

Factors related to colonic fermentation of nondigestible carbohydrates of a previous evening meal increase tissue glucose uptake and moderate glucose-associated inflammation^{1–4}

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ABSTRACT

Background: Evening meals that are rich in nondigestible carbohydrates have been shown to lower postprandial glucose concentrations after ingestion of high-glycemic-index breakfasts. This phenomenon is linked to colonic fermentation of nondigestible carbohydrates, but the underlying mechanism is not fully elucidated.

Objective: We examined the way in which glucose kinetics and related factors change after breakfast as a result of colonic fermentation.

Design: In a crossover design, 10 healthy men ingested as an evening meal white wheat bread (WB) or cooked barley kernels (BA) that were rich in nondigestible carbohydrates. In the morning after intake of 50 g ¹³C-enriched glucose, the dual-isotope technique was applied to determine glucose kinetics. Plasma insulin, free fatty acid, interleukin-6, tumor necrosis factor- α , and short-chain fatty acid concentrations and breath-hydrogen excretion were measured.

Results: The plasma glucose response after the glucose drink was 29% lower after the BA evening meal ($P = 0.019$). The insulin response was the same, whereas mean (\pm SEM) tissue glucose uptake was 30% higher (20.2 ± 1.9 compared with 15.5 ± 1.8 mL/2 h; $P = 0.016$) after the BA evening meal, which indicated higher peripheral insulin sensitivity ($P = 0.001$). The 4-h mean postprandial interleukin-6 (19.7 ± 5.1 compared with 5.1 ± 0.7 pg/mL; $P = 0.024$) and tumor necrosis factor- α (7.8 ± 2.1 compared with 5.3 ± 1.6 pg/mL; $P = 0.008$) concentrations after the glucose drink were higher after the WB evening meal. Butyrate concentrations ($P = 0.041$) and hydrogen excretion ($P = 0.005$) were higher in the morning after the BA evening meal.

Conclusion: In healthy subjects, factors related to colonic fermentation of nondigestible carbohydrates increase peripheral insulin sensitivity and moderate glucose-associated inflammation. *Am J Clin Nutr* 2010;91:90–7.

INTRODUCTION

In observational studies, whole-grain foods and foods with a low glycemic index (GI) and rich in nondigestible carbohydrates have been associated with a decreased risk of type 2 diabetes (1) and cardiovascular disease (2). These foods are thought to exert their beneficial effect by decreasing the postprandial blood glucose response by slowing gastric emptying and/or delaying starch digestion and starch-derived glucose absorption. Besides this immediate postprandial effect, these foods have also been shown to influence the blood glucose response after a subsequent meal. In several studies, evening meals rich in

nondigestible carbohydrates have been shown to have the ability to reduce postprandial glucose to a high-GI breakfast (3–5). This overnight second meal effect is purported to be due to short-chain fatty acids (SCFAs; acetate, propionate, and butyrate) produced by fermentation of nondigestible carbohydrates by the colonic microbiota, even though other yet unknown mechanisms cannot be excluded. These SCFAs are rapidly absorbed from the colonic lumen and metabolized by colonic epithelial cells, but part of them also enter the portal and peripheral circulation (6). Effects of SCFAs on liver metabolism have been reported (7) and implicated with effects on the adipose tissue metabolism (8) and secretome (9). Although the liver extracts $\approx 75\%$ of acetate, 90% of propionate, and 95% of butyrate from the portal vein (10), higher concentrations of SCFAs in the peripheral circulation have been observed after ingestion of nondigestible carbohydrates (8, 11). Several hypotheses as to how SCFAs mediate the glucose-lowering effect have been postulated. SCFAs may delay gastric emptying (12), have insulin-like properties (8), increase insulin sensitivity by decreasing free fatty acid (FFA) concentrations (13), have antiinflammatory effects (14), or promote insulin-independent glucose sparing.

Until now, mainly total blood glucose concentrations have been reported after a high-GI breakfast. This makes it difficult to examine in detail the underlying mechanisms of the second-meal effect, because total blood glucose is the net result of influx of exogenous glucose from the small intestine into the systemic circulation, endogenous glucose production, and uptake of glucose into peripheral tissue. Therefore, we conducted a study to

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investigate the underlying glucose kinetics using the dual-isotope technique. In addition, several factors related to the regulation of glucose homeostasis, such as FFA concentrations, inflammation markers, adiponectin, and plasma concentrations of SCFAs were measured.

SUBJECTS AND METHODS

Experimental design

This study was performed in a randomized crossover manner, with each subject studied on 2 occasions 1 wk apart. The order in which the evening meal was administered first was determined by the order of recruitment. The subjects were asked to refrain from consuming foods enriched in ^{13}C , such as cane sugar, corn, corn products, and pineapple, for the 3 d preceding the experiments and from alcohol and strenuous exercise for 24 h before each study day. Each subject recorded his food consumption on the first study day and consumed the same food on the second study day. On both study days, the subjects were present at the clinical research unit from 2130 the evening before the experiment. The evening meal was taken at 2200, and the subjects stayed overnight. In this way, food intake could be controlled and physical activity reduced. After the evening meal, the subjects fasted until the oral-glucose-tolerance test (OGTT), which started at 0800 in the morning. Catheters were inserted into veins in both forearms: one for collection of the blood sample, kept patent with heparin (50 IE/mL), and the other for infusion of D-[6,6- $^2\text{H}_2$] glucose (98% ^2H atom percent excess) (Isotec Inc, Miamisburg, OH). At the start of the experiment at 0600 ($t = -120$ min), one bolus of D-[6,6- $^2\text{H}_2$]glucose solution (80 times the amount infused per minute) was infused and thereafter a continuous infusion of D-[6,6- $^2\text{H}_2$]glucose solution ($0.07 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$) was started. Two hours after the start of the continuous infusion ($t = 0$), the OGTT was started with 55 g glucose (90% carbohydrates) (glucose-monohydrate, Natufood; Natuproduits BV, Harderwijk, Netherlands) dissolved in 250 mL drinkable tap water. The glucose used was corn derived and therefore naturally labeled with ^{13}C , which was necessary to be able to apply the dual-isotope technique. The ^{13}C abundance of glucose was 1.09837 atom% ^{13}C . Throughout the study, subjects relaxed by reading or watching videos, to restrict physical activity.

Subjects

Ten healthy white men with a mean (\pm SEM) age of 21 ± 2.0 y and a body mass index (in kg/m^2) of 21.4 ± 1.0 were recruited in Groningen, Netherlands, by advertising beginning in October 2006. The criteria for exclusion were use of medications, blood donation in the previous 6 mo, use of antibiotics in the past 3 mo, gastrointestinal symptoms, diabetes mellitus, and gastrointestinal surgery. Approval was obtained from the Medical Ethics Committee of the University Medical Centre in Groningen, and each subject gave written informed consent for the study.

Evening meals

The evening meals consisted of either 105 g white wheat bread (WB) or 86 g (dry weight) of cooked whole barley kernels (BA). WB (Zaanse Snijder wit) was obtained at a local supermarket

(Albert Hein, Groningen, Netherlands). BA (whole-grain brown barley; Nature's Harvest, Burton-on-Trent, United Kingdom) was cooked in 250 g water with 1 g salt for 32 min until all the water was absorbed. Each test meal provided 50 g available carbohydrate and was taken with 250 mL drinkable tap water. It was estimated that the WB and BA contained 2 and 15 g non-digestible carbohydrates, respectively, as the degree of pearling of BA was the same as in the study of Nilsson et al (15).

Sample collection

Blood was collected throughout the experiment into tubes containing sodium fluoride/potassium oxalate. After centrifugation ($1000 \times g$; 10 min) at 4°C , the samples were stored at -20°C until assayed. Breath samples were collected by breathing through a straw into 10-mL Exetainers (Labco Limited, Buckinghamshire, United Kingdom). Before ingestion of the breakfast, 6 basal breath samples (-120 , -90 , -60 , -30 , -15 , and 0 min) and 5 basal blood samples (-90 , -60 , -30 , -15 , and 0 min) were collected. The sample of time point 0 was collected directly before the start of the OGTT. During the OGTT, blood samples were taken every 15 min for 2 h and every 30 min for an additional 2 h. After a blood sample was taken, 0.6 mL heparin (50 E/mL) was injected into the catheter to prevent blocking of the tube by clotting. Before the next blood sample was taken, the heparin was withdrawn with a small amount of blood and discarded.

Analytic procedures

Glucose was measured with an ECA-180 glucose analyzer (Medingen, Dresden, Germany). The interassay and intraassay CVs were 3% and 1%, respectively. Insulin concentrations were measured in duplicate with a commercially available radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). The interassay and intraassay CVs were 9.9% and 4.5%, respectively. The derivatization of plasma glucose to glucose penta-acetate for the analysis of the isotopic enrichment of plasma glucose is described in detail elsewhere (16). The $^{13}\text{C}/^{12}\text{C}$ isotope ratio measurement of the glucose pentaacetate derivative was determined by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) (TracerMAT; Thermo Finnigan, Bremen, Germany), and ^2H enrichment was measured by gas chromatography/mass spectrometry (GC/MS) under conditions previously described (17, 18). SCFAs were measured by GC/MS as described by Morrison et al (19). All plasma samples of one subject were analyzed in one batch to eliminate the effects of interbatch variation. Breath hydrogen analysis was performed by GC (HP 6890 Agilent; Hewlett-Packard Co, Palo Alto, CA) with a CP-Molsieve 5A column of $25 \text{ m} \times 0.53 \text{ mm}$ ($50\text{-}\mu\text{m}$ film thickness) (Chrompack International BV, Bergen op Zoom, the Netherlands). Plasma FFA concentrations were measured with an enzymatic colorimetric method (NEFAC ACS-ACOD Method; Wako Chemicals, Richmond, VA); samples were read at 540 nm on a BioTek EL 800 microplate reader (BioTek, Winooski, VT). Plasma interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were measured with the Luminex Bio-plex Cytokine Assay (Bio-Rad Laboratories, Hercules, CA), adiponectin was measured with the Luminex Bio-Plex Pro Assay Diabetes (Bio-Rad Laboratories), and samples were read on the Luminex 100 Total System (Luminex, Austin, TX).

Calculations

Glucose kinetics

The molar percentage enrichment of [6,6-²H₂]glucose and the ¹³C atom% were calculated as previously described (17) and smoothed by using the Optimized Optimal Segments (OOPSEG) program developed by Bradley et al (20). The rate of appearance (Ra) of total glucose in plasma (RaT) from exogenous (meal) and endogenous (hepatic) sources was calculated by using the nonsteady state equation of Steele et al (21) as modified by De Bodo et al (22). The identical behavior of labeled and unlabeled glucose molecules was assumed. The effective volume of distribution was assumed to be 200 mL/kg and the pool fraction to be 0.75 (23). The systemic Ra of exogenous glucose (RaE) was estimated as described by Tissot et al (23), and endogenous glucose production (EGP) was calculated by subtracting RaE from RaT (23). The glucose clearance rate (GCR), which reflects the insulin-mediated glucose uptake of tissue, was calculated as described by Schenk et al (24).

Incremental areas under the curve

To determine differences in glucose kinetics and in plasma glucose, insulin, FFA, and SCFA concentrations, the incremental areas under the curve (iAUCs) of 0–2 h and 0–4 h were calculated and compared. For these measurements also, peak (nadir) concentrations or rates and time to peak (nadir) were compared. The time to peak (nadir) was defined as the time period between the intake of the glucose drink and the appearance of peak (nadir) plasma concentrations or rate. For the iAUC calculations, the value of the fasting measurements was taken as the baseline value. Areas below baseline were not included. For the iAUC calculations of GCR, RaT, and RaE, the values were multiplied by body weight. The iAUC of RaE was expressed as a percentage of the administered dose of glucose equivalents (cumulative dose %). EGP and FFA concentrations were suppressed after the OGTT. Therefore, the data were symmetrically transformed with the baseline value as the symmetric axis to calculate the iAUC. For the other measurements, which showed no obvious rise in concentration on one occasion (breath hydrogen and plasma cytokines), the mean fasting and mean 0–2 h and 0–4 h postprandial values were calculated and compared.

Insulin sensitivity

An index for peripheral insulin sensitivity (SI) for use after the OGTT was calculated based on the method developed by Cederholm and Wibell (25) with some modifications. SI was expressed as the ratio of metabolic glucose clearance rate (MCR) to log mean postprandial plasma insulin. We calculated MCR as the 0–2 h iAUC of GCR (including areas below baseline) times body weight and divided by 1000. Cederholm and Wibell (25) derived the MCR from plasma glucose measurements during the OGTT and estimates of the glucose space.

Statistical analyses

Data are presented as means \pm SEMs. The univariate procedure of the general linear model was applied to test statistical differences, with test meal and sequence of the evening meal as a fixed factor and subject as a random factor. All analyses were performed with SPSS 14.0 for Windows (release 14.0.2; SPSS

Inc, Chicago, IL). A *P* value <0.05 was considered statistically significant.

RESULTS

Plasma glucose and insulin

The results are summarized in **Table 1**. In the morning after the BA evening meal, the 0–2 h glucose iAUC during the OGTT was 29% smaller (*P* = 0.019) than that after the WB evening meal. Also, the 0–4 h iAUC and peak concentrations (**Figure 1A**) were significantly different (*P* = 0.014 and *P* = 0.041, respectively). However, no differences were found between the insulin responses after both evening meals in any of the indexes compared (Table 1, Figure 1B).

Blood glucose kinetics

The results are summarized in Table 1. RaT, RaE, and EGP did not differ in any of the compared indexes in the morning after the BA and WB evening meals. In the morning after the BA evening meal, the 0–2 h iAUC of GCR was 30% greater (*P* = 0.016) than that after the WB evening meal. The GCR reached a peak value earlier after the BA than after the WB evening meal (*P* = 0.011), but the peak values were the same (*P* = 0.059) (Table 1, **Figure 2**).

Plasma FFAs

No differences were found in any of the compared indexes for FFA concentrations (Table 1).

Plasma SCFAs

Propionate, acetate, and total SCFA plasma concentrations did not differ in any indexes compared (Table 1). However, the 0–2 h iAUC of butyrate was significantly higher in the morning after the BA evening meal than after the WA evening meal (*P* = 0.041) (**Figure 3**).

Breath hydrogen

The mean 0–2 h and 0–4 h concentrations of hydrogen during the OGTT in the morning after the BA evening meal were significantly higher than that after the WB evening meal (*P* = 0.005 for both) (**Table 2**, **Figure 4**).

Plasma cytokines

The results are summarized in Table 2. Mean postprandial 0–4 h concentrations of IL-6 (**Figure 5A**) and TNF- α (**Figure 5B**) were significantly higher in the morning after the WB evening meal than after the BA evening meal (*P* = 0.024 and *P* = 0.008, respectively); however, mean postprandial 0–2 h concentrations were not (*P* = 0.079 and *P* = 0.054, respectively). No significant differences were observed for adiponectin (Table 2).

Insulin sensitivity index

Insulin sensitivity was significantly (*P* = 0.001) impaired in the morning after the WB evening meal (24.5 ± 1.5) compared with that after the BA evening meal (30.1 ± 1.9).

TABLE 1

Indexes showing the response of plasma glucose, insulin, free fatty acids (FFAs), short-chain fatty acids (SCFAs), glucose clearance rate (GCR), systemic rate of appearance of total glucose (RaT), rate of appearance of exogenous glucose (RaE), and endogenous glucose production (EGP) to a 50-g oral-glucose-tolerance test in the morning after a barley kernel (BA) or white bread (WB) evening meal¹

	Fasting value	Time to peak (min)	Peak value	iAUC ²	
				0–2 h	0–4 h
Glucose (mmol/L)					
BA	5.1 ± 0.1	40.5 ± 3.2	8.5 ± 0.3 ³	167.1 ± 18.9 ³	170.2 ± 21.1 ³
WB	5.0 ± 0.1	39.0 ± 2.5	9.1 ± 0.4	234.9 ± 29.9	241.4 ± 30.9
Insulin (mU/L)					
BA	6.3 ± 0.7	40.5 ± 3.9	72.5 ± 11.4	3035 ± 417	3121 ± 431
WB	5.5 ± 0.6	43.5 ± 3.5	70.1 ± 6.5	3753 ± 392	4057 ± 481
RaT (mg · kg ⁻¹ · min ⁻¹)					
BA	2.5 ± 0.1	54.0 ± 3.3	7.1 ± 0.2	27.0 ± 1.2	35.7 ± 1.5
WB	2.5 ± 0.1	51.0 ± 3.3	6.6 ± 0.4	24.7 ± 1.5	33.0 ± 1.7
RaE (mg · kg ⁻¹ · min ⁻¹)					
BA	0	60.0 ± 5.5	5.3 ± 0.2	66.2 ± 2.4	89.7 ± 2.4
WB	0	57.0 ± 7.7	4.8 ± 0.4	61.5 ± 3.1	86.7 ± 1.7
EGP (mg · kg ⁻¹ · min ⁻¹)					
BA	2.5 ± 0.1	90.0 ± 10.5	1.5 ± 0.1	86.3 ± 9.4	142.0 ± 22.7
WB	2.5 ± 0.1	99.0 ± 10.1	1.4 ± 0.1	85.5 ± 9.3	148.1 ± 25.0
GCR (mL · kg ⁻¹ · min ⁻¹)					
BA	2.8 ± 0.1	84.0 ± 7.1 ³	8.1 ± 0.5	20.2 ± 1.9 ³	33.4 ± 2.6
WB	2.8 ± 0.1	118.5 ± 9.1	7.0 ± 0.3	15.5 ± 1.8	29.0 ± 2.1
FFAs (mmol/L)					
BA	0.39 ± 0.04	94.5 ± 3.2	0.03 ± 0.01	28.6 ± 3.7	43.9 ± 6.7
WB	0.48 ± 0.04	103.5 ± 6.9	0.04 ± 0.01	36.3 ± 3.7	57.6 ± 6.6
Acetate (μmol/L)					
BA	479.7 ± 61.2	36.0 ± 6.0	864.4 ± 207.3	11,934 ± 4601	13,480 ± 5259
WB	418.6 ± 47.8	78.0 ± 19.6	732.3 ± 117.1	15,389 ± 5942	19,782 ± 7296
Propionate (μmol/L)					
BA	10.3 ± 0.6	51.0 ± 11.9	16.0 ± 1.5	223.8 ± 41.9	258.3 ± 44.9
WB	9.9 ± 0.7	36.0 ± 4.0	15.3 ± 1.3	245.5 ± 39.8	264.7 ± 42.9
Butyrate (μmol/L)					
BA	1.8 ± 0.2	60.0 ± 23.7	2.9 ± 0.3	48.6 ± 10.1 ³	74.7 ± 17.7
WB	1.6 ± 0.1	84.0 ± 35.2	2.6 ± 0.2	23.9 ± 6.2	42.4 ± 11.4
Total SCFAs (μmol/L)					
BA	491.8 ± 61.5	36.0 ± 6.0	882.7 ± 207.3	12,330 ± 4582	13,883 ± 5267
WB	430.1 ± 48.1	78.0 ± 19.6	747.4 ± 117.3	15,615 ± 5969	20,007 ± 7311

¹ All values are means ± SEMs; *n* = 10 healthy men. iAUC, incremental area under the curve.

² Units of the 0–2-h and 0–4-h iAUC for glucose, insulin, FFAs, acetate, propionate, butyrate, and total SCFAs are the unit of the corresponding variable per 2 or 4 h, respectively; units of 0–2 h and 0–4 h iAUC of RaT and EGP are grams per 2 h and grams per 4 h, respectively; units of 0–2 h and 0–4 h iAUC of GCR are milliliters per 2 h and milliliters per 4 h, respectively; units of 0–2 h and 0–4 h iAUC of RaE are dose percentage per 2 h and dose percentage per 4 h, respectively.

³ Significantly different from WB, *P* < 0.05 (univariate general linear model).

DISCUSSION

The findings of this study elucidate in which way glucose kinetics after a high-GI breakfast are altered because of the ingestion of an evening meal rich in nondigestible carbohydrates and show that a glucose-associated rise in proinflammatory cytokines can be moderated by the previous evening meal.

With the dual-isotope technique, we were able to show that reduced glucose response to a 50-g OGTT after a BA evening meal was due to higher glucose uptake into peripheral tissue, reflected by the GCR. It also excludes possible effects mediated by SCFAs on gastric emptying as proposed previously (12), because the RaE was not different. In addition, modulation of EGP does not seem to contribute to the reduced glucose response.

So far, only one study investigated postprandial glucose kinetics after the breakfast in an overnight second meal study

design with the dual-isotope technique (26). In this study, no change in GCR was found after 75 g glucose in the morning after an evening meal of BA as compared with brown rice. However, these data were only reported for one postprandial time point (60 min), which makes comparison with our results difficult. In the study by Thorburn et al (26), lower EGP was observed after the BA evening meal. The difference in EGP was mainly due to an initial pronounced increase of EGP 20 min after the start of the OGTT, which occurred after the rice but not after the BA evening meal. In our study, as well as in other studies using the same technique to determine EGP (27, 28), an initial increase in EGP was not observed. The differences in results could be due either to the characteristics of the subjects or to the characteristics of the evening meals low in non-digestible carbohydrates.

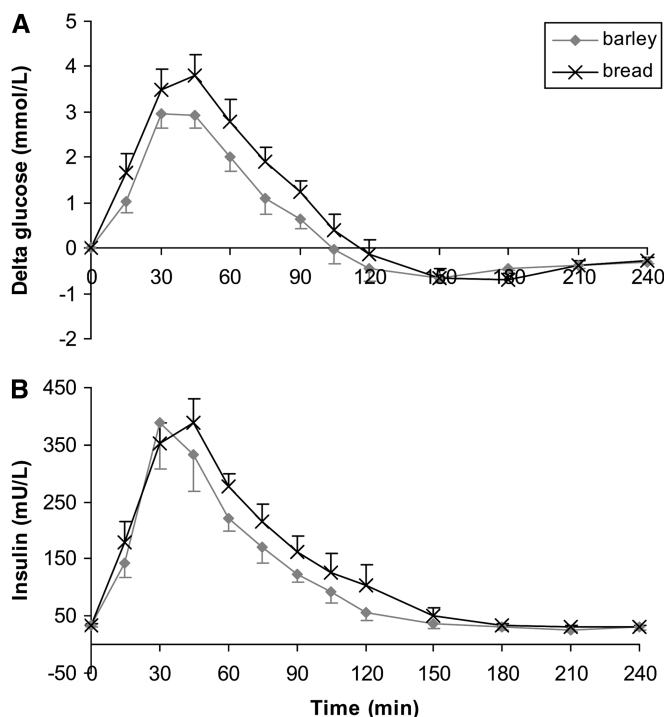


FIGURE 1. Mean (\pm SEM) incremental plasma glucose changes (A) and plasma insulin concentrations (B) after a 50-g oral-glucose-tolerance test in the morning after a barley kernel or white bread evening meal. The 0–2-h and 0–4-h glucose incremental areas under the curve were lower ($P = 0.019$ and $P = 0.014$, respectively) after the barley evening meal, whereas those of insulin were the same (univariate general linear model). $n = 10$ healthy men.

The higher GCR in our study was not accompanied by higher insulin concentrations, which implies that tissue sensitivity to insulin was higher after the BA evening meal. EGP was the same after both evening meals, which suggests that only peripheral and not hepatic insulin sensitivity was altered. We tested this assumption by calculating an index of peripheral insulin sensitivity (25) by using GCR, which was indeed significantly different. Other overnight second-meal studies, thus far, have not reported insulin sensitivity. However, the results of one study with a similar design are consistent with our results. Higher insulin sensitivity (calculated with the minimal model) in healthy subjects was found after a standard breakfast after administering 3

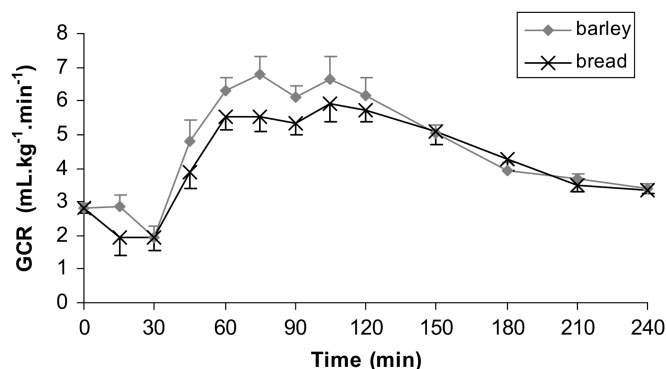


FIGURE 2. Mean (\pm SEM) glucose clearance rate (GCR) after a 50-g oral-glucose-tolerance test in the morning after a barley kernel or white bread evening meal. The 0–2 h-incremental area under the curve of GCR was significantly higher ($P = 0.016$) after the barley evening meal (univariate general linear model). $n = 10$ healthy men.

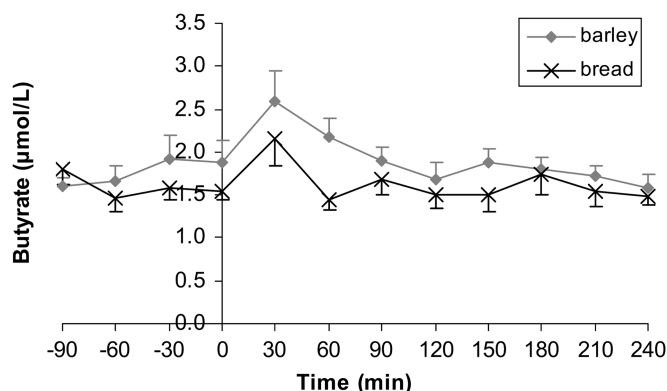


FIGURE 3. Mean (\pm SEM) plasma butyrate concentrations before and after a 50-g oral-glucose-tolerance test in the morning after a barley kernel or white bread evening meal. The 0–2-h incremental area under the curve was significantly higher ($P = 0.041$) after the barley evening meal, whereas the 0–4-h incremental area under the curve was the same after both evening meals (univariate general linear model). $n = 10$ healthy men.

high-resistant-starch meals on the previous day compared with meals without resistant starch (29). Our findings are also in line with the results of more longer-term interventions, because supplementation of a diet with either cereal fiber-enriched bread for 3 d (30) or 30 g resistant starch per day for 4 wk (8) increased insulin sensitivity (measured with the euglycemic hyperinsulinemic clamp) in healthy subjects. The results of our study thus indicate that food-associated factors can acutely influence peripheral insulin sensitivity. This is even more remarkable because the observation was made in healthy lean volunteers, in whom glucose homeostasis can be expected to be optimally regulated.

Breath hydrogen was higher in the morning after the BA evening meal, which supports the hypothesis of involvement of colonic fermentation in the overnight second-meal effect. Furthermore, we observed 103% higher butyrate concentrations in the morning after the BA evening meal, which is consistent with the results of Nilsson et al (4) but not in agreement with the findings of 2 other studies (15, 29). These inconsistencies in results could be due to differences in the time points of sampling, characteristics of the evening meal, or analytic methods. The study by Gao et al (31) gives an indication as to the possible underlying mechanisms of the insulin-sensitizing effect of butyrate. In that study, oral supplementation with butyrate ($5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), which increased butyrate concentrations in serum in the fed condition by 71%, prevented development of insulin resistance and obesity in C57BL/6J mice consuming a high-fat diet. Cell culture experiments to explore the underlying molecular mechanisms of their observation showed the potency of butyrate to directly activate, among others, AMP activated protein kinase (AMPK). The activation of AMPK by pharmacologic means was previously shown to increase glucose transport and the cell-surface GLUT 4 content in skeletal muscle from nondiabetic men (32). Thus, the results of our study suggest that butyrate, derived from colonic fermentation of non-digestible carbohydrates, could be involved in the overnight second-meal effect. To prove a causal relation, however, intervention studies with butyrate are necessary.

Another novel finding of our study concerns the ability of an evening meal of BA to prevent the late postprandial rise in the

TABLE 2

Breath-hydrogen, plasma interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and adiponectin concentrations before and after a 50-g oral-glucose-tolerance test in the morning after a barley kernel (BA) or white bread (WB) evening meal¹

	Fasting concentration	Postprandial concentration	
		0–2 h	0–4 h
Hydrogen (ppm)			
BA	31.5 \pm 3.4	40.0 \pm 6.5 ²	39.5 \pm 7.0 ²
WB	24.4 \pm 3.5	15.2 \pm 1.4	14.2 \pm 1.2
IL-6 (pg/mL)			
BA	7.0 \pm 0.8	5.2 \pm 0.8	5.1 \pm 0.7 ²
WB	13.1 \pm 4.2	15.0 \pm 4.5	19.7 \pm 5.1
TNF- α (pg/mL)			
BA	5.7 \pm 1.9	5.5 \pm 1.8	5.3 \pm 1.6 ²
WB	6.6 \pm 1.8	6.7 \pm 1.7	7.8 \pm 2.1
Adiponectin (μ g/mL)			
BA	27.0 \pm 5.4	26.5 \pm 4.4	28.3 \pm 4.5
WB	32.0 \pm 5.8	30.4 \pm 4.5	30.6 \pm 4.8

¹ All values are means \pm SEMs; n = 10 healthy men.

² Significantly different from WB, P < 0.05 (univariate general linear model).

proinflammatory cytokines IL-6 and TNF- α after the glucose load in the morning. Recently, much attention has been paid to inflammatory processes associated with acute hyperglycemia in relation to insulin sensitivity and atherosclerotic processes. Hyperglycemia induced by a hyperglycemic clamp, oral glucose, or a high-GI meal has been shown to increase nuclear transcription factor κ B activity (NF- κ B) in peripheral blood mononuclear cells in healthy lean volunteers (33–35). NF- κ B plays a central role in inflammatory responses and is involved in transcriptional regulation of many cytokines (36). In vitro, high glucose concentrations induce Toll-like receptor 2 and 4 expression in human monocytes, which leads to higher NF- κ B activity and IL-6 and TNF- α secretion (37). Higher plasma concentrations of IL-6 and TNF- α were observed after plasma glucose concentrations were acutely raised in a glucose clamp study in healthy volunteers (38) and in a hyperinsulinemic euglycemic clamp study (39). In addition, IL-6 was higher postprandially after a high-GI meal, with no change in TNF- α (40). It may be of interest to note that similar activation of NF- κ B or an increase in IL-6 and TNF- α have also been observed after fat-

containing meals (40–42). The high glucose or meal-induced inflammatory response could be prevented by the concomitant intake of antioxidants (glutathione and vitamin C) (34, 38) or antioxidant-rich food (olive oil and red wine) (41, 43). Our observation that an evening meal rich in nondigestible carbohydrates

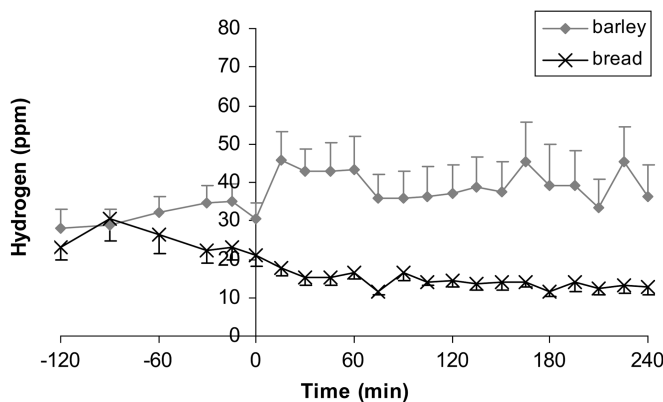


FIGURE 4. Mean (\pm SEM) breath-hydrogen concentrations before and after a 50-g oral-glucose-tolerance test in the morning after a barley kernel or white bread evening meal. The breath-hydrogen concentrations (0–2 and 0–4 h postprandially) were significantly higher (P = 0.005 both) after the BA evening meal (univariate general linear model). n = 10 healthy men.

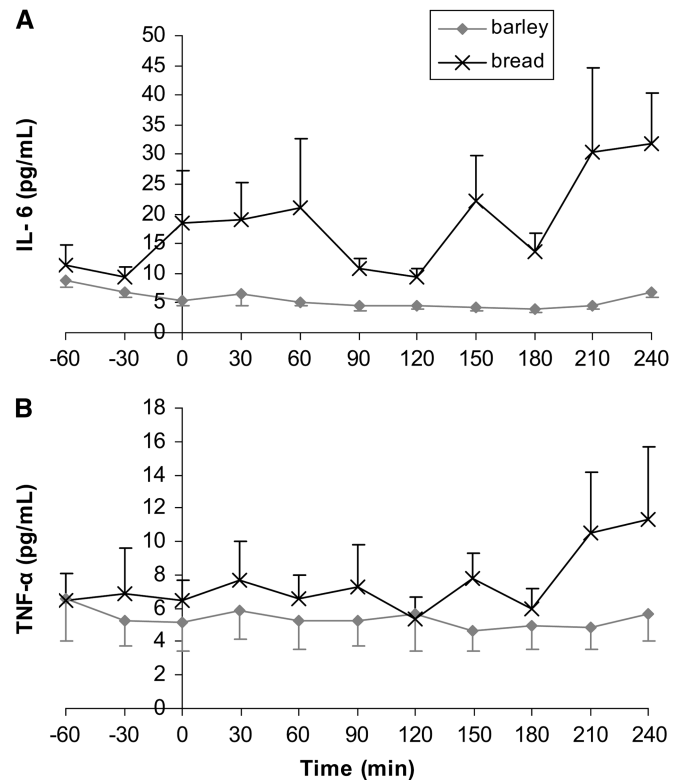


FIGURE 5. Mean (\pm SEM) plasma interleukin-6 (IL-6) concentrations (A) and tumor necrosis factor- α (TNF- α) concentrations (B) before and after a 50-g oral-glucose-tolerance test in the morning after a barley kernel or white bread evening meal. The 0–2-h mean postprandial IL-6 and TNF- α concentrations were the same after both evening meals, whereas the mean 0–4-h mean postprandial IL-6 and TNF- α concentrations were lower (P = 0.024 and P = 0.008, respectively) after the BA evening meal (univariate general linear model). n = 10 healthy men.

can also prevent the meal-associated rise in IL-6 the following morning is in line with the results from Nilsson et al (44); however, the effect of a previous meal on the rise in TNF- α after a subsequent meal has not been examined before. Thus, our finding suggests that not only the concomitant intake of antioxidants but also so far unknown antioxidant or antiinflammatory mechanisms or factors derived from a previous evening meal can moderate meal-associated inflammation.

We can only speculate what governs the factors responsible for suppression of the inflammatory response. On the one hand, this could be due to the antiinflammatory properties of SCFAs. Butyrate has been shown in vitro to decrease proinflammatory cytokine expression via inhibition of NF- κ B activation in peripheral blood mononuclear cells (45), and all 3 SCFAs decreased lipopolysaccharide-stimulated TNF- α release from human neutrophils (46). On the other hand, the presence of bioactive compounds with antioxidative capacity could play a role in the observed effect. Many classes of phenolic compounds are present in BA, which have been shown to have strong antioxidative capacity in vitro (47). Many of these bioactive components are bound to cell walls of the grain and reach the colon, where they are released during the fermentation process (48). Currently, however, little information is available about their antioxidant effects in vivo.

In summary, the results of our study highlight the potency of an evening meal rich in nondigestible carbohydrates to increase tissue glucose uptake the next morning and suggest a possible role for butyrate or butyrate-associated factors. The observation of the late antiinflammatory effect of BA is valuable with regard to the exploration of new strategies to prevent high-glucose or meal-induced postprandial inflammation.

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