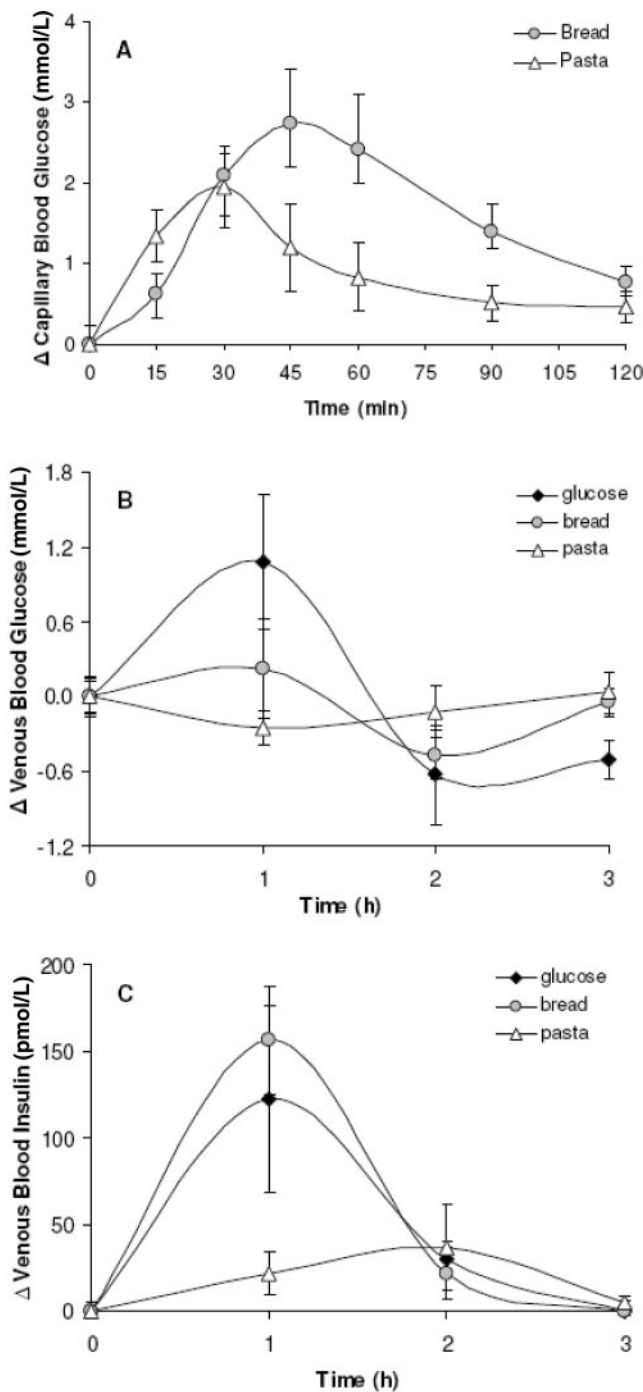


FIGURE 1. A: Changes in plasma glucose concentrations in fingertip capillary blood after 50 g available carbohydrate as white bread or pasta (mean \pm SEM, $n = 6$). B: Changes in plasma glucose concentration in venous blood after 50 g available carbohydrate as glucose, white bread, or pasta (mean \pm SEM, $n = 10$). C: Changes in plasma insulin in venous blood after 50 g available carbohydrate as glucose, white bread, or pasta (mean \pm SEM, $n = 10$).

(Figure 3B). Fasting insulin concentrations, but not fasting glucose, glucose AUC, or insulin AUC, predicted NF- κ B AUC ($R^2 = 0.26$, $P = 0.007$).

Changes in nitrotyrosine

Changes in nitrotyrosine concentrations are shown in **Figure 4**. Peak concentrations occurred at 3 h following the glucose

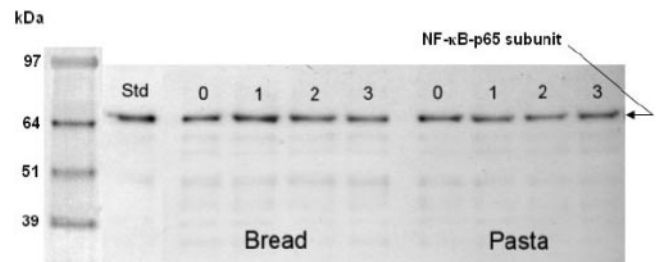


FIGURE 2. Representative Western blot showing changes in intranuclear nuclear factor- κ B (NF- κ B)-p65 antigen in peripheral blood mononuclear cells after a 50-g carbohydrate load of bread and pasta. Lanes are shown at 0–3-h time intervals for each food. A standard was run with all samples for comparative purposes. After the bread and pasta meals 1 wk apart, nuclear extracts were run on the same gel for each subject. Absorption of the bands was analyzed with IMAGEQUANT software. Std, standard.

challenge and bread meal (glucose: 0.47 ± 0.49 nM, bread: 0.67 ± 0.49 nM). In contrast, nitrotyrosine concentrations declined after the pasta meal reaching a nadir of -0.81 ± 0.3 nM at 2 h.

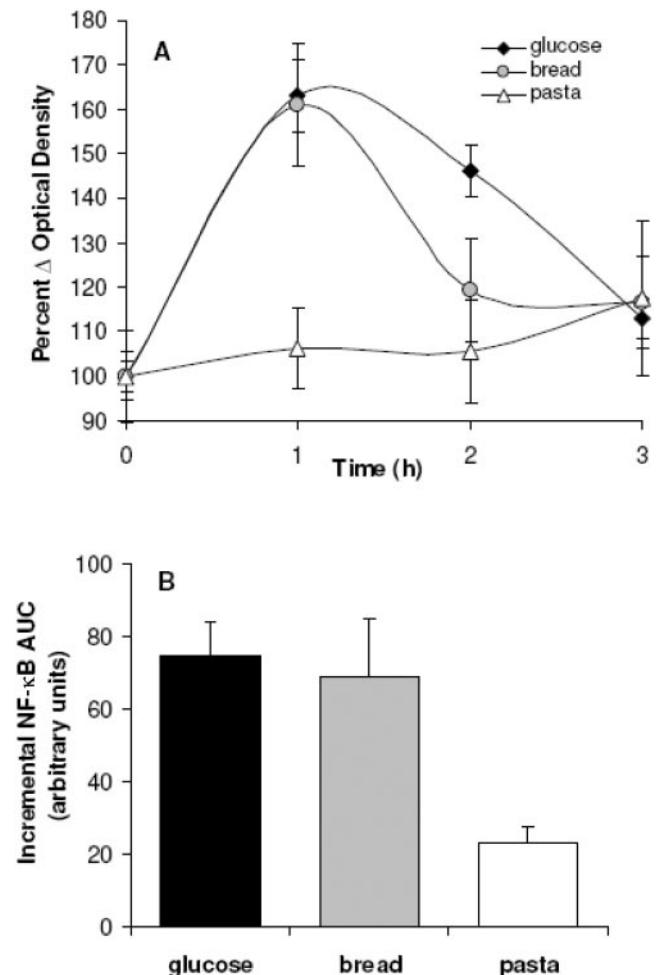


FIGURE 3. A: Changes in intranuclear nuclear factor- κ B (NF- κ B)-p65 antigen in peripheral blood mononuclear cells after 50 g available carbohydrate as glucose, white bread, or pasta (mean \pm SEM, $n = 10$). The signal of NF- κ B-p65 antigen obtained at time zero (fasting level) was defined as 100%. B: Changes in postprandial NF- κ B-p65 antigen concentrations shown by the area under the curve (AUC). At each time point, results are expressed as mean \pm SEM ($n = 10$). There was a significant difference among the 3 foods, $P = 0.02$.

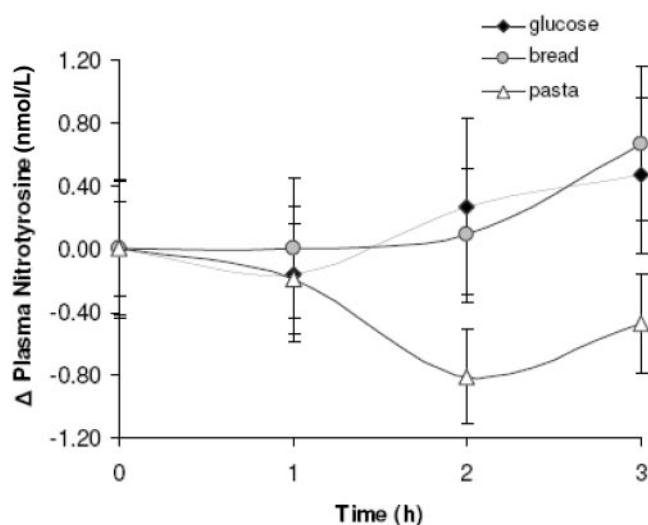


FIGURE 4. Changes in nitrotyrosine concentrations after consumption of a 50-g carbohydrate portion of glucose, white bread, or pasta. At each time point, results are expressed as mean \pm SEM ($n = 10$). Differences among the foods were significant at 2 h ($P = 0.01$) but not at 1 h or 3 h.

Differences among the foods were significant at 2 h ($P = 0.01$) but not at 3 h ($P = 0.06$).

Soluble intercellular adhesion molecule-1

Changes in sICAM-1 concentrations are shown in **Figure 5**. The highest concentrations were recorded at 3 h for all meals. Differences among the foods were not significant. ($P = 0.27$).

DISCUSSION

The results of the present study show that a high-GI source of carbohydrate in the form of white bread has the same capacity to acutely activate the redox-sensitive transcription factor NF- κ B as does a 50-g oral glucose challenge. By contrast, a low-GI source of carbohydrate in the form of pasta had an almost negligible effect on NF- κ B activation. All 3 challenges contained the same amount of absorbable carbohydrate, and the pasta and bread meals were matched for protein, starch, fiber, and most micronutrients. The changes in NF- κ B paralleled changes in blood glucose and insulin concentration, which suggests that slower rates of digestion and absorption reduced the oxidative stress created by high-carbohydrate meals. The findings therefore provide a molecular mechanism linking fast digestion and absorption, high-normal postprandial glycemia, and cellular dysfunction. Some observational studies suggest that high-GI foods and diets with a high glycemic load increase the risk of type 2 diabetes (19), cardiovascular disease (8), age-related macular degeneration (20), and some forms of cancer (21, 22). Nonetheless, not all studies have found a relation (23, 24).

NF- κ B activation appears to be regulated by oxidation-sensitive mechanisms (25). Previous studies showed a direct effect of glucose on the generation of reactive oxygen species, the induction of the ras- and p44/p42-MAP-kinase pathway, and the downstream activation of NF- κ B in peripheral blood monocytes. Maintenance of 10 mmol/L glucose concentrations in normal subjects using a hyperglycemic clamp was shown to cause a significant increase in carboxy-methyllysine, p21ras, and p42/44

mitogen-activated protein kinase phosphorylation and a concomitant increase in NF- κ B activation (13). Two recent studies showed elevated concentrations of intranuclear NF- κ B after an oral 75-g glucose challenge but not after water or alcohol (26, 27), whereas a previous study showed that an 8-wk reduction of postprandial hyperglycemia by the anti-diabetic drug acarbose (an alpha glucosidase inhibitor) reduced postprandial mononuclear NF- κ B activation (28).

We also explored the possible association between NF- κ B with glucose and insulin concentrations by use of correlational analysis. There was no correlation between NF- κ B concentrations and venous glucose concentrations (peak, 2 h, or AUC), but it is important to note that the venous glucose changes were very much attenuated compared with the capillary glucose changes (compare Figure 1A and 1B), and this could explain the lack of correlation. Unfortunately, we were unable to compare the capillary data directly with the NF- κ B data because different subjects were used. The lack of correlation may also be a matter of timing. Most of the glucose fluctuation occurs in the first hour, but the first venous sample was taken at the 1-h time point.

Although fasting insulin concentrations fell within the normal range, we found a significant positive relation between individual NF- κ B concentrations (AUC) and fasting insulin ($R^2 = 0.26$, $P = 0.007$), a surrogate marker for insulin sensitivity (29), but not with postprandial insulin concentrations. The independent relation of NF- κ B with fasting insulin suggests that insulin sensitivity may be a key determinant of the glycemia-induced inflammatory response.

The findings suggest that NF- κ B activation was not due to increased plasma insulin concentrations per se. Indeed, at physiologically relevant concentrations, insulin has been shown to inhibit NF- κ B and other inflammatory mediators including ICAM-1, MCP-1, AP-1, and Egr-1 in both human aortic cells and in test subjects (26, 30). Even at a concentration of 250 μ U/mL, insulin does not affect NF- κ B activation (13). In studies using

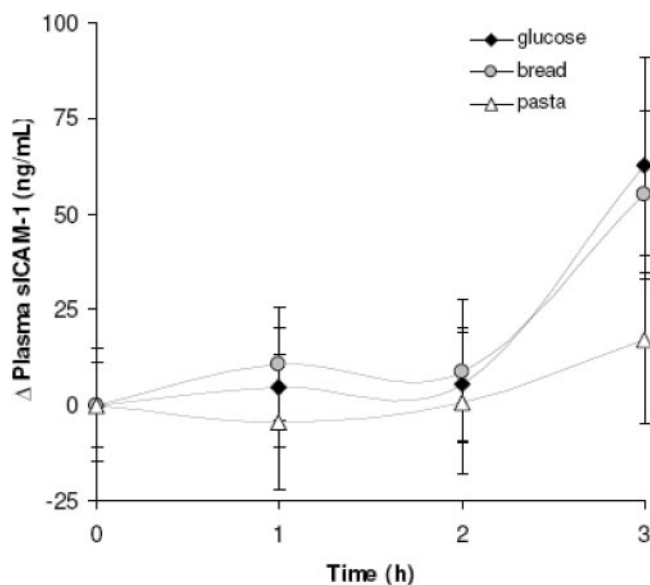


FIGURE 5. Changes in soluble intercellular adhesion molecule-1 (sICAM-1) concentrations after consumption of a 50-g carbohydrate portion of glucose, white bread, or pasta. Fasting concentrations were normalized to zero. Results are expressed as mean \pm SEM ($n = 10$). Differences among foods were not significantly different.

controlled glucose infusions, the euglycemic hyperinsulinemic clamp does not yield an increase in NF- κ B activation, whereas the hyperglycemic clamp does (13). In The United Kingdom Prospective Diabetes Study, insulin therapy was not associated with increased incidence of atherosclerosis in subjects with type 2 diabetes (31). Indeed, chronic insulin therapy may improve endothelium-mediated vasodilation and suppress C-reactive protein (32, 33).

NF- κ B is a regulator of adhesion molecules such as sICAM-1 (34), which mediate lymphocyte binding to endothelial cells and play an important part in the initiation of atherosclerosis (35). Although changes in sICAM-1 in the present study were not significant, sICAM-1 concentrations were higher at the 3-h mark after both the glucose and the bread meals. Larger subject numbers may be necessary to show that increased activation of NF- κ B has an effect on sICAM-1 transcription. Marfella et al (36) showed a similar but significant increase in sICAM-1 using the hyperinsulinemic clamp in 10 normal subjects. It is also possible that the glycemic response elicited by the bread meal was not sufficient in these young and relatively insulin-sensitive subjects to elicit a significant change in production of adhesion molecules. Studies using higher GI products (such as potatoes and Jasmine rice) in persons with greater degrees of glucose intolerance may be valuable.

In endothelial cells, hyperglycemia has been shown to increase superoxide, stimulating peroxynitrite and nitrotyrosine production. Nitrotyrosine is a marker of oxidative stress, proportional to prevailing (or ambient) glycemia in people with diabetes (37). In the present study, there were significant differences in nitrotyrosine concentration among the meals at 2 h (but not at 1 h or 3 h) with lower levels after the pasta than after bread and glucose. The changes in nitrotyrosine concentrations for the glucose challenge and bread meal were consistent with previous studies in which either a mixed meal was given or subjects underwent a hyperglycemic clamp (18, 38) causing a rise in nitrotyrosine concentrations of around 0.2–0.4 μ mol/L. However, these studies detected differences in subjects with either established IGT and diabetes or used a hyperglycemic clamp so that levels of glycemia were maintained at nonphysiologic levels in normal subjects. Some of the toxic effects of hyperglycemia may in fact be modulated by peroxynitrite and may be responsible for damaging effects previously associated with superoxide and nitric oxide (39).

In persons with diabetes, there is considerable evidence that hyperglycemia per se drives the development and progression of microvascular and macrovascular complications. Hyperglycemia reduces flow-mediated endothelium-dependent vasodilation (40), increases the production of adhesion molecules (36), and induces mitochondrial superoxide production. High concentrations of glucose have been shown to activate 4 biochemical pathways implicated in the pathogenesis of atherosclerosis (41). Even in the nondiabetic range, high glucose concentrations are associated with reactive oxygen species generation and changes in vascular function that promote macrovascular damage (42, 43).

Our study has limitations. The present findings apply to foods tested in isolation and may not reflect normal daily metabolic responses to realistic meals. Further studies of daylong profiles in response to consecutive mixed meals (breakfast, morning tea, lunch, dinner, etc) are warranted. The physiologic relevance of the magnitude of the increases of NF- κ B activation and oxidative

stress markers is not known. Nonetheless, our findings are novel and have important implications. High-GI carbohydrates including potatoes, wholegrain breads, and many varieties of breads and breakfast cereals dominate modern diets. Although antioxidants and phytonutrients in whole grains may quench free radicals and help to protect cells from oxidative damage, it may be prudent to also reduce the source of oxidative stress (ie, reduce postprandial hyperglycemia). Longer term studies comparing conventional and low-GI diets on the development of both type 2 diabetes and CVD in high-risk groups are warranted.

The contributions of the authors were as follows—JB-M and SD: conceived and designed the study, analyzed and interpreted the findings, and contributed to the writing of the manuscript; DPH: contributed to the design and conduct of the study and the interpretation of the findings; PP: analyzed the findings and contributed to their interpretation; and AC: conducted parts of the study and contributed to the interpretation of the findings and the writing of the final article.

JB-M is a co-author of *The Low GI Diet* (Marlowe & Co, New York, 2005) and a co-author of *The New Glucose Revolution* book series (Marlowe and Co, New York; Hodder Headline, Sydney and elsewhere). None of the other authors had any potential conflicts of interest relevant to the conduct of this research.

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