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ORIGINAL ARTICLE

Effects of GI and content of indigestible carbohydrates of cereal-based evening meals on glucose tolerance at a subsequent standardised breakfast

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Objective: To evaluate the impact of four low-glycaemic index (GI) and one high-GI cereal-based evening meals on glucose tolerance at a subsequent standardised breakfast.
Design: Wheat kernels, barley kernels, spaghetti, spaghetti with added wheat bran and white wheat bread (WWB) were consumed in the evening in a random order at five different occasions. At the subsequent breakfast, blood glucose, serum insulin, plasma short chain fatty acid, plasma free fatty acid (FFA) and breath hydrogen were measured.
Setting: The study was performed at Applied Nutrition and Food Chemistry, Lund University, Sweden.
Subjects: Fifteen healthy volunteers were recruited. One subject was later excluded owing to abnormal blood glucose values.
Results: The blood glucose response (0–120 min) to the standardised breakfast was significantly lower after consuming barley kernels in the evening compared with evening meals with WWB ($P=0.019$) or spaghetti + wheat bran ($P=0.046$). There were no significant differences in insulin concentrations at breakfast. Breath hydrogen excretion at breakfast was significantly higher after an evening meal with barley kernels compared with WWB, wheat kernels or spaghetti ($P=0.026$, 0.026 and 0.015, respectively), and the concentration of plasma propionate at breakfast was significantly higher following an evening meal with barley kernels compared with an evening meal with WWB ($P=0.041$). In parallel, FFA concentrations were significantly lower after barley kernels compared with WWB ($P=0.042$) or spaghetti evening meals ($P=0.019$).
Conclusions: The improved glucose tolerance at breakfast, following an evening meal with barley kernels appeared to emanate from suppression of FFA levels, mediated by colonic fermentation of the specific indigestible carbohydrates present in this product, or, to the combination of the low-GI features and colonic fermentation.
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Keywords: glycaemic index; glucose tolerance; second-meal effect; colonic fermentation; indigestible carbohydrates; SCFA

Introduction

The glycaemic index (GI) is a concept used to classify carbohydrate-rich foods according to their effects on the postprandial blood glucose response. Owing to an accumulating body of data showing beneficial effects of low-GI foods on glucose and lipid metabolism (Jenkins *et al*., 2002; Brand-Miller, 2003), a diet rich in such foods is increasingly being considered advantageous. Epidemiological data also indicate that a low-GI diet has a protective role against development of type II diabetes (Salmeron *et al*., 1997a, b), coronary heart disease (Liu *et al*., 2000; Jenkins *et al*., 2002) and the metabolic syndrome (McKeown *et al*., 2004). Insulin

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resistance is a characteristic feature of the metabolic syndrome (Arner, 2001, 2002), and impaired sensitivity to insulin at the level of adipose tissue plays a central role for the genesis of the metabolic abnormalities associated with this syndrome. An improved adipocyte insulin sensitivity has been demonstrated following a low-GI diet in patients with coronary heart disease (Frost *et al*., 1996, 1998). Consequently, a low-GI diet appears to have a therapeutic potential in subjects with the metabolic syndrome, and a preventive potential by reducing insulin resistance and other risk factors associated with the genesis of the metabolic syndrome.

The mechanisms whereby low-GI foods exert long-term metabolic benefits are not fully understood. However, the lowered insulin surge, and the more distal absorption of carbohydrates have been implemented (Jenkins *et al*., 1982). As judged from the literature, low-GI foods appear to have the capacity to improve glucose tolerance at a 'second meal' ingested 4 h after the low-GI meal, hence, reducing the glycaemic and insulinaemic responses also over two consecutive meals (Jenkins *et al*., 1982; Trinick *et al*., 1986; Liljeberg *et al*., 1999; Liljeberg and Bjorck, 2000). An improvement in second-meal glucose tolerance, following low-GI foods has also been observed in the perspective from a late dinner to breakfast the following morning (Wolever *et al*., 1988; Thorburn *et al*., 1993; Granfeldt *et al*., 2006). The mechanisms for a 'second-meal effect' in the perspective from breakfast to lunch (4 h) is probably mediated by the slow absorption and digestion, resulting in a postponed in-between-meal fasting and a suppression of free fatty acids (FFA), hence improving glucose tolerance at the time of the subsequent lunch-meal. Also the mechanism for the second-meal effect from dinner to breakfast has been suggested to be due to the lente features of a low-GI evening meal and a concomitant suppression of FFA (Thorburn *et al*., 1993; Wolever *et al*., 1995). The mechanisms for the suppression of FFA at the time of the next meal might vary depending on the time frame in between meals and other food properties for example, presence of fermentable dietary fibre (DF) (Thorburn *et al*., 1993) is probably also involved in achieving the second-meal effect from evening to breakfast. It has been suggested that the GI of the dinner meal *per se* can predict the glucose response to a standardised test meal the following morning (Wolever *et al*., 1988). However, in a recent study, a low-GI evening meal *per se* was not enough to improve glucose tolerance the following morning (Granfeldt *et al*., 2006) and only a low-GI meal rich in indigestible carbohydrates significantly lowered blood glucose and insulin responses at a subsequent breakfast. Indigestible carbohydrates, that is resistant starch (RS) and DF, reach the colon and constitute a substrate for fermentation by colonic bacteria (Rumessen, 1992). Colonic fermentation of indigestible carbohydrates results in formation of microbial metabolites, particularly short-chain fatty acids (SCFA), mainly acetic, propionoic and butyric acid, and gases (e.g. carbon dioxide and hydrogen) (Cummings *et al*., 1987, 2001). It has been suggested that SCFA produced during colonic fermentation may have beneficial implications on glucose metabolism (Anderson and Bridges, 1984; Thorburn *et al*., 1993; Berggren *et al*., 1996). Propionate has been shown to affect hepatic carbohydrate metabolism in rats (Anderson and Bridges, 1984) and improve glucose tolerance in man (Venter *et al*., 1990), implicating that indigestible carbohydrates, in addition to low-GI features, might modulate second-meal glucose metabolism. In accordance, epidemiological data are at hand suggesting that a diet characterised by a low GI, and rich in cereal fibre, is particularly efficient in reducing the risk of type II diabetes (Salmeron *et al*., 1997a, b). Furthermore, a whole-grain diet has been implicated as protective against development of type II diabetes and cardiovascular disease (Anderson *et al*., 2000; Jenkins *et al*., 2000; McKeown *et al*., 2002; Anderson, 2003; Schulze *et al*., 2004).

Currently, there is a lack of low-GI cereal foods on the market. In the formulation of such foods, more knowledge is needed concerning the mechanisms for observed long-term metabolic benefits of low-GI diets, and concerning the possibilities to enhance further the metabolic benefits by formulating low-GI foods that are also rich in specific indigestible carbohydrates. The aim of this study was to evaluate the key product features responsible for benefits on 'second-meal' glucose tolerance occurring in the perspective from a late evening meal to a subsequent breakfast. For this purpose, the effect of four different low-GI cereal evening meals and a high-GI white wheat bread (WWB) evening meal on glucose tolerance at a standardised breakfast meal the following morning was evaluated in healthy subjects. The low-GI evening meals were similar with respect to GI, but differed in type and/or amount of DF and RS. In addition to the determination of blood glucose and insulin responses at the standardised breakfast, FFA levels and markers of colonic fermentation were registered in blood and urine (SCFA) and in expired air (H_2).

Materials and methods

Evening test meals

Composition. The quantity of the evening test meals was calculated based on 50 g of potentially available starch, analysed according to Holm *et al*. (1986). The test meals were WWB (reference product), boiled wheat kernels, boiled barley kernels, spaghetti and spaghetti with added wheat bran. A measure of 150 ml water was consumed with the test products and an additional 150 ml coffee/tea (without milk and sugar) or 150 ml water was consumed directly there after.

Recipes and preparation of test products included in the evening test meals. *White wheat bread;* A standardised WWB was baked in a home baking machine (ide line®, model no. TS-128S, normal program). The bread was made from 450 g of white wheat flour (Kungsörnen AB, Järna, Sweden), 300 g

water, 4 g dry yeast, 4 g NaCl and 10 g monoglycerides. After cooling, the crust was removed and the bread was sliced and portions of 119.9 g were wrapped in aluminium foil, put into plastic bags and stored in a freezer. The day before the experiment, the bread was taken from the freezer in the morning and was thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

Boiled wheat kernels; One portion (86.0 g) of dried whole-wheat kernels (Durum wheat, Hven-durum HB, Hven, Sweden) was boiled at low temperature for 20 min in 100 ml water containing 1 g NaCl. All water was absorbed into the kernels.

Boiled barley kernels; One portion (83.7 g) of dried whole pearled barley kernels (Pot barley, Goudas Food Products, Concord, Ontario, Canada) was boiled at low temperature for 40 min in 250 ml water containing 1 g NaCl. All water was absorbed into the kernels.

Spaghetti; The spaghetti (Kungsörnen, Järna, Sweden) was made from Durum wheat flour. One portion of 76.6 g uncooked spaghetti was boiled for 8 min in 1 l water with addition of 1 g NaCl.

Spaghetti with added wheat bran; In this spaghetti meal, purified wheat bran (Fiberform®, Tricum) was sprinkled over the boiled spaghetti. For one portion of spaghetti (76.6 g), 11.7 g (ambient dry) wheat bran was added.

Analysis of starch, resistant starch and dietary fibre. Potentially available starch in the test products was analysed with an enzymatic method according to Holm *et al*. (1986), and DF (insoluble and soluble) with a gravimetric, enzymatic method according to Asp *et al*. (1983). Before analysis, the WWB, boiled barley kernels and boiled wheat kernels were air dried and milled (Cyclotec, Foss Tecator AB, Höganäs, Sweden), whereas the spaghetti was boiled and homogenised in a buffer. Resistant starch was analysed according to Åkerberg *et al*. (1998) and included chewing as a pre-step before incubation with enzymes.

GI characterisation. The GI of the boiled whole-wheat and whole-barley kernels was predicted using an *in vitro* method with an initial chewing step to mimic eating conditions (Granfeldt *et al*., 1992), whereas GI of the spaghetti was determined in healthy subjects (Liljeberg *et al*., 1999). Except for the WWB, the test meals were matched to have similar and low GI (Table 1). Glycaemic index was considered to be similar for the spaghetti meals with and without wheat bran.

Standardised breakfast

The breakfast contained 109.0 g (wet weight) of WWB (Jätterasken, Pågen AB, Malmö, Sweden) with the crust removed, equivalent to 50 g of potentially available starch analysed according to Holm *et al*. (1986). A 150 ml portion of water was served with the breakfast and an additional 150 ml tea/coffee (without milk or sugar) or 150 ml water was served directly after breakfast.

Experimental design

Test subjects and procedure. Fifteen healthy volunteers, nine men and six women aged 20–30 years, with normal body mass indices (mean $\pm$ s.d = 21.9 $\pm$ 2.6 kg/m^2) participated in the study. One man was later excluded owing to abnormal blood glucose values. Each subject participated in the experiment on five different occasions, with approximately 1 week apart. They were told to avoid food rich in DF the entire day before the experiment day, and to avoid alcohol and excessive physical exercise in the evening. At 0930, the evenings before the experimental days, the subjects prepared and consumed in a random order one of the five test products in their home. The meals were prepared according to a detailed written description of the cooking procedure and were consumed directly after preparation. After the late evening test meal, the subjects were fasting until the standardised breakfast. In the morning of the experimental day, an intravenous cannula (BD Venflon, Becton Dickinson,

Table 1 GI characteristics and contents of RS and insoluble-, soluble-, and total DF in each test meal

Evening test meals	*GI characteristics*		*Content in the test meal*				
	g/meal	*(%)*	*RS (g/meal)*	*DF*			*RS + DF (g/meal)*
				Insoluble (g/meal)	*Soluble (g/meal)*	*Total (g/meal)*	
WWB	119.9	100	0.5 $\pm$ 0.0	1.6 $\pm$ 0.0	1.4 $\pm$ 0.1	3.0 $\pm$ 0.1	3.6
Wheat kernels	86.0	54 $\pm$ 1.0[a]	10.4 $\pm$ 0.8	8.5 $\pm$ 0.2	1.6 $\pm$ 0.1	10.1 $\pm$ 0.4	20.5
Barley kernels	83.7	54 $\pm$ 1.0[a,b]	7.3 $\pm$ 0.7	5.5 $\pm$ 0.1	4.3 $\pm$ 0.2	9.8 $\pm$ 0.3	17.0
Spaghetti	76.6	52[c]	2.6 $\pm$ 0.4	2.2 $\pm$ 0.1	0.9 $\pm$ 0.1	3.1 $\pm$ 0.2	5.7
Spaghetti + wheat bran	88.3	52	2.6 $\pm$ 0.4	11.4 $\pm$ 0.4	1.1 $\pm$ 0.1	12.5 $\pm$ 0.5	15.1

Abbreviations: DF, dietary fibre; GI, glycaemic index, RS, resistant starch.
[a]GI of the wheat and barley kernels was predicted using an *in vitro* method according to Granfeldt *et al*. (1992).
[b]The predicted GI of the barley kernels agreed well with GI (53) previously determined by Granfeldt *et al*. (2006).
[c]GI of spaghetti determined in healthy subjects (Liljeberg *et al*., 1999).

Helsingborg, Sweden) was inserted into an antecubital vein to be used for blood sampling. The standardised breakfast was served at 0800 and the subjects were told to eat the breakfast and drink the water within 10–12 min. The additional coffee/tea or water was served 15 min after start of the breakfast. Each subject maintained the same drink throughout the study. During the 3 h blood sampling, the subjects were told to sit and rest as much as possible.

Approval of the study was obtained by the Ethics Committee at the Faculty of Medicine at Lund University, Sweden.

Sampling and analysis of blood, urine and expired air. Venous blood samples were obtained for analysis of glucose, insulin, FFA and SCFA (acetate, propionate and butyrate). Short chain fatty acids were also analysed in morning urine. Blood samples for determination of glucose and insulin were taken immediately before the breakfast meal (0 min) and then at 15, 30, 45, 60, 90, 120 and 180 min after commencing of the breakfast. Blood samples for detection of FFA and SCFA were taken at 0, 60, 120 and 180 min and at 0 and 180 min, respectively. Urinary SCFA were analysed on the first urine collected in the morning, and acetate, propionate and butyrate concentration and their total was quoted with respect to urinary creatinine. Breath hydrogen (H_2) concentrations were measured as a marker of colonic fermentation and sampled before the test meals in the evening before the experimental day (baseline value), before bed time, when waking in the morning, and directly before and directly after the standardised breakfast.

Blood glucose concentrations were determined with a glucose oxidase–peroxidase reagent and serum insulin with a solid-phase two-site enzyme immunoassay kit (Insulin ELISA 10-1113-01, Mercodia AB, Uppsala, Sweden). Plasma FFA concentrations were analysed with an enzymatic colorimetric method (NEFA C 994–75409, WAKO Chemicals GMbH, Germany). Concentrations of SCFA in plasma and urine were analysed with GC/MS (Hewlett Packard 5890 II GC with an Optic II PTV and CTC A200S autosampler and VG Trio-1000 quadrupole MS with EI ionisation) following purification by ultrafiltration (Amicon Centriplus centrifugal filter device YM-30, 30 kDa cutoff, Millipore (UK) Ltd, Watford, UK) and solid-phase extraction (Bakerbond SPE column, 200 mg SDB-2, 3 ml capacity, Mallinckrodt Baker Uk, Milton Keynes, UK) (Morrison *et al*., 2004). H_2 was sampled by blowing gently through a straw into a glass vial. The breath samples were analysed with an automated continuous-flow isotope ratio mass spectrometer (HYDRA, PDZ Europe, Crewe) (Slater and Preston, 2004).

Calculations and statistical methods. The results are expressed as means ± s.e.m. The statistical significance of difference was assessed by analyses of variance followed by Tukey's pairwise multiple comparison method for means (glucose, insulin and H_2) and/or Dunnetts comparisons with the reference WWB as a control (FFA respective plasma SCFA) (ANOVA, General Linear Model, MINITAB™ release 13 for windows). Incremental areas under the glucose and insulin curves (IAUC) were calculated by use of GraphPad Prism® 3.0. A value of $P<0.05$ vas considered statistically significant. Blood samples for determination of SCFA was taken in the beginning and in the end of the experiment, and a mean of the two values were used for statistical analysis. The peak values for glucose and insulin were estimated from each subject's highest value and could therefore have emanated from different time points.

Results

Glycaemic indices and contents of resistant starch and dietary fibre in the evening test meals

The GI and contents of RS and DF in the evening test meals are presented in Table 1.

Postprandial blood glucose and insulin levels following the standardised breakfast

There were no significant differences in fasting blood glucose or serum insulin in the morning after consuming the different evening test meals.

The blood glucose responses to the standardised breakfast after the different test evening meals are displayed in Figure 1 and Table 2. The postprandial blood glucose increment to the standardised breakfast meal was significantly lower at 45 min after the barley kernel evening meal compared with WWB or spaghetti. At 60 min, the postprandial blood glucose increment to the standardised breakfast meal was significantly lower after the barley kernel evening meal compared with WWB. The second meal incremental area under the glucose curve at 0–120 min was significantly lower at breakfast after the evening meal with barley kernels compared with both the WWB and pasta with wheat bran.

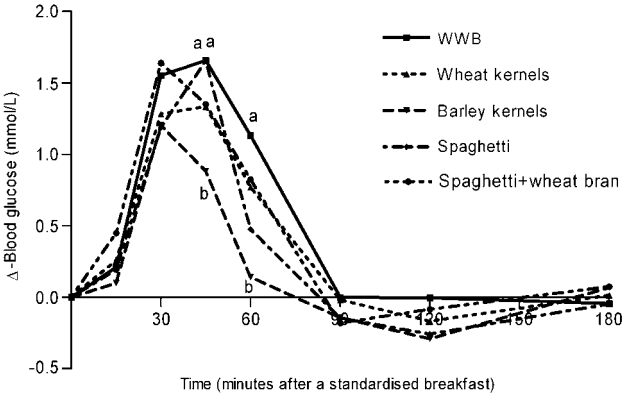

Figure 1 Postprandial blood glucose curves after a standardised breakfast given ≈10.5 h after consuming different evening meals. Values with different letters are significantly different ($P<0.05$).

Table 2 Fasting blood glucose concentrations, postprandial incremental blood glucose areas and delta glucose peak values after a standardised breakfast, following different evening meals

Evening test meals	*Fasting blood glucose (mmol/l)*	*IAUC after a standardised breakfast*		*Δ blood glucose peak*[1] *(mmol/l)*
		0–120 min[1] *(mmol · min/l)*	*Change*[2] *(%)*	
WWB	4.4±0.2	90.4±15.6[a]	0	2.1±0.2[a]
Wheat kernels	4.5±0.1	79.1±16.2[a,b]	−13	1.8±0.2[a,b]
Barley kernels	4.6±0.1	48.9±9.1[b]	−46	1.4±0.2[b]
Spaghetti	4.5±0.1	67.3±10.1[a,b]	−26	1.8±0.2[a,b]
Spaghetti + wheat bran	4.4±0.1	86.0±15.1[a]	−5	1.9±0.2[a,b]

Abbreviations: WWB, white wheat bread, IAUC, incremental areas under the curves.
[1]Values in a column with different superscript letters are significantly different ($P<0.05$).
[2]Change in postprandial IAUC compared with the reference white bread.

The blood glucose peak increment to the standardised breakfast after consuming the evening test meal with barley was significantly lower compared with the evening meal with WWB.

No significant differences were seen in serum insulin levels, with respect to delta values, areas under the curves or peak values following the standardised breakfast, irrespective of the preceding evening meal (Figure 2).

Breath hydrogen in the evening and at the standardised breakfast

There were no significant differences in H_2 concentrations in expired air in the evenings before ingestion of the test and reference meals. At the time of the standardised breakfast (mean of the H_2 values immediately before and 20 min after commencing the breakfast), H_2 excretion was significantly higher after consuming a barley kernel evening test meal than after all the other test products, with the exception of spaghetti + wheat bran ($P=0.08$) (Figure 3).

Fasting and postprandial plasma FFA-levels at the standardised breakfast

Fasting plasma FFA levels in the morning were significantly lower after consuming barley kernels compared with spaghetti as an evening meal (Figure 4). Fasting plasma FFA concentration was also lower after the barley kernel evening meal compared with the reference WWB.

Short chain fatty acids in urine and plasma before and following the standardised breakfast

The mean concentration of plasma propionate was significantly higher in the morning following an evening meal with barley kernels compared with an evening meal with WWB (Table 3). There were no significant differences in plasma levels of total SCFA, acetate or butyrate nor in SCFA concentrations in the first morning urine, irrespective of the

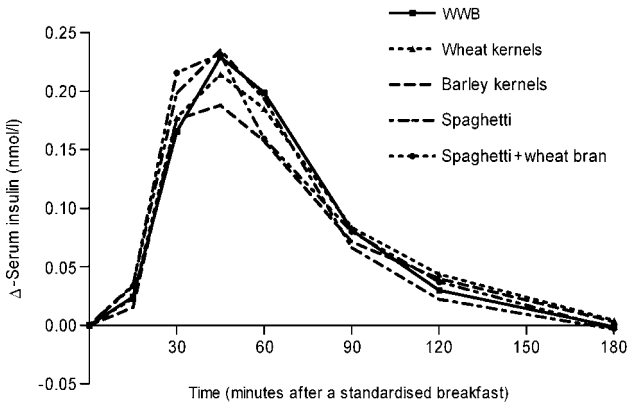

Figure 2 Postprandial serum insulin curves after a standardised breakfast given ≈10.5 h after consuming different evening meals.

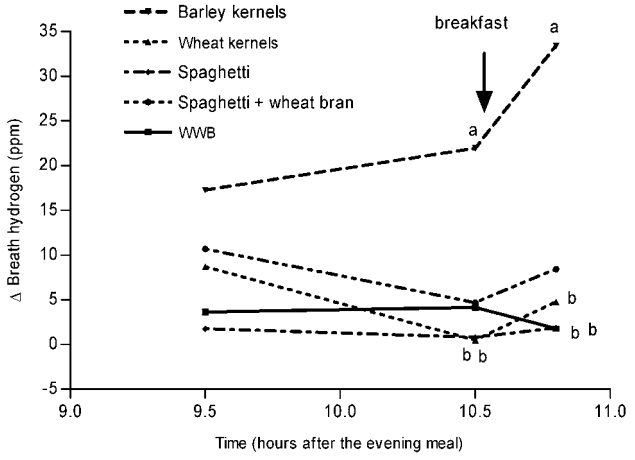

Figure 3 Breath hydrogen excretion in the morning after consuming different evening meals. A standardised breakfast was given ≈10.5 h after the evening meals. Values with different letters are significantly different ($P<0.05$).

GI character or indigestible carbohydrate content of the evening meal.

Discussion

In the present study, we examined the effect of five evening meals (four low-GI and one high-GI reference), containing different amounts of RS and DF, on the glucose tolerance at a subsequent standardised high-GI breakfast. This study showed a reduction in glycaemic response to a standardised breakfast following an evening meal of boiled barley kernels compared with an evening meal of WWB, spaghetti or spaghetti with added wheat bran. The reduction in blood glucose IAUC (0–120 min) at the breakfast was approximately 46%, following the evening meal with boiled barley kernels as compared with the evening meal with WWB. No significant effects on insulin response were seen after the standardised breakfast, irrespective of the preceding evening meal. The findings from this study indicate that the characteristics of the evening meal may affect the glycaemic response to a subsequent standardised high-GI breakfast ingested 10.5 h later. The results are in line with previous findings (Granfeldt *et al*., 2006), showing 23% lower blood glucose responses to a standardised breakfast meal following an evening meal with barley kernels compared with an evening meal with WWB. In that study, there was also a concomitant reduction in insulinaemic area at breakfast when preceded by an evening test meal with barley kernels (Granfeldt *et al*., 2006).

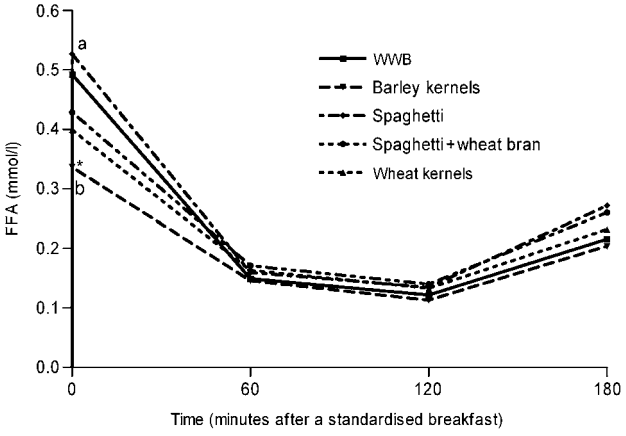

Figure 4 Fasting plasma free fatty acid concentration (time = 0) and concentrations at 60, 120 and 180 min after a standardised breakfast, consuming different evening meals. Values with different letters are significantly different ($P<0.05$, analyses of variance followed by Tukey's pairwise multiple comparison method for means). *Significantly lower compared with white wheat bread (WWB) ($P<0.05$, analyses of variance followed by Dunnett's comparisons with the reference WWB as a control).

Except for the WWB, the evening meals all consisted of low-GI products, matched to have similar GI. However, only the low-GI test meal consisting of barley kernels affected the glucose tolerance at the subsequent standardised breakfast. The present study thus shows that the GI of the evening meal *per se* is a poor predictor of the overnight effect on glucose tolerance.

It has been shown that the improved glucose tolerance seen in the perspective from breakfast to lunch after some low-GI products is related to the suppression of plasma FFA, probably mediated by a prolonged net increment in blood glucose (Wolever *et al*., 1995). It is, however, not likely that the prolonged digestive phase and maintenance of a net increment in late postprandial glycaemia is a main reason for the improved glucose tolerance seen at breakfast in the case of the present study. The time period in between the meals was long enough to ensure that the digestive phase was completed. Moreover, the pasta evening meal did not improve overnight glucose tolerance despite the fact that this particular low-GI product was shown to be very effective in improving glucose tolerance and insulin economy in the 4 h perspective from breakfast to lunch (Liljeberg and Bjorck, 2000).

The test meals with barley kernels, wheat kernels and spaghetti with added wheat bran all contained high amounts of indigestible carbohydrates. However, they differed in the distribution of RS and soluble and insoluble DF. Hence, the barley kernels contained the highest amount of soluble DF, whereas wheat kernels and spaghetti with wheat bran were particularly rich in insoluble DF. Both barley kernels and wheat kernels also contained high amounts of RS. The result indicates that not only the quantity but also the quality of indigestible carbohydrates may play a role for the over-night

Table 3 Plasma short-chain fatty acids in the morning after consuming different evening meals

Evening test meals	*SCFA*			
	Total P-SCFA (μM)	*P-acetate (μM)*	*P-propionate (μM)*	*P-butyrate (μM)*
WWB (control)	144.8±9.5	137.1±9.5	6.4±0.2	1.4±0.3
Wheat kernels	168.5±11.6	160.3±11.4	6.9±0.4	1.5±0.4
Barley kernels	160.1±6.9	151.6±7.0	7.7±0.5*	1.6±0.3
Spaghetti	159.3±8.1	151.5±8.2	6.3±0.3	1.4±0.4
Spaghetti + wheat bran	155.5±8.9	146.7±8.7	7.2±0.3	1.6±0.4

Abbreviations: SCFA, short chain fatty acid; WWB, white wheat bread.
*$P<0.05$. Statistical significance of difference assessed by analyses of variance followed by Dunnett's comparisons with the WWB as a control.

effect, possibly by differing in site of colonic fermentation and/or differences in the pattern of SCFA formed. Increased hydrogen excretion in expired air is a marker of increased bacterial fermentation in colon (Rumessen, 1992). The results from the present study show a significantly higher hydrogen excretion in the morning following an evening meal with barley kernels compared with WWB, spaghetti and wheat kernels, indicating a higher fermentative activity at the time of the breakfast following the barley kernel evening meal.

Also SCFA are indicators of colonic fermentation and can be measured in venous blood (Muir *et al*., 1995). It has been demonstrated that the presence of SCFA in the colon can modify the gastric emptying rate (Cherbut, 2003). The effect on motility seems to depend on the concentration of SCFA in the colonic lumen, and most likely sufficient quantities of SCFA can be reached during colonic fermentation of indigestible carbohydrates (Cherbut, 2003). This may provide one mechanism for a reduced glycaemic response following ingestion of a meal containing fermentable carbohydrates. Further, propionate has been shown to reduce FFA synthesis in rat hepatocytes (Wright *et al*., 1990) and affect hepatic glucose metabolism in isolated rat hepatocytes by increasing glycolysis and decreasing glucose production (Anderson and Bridges, 1984). Propionate and acetate also appear to reduce venous FFA levels in humans (Venter *et al*., 1990; Wolever *et al*., 1991). Circulating FFA levels have implication for glucose utilisation, and FFA compete with glucose uptake in peripheral insulin-sensitive tissue and induce peripheral insulin resistance (Ferrannini *et al*., 1983; Homko *et al*., 2003; Jensen *et al*., 2003). Free fatty acid may also induce hepatic insulin resistance, resulting in an increased endogen glucose production and inhibition of glycogen production (Ferrannini *et al*., 1983; Homko *et al*., 2003). Thorburn *et al*. (1993) have previously demonstrated that ingestion of pearled barley in the evening enhances suppression of FFA and causes reduced glycaemia at a glucose tolerance test performed the following morning, compared with an evening meal of brown rice. The effect was suggested to be owing to a decreased hepatic glucose output and suppression of FFA, resulting from an increased colonic production of SCFA from carbohydrate fermentation. The present study showed a significantly higher concentration of plasma propionate in the morning after a barley kernel evening meal compared with an evening meal with WWB. This supports a mechanism related to colonic fermentation and implicates propionate as being involved in the 'overnight' effects on glucose tolerance. In addition to increased plasma levels of propionic acid, the present study shows a significant reduction of fasting plasma FFA concentrations at breakfast after the barley kernel evening meal, which supports a role of plasma FFA levels in the modulation of glycaemia in the perspective of evening meal to breakfast.

In conclusion, this study shows that the blood glucose response to a high-GI breakfast can be importantly reduced by certain indigestible carbohydrates included in the previous evening meal. The finding of increased levels of H_2 in expired air at the time of the breakfast in the case of the evening meal with barley kernels indicates that the colonic fermentation is likely to be involved. Higher concentration of plasma propionate in the morning after the evening meal with barley kernels supports the involvement of this particular SCFA in the observed benefits on blood glucose regulation. Although literature data suggest that low-GI foods, irrespective of content of indigestible carbohydrates, improve glucose tolerance in a 4 h perspective, other characteristics of low-GI foods, such as the presence of specific indigestible and fermentable carbohydrates, appear to be necessary to achieve improvements in glucose tolerance in the perspective from an evening meal to breakfast. The beneficial second-meal effects seen with most low-GI foods in the perspective of breakfast to lunch, and with certain low-GI foods in the perspective of evening meal to breakfast may contribute to the long-term metabolic benefits observed with low-GI diets (Salmeron *et al*., 1997a; Liu *et al*., 2000; Leeds, 2002; Schulze *et al*., 2004). Further studies are necessary to clarify the relation between colonic fermentation and glucose metabolism, and to what extent the overnight effect seen with certain low-GI foods can be optimised by choice of specific indigestible carbohydrate sources.

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