Effect of cereal test breakfasts differing in glycemic index and content of indigestible carbohydrates on daylong glucose tolerance in healthy subjects1–3

Anne C Nilsson, Elin M Östman, Yvonne Granfeldt, and Inger ME Björck

ABSTRACT

Background: Frequent hyperglycemic episodes are increasingly being associated with an increased risk of type 2 diabetes and cardiovascular disease.

Objective: We studied the extent to which acute glycemia and glycaemia after subsequent meals can be modulated by the characteristics of cereal foods, such as glycemic index (GI) and content of indigestible carbohydrates.

Design: Twelve healthy subjects consumed test meals in a random order. In series 1, the test meals were consumed at breakfast, and postprandial blood glucose incremental areas under the curve (IAUCs) were calculated after the test breakfast, standardized lunch, and standardized dinner. In series 2, the subjects consumed test evening meals and IAUCs were calculated after a subsequent standardized breakfast. Breath hydrogen was measured as an indicator of colonic fermentation.

Results: Barley or rye kernel breakfasts lowered the blood glucose IAUC (0–120 min) at breakfast, at a subsequent lunch, and the cumulative IAUCs (breakfast + lunch + dinner) when compared with white-wheat bread ($P < 0.05$). The lunch blood glucose IAUCs were positively correlated with breakfast IAUCs ($r = 0.80$, $P < 0.05$). Breath hydrogen excretion was negatively correlated with blood glucose IAUCs after lunch ($r = -0.33$, $P < 0.05$) and dinner ($r = -0.22$, $P < 0.05$). A barley kernel evening meal resulted in lower IAUCs ($P < 0.05$) and higher breath hydrogen ($P < 0.001$) after a subsequent breakfast compared with white-wheat bread.

Conclusions: Glucose tolerance at subsequent meals can be notably improved during the course of a whole day or overnight by choosing specific low-GI, whole-grain cereal products. A low GI may be sufficient to achieve a second-meal effect from breakfast to lunch. A specific indigestible carbohydrate mixture appears to be required to show benefits on glucose tolerance in a longer time frame (9.5 h), most likely mediated through colonic fermentation.


KEY WORDS GI, glycemic index, indigestible carbohydrates, second-meal effect, colonic fermentation, breath hydrogen

INTRODUCTION

An accumulating body of data shows beneficial effects of low–glycemic index (GI) foods on chronic diseases related to impairments of glucose and lipid metabolism (1, 2). Epidemiologic data suggest that a low-GI diet plays a protective role against the development of type 2 diabetes (3, 4), coronary artery disease (1, 5), and the metabolic syndrome (6). Similar preventive effects have also been observed with foods rich in whole grains (7, 8); therefore, it could be hypothesized that low-GI foods that are additionally rich in whole-grain constituents could be particularly advantageous. The factors underlying the protective effects of low-GI or whole-grain properties are not fully clarified but likely include improvements in insulin sensitivity (6, 9, 10). Recent findings (11–13) suggest that oxidative stress is a possible mechanism underlying the pathophysiology of diabetes and cardiovascular disease. Hyperglycemia, and probably also elevated concentrations of free fatty acids, induce increased concentrations of reactive oxygen and nitrogen species (12), resulting in cell damage, endothelial dysfunction, and vascular complications (11), as well as β-cell dysfunction (14, 15). A meta-analysis has shown that adults with the metabolic syndrome have lower concentrations of several antioxidants (16), which suggests either a deficiency in dietary supply or that antioxidants have been used to scavenge reactive species emanating from hyperglycemia. Oxidative stress is likely a key mediator of increased cytokine concentrations and low-grade systemic inflammation (17), suggesting its role in the genesis of vascular damage (18).

Certain low-GI foods can lower glycemia not only in direct connection to a meal (acutely), but also at a consecutive standardized second meal, ie, lunch after a test breakfast (19–22) or breakfast after a test dinner (23–26), indicating improvements in insulin sensitivity or insulin economy also within a semi-acute time frame. In the case of benefits in the breakfast to lunch scenario, the key factor involved is likely the lente carbohydrate (ie, carbohydrates capable of maintaining a low but sustained net increment in blood glucose) features of low-GI foods per se (19,
21, 22). However, in the evening meal to breakfast scenario, other properties of the low-GI foods, such as the specific amount or type of indigestible carbohydrates, might contribute to the improvement in glucose tolerance (24–26). Colonic fermentation of indigestible carbohydrates [dietary fiber (DF) and resistant starch (RS)] results in the formation of colonic metabolites, particularly short-chain fatty acids (mainly acetate, propionate, and butyrate) (27, 28). The colonic metabolites may enter the circulation, and it has been suggested that certain short-chain fatty acids produced during colonic fermentation may exert systemic effects, including benefits on glucose metabolism (24, 25, 29, 30).

The purpose of the present study was to investigate the effects of some cereal-based test meals that varied in GI features and content of indigestible carbohydrates (DF and RS) on acute postprandial glucose tolerance as well as on glucose tolerance at subsequent standardized meals. The postprandial glycemia at subsequent meals was related to the magnitude of colonic fermentation of indigestible carbohydrates using breath hydrogen as an indicator.

SUBJECTS AND METHODS

Meals

Test meals

The test meals included in the study were white-wheat bread (WWB, reference product), wheat kernels, rye kernels, oat kernels, barley kernels, whole-grain barley flour porridge (made from flour of the same barley kernels), and WWB enriched with barley DF (Lyckeby Stärkelsen, Kristianstad, Sweden) (WWB+barley DF). The cereal kernels used were commercially available, nonspecified Swedish varieties. The barley and oat kernels were generously provided by Finax AB, Helsingborg, Sweden, and the wheat and rye kernels by Nord Mills AB, Malmö, Sweden. The amount of barley DF added to the WWB meal was intended to correspond to the total DF content naturally occurring in an equivalent starch portion of boiled barley kernels. The size of the test meals corresponded to 50 g available starch (calculated by subtracting RS from total starch). The portion sizes and contents of available starch, RS, and DF (soluble and insoluble) are shown in Table 1. The wheat, rye, and barley kernels were boiled for 30, 35, and 23 min, respectively, in 110 mL water. The oat kernels were boiled for 18 min in 100 mL water. Salt (0.5 g) was added to the boiling water. All water was absorbed into the kernels when cooked. The whole-grain barley flour used for the porridge was suspended in 400 mL water (with 0.5 g salt) and then cooked in a microwave oven for 5 min (600 W) with 2 intermittent breaks for stirring. The products were served immediately after preparation. The WWB was baked according to a standardized procedure (31) in a home baking machine (Severin model no. BM 3983; Severin Svenakal AB, Djursholm, Sweden). After cooling, the crust was removed and the bread was sliced and wrapped into aluminum foil in portion sizes, put into plastic bags, and stored in a freezer. On the day before the experiment, the bread was removed from the freezer and thawed at an ambient temperature in its aluminum foil and plastic bag wrapping. The barley DF extract in the meal with WWB+barley DF was mixed with 250 mL water and consumed with the WWB. A 250-mL portion of water was served with all meals.

Standardized meals

In series 1 (test breakfast series), a standardized lunch was included, consisting of mashed potatoes (Felix Basmos, Procordia Food AB, Eslöv, Sweden) and 100 g meatballs (ICA frozen meatballs, ICA AB, Solna, Sweden). The meatballs were heated in a microwave oven according to the manufacturer’s instructions. The mashed potato powder was mixed with boiled water (68 g mashed potato powder + 3.5 dL water). A standardized

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Portion sizes and contents of available starch, dietary fiber (DF; soluble and insoluble), and resistant starch (RS) in the test products and meals.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test products (%)</td>
<td>Portion weight</td>
</tr>
<tr>
<td>WWB</td>
<td>118.9</td>
</tr>
<tr>
<td>Wheat kernels</td>
<td>90.5</td>
</tr>
<tr>
<td>Rye kernels</td>
<td>93.0</td>
</tr>
<tr>
<td>Oat kernels</td>
<td>118.9</td>
</tr>
<tr>
<td>Barley kernels</td>
<td>95.5</td>
</tr>
<tr>
<td>White WB + barley DF</td>
<td>95.3</td>
</tr>
<tr>
<td>Barley porridge</td>
<td>115.5 + 15.8</td>
</tr>
</tbody>
</table>

1 WWB, white wheat bread (reference product).
2 The portion weight of the white WB was measured as eaten. Cereal kernels, barley DF, and barley flour were ambient dry and uncooked.
3 Available starch calculated by difference between total starch (33) and RS (34).
4 Values are presented as % of dry matter.
WWB (122.9 g fresh weight, Jättersken, Pågen AB, Malmö, Sweden), with the crust removed, was included as a standardized dinner. The WWB and mashed potato portions were equivalent to 50 g available starch (32). At 2 h after commencing the test breakfast and the standardized lunch, respectively, the participants were served 150 mL water and 150 mL coffee or tea without milk or sugar (individually standardized to the subjects).

In series 2 (test evening meal series), a standardized breakfast was provided that was identical in content to the standardized dinner in the test breakfast series. Water (250 mL) was consumed with all meals.

Analysis of total starch, resistant starch, and dietary fiber in the test products

The test products were analyzed with respect to total starch (33), RS (34), and DF (soluble and insoluble) (35). To analyze total starch and DF, the cereal kernels were boiled, air dried, and milled (Cyclotec, Foss Tecator AB, Höganäs, Sweden); the WWB was air dried and milled; and the whole-grain barley porridge was analyzed in the same form as when it was consumed. To analyze RS, all test products were analyzed in the same form as when eaten. Available starch was calculated by subtracting RS from total starch.

Experimental design

Test subjects and procedure

Twelve healthy subjects (5 women and 7 men) aged 21–41 y (mean ± SD = 28.3 ± 5.1 y) and with normal body mass indexes (mean ± SD: 22.1 ± 2.0, in kg/m²) participated in the study. Approval of the study was given by the Regional Ethical Review Board in Lund, Sweden. The subjects were encouraged to standardize their meal patterns and to avoid foods rich in DF the day before an experimental day. Furthermore, they were asked to avoid alcohol and excessive physical exercise in the evening or morning before an experiment. Finally, they were not to have consumed antibiotics or probiotics for 2 wk before the experiment. The test meals were consumed in a random order.

In series 1 (test breakfast series; Figure 1), the test meals included were WWB, wheat kernels, barley kernels, oat kernels, rye kernels, whole-grain barley flour porridge, and WWB+barley DF. Each subject participated at 7 occasions, once or twice a week, with each occasion being ≥3 d apart. At 2030 in the evening before the experimental days, the subjects consumed an individually standardized evening meal composed of WWB and water. After this meal, they were fasting for ≈10 h until breakfast the next morning. On the experimental days, the subjects arrived at 0745 and sat resting until 0800. At that point, fasting blood glucose and breath hydrogen were measured, and subsequently one of the cereal test meals was served. The postprandial blood glucose concentration was measured repeatedly during a 2-h period after the start of the test breakfast, standardized lunch (served at 1200), and standardized dinner (served at 1730), respectively. Breath hydrogen was measured every half-hour during the 11.5 h after the cereal test meals. Coffee or tea and water were served 2 h after the start of the breakfast and lunch, respectively. The subjects were told to maintain a low but consistent level of physical activity throughout the experimental day.

In series 2 (test evening meal series; Figure 2), the test meals were consumed as late evening meals at 2230 in a random order, once or twice a week and at least 3 days apart. The time point for the evening meal was chosen to achieve the same time interval between the test meal and breakfast in series 2 as between the test breakfast and evening meal in series 1. The test evening meal consisted of either WWB, barley kernels, or WWB+barley DF. On the subsequent morning, the subjects consumed a standardized WWB breakfast. Each subject participated once or twice a week at 3 separate occasions. Blood glucose and breath hydrogen were determined at fasting and repeatedly during the 2-h period after the standardized breakfast. In all other respects, the procedure was similar to that of series 1.

Sampling and analyses of blood glucose and hydrogen in expired air

To measure the blood glucose concentration, finger-prick capillary blood samples (HemoCueB-glucose; HemoCue AB, Angelholm, Sweden) were taken before the breakfast, lunch, and evening meals and again at 15, 30, 45, 60, 90, and 120 min in the postprandial phase of the respective meals. The blood glucose concentration before each meal (time = 0) was used as the basal value in the statistical calculations. Breath hydrogen was measured as a marker of colonic fermentation, and was measured at fasting and at half-hour increments during the experimental day with an EC 60 gastrolyzer (Bedfont EC60 Gastrolyzer, Rochester, England). In series 1, the statistical calculations were based on breath hydrogen samples taken 4 h after the breakfast.

Calculations and statistical methods

The GIs of the test products were determined after the breakfast test meals. GIs and glucose areas were determined by using the 0–120-min incremental blood glucose areas under the curves (IAUCs), ignoring the negative areas. The GIs were calculated as the mean of individual ratios (36). GraphPad Prism (version 4.03; GraphPad Software, San Diego, CA, USA) was used for graph plotting and calculating glucose IAUCs. WWB was used as a reference product in the GI calculations. Significant differences among glucose IAUCs and breath hydrogen means were determined with analysis of variance (ANOVA general linear model).
followed by Tukey’s multiple-comparison test (MINITAB Statistical Software, release 13.32 for WINDOWS; Minitab Inc, State College, PA). Participants acted as their own control.

With the exception of postprandial blood glucose concentrations after the standardized meals in test series 1 and in test series 2, the differences in test parameters in a series of time points were analyzed by using a mixed model (PROC MIXED in SAS release 8.01; SAS Institute Inc, Cary, NC) with repeated measures and an autoregressive covariance structure. When significant interactions between treatment (i.e., test meals) and time were found, Tukey’s tests were performed at each instance (MINITAB, release 13.32; Minitab Inc). The blood glucose concentrations before and after the standardized meals in test series 1 and in test series 2 were dependent on the preceding test meals. To achieve a measure of the incremental blood glucose response after the standardized meals with basal values immediately before the meals set to 0 mmol/L, analyses were performed with ANOVA followed by Tukey’s test at the specific test points.

Spearman’s rank correlation was used to study relations between test variables. A correlation for each subject was calculated, and from these values the mean value of Spearman’s correlation coefficient was obtained. To determine the P value, a permutation test was performed by using MATLAB with the null hypothesis that no correlations existed (the alternative hypothesis was that the data were correlated). The results are expressed as means ± SEMs. Values P ≤ 0.05 were considered statistically significant throughout the study.

RESULTS

Series 1 (test breakfast series): course of the day perspective

Effects of different cereal test products consumed at breakfast on postprandial blood glucose increments after the breakfast, a standardized lunch, and a standardized dinner

An overview of the postprandial blood glucose increments during the course of the experimental day (test breakfast, standardized lunch, and standardized dinner) is shown in Figure 3.

FIGURE 3. A significant effect of time (P < 0.0001) and a significant treatment × time interaction (P < 0.0001) were found on basal blood glucose concentrations throughout the test day (n = 12 healthy subjects). WWB, white-wheat bread; DF, dietary fiber.

### TABLE 2
Blood glucose concentrations before each meal after different test breakfasts and mean blood glucose concentrations before the breakfast, lunch, and dinner meals

<table>
<thead>
<tr>
<th>Test breakfast meals</th>
<th>Breakfast (fasting)</th>
<th>Lunch</th>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>4.7 ± 0.1</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Barley porridge</td>
<td>4.8 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Wheat kernels</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Oat kernels</td>
<td>4.6 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Rye kernels</td>
<td>4.6 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Barley kernels</td>
<td>4.7 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>White WB + barley DF</td>
<td>4.6 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Mean blood glucose concentrations</td>
<td>4.6 ± 0.0</td>
<td>4.4 ± 0.1</td>
<td>4.0 ± 0.1</td>
</tr>
</tbody>
</table>

*All values are x ± SEM; n = 12 healthy subjects. WWB, white-wheat bread; DF, dietary fiber. A significant effect of time (P < 0.0001) and a significant treatment × time interaction were found (P < 0.0001). Values in a column not sharing the same lowercase letter are significantly different, and values in a row not sharing the same capital letter are significantly different, P < 0.05 (Tukey’s test).

The mean baseline blood glucose concentrations showed a tendency to decrease during the course of the experimental day. Accordingly, when we investigated the blood glucose concentrations immediately before the meals (time = 0 at breakfast, lunch, and dinner, respectively), a significant effect of time (P < 0.0001) was found throughout the test day as well as a significant treatment × time interaction (P < 0.0001). The mean blood glucose concentrations before the lunch and dinner meals were lower than the mean fasting glucose concentrations before breakfast (P < 0.001 and P < 0.0001, respectively). The mean glucose concentration before dinner was also lower than the corresponding value before lunch (P < 0.0001; Table 2). There were no significant differences in fasting blood glucose concentrations before the start of the test breakfasts. However, before the standardized lunch, the barley kernel breakfast resulted in significantly higher glucose concentrations than did the WWB breakfast (P < 0.05; Table 2). Furthermore, before the standardized dinner meal, blood glucose concentrations were higher after the barley kernel breakfast than after the breakfast of boiled oat kernels (P < 0.05).

The boiled barley and rye kernel breakfasts resulted in lower Glis than did the breakfast composed of WWB (P < 0.001 and P < 0.01, respectively; Table 3) or whole-grain barley porridge (P < 0.01 and P < 0.05, respectively). In addition, the early postprandial glucose response (IAUC 0–60 min) was lower (−33%) after the WWB + barley DF test breakfast than after the WWB (P < 0.05).

When the blood glucose responses after the breakfast, lunch, and dinner meals were expressed as mean IAUCs (0–120 min), a significant effect was found of treatment along the test day (P < 0.0001), whereas no treatment × time interaction was observed (Table 3). The blood glucose increments during the whole test day, as measured with IAUCs 0–120 min after the breakfast, lunch, and dinner, were significantly lower after the barley kernel or the rye kernel breakfasts than after the WWB breakfast (P < 0.001 and P < 0.05, respectively). The blood glucose IAUCs...
during the test day were also lower after the barley kernel breakfast than after the breakfasts consisting of whole-grain barley porridge or WWB + barley DF ($P < 0.01$). In addition, a significant effect of time on blood glucose IAUCs (0–120 min) was seen throughout the test day; that is, the blood glucose IAUCs increased significantly during the day ($P < 0.0001$). Consequently, the mean blood glucose IAUCs (0–120 min) after the dinner (356.6 ± 11.3 mmol · min/L) were significantly larger ($P < 0.0001$) than both the mean blood glucose IAUCs after breakfast (107.7 ± 6.7 mmol · min/L) and those after lunch (144.1 ± 7.8). The blood glucose IAUCs after lunch were also significantly higher than those after the breakfast ($P < 0.01$).

A significant treatment effect ($P < 0.0001$) and a significant interaction (treatment × time, $P < 0.0001$) were found after the breakfast meal (0–120 min) (Figure 4). The lowest postprandial blood glucose increments were achieved after a barley or rye kernel breakfast. The barley or rye kernel breakfasts resulted in significantly lower blood glucose peaks (1.5 ± 0.2 mmol/L and 1.6 ± 0.2 mmol/L, respectively, based on individual peak increment) than did breakfasts of WWB (3.2 ± 0.4 mmol/L, $P < 0.0001$), whole-grain barley porridge (2.9 ± 0.3 mmol/L, barley/rye; $P < 0.01$ and $P < 0.01$, respectively), or oat kernels (2.7 ± 0.2 mmol/L, barley/rye; $P < 0.01$ and $P < 0.05$, respectively). The breakfast consisting of whole-grain barley flour porridge resulted in the fastest rise in blood glucose concentration after breakfast. Consequently, the blood glucose increment at 15 min was significantly higher after the whole-grain barley porridge breakfast than after a breakfast of boiled barley kernels or WWB + barley DF ($P < 0.01$). The breakfast with WWB + barley DF showed a delay in the mean blood glucose peak (Figure 4). Moreover, the drop in blood glucose concentrations in the later postprandial phase (90–120 min after the breakfast) was less steep after the consumption of WWB + barley DF. Consequently, the blood glucose increment remained higher at 120 min after the WWB + barley DF breakfast than after the WWB, barley kernel, and barley porridge ($P < 0.05$). In the postprandial period after the standardized lunch with the highest blood glucose increments, that is, 30–60 min after the standardized lunch, a breakfast of barley kernels resulted in significantly lower blood glucose increments than did the WWB (30 min, $P < 0.001$; 45 min, $P < 0.05$; 60 min, $P = 0.001$) (Figure 5). At 30 min after the start of the standardized lunch, a barley kernel breakfast also resulted in lower blood glucose increments than did breakfasts consisting of whole-grain barley porridge or WWB + barley DF ($P < 0.05$). At 60 min after the start of the lunch, all the kernel-based breakfasts resulted in lower blood glucose increments than did a breakfast of WWB ($P < 0.05$). The blood glucose IAUCs (0–120 min) after the standardized lunch were positively correlated with the blood glucose IAUCs (0–120 min) after the cereal test breakfast ($r = 0.30$, $P < 0.05$).

No significant effects of the type of breakfast consumed were seen after the standardized dinner meals, at 9.5–11.5 h after the test breakfasts (Figure 6). However, the cumulative postprandial glucose IAUCs (0–120 min) over the entire test period (breakfast + lunch + dinner) were significantly lower after the barley kernel or the rye kernel breakfasts than after the WWB breakfast.

**TABLE 3**

Glycemic index (GI) characteristics and postprandial blood glucose incremental areas under the curve (IAUCs; 0–120 min) after the test breakfast and the following standardized lunch and standardized dinner, respectively, and also the total blood glucose IAUCs (breakfast + lunch + dinner) after the different test breakfasts.

<table>
<thead>
<tr>
<th>Test meal</th>
<th>GI</th>
<th>Breakfast IAUC (0–120 min) mmol · min/L</th>
<th>Lunch IAUC (0–120 min) mmol · min/L</th>
<th>Dinner IAUC (0–120 min) mmol · min/L</th>
<th>Total IAUC (0–120 min) mmol · min/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>100a</td>
<td>145.4 ± 22.4</td>
<td>192.5 ± 28.9</td>
<td>360.6 ± 36.3</td>
<td>698.5 ± 70.7a</td>
</tr>
<tr>
<td>Barley porridge</td>
<td>112a</td>
<td>134.6 ± 20.6</td>
<td>157.0 ± 17.8</td>
<td>366.9 ± 23.9</td>
<td>658.5 ± 41.8a</td>
</tr>
<tr>
<td>WWB + barley DF</td>
<td>93b</td>
<td>116.0 ± 14.8</td>
<td>156.2 ± 21.9</td>
<td>379.7 ± 17.6</td>
<td>651.9 ± 42.0b</td>
</tr>
<tr>
<td>Oat kernels</td>
<td>85b</td>
<td>105.0 ± 15.1</td>
<td>137.1 ± 17.0</td>
<td>372.6 ± 34.7</td>
<td>614.7 ± 49.3b</td>
</tr>
<tr>
<td>Wheat kernels</td>
<td>79b</td>
<td>100.4 ± 17.5</td>
<td>127.4 ± 14.2</td>
<td>361.0 ± 33.5</td>
<td>588.8 ± 40.5b</td>
</tr>
<tr>
<td>Rye kernels</td>
<td>73b</td>
<td>80.6 ± 11.3</td>
<td>130.7 ± 21.2</td>
<td>345.8 ± 36.0</td>
<td>557.1 ± 58.2b</td>
</tr>
<tr>
<td>Barley kernels</td>
<td>49b</td>
<td>68.2 ± 11.8</td>
<td>107.7 ± 14.4</td>
<td>309.6 ± 25.6</td>
<td>485.5 ± 40.1c</td>
</tr>
</tbody>
</table>

All values are x ± SEM; $n = 12$ healthy subjects. WWB, white-wheat bread; DF, dietary fiber. Significant effects of treatment (type of breakfast) ($P < 0.001$) and of time ($P < 0.0001$) were found along the test day, whereas no treatment × time interaction was seen. Means in a column not sharing the same superscript letter are significantly different, $P < 0.05$ (ANOVA followed by Tukey’s test).

The combined blood glucose IAUCs after the breakfast, lunch, and dinner.

FIGURE 4. Mean acute incremental blood glucose changes (Δ) after the different cereal breakfasts. A significant treatment (type of breakfast) effect ($P < 0.001$) and a significant interaction (treatment × time, $P < 0.0001$) were found over the test period. At a given point in time, values not sharing the same letters are significantly different, $P < 0.05$ (ANOVA followed by Tukey’s test). $n = 12$ healthy subjects. WWB, white-wheat bread; DF, dietary fiber.
The cumulative glucose IAUCs (breakfast + lunch + dinner) after the barley kernel breakfast were also lower than after breakfasts consisting of whole-grain barley porridge or WWB + barley DF, respectively. The cumulative postprandial glucose IAUCs after the breakfast (0–120 min) and lunch (0–120 min) were lower after all kernel-based breakfasts than after the WWB breakfast (barley, \( P < 0.0001 \); rye, \( P < 0.001 \); wheat, \( P < 0.01 \); oat, \( P < 0.05 \), respectively). In addition, the barley kernel breakfast resulted in lower cumulative blood glucose IAUCs (breakfast + lunch) than did whole-grain barley porridge (\( P < 0.01 \)) or WWB + barley DF (\( P < 0.05 \)). The blood glucose IAUCs (0–120 min) at the standardized lunch and dinner meals were inversely correlated to the blood glucose value before the start of the meals (\( r = -0.64, P < 0.001 \), and \( r = -0.39, P < 0.01 \), respectively).

Breath hydrogen excretion

The mean breath hydrogen excretions after the different test breakfasts (at lunch, dinner, and total) are shown in Figure 8. With respect to mean breath hydrogen excretion at lunch and dinner, significant effects of product (\( P < 0.0001 \)) and time (\( P < 0.05 \)) were found, as well as a significant treatment × time interaction (\( P < 0.001 \); Table 4). At the standardized lunch, 4–6 h after the test breakfast, the mean breath hydrogen excretion was higher after the rye kernel breakfast than after all the other breakfasts except for the barley kernel breakfast. The barley kernel breakfast resulted in higher mean breath hydrogen excretion at lunch in comparison with WWB, and after the standardized dinner (9.5–11.5 h), barley kernels resulted in higher mean hydrogen excretion compared with all other breakfast meals except for oat kernels. The mean hydrogen excretion at dinner was higher after the oat kernel breakfast than after the WWB and whole-grain barley porridge breakfasts. Breath hydrogen excretion at the standardized lunch and dinner meals was negatively correlated to the blood glucose IAUCs (0–120 min) at the same meals (lunch, \( r = -0.33, P < 0.05 \); dinner, \( r = -0.22, P < 0.05 \)).
A significant treatment effect of the preceding evening test meal was noted in blood glucose increments in the 45–60-min postprandial period after the standardized breakfast meal was noted in blood glucose increments in the 45–60-min postprandial period after different cereal-based evening meals.

**Breath hydrogen excretion after the standardized breakfast**

The mean breath hydrogen concentration after the standardized breakfast (0–120 min) was significantly higher after an evening meal of barley kernels than after an evening meal of WWB (54.9 ± 9.9 and 18.4 ± 2.9 ppm, respectively; Figure 10) (P < 0.001). There were no significant differences (P = 0.09) in mean breath hydrogen concentrations in the morning after an evening meal of barley kernels compared with WWB + barley DF (37.4 ± 6.8 ppm), and no significant differences (P = 0.06) were obtained in mean hydrogen excretion at breakfast after the evening meal of WWB + barley DF compared with WWB.

**Comparisons between series 1 and series 2**

The blood glucose response (0–120 min IAUCs) at a standardized WWB meal was significantly higher at 9.5 h after the test breakfasts than the corresponding area obtained at 9.5 h after the various test evening meals (Figure 11). That is, the blood glucose response was higher in the evening than in the morning (P < 0.0001). There were, however, no corresponding differences in mean hydrogen concentrations at 9.5–11.5 h after the test meals depending on whether the test period was from evening meal to breakfast or from breakfast to evening meal (Figure 12).

### Table 4

Mean breath hydrogen concentrations during 2 h after the standardized lunch (4–6 h) and dinner meals (9.5–11.5 h), respectively, and mean breath hydrogen concentrations (4–11.5 h) after consuming the different cereal test breakfasts.

<table>
<thead>
<tr>
<th>Test meals</th>
<th>Lunch (4–6 h)</th>
<th>Dinner (9.5–11.5 h)</th>
<th>Total (4–11.5 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>18.0 ± 3.6a</td>
<td>22.9 ± 3.1a</td>
<td>17.2 ± 2.6a</td>
</tr>
<tr>
<td>Barley porridge</td>
<td>28.6 ± 4.9b</td>
<td>26.1 ± 3.2a</td>
<td>25.0 ± 3.5b</td>
</tr>
<tr>
<td>Wheat kernels</td>
<td>28.7 ± 5.1ab</td>
<td>33.8 ± 6.0b</td>
<td>29.9 ± 5.5b</td>
</tr>
<tr>
<td>Oat kernels</td>
<td>31.7 ± 5.6bc</td>
<td>42.7 ± 4.2bc</td>
<td>36.4 ± 4.4bc</td>
</tr>
<tr>
<td>Rye kernels</td>
<td>52.6 ± 8.4c</td>
<td>37.2 ± 4.6bc</td>
<td>41.0 ± 5.1bc</td>
</tr>
<tr>
<td>Barley kernels</td>
<td>39.3 ± 7.6cd</td>
<td>52.7 ± 6.8d</td>
<td>46.3 ± 6.5d</td>
</tr>
<tr>
<td>WWB + barley DF</td>
<td>27.1 ± 4.1ab</td>
<td>36.4 ± 4.0b</td>
<td>31.7 ± 4.1bc</td>
</tr>
</tbody>
</table>

All values are ± SEM; n = 12 healthy subjects. WWB, white-wheat bread; DF, dietary fiber. Significant effects of product (P < 0.0001) and of time (P < 0.05) and a significant treatment × time interaction were found (P ≤ 0.001). Values in a column not sharing the same superscript letter are significantly different, P < 0.05 (ANOVA followed by Tukey’s test).

### Series 2 (test evening meal series): overnight perspective

**Postprandial blood glucose increments after a standardized breakfast after different cereal-based evening meals**

A significant treatment effect of the preceding evening test meal was noted in blood glucose increments in the 45–60-min postprandial period after the standardized breakfast (Figure 9). At 45 min after the start of the standardized breakfast, the blood glucose increment was significantly lower after an evening meal with barley kernels than after both an evening meal of WWB (P < 0.01) and of WWB + barley DF (P < 0.05). When expressed as IAUCs (0–120 min), the postprandial blood glucose response after the standardized breakfast was lower after an evening meal of barley kernels than after a corresponding evening meal of WWB (IAUCs of 80.9 ± 12.4 and 121.6 ± 10.7 mmol · min/L, respectively; P < 0.05). There were no significant differences (P = 0.06) in the 0–120-min blood glucose IAUCs at breakfast after

![Test meals the previous evening](image)

**FIGURE 9.** Mean blood glucose incremental changes (A) after a standardized breakfast, 9.5–11.5 h after different test evening meals. At a given point in time, values not sharing the same letter are significantly different, P < 0.05 (ANOVA followed by Tukey’s test). n = 12 healthy subjects. WWB, white-wheat bread; DF, dietary fiber.

![Breath hydrogen excretion after the standardized breakfast](image)

**FIGURE 10.** Mean breath hydrogen concentrations after a standardized breakfast, 9.5 h after different test evening meals. A significant treatment (type of breakfast) effect (P < 0.001, Tukey’s test) was found, whereas no treatment × time interaction was seen along the test period, n = 12 healthy subjects. WWB, white-wheat bread; DF, dietary fiber.
DISCUSSION

The lack of difference in acute glycemic area after breakfasts consisting of whole-grain barley flour porridge compared with the WWB makes us conclude that the low-GI features observed in the present work with barley kernels are probably not related to the amounts of cereal DF present in barley kernels per se. This supports previous observations (37–39) showing that disruption of the botanical structure of cereal kernels before heat treatment, for example, by grinding to a whole-grain flour, renders the starch highly susceptible to digestive enzymes. The barley DF used in the WWB + barley DF meal contained a substantial fraction of soluble DF (28% dry weight basis), which was made up almost exclusively of β-glucans, yielding ≈4 g β-glucans/serving. The beneficial effect of β-glucans on the acute glucose response in the present study (IAUC 0–60 min) is in line with previous studies with oat β-glucans, showing that 4 g β-glucans seems to be a critical amount to elicit a significant decrease in glucose response to a breakfast based on β-glucan-enriched muesli served with yogurt and WWB (40). Clearly, β-glucans from oats and barley appear to have similar benefits on acute glycemia. The lack of effect of WWB + barley DF when calculating the 0–120-min blood glucose IAUCs was due to a higher blood glucose increment in the later (60–120 min) postprandial phase. A prolonged net increment in blood glucose concentrations in the late postprandial phase was observed also after enrichment of the meal with 4 g oat β-glucans (40) and can be considered beneficial in that a hypoglycemic state in between meals is avoided. The low-GI features of barley and rye kernels in the present study are consistent with previous observations, as are the findings in the present work of high-GI features of whole-grain barley flour and boiled oat kernels (37).

In the present study, the glycemic response to the breakfast products seemed to be an important factor in predicting the glucose response at the subsequent lunch. Consequently, a significant positive correlation was observed between the blood glucose responses (IAUCs 0–120 min) after the test breakfasts and the glucose responses (IAUCs 0–120 min) after the subsequent standardized lunch. This finding agrees with previous studies showing that a low-GI breakfast with lente carbohydrates may significantly reduce postprandial glycemia after a standardized lunch meal (19, 21, 22), independent of the content of indigestible carbohydrates. However, although the low-GI feature per se probably is the most important factor for the benefits on glucose tolerance seen in a shorter in-between meal period (from breakfast to lunch), additional mechanisms deriving from colonic fermentation cannot be excluded in the case of rapidly fermentable carbohydrates, such as lactulose (41). In the present study of using breath hydrogen excretion as an indicator, signs of colonic fermentation were seen already at the time of the lunch meal, especially after the rye kernel breakfast. However, the conclusive results from previous studies and the positive correlation between glycemia at breakfast and glycemia at lunch in the present study make us conclude that the glycemic features of a cereal-based breakfast are the major determinant of glycemia at the subsequent lunch.

The cumulative glycemic response (IAUCs of breakfast + lunch + dinner) was highly positively correlated to the GI of the breakfast product (r = 0.63, P < 0.0001). Nevertheless, no significant correlation was seen between the glucose IAUCs after breakfast and after the standardized dinner (r = 0.16, P = 0.18) (test breakfast series). Previous studies have shown that a low-GI spaghetti evening meal has no effect on the glucose tolerance at a standardized breakfast consumed 10.5 h later (25, 26), whereas boiled barley kernels significantly lower the glucose response. This indicates that in a longer time perspective, eg, from breakfast to the evening meal, the GI of the food per se is probably not effective. The fact that a significant negative correlation between breath hydrogen excretion and glucose response was observed at the standardized dinner supports previous suggestions of an important additional effect on glucose tolerance mediated by metabolites produced during colonic fermentation of specific indigestible carbohydrates (24, 25) at that point. Considering the lack of effect of whole-grain barley porridge or WWB + barley DF in

![FIGURE 11. Blood glucose responses to a standardized meal consumed 9.5 h after test meals consumed either at dinner (filled symbols) or at breakfast (open symbols). The blood glucose responses to all test meals were higher 9.5 h after a breakfast compared with 9.5 h after an evening meal, P < 0.0001 (ANOVA followed by Tukey’s test). n = 12 healthy subjects. WWB, white-wheat bread; DF, dietary fiber.](https://academic.oup.com/ajcn/article-abstract/87/3/645/4633269/fig11)

![FIGURE 12. Breath hydrogen concentrations after a standardized meal consumed 9.5 h after test meals consumed either at dinner (filled symbols) or at breakfast (empty symbols). There were no significant differences depending on when the test meals were consumed (breakfast or dinner). n = 12 healthy subjects. WWB, white-wheat bread; DF, dietary fiber.](https://academic.oup.com/ajcn/article-abstract/87/3/645/4633269/fig12)
the lowering of the cumulative glucose IAUCs, it can be suggested that the intact structure and the concomitant elevation in content of RS in the barley and rye kernels may have contributed to the effect. It was previously shown that intake of RS, 30 g/d for 4 wk, or 60 g RS the day before the test day (type 2 RS), improves insulin sensitivity in healthy subjects (42, 43).

In series 2, the time period in between the test evening meal and the standardized breakfast was 9.5 h and thus similar to the time interval between the test breakfast and the standardized dinner in series 1. When comparing the glucose IAUCs 9.5 h after the test meals in series 1 and series 2, the glucose areas were larger after the standardized WWB dinner (series 1) than after the corresponding standardized WWB breakfast (series 2). This indicates a lower glucose tolerance in the evening than in the morning. The decrease in glucose tolerance seen in healthy subjects over the course of the day was reported previously (44, 45) and is the opposite of what could be expected in diabetic (type 2 diabetes and type 1 diabetes) or obese subjects (44). More knowledge about potential differences in glucose tolerance during the course of a day is of importance and may have implications for diagnosis based on oral-glucose-tolerance tests and measurement of GI, to name a few. In the present study, the baseline glucose concentrations before the meals dropped from breakfast to lunch and dropped again from lunch to dinner. The drop was, however, less pronounced after the barley kernel breakfast, which might be associated with benefits on insulin sensitivity through a mechanism related to a prolonged suppression of free fatty acid concentrations in the blood. This proposal could be strengthened by the negative correlations observed between blood glucose IAUCs (0–120 min) and the basal blood glucose concentrations before the lunch and dinner meals, respectively. It was previously reported that fasting glucose concentrations, when measured repeatedly during daytime, decline from the morning to the evening in healthy subjects (44, 46). It should be noted that the total caloric intake during the experimental days in this study was between 1000 and 1060 kcal per person and consequently lower than the recommended daily caloric intake for most healthy adults.

Prospective studies that include a large number of subjects have indicated that dietary supplementation with acarbose, an α-glucosidase inhibitor, importantly delays the onset of type 2 diabetes (47) and reduces the risk of cardiovascular disease and hypertension (48) in glucose-intolerant subjects. The beneficial effect of acarbose is proposed to derive from a reduced oxidative stress and subclinical inflammation due to lower postprandial glucose excursion (47, 49). The effect of acarbose is thus similar to that of starchy foods with a low GI. It could therefore be hypothesized that a low-GI diet, by virtue of maintaining tighter glucose regulation, decreases oxidative stress and low-grade inflammation. Acute hyperglycemia also induces increased concentrations of circulating cytokines (tumor necrosis factor-α, interleukin-6, interleukin-18) in healthy subjects (17). Low-grade inflammation in healthy subjects has been connected to an adverse effect on insulin sensitivity, glucose and lipid metabolism, and blood pressure (50). In particular, frequent postprandial hyperglycemic episodes appear to be more prone to initiate cytokine production (17), indicating that tight glucose regulation may protect against cardiovascular disease (51). Consequently, low-GI foods capable of reducing blood glucose excursions during the course of a whole day, or in an overnight perspective, could be expected to further add to the beneficial effects of low-GI diets. As judged from the present work, low-GI cereals rich in fermentable carbohydrates might be advantageous in this respect. In previous studies (25), it was shown that the overnight effect seen on glucose tolerance at breakfast after a barley kernel evening meal corresponded to a higher concentration of plasma propionate (derived from colonic fermentation) and a lower concentration of free fatty acids in the morning. In addition, in a recent study (52), it was found that after different cereal-based evening meals, the glucose response to a standardized high-GI breakfast was negatively correlated to the concentration of plasma propionate and positively correlated to the concentration of fasting plasma free fatty acids. Such benefits mediated through colonic fermentation might actually contribute to the protective role of whole-grain diets adjunct to the development of type 2 diabetes (3, 4), coronary artery disease (1, 5), and the metabolic syndrome (6). However, whole-grain foods are additionally rich in associated bioactive components such as choline, betaine (53), minerals, plant sterols, vitamins, antioxidants, and lignans (54, 55), which may add to the beneficial effects.

In conclusion, the present study shows that certain cereal products with low GIs and high contents of specific indigestible carbohydrates (RS + DF) can modulate the glucose response not only in the acute phase but also during the course of a whole day, as well as in the perspective from a late evening meal to the subsequent breakfast. Taken together, this information provides additional support for metabolic benefits of foods that induce lower acute glycemic excursions and provides a new dimension for the design of low-GI foods with optimal contents of indigestible carbohydrates for magnified metabolic benefits on blood glucose regulation.

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The contributions of the authors were as follows—ACN: coordinated the study and was responsible for the study design, for the collection and analysis of the data, for statistical analysis, and for writing the paper; EMO: was involved in the evaluation and writing of the paper; YG: provided comments to the results and to several practical details; IMEB: was the guarantor for the funding of the study and was involved in the study design and writing of the paper. None of the authors had any conflicts of interest.

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