Including Indigestible Carbohydrates in the Evening Meal of Healthy Subjects Improves Glucose Tolerance, Lowers Inflammatory Markers, and Increases Satiety after a Subsequent Standardized Breakfast1,2

Anne C. Nilsson,1* Elin M. Östman,3 Jens J. Holst,4 and Inger M. E. Björck3

1Division of Applied Nutrition and Food Chemistry, Department of Food Technology, Engineering and Nutrition, Lund University, SE-22100 Lund, Sweden and 3Department of Biomedical Sciences, University of Copenhagen, the Panum Institute, DK-2200 Copenhagen, Denmark

Abstract

Low-glycemic index (GI) foods and foods rich in whole grain are associated with reduced risk of type 2 diabetes and cardiovascular disease. We studied the effect of cereal-based bread evening meals (50 g available starch), varying in GI and content of indigestible carbohydrates, on glucose tolerance and related variables after a subsequent standardized breakfast in healthy subjects (n = 15). At breakfast, blood was sampled for 3 h for analysis of blood glucose, serum insulin, serum FFA, serum triacylglycerides, plasma glucagon, plasma gastric-inhibitory peptide, plasma glucagon-like peptide-1 (GLP-1), serum interleukin (IL)-6, serum IL-8, and plasma adiponectin. Satiety was subjectively rated after breakfast and the gastric emptying rate (GER) was determined using paracetamol as a marker. Breath hydrogen was measured as an indicator of colonic fermentation. Evening meals with barley kernel based bread (ordinary, high-amylose- or β-glucan-rich genotypes) or an evening meal with white wheat flour bread (WWB) enriched with a mixture of barley fiber and resistant starch improved glucose tolerance at the subsequent breakfast compared with unsupplemented WWB (P < 0.05). At breakfast, the glucose response was inversely correlated with colonic fermentation (r = −0.25; P < 0.05) and GLP-1 (r = −0.26; P < 0.05) and positively correlated with FFA (r = 0.37; P < 0.001). IL-6 was lower (P < 0.01) and adiponectin was higher (P < 0.05) at breakfast following an evening meal with barley-kernel bread compared with WWB. Breath hydrogen correlated positively with satiety (r = 0.27; P < 0.01) and inversely with GER (r = −0.23; P < 0.05). In conclusion, the composition of indigestible carbohydrates of the evening meal may affect glycemic excursions and related metabolic risk variables at breakfast through a mechanism involving colonic fermentation. The results provide evidence for a link between gut microbial metabolism and key factors associated with insulin resistance. J. Nutr. 138: 732–739, 2008.

Introduction

Foods with low glycemic index (GI)1 have proven beneficial in the treatment and prevention of the metabolic syndrome, diabetes,

1 Supported by the European Commission in the Communities 6th Framework Program, Project HEALTHGRAIN (FP6-514008). This publication reflects only the authors’ views and the Community is not liable for any use that may be made of the information contained in this publication.
3 Abbreviations used: AUC, total area under the curve; CutOB, ordinary barley kernels cut 1–2 times; DF, dietary fiber; 1-FFA, fasting FFA; GER, gastric emptying rate; GI, glycemic index; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; HAB, high amylose barley kernels; HBB, high β-glucan barley kernels; IAUC, incremental area under the curve; IL, interleukin; OB, ordinary barley kernels; 1/2OB, 1/2 portion of ordinary barley kernels; RS, resistant starch; WWB, white wheat flour bread; WWB + RS, white wheat bread and resistant starch; WWB + RS + DF, white wheat bread with resistant starch and dietary fiber.
4 To whom correspondence should be addressed. E-mail: anne.nilsson@appliednutrition.thh.se.

and cardiovascular disease (1–4). Similarly, preventive effects adjunct to development of type 2 diabetes, cardiovascular disease, and obesity are also reported from observational studies with foods rich in whole grain (5,6). It could therefore be hypothesized that low-GI foods, which in addition are rich in whole-grain constituents, could be particularly advantageous. The mechanisms underlying the beneficial effects of low-GI and whole-grain foods are, however, not completely explained. It is known that hyperglycemia, and probably also elevated levels of FFA, induce increased concentrations of reactive oxygen and nitrogen species (7). Oxidative stress is probably a key mediator of increased cytokine concentrations and low grade systemic inflammation (8), suggesting a role in the genesis of vascular damage (9), and a possible mechanism underlying the pathophysiology of type 2 diabetes and cardiovascular disease (7,10,11). It could be hypothesized that a low-GI diet decreases oxidative stress and lower inflammation by maintaining a more tight blood glucose regulation.
In addition to lowering the acute blood glucose response, several studies show that most low-GI foods consumed at breakfast may lower glycemia at a "second-meal" standardized lunch after 4 h (12), indicating improvements on glycemic regulation, insulin sensitivity, and/or insulin economy also within a semiacute time frame. Benefits on glycemic excursions are also reported with certain low-GI products in the perspective from a late evening meal to a subsequent standardized breakfast (13,14). However, within this longer time frame, low-GI features per se were not sufficient to achieve a second-meal effect on glucose tolerance. Instead, low-GI foods that were also rich in indigestible carbohydrates induced benefits to glucose tolerance in an overnight perspective. Accordingly, it appears plausible that the second-meal effect on glucose tolerance may be mediated through different mechanisms depending on the in-between meal time period both with respect to the food factors and the physiological mechanisms involved.

The aim of this work was to study the potential importance of GI and indigestible carbohydrates [dietary fiber (DF) and resistant starch (RS)] of tailored whole-grain evening meals on glucose tolerance the subsequent morning and to study possible physiological mechanisms underlying the improved glucose regulation in this longer period of time (10.5 h). For this purpose, barley varieties differing in contents of DF or amylose:amyllopectin ratio were used in baking kernel-based bread (whole- or cut cereal kernels), thus enabling production of low-GI barley bread products differing in the amount and distribution of DF and RS. A white wheat bread (WWB) was used as a reference evening meal and a WWB meal enriched with barley DF and RS, or RS only, were included as controls (high-GI products). Healthy subjects ingested carbohydrate-equivalent evening test meals and blood was sampled prior to and following a standardized breakfast to measure blood glucose, serum insulin, plasma glucagon, serum lipids (FFA and triacylglycerides), plasma incretins [glucagon-like peptide-1 (GLP-1) and gastric-inhibitory peptide (GIP)], plasma adiponectin, and markers of inflammation [interleukin (IL)-6 and IL-8]. Breath hydrogen was measured as an indicator of colonic fermentation and gastric emptying rate (GER) was measured using serum paracetamol as a marker. Finally, subjective rating of satiety was measured after the standardized breakfast.

Materials and Methods

Test subjects
Healthy volunteers, 6 women and 11 men, aged 22 – 32 y (mean ± SD = 25.9 ± 3.2 y) with normal BMI (mean ± SD = 22.5 ± 2.1 kg/m²) participated in the study. Subjects were recruited by e-mail or telephone calls to people who were previously involved in similar studies or friends of such persons that asked to be included in the study. The subjects were carefully informed that they could quit the experiment at any time, without feeling forced to give any explanation. One male was excluded because the breakfast bread made him nauseous and 1 woman dropped out due to gastrointestinal symptoms. A total of 15 subjects completed the study. Approval of the study was given by the Regional Ethical Review Board in Lund, Sweden.

Evening test meals and the standardized breakfast
Eight cereal-based test meals with different GI and contents of indigestible carbohydrates were included in the study (Tables 1 and 2). The test meals consisted of bread with the main proportion of carbohydrates derived from: white wheat flour (WWB; reference product), ordinary barley kernels (OB; unspecified ordinary Swedish barley provided by Lantmännen Food), OB that were cut 1–2 times (CutOB), kernels from a barley variety with elevated amounts of amylose yielding increased levels of RS in the finished product (HAB; 31% RS; starch basis, Karmös, Svalöf Weibull), kernels from a barley variety with elevated amounts of β-glucans (HBB; ~14%, dry basis, mutant 13, Svalöf Weibull), WWB added with RS (RS 2) from a high-RS corn starch (WWB + RS; Hi-maize 1043, Bionom), or WWB added with the same amount of RS and also with DF from barley (WWB + RS + DF; Lyckebäck Ståkelsen) to match the content of indigestible carbohydrates in the OB product. To investigate a possible dose-response effect, 1 test meal consisted of a one-half portion of OB bread (1/2OB bread). The size of all test meals (except for 1/2OB) corresponded to 50 g available carbohydrates, calculated by subtracting RS (15) from the total starch content (16). White wheat flour was added to the cereal kernel breads in proportions (weight percent) of 10:90 white wheat flour:kerneis. Water (unlimited amounts) was consumed with the test evening meals.

The standardized breakfast consisted of 116.7 g WWB, corresponding to 50 g available starch. The bread was baked similarly to the WWB reference bread described in the next paragraph, except that 8.25 g paracetamol was added to each bread. The paracetamol was included in the bread (1 g per portion) as a tool to measure the GER. Water (250 mL) was served with the standardized breakfast meal.

Recipes

WWB. The WWB was baked according to a standardized procedure in a home baking machine (Severin model no. BM 3983; Menu choice, program 2 [white bread, 1000 g, quick (time: 2:35)]. The bread was made from 540 g white wheat flour (Kungsörnen), 360 g water, 4.8 g dry yeast, and 4.8 g salt.

OB bread. A total of 630 g OB was boiled in 550 g water for 12 min and was then cooled for 30 min in ambient temperature. All water was

---

**TABLE 1** GI characteristics and percentages of starch (total, resistant, and available) and DF (soluble and insoluble) in the test products

<table>
<thead>
<tr>
<th>Products</th>
<th>GI</th>
<th>Starch</th>
<th>RS</th>
<th>Available</th>
<th>Insoluble</th>
<th>Soluble</th>
<th>Total RS + DF</th>
<th>% dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>100</td>
<td>84.4</td>
<td>2.1</td>
<td>82.3</td>
<td>3.3</td>
<td>1.1</td>
<td>4.4</td>
<td>6.5</td>
</tr>
<tr>
<td>OB</td>
<td>52</td>
<td>77.2</td>
<td>12.3</td>
<td>64.9</td>
<td>10.1</td>
<td>3.8</td>
<td>13.9</td>
<td>26.2</td>
</tr>
<tr>
<td>CutOB</td>
<td>55</td>
<td>76.3</td>
<td>11.5</td>
<td>64.9</td>
<td>10.5</td>
<td>3.2</td>
<td>13.7</td>
<td>25.2</td>
</tr>
<tr>
<td>1/2OB</td>
<td>52</td>
<td>77.2</td>
<td>12.3</td>
<td>64.9</td>
<td>10.1</td>
<td>3.8</td>
<td>13.9</td>
<td>26.2</td>
</tr>
<tr>
<td>HAB</td>
<td>52</td>
<td>72.1</td>
<td>22.0</td>
<td>50.1</td>
<td>10.9</td>
<td>5.3</td>
<td>16.2</td>
<td>38.2</td>
</tr>
<tr>
<td>HBB</td>
<td>50</td>
<td>48.5</td>
<td>18.5</td>
<td>30.0</td>
<td>16.0</td>
<td>14.0</td>
<td>30.0</td>
<td>48.5</td>
</tr>
<tr>
<td>WWB + RS</td>
<td>76</td>
<td>84.6</td>
<td>11.7</td>
<td>72.9</td>
<td>3.6</td>
<td>1.4</td>
<td>5.0</td>
<td>18.7</td>
</tr>
<tr>
<td>WWB + RS + DF</td>
<td>88</td>
<td>70.4</td>
<td>10.5</td>
<td>59.9</td>
<td>6.5</td>
<td>5.9</td>
<td>12.4</td>
<td>22.9</td>
</tr>
</tbody>
</table>

---

1 Values of total starch are means of 2 replications, RS means of 6 replications, DF means of 3 replications.

---

**TABLE 2** Portion sizes and the content of starch (total, resistant, and available) and DF (soluble and insoluble) in the test meals

<table>
<thead>
<tr>
<th>Test meals</th>
<th>Starch</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g/portion</td>
</tr>
<tr>
<td>WWB</td>
<td>116.7</td>
<td>51.3</td>
</tr>
<tr>
<td>OB</td>
<td>161.0</td>
<td>59.5</td>
</tr>
<tr>
<td>CutOB</td>
<td>190.2</td>
<td>58.8</td>
</tr>
<tr>
<td>1/2OB</td>
<td>80.5</td>
<td>29.7</td>
</tr>
<tr>
<td>HAB</td>
<td>213.0</td>
<td>72.0</td>
</tr>
<tr>
<td>HBB</td>
<td>388.2</td>
<td>80.9</td>
</tr>
<tr>
<td>WWB + RS</td>
<td>130.0</td>
<td>58.0</td>
</tr>
<tr>
<td>WWB + RS + DF</td>
<td>181.8</td>
<td>58.8</td>
</tr>
</tbody>
</table>

1 Values of total starch are based on means of 2 replications, RS means of 6 replications, DF means of 3 replications.
absorbed into the kernels when cooked. Added to the kernels were: 70 g white wheat flour, 12 g dry yeast, 5 g salt, and 240 g water. The dough was kneaded for 4 min (Electrolux AKM 3110n N 25) and proofed for 30 min in the dough mixing bowl, followed by another proofing (35 min) in a baking tin. Bread was baked in a household oven at 205°C for 40 min, covered with aluminum foil during the last 20 min.

**CutOB bread.** A total of 630 g CutOB kernels was boiled in 650 g water for 10 min. The other ingredients,proofing, and baking procedure were similar to the OB bread.

**HAB bread.** The recipe was similar to the OB bread, except the amount of water added to the dough was 190 g.

**HBB bread.** A total of 630 g kernels was boiled in 550 g water for 10 min. The other ingredients,proofing, and baking were similar to the OB bread.

**WWB+RS+DF.** The bread was made from 465 g white wheat flour, 100 g High-maize starch, 125 g barley DF extract, 5 g salt, 5 g yeast, and 650 g water. The dry ingredients were mixed with water in the dough mixing bowl (Electrolux AKM 3110n N 25) and kneaded for 6 min. The dough was proved for 30 min in the dough mixing bowl, kneaded for 2 min, and proved again (45 min) in a baking tin. Bread was baked at 225°C for 45 min.

**WWB+RS.** The bread was made of 490 g white wheat flour, 100 g High-maize starch, 5 g salt, 5 g yeast, and 400 g water. The bread was baked as described for the WWB.

After cooling, the crusts were removed and the breads were sliced and wrapped in aluminum foil in portion sizes, put into plastic bags, and stored in a freezer (−20°C). Bread slices consumed in the evening were removed from the freezer in the morning and thawed at an ambient temperature in their aluminum foil and plastic bag wrapping.

**Chemical analyses and GI characteristics of the test products**

The test products were analyzed with respect to total starch (16), RS (15), and DF (soluble and insoluble) (17). Before analysis of total starch and DF, the bread products were air dried and milled (Cyclotec, Foss Tecator). RS in all test products were analyzed on products as eaten. The available starch content was calculated by subtracting RS from total starch. The GI of the products were predicted from hydrolysis indices using an in vitro method (18) and GI calculated from a modified equation: GI = 6.272 + 0.912 × hydrolysis indices (19).

**Experimental design**

**Procedure.** The subjects were encouraged to standardize their meal pattern and avoid foods rich in DF the day prior to each experimental day. Furthermore, they should avoid alcohol and excessive physical exercise in the evening and should not have consumed antibiotics or probiotics during the previous 2 wk. The subjects participated approximately once per week and the test meals were consumed in a randomized order. Subjects consumed the test meals at 2130 and then fasted until the approximate once per week and the test meals were consumed in a random pattern and avoid foods rich in DF the day prior to each experimental Procedure.

**Physiological variables.** Finger-prick capillary blood samples were withdrawn repeatedly for determination of blood glucose concentrations (HemoCue B-glucose, HemoCue). Venous blood was withdrawn repeatedly for determination of serum insulin, FFA, IL-6, IL-8, triacylglycerol, and paracetamol (marker of GER), and plasma adiponectin, GIP, GLP-1, and glucagon. Breath H₂ was measured as an indicator of colonic fermentation using an EC 60 gas analyzer (Bedfont EC60 Gastrolyzer). In addition, pre- and postbreakfast satiety was obtained with a bipolar rating scale, marked with 9 statements that describe the feeling of hunger or satiation, with the statement “no specific feeling” in the middle of the scale. A time schedule for determination of the physiological variables is presented in **Table 3**.

**Calculations and statistical methods.** The results are expressed as means ± SEM. The positive incremental area under the curve (IAUC) was used for expressing the results of glucose, insulin, and paracetamol responses. Total areas under the curve (AUC; basal value = 0) were used in the statistical calculations of plasma glucagon, GLP-1, GIP, and satiety. Statistical evaluation of serum IL-6 was based on data after the WWB, OB bread, and HBB bread and evaluations of serum IL-8 and plasma adiponectin were based on data after the WWB and OB bread. GraphPad Prism (version 4.03; GraphPad Software) was used for graph plotting and calculation of areas. Significant differences in test variables after the different test meals were assessed with ANOVA (general linear model) followed by Tukey’s pairwise multiple comparison method for means, in MINITAB Statistical Software (release 13.32; Minitab). Differences between the products at different time points were analyzed using a modified model (PROC MIXED) in SAS release 8.01 (SAS Institute) with repeated measures and an autoregressive covariance structure. Spearman rank correlation was used to study relations between some of

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Glucose</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>S-insulin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>S-FFA²</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>S-triacylglycerol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P-glucagon</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P-GIP, P-GLP-1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>S-IL-6, S-IL-8</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P-adiponectin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>S-paracetamol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Satiety</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Breath H₂</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

1 B, Blood, S, serum, P, plasma.
2 Time = 0; variables determined immediately prior to breakfast.
3 S-FFA were analyzed on 3 separate blood samples taken within 3 min.
the test variables. We calculated a correlation for each subject and from these values we obtained the mean value of Spearman correlation coefficient. To determine the $P$-value, a permutation test was performed using MATLAB with the null hypothesis that no correlations existed (the alternative hypothesis was that the data were correlated). Pearson correlation was used to assess relations involving serum IL-6 and plasma adiponectin, respectively. Generally, $n = 15$ in all calculations except that $n = 14$ after consumption of the HBB bread. Values of $P < 0.05$ were considered significant.

**Results**

**Blood glucose, serum insulin, and plasma glucagon responses following the standardized breakfast.** The different cereal evening meals did not result in significant differences in fasting values of blood glucose, serum insulin, or plasma glucagon by the subsequent morning. Expressed as IAUC (0–120 min), all evening meals, with the exception of WWB + RS and 1/2OB bread, resulted in lower glucose response at breakfast compared with the WWB evening meal ($P < 0.05$) (Fig. 1A; Table 4). In addition, all kernel-based evening meals (whole or cut kernels) based on 50 g available carbohydrates resulted in lower blood glucose peak increments after the standardized breakfast (based on peak values for each individual) compared with peak increments after the WWB evening meal ($P < 0.05$). The evening meals with OB bread and CutOB bread resulted in similar mean glucose increments 0–60 min after the start of the standardized breakfast (Fig. 1A). Thereafter, the glucose increment declined more rapidly following the evening meal with CutOB bread compared with OB bread.

When expressed as IAUC (0–120 min), the insulin response after the standardized breakfast was lower following an evening meal with OB bread compared with an evening meal composed of WWB ($P < 0.05$; Fig. 1B; Table 4).

After a slight increase in plasma glucagon concentrations in the early postprandial phase after the standardized breakfast (up to 15 min), the glucagon concentrations were suppressed to a level beneath the fasting value from ~45 min and throughout the 120-min postprandial period (Fig. 2A; Table 4). Expressing the result as AUC (0–120 min), the plasma glucagon concentration after the standardized breakfast was lower following the WWB evening meal compared with after the evening meals with HBB bread, OB bread, or CutOB bread, respectively ($P < 0.05$).

**Plasma adiponectin concentrations following the standardized breakfast.** Plasma adiponectin concentrations were measured at breakfast following the OB bread and the WWB evening meals. The fasting concentrations of plasma adiponectin were higher following the evening meal containing OB bread (83.9 ± 8.3 µg/L) compared with the evening meal with WWB (77.0 ± 7.3 µg/L; $P < 0.05$). At 45 min after the standardized breakfast, plasma adiponectin did not differ between WWB (78.5 ± 7.5 µg/L) and OB bread trials (82.0 ± 8.6 µg/L).

**Serum FFA and triacylglycerols following the standardized breakfast.** The fasting FFA (f-FFA) concentration was lower in the morning after the OB bread evening meal compared with WWB, 1/2OB bread, or HBB bread ($P < 0.05$) (Table 4). In addition, the evening meal bread products composed of HAB, CutOB, and WWB+RS resulted in lower f-FFA concentrations compared with the WWB evening meal ($P < 0.01$). In contrast, at breakfast, the serum triacylglycerols did not differ depending on the test meal consumed the previous evening.

**Plasma GLP-1 and GIP concentrations following the standardized breakfast.** The fasting plasma GLP-1 (Fig. 2B) and GIP concentrations (Fig. 2C) did not differ significantly after the different evening meals. The total GLP-1 AUC (0–120 min) after the standardized breakfast was higher following the OB bread evening meal (3513 ± 333 pmol-min/L) in comparison with after the WWB evening meal (2843 ± 265 pmol-min/L) ($P < 0.05$).

Expressed as 0–120 min AUC, the plasma GIP response did not differ after the standardized breakfast depending on test evening meals. However, the differences in plasma GIP concentrations after the breakfast tended to increase toward the late postprandial phase. Consequently, the plasma GIP AUC 60–120 min after the breakfast was higher following the evening meal with HBB bread (2610 ± 340 pmol-min/L) compared with after the evening meal with WWB (2030 ± 225 pmol-min/L) ($P < 0.01$).

**Serum IL-6 and IL-8 concentrations following the standardized breakfast.** Serum IL-6 concentrations were analyzed post breakfast following the evening meals with WWB, OB bread, and HBB bread. Expressing the results as mean values (0–180 min), the plasma IL-6 concentration after the standardized breakfast was lower following the OB bread evening meal (13.6 ± 1.0 ng/L) compared with following the WWB evening meal (16.3 ± 1.0 ng/L; $P < 0.01$). There were no differences in plasma...
IL-8 concentrations following the standardized breakfast were higher than those following the evening meal irrespective of the preceding evening meals.

**Breath H₂ excretion following the standardized breakfast.** Expressed in terms of breath H₂ excretion (mean 0–180 min), the HBB bread evening meal induced higher colonic activity at breakfast the following morning compared with all the other evening meals (P < 0.01) except the HAB bread (Fig. 3A). The HAB bread evening meal resulted in higher colonic activity at breakfast compared with the WWB and the 1/2OB bread evening meal tests (P < 0.05). In addition, the colonic fermentation was higher in the late postprandial phase post the breakfast (180 min) after the OB bread evening meal (23.3 ± 4.5 ppm) compared with the WWB evening meal (6.6 ± 2.0 ppm) (P < 0.05).

**GER and satiety score following the standardized breakfast.** As judged from the serum paracetamol IAUC (0–90 min), the GER after the standardized breakfast was lower following the evening meal with HBB bread (IAUC 2.23 ± 0.3 mmol min/L) compared with following evening meals with WWB (IAUC 3.43 ± 0.4 mmol min/L), WWB + RS (IAUC 3.42 ± 0.3 mmol min/L), and 1/2OB bread (IAUC 3.34 ± 0.2 mmol min/L) (P < 0.05) (Fig. 3B). After the evening meal with WWB, the serum paracetamol concentration after the standardized breakfast reached a peak value at 60 min, whereas the rest of the evening test meals resulted in a continuous increase of this marker throughout the test period (0–90 min).

The evening meal with HBB bread resulted in a higher satiety score (IAUC 0–180 min) after the standardized breakfast compared with after all the other evening meals (P < 0.05; Fig. 3C).

**Relations between test variables.** The glucose response after the standardized breakfast (IAUC 0–120 min) was inversely related to colonic fermentation, as determined by breath H₂ excretion (0–180 min) (r = −0.23; P < 0.05). Furthermore, the postprandial glucose response (IAUC 0–120 min) after the standardized breakfast was positively correlated to the f-FFA (r = 0.37; P < 0.001). The serum insulin response (IAUC 0–120 min) correlated to f-FFA in a similar manner (r = 0.23; P < 0.05). In addition, serum IL-6 concentrations (mean 0–180 min) were positively correlated with the f-FFA concentration (r = 0.32; P < 0.05).

The blood glucose response (IAUC 0–120 min) after the standardized breakfast was inversely related to plasma GLP-1 (AUC 0–120 min) (r = −0.26; P < 0.05) and glucagon (AUC 0–120 min) (r = −0.23; P < 0.05)

The mean concentration (0–45 min) of adiponectin was positively correlated to satiety (AUC 0–180 min) (r = 0.43; P < 0.05) and inversely correlated to the total GLP-1 concentration (AUC 0–120 min) (r = −0.38; P < 0.05). At breakfast, the H₂ excretion (mean 0–180 min) correlated positively to satiety (AUC 0–180 min; r = 0.27; P < 0.01), whereas there was an inverse relation between H₂ and GER (serum paracetamol IAUC 0–90 min; r = −0.24; P < 0.05). Further, there was an inverse relation between satiety at breakfast and the GER (r = −0.23; P < 0.05).

**Discussion**

An evening meal of barley kernel bread (OB bread) significantly improved glucose tolerance at a following standardized breakfast in healthy subjects. Consequently, the IAUC 0–120 at breakfast was reduced by 28% after the OB bread evening meal compared with the corresponding area following ingestion of a WWB evening meal. Cutting the barley kernels 1–2 times did not deteriorate the “overnight” benefits to glucose tolerance. Also, the evening meal bread made from white wheat flour enriched with both barley DF and RS in amounts corresponding to that in the OB bread lowered the blood glucose IAUC (0–120 min) at breakfast compared with the WWB evening meal by ~26%. This is an important finding when aiming at exploiting the benefits of cereal products in new and palatable low-GI foods with optimal benefits to blood glucose regulation. In previous studies, no significant effects on overnight glucose tolerance were observed when adding DF (with no added RS) from wheat, barley, or oats to a high- or low-GI evening meal in quantities similar to that in barley kernels (13, 21).

Predicted from the rate of in vitro hydrolysis of starch (WWB as a reference), all test products based on whole or cut cereal kernels had low GIs (≤55). In contrast, the WWB+DF+RS had a predicted GI of 85. The results from this study therefore indicate that low-GI features per se do not seem to be necessary for overnight benefits to glucose tolerance. The HAB bread evening meal displayed a more pronounced capacity to lower the breakfast glucose response (IAUC 0–120 min) compared with WWB (~36%; P = 0.001) than did the evening meal with OB bread (~28%; P < 0.05). This indicates that the elevated level of indigestible carbohydrates, and particularly RS, in the HAB

---

**TABLE 4** B-glucose, S-insulin, P-glucagon, S-FFA, and breath H₂ of subjects after they consumed the standardized breakfast following cereal-based test evening meals

<table>
<thead>
<tr>
<th>Evening meal</th>
<th>B-glucose (mmol×L⁻¹×min⁻¹) IAUC (0–120 min)</th>
<th>Peak</th>
<th>S-insulin (pmol×L⁻¹×min⁻¹) IAUC (0–120 min)</th>
<th>Peak</th>
<th>P-glucagon (ng×L⁻¹×min⁻¹) AUC (0–120 min)</th>
<th>S-FFA (nmol/L) Mean (0–180 min)</th>
<th>Breath H₂ (ppm)</th>
<th>Mean (0–180 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>211.6 ± 23.8b</td>
<td>4.0</td>
<td>0.3b</td>
<td>17.3</td>
<td>2.9b</td>
<td>6520 ± 536b</td>
<td>0.29 ± 0.05</td>
<td>0.04b</td>
</tr>
<tr>
<td>WWB + RS</td>
<td>167.2 ± 21.3ab</td>
<td>3.5</td>
<td>0.2ab</td>
<td>12.5</td>
<td>1.5ab</td>
<td>6870 ± 542ab</td>
<td>0.24 ± 0.02</td>
<td>0.07ab</td>
</tr>
<tr>
<td>1/2OB</td>
<td>160.6 ± 16.4ab</td>
<td>3.2</td>
<td>0.2ab</td>
<td>13.3</td>
<td>1.5ab</td>
<td>7306 ± 516ab</td>
<td>0.25 ± 0.03</td>
<td>0.03ab</td>
</tr>
<tr>
<td>WWB + RS + DF</td>
<td>156.8 ± 17.0b</td>
<td>3.3</td>
<td>0.3ab</td>
<td>14.8</td>
<td>1.7ab</td>
<td>7086 ± 536ab</td>
<td>0.29 ± 0.04</td>
<td>0.13ab</td>
</tr>
<tr>
<td>OB</td>
<td>152.1 ± 17.0b</td>
<td>3.0</td>
<td>0.3b</td>
<td>11.6</td>
<td>1.3b</td>
<td>7596 ± 492ab</td>
<td>0.23 ± 0.03</td>
<td>0.07ab</td>
</tr>
<tr>
<td>HBB</td>
<td>149.5 ± 19.3b</td>
<td>2.7</td>
<td>0.3b</td>
<td>12.2</td>
<td>2.3ab</td>
<td>7623 ± 478ab</td>
<td>0.22 ± 0.06</td>
<td>0.03ab</td>
</tr>
<tr>
<td>CutOB³</td>
<td>142.2 ± 14.1b</td>
<td>3.0</td>
<td>0.2b</td>
<td>12.2</td>
<td>1.3ab</td>
<td>7571 ± 641ab</td>
<td>0.24 ± 0.03</td>
<td>0.04bc</td>
</tr>
<tr>
<td>HAB</td>
<td>135.5 ± 11.6b</td>
<td>2.9</td>
<td>0.2b</td>
<td>12.6</td>
<td>1.2b</td>
<td>7265 ± 573ab</td>
<td>0.22 ± 0.02</td>
<td>0.04bc</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 15. Means in a column with superscripts without a common letter differ, P < 0.05 (ANOVA followed by Tukey’s test). B, blood; S, serum; P, plasma.

2 Glucose and insulin peak values are based on the mean of the individual peaks.

3 CutOB vs. WWB, P = 0.06.
bread meal (22.0 g vs. 9.5 g in the OB bread meal) resulted in additional benefits with respect to overnight glucose tolerance. The serving of HBB bread was in addition to its elevated content of β-glucans also rich in RS and insoluble DF. Due to the low concentration of available starch in this bread, the size of the HBB serving (equivalent to 50 g available starch) became large (388 g). One of the subjects failed to eat this meal and another 3 finished only ~75% of the serving. The HBB bread evening meal resulted in a low and prolonged net increment in blood glucose concentration after the standardized breakfast. The appearance of the 0–120-min blood glucose curve at breakfast after the HBB evening meal bread is probably related to a decreased GER at the time of the breakfast (measured as appearance of paracetamol in serum after the breakfast). The fact that a significant negative correlation was observed between the GER and subjective rating of satiety after the standardized breakfast suggests that the

**FIGURE 2** Concentrations of subjects’ plasma glucagon (A), plasma GLP-1 (B), and plasma GIP (C) after the standardized breakfast following different cereal-based evening meals. The evening meals differed in GI and contents of indigestible carbohydrates. All meals were based on 50 g available starch. Values are means ± SEM, n = 15.

**FIGURE 3** Concentrations of subjects’ breath H2 excretion (indicator of colonic fermentation) (A), serum paracetamol (measure of the GER) (B), and satiety scores (C) after the standardized breakfast following different cereal-based evening meals. The evening meals differed in GI and contents of indigestible carbohydrates. Values are means ± SEM, n = 15.
higher satiety obtained after the HBB evening meal was the result of a lower GER. Such a relation between increased satiety and lowered GER is in concordance with observations by Verdich et al. (22).

A limitation of the conclusions from this study could be that the subjects consumed the test meals in their home and, except from a form to be answered by the subjects, it was not possible to fully control adherence to the written instructions. For example, not following the instructions regarding the time point for intake of the evening test meals or fasting prior to ingestion of the standardized breakfast may have influenced the results. Another study limitation regarding relations between GI and overnight effects could be that the GI of the test products were not determined but were predicted in vitro. However, for cereal products, the predicted and measured GI have been shown to correlate well (18). Moreover, the range of predicted GI are coherent with previously published data for similar products (23).

In this study, the blood glucose response to the standardized breakfast (IAUC 0–120 min) was positively correlated to the f-FFA concentrations. Such a relation has also been observed in previous studies examining the effects of various test breakfasts at a standardized lunch (24) or from an evening test meal to a standardized breakfast (13,25), respectively. The concentration of circulating FFA is known to affect insulin sensitivity in a dose-dependent manner also within a modest range (440–695 μmol/L, healthy lean subjects) (26) and we strongly suggest that suppression of FFA concentration is a main cause for the overnight benefits seen on glucose tolerance at the breakfast meal in this study.

The reason for the overnight decreased serum FFA with some cereal-based evening meals is not clear, but several factors may be involved. First, a prolonged fed state after low-GI foods related to their improved capacity to reduce the rate of digestion and absorption, thereby maintaining FFA suppressed for a longer time period, has previously been suggested as a mechanism for the improved glucose tolerance from breakfast to a standardized lunch (24). However, in this study, the period between the meals was 10.5 h, which probably is too long for a substantial suppression of FFA concentrations due to a reduced fasting state. Second, it is possible that the higher concentration of fasting plasma adiponectin, as seen in the morning after the OB bread evening meal, might have affected the FFA concentrations. Thus, it has been shown that adiponectin promote an increased rate in clearance of FFA from the blood, hence improving insulin sensitivity (27,28).

Third, it has been shown that IL-6 stimulates an increase in FFA concentration that may persist for several hours after an acute increase in IL-6 (29). The positive correlation between serum IL-6 and FFA in this study further supports such a relation. Finally, metabolites produced during colonic fermentation of indigestible carbohydrates may enter the systemic circulation and it has been suggested that SCFA, particularly propionate, may exert systemic effects, including benefits to glucose metabolism (13,25,30,31) and lowered plasma FFA concentrations in humans (32,33). In this study, a negative correlation existed between breath H2 excretion and lowered plasma FFA concentrations. Such a relation has also been observed in previous studies (440–695 μmol/L, healthy lean subjects) (26) and we strongly suggest that suppression of FFA concentration is a main cause for the overnight benefits seen on glucose tolerance at the breakfast meal in this study.

In conclusion, a cereal-based evening meal with adequate amounts of specific indigestible carbohydrates (barley DF and RS) was capable of improving glucose excursion and increased the satiety after a subsequent standardized high-GI breakfast. Of particular interest is the finding that a mixture of DF and RS added to WWB, simulating the content of these components in the OB bread evening meal, induced similar benefits to overnight glucose tolerance as did the OB bread. These results suggest that it may be possible to tailor novel low-GI whole-grain foods capable of facilitating glycemic regulation not only at the acute meal but also at a meal ingested 10 h thereafter. The overnight benefits to glucose tolerance and satiety are probably mediated by mechanisms emanating from colonic fermentation and suggest that specific combinations of indigestible carbohydrates may have probiotic effects. The benefits were associated with decreased concentrations of FFA and IL-6 and increased GLP-1 and adiponectin at the time of the breakfast, thus providing evidence for a link between the gut microbial metabolism and key factors associated with insulin resistance.

Acknowledgment

We thank L. Persson at Applied Nutrition and Food Chemistry, Lund University, Sweden for her skillful technical assistance.

Literature Cited