Postprandial glucose, insulin, and incretin responses to grain products in healthy subjects1–3

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ABSTRACT
Background: Various botanical and structural characteristics of starchy food modify the postprandial glucose and insulin responses in humans.

Objective: We investigated what factors in grain products affect human glucose and insulin responses and elucidated the mediating mechanisms.

Design: Ten men and 10 women [mean age: 28 ± 1 y; mean body mass index (in kg/m²): 22.9 ± 0.7] with normal glucose tolerance were recruited. The test products were whole-kernel rye bread, whole-meal rye bread containing oat β-glucan concentrate, dark durum wheat pasta, and wheat bread made from white wheat flour. Paracetamol, a marker of the rate of gastric emptying, was added to the breads during baking. Each product provided 50 g available carbohydrate and was served in random order with breakfast (except for the β-glucan rye bread, which was served at the last visit). Fasting and 8 postprandial blood samples were collected at intervals of 15–30 min for 3 h to determine plasma glucose, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP-1), serum insulin, and paracetamol concentrations. The in vitro starch hydrolysis, the structural characteristics (by light microscopy), and the molecular weight of β-glucan in the test products were analyzed.

Results: Glucose responses and the rate of gastric emptying after consumption of the 2 rye breads and pasta did not differ from those after consumption of white wheat bread. However, insulin, GIP, and GLP-1 responses, except for GLP-1 responses to the rye bread containing oat β-glucan concentrate, were lower after the consumption of rye breads and pasta than after consumption of white wheat bread.

Conclusions: Postprandial insulin responses to grain products are determined by the form of food and botanical structure rather than by the amount of fiber or the type of cereal in the food. These effects may be mediated through GIP and GLP-1.

INTRODUCTION
Cereal products are the major source of carbohydrate in the diet, and pasta and breads are probably the most studied cereal products regarding the glycemic response they produce when consumed. These lente properties have been studied both in healthy subjects (1–6) and in diabetic patients (7–11). Carbohydrates that produce both low postprandial blood glucose and insulin responses are considered beneficial for health (11–15).

Many characteristics of pasta products have been studied with respect to the glycemic response they produce. Such characteristics include the type of flour (ie, durum compared with nondurum wheat) used for making pasta (2) and the amount and type of fiber (5, 14) and protein (16) in the final product. The compact structure of pasta achieved during processing appears to contribute more substantially to starch hydrolysis and glucose response than do cereals of a similar chemical composition and type, as indicated by the finding that bread made from durum wheat gives a higher glucose response than does pastas made from durum wheat (17). The importance of food structure (ie, thickness, particle size, and shape) and processing in relation to postprandial responses is further elucidated by studies that compared the lente properties of spaghetti with macaroni (3, 16) and extruded with homemade nonextruded pasta (5). Cooking time showed no influence on glycemic responses in either healthy subjects (1) or diabetic patients (16) or on insulin responses in healthy subjects (1).

In breads, the data accumulated thus far on the significance of the quantity and quality of fiber in the regulation of postprandial glycemia is controversial. Despite their different fiber contents, white and whole-meal breads showed similar postprandial glycemic responses both in healthy volunteers (18, 19) and in diabetics (7, 20). In some studies, low glycemic responses were explained by the viscous fiber content of bread (21), which may slow the gastric emptying rate or the absorption of nutrients in the small intestine (22–24). We also showed in healthy subjects that less insulin is required postprandially for the regulation of

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plasma glucose after the consumption of whole-kernel rye bread than after the consumption of white wheat bread (6). Various processing methods (eg, parboiling bread ingredients) may also be essential in lowering the glycemic response produced after the consumption of breads (12).

The hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like-peptide 1 (GLP-1) were shown to be potent determinants of the postprandial insulin release that occurs after increases in blood glucose (25). As a result, these incretin hormones are also essential in the regulation of postprandial glycemia. Controlled studies in humans on the effect of fiber and cereal consumption on the responses of these hormones are scanty and discrepant. GIP showed either low responses (26, 27) or no effect (28) after fiber consumption, and rye flaxes produced smaller GIP responses than did white wheat bread (8). In experimental animals, the addition of fermentable fiber to the diet reduced glucose, insulin, and GIP responses in pigs (23); increased GLP-1 and insulin responses and decreased glucose responses in dogs (29); and improved glycemic control in rats (30).

In the present study, we made 2 types of rye bread to elucidate the bread characteristics that are important for postprandial glycemic response: one bread had a high content of whole kernels and the other bread had a high content of soluble fiber (ie, oat β-glucan). In addition, the postprandial effects of dark durum wheat pasta (positive control) and white wheat bread (reference food) were determined. Paracetamol was baked into the test breads and its appearance in circulation was used as a marker for the rate of gastric emptying (9, 3). To study the possible mechanisms behind the glycemic and insulimemic responses to the consumption of these grain products, plasma GIP and GLP-1 concentrations were measured after each test meal.

SUBJECTS AND METHODS

Subjects

Twenty healthy subjects (10 men and 10 women) with normal oral glucose tolerance were recruited for the study. The men and women had a mean age of 29 ± 1.8 and 28 ± 1.8 y, a mean body mass index (in kg/m²) of 24.3 ± 1.0 and 21.5 ± 0.6, and a mean energy intake of 10130 kJ/d (2412 kcal/d) and 6964 kJ/d (1658 kcal/d), respectively.

Test products

The test products for the in vivo study were chosen by using the in vitro starch hydrolysis method (see below). The rye breads chosen for the present study were whole-kernel rye bread, composed of 60% whole rye kernels and 40% rye flour, and β-glucan rye bread, composed of 20% oat β-glucan concentrate (Swedish Oat Fiber, Väröbacka, Sweden) and 80% rye flour. The white wheat bread was used as the reference food. Paracetamol was added to the flours before making the dough of the rye and white wheat breads to study the rate of gastric emptying after consumption of the test breads (9, 31). Each test bread portion was intended to contain 1 g paracetamol. Commercial dark durum wheat pasta (Melia Ltd, Raisio, Finland) was used as a positive control because it has been shown to be a food that produces a low glucose response.

Recipes for the breads and pasta

White wheat bread

The white wheat breads were made from 6250 g white wheat flour, 4313 g water, 93.8 g dry yeast, 93.8 g NaCl, 93.8 g margarine, 77.6 g paracetamol, and 30 g bread improver (Panodan M2020; Danisco Ingredients, Copenhagen). The dough was mixed for 7 min. After a floor time of 20 min at 28°C (relative humidity: 80%), the dough was divided into 400-g pieces, which were manually moulded and panned before proofing for 50 min at 34°C (Rh: 80%). Loaves were baked at 200°C for 20 min and then cooled for 2 h. The loaves were stored in a freezer at −20°C until used.

Whole-kernel rye bread

Rye kernels (4200 g) were autoclaved with 2100 g water (101325 Nm⁻² for 50 min) and then cooled, after which 3290 g H₂O, 1400 g sifted rye flour, 1400 g whole-meal rye flour, 70 g dry yeast, 105 g NaCl, 76.5 g paracetamol, and 70 mL lactic acid were added. The dough was mixed for 5 min. After a floor time of 45 min at 28°C (Rh: 80%), the dough was divided into 400-g pieces, which were manually moulded and panned before proofing for 1 h and 30 min at 34°C (Rh: 80%). Loaves were baked at 175°C for 35 min and then cooled for 2 h. The loaves were stored in a freezer at −20°C until used.

β-glucan rye bread

The β-glucan rye breads were made from 1000 g sifted rye flour, 1000 g whole-meal rye flour, 500 g oat β-glucan concentrate, 2325 g H₂O, 25 g dry yeast, 37.5 g NaCl, 26.1 g paracetamol, and 25 mL of a mixture of lactic and acetic acids (5:1). Loaves were baked at 175°C for 35 min and then cooled for 2 h.

Pasta

Eighty grams of pasta was boiled for 10 min in 0.64 L water that contained 2.3 g salt. After boiling, the pasta was rinsed with 2.4 L cold water and strained for 5 min in a colander. The test portion of the pasta was weighed and the cooked pasta was kept under a dome and heated for 90 sec in a microwave oven just before serving. The pasta was served within 1 h of being boiled.

Characterization of the test products

The rate of in vitro starch hydrolysis of the test products was determined by the enzymatic hydrolysis method of Granfeldt et al (32). The amount of available starch was measured with the use of a specific enzymatic kit (Megazyme, Bray, United Kingdom). An equivalent amount of available starch (1 g on the basis of analyzed data) from each test product was chewed for 15 s by 6 subjects and each subject chewed all test products. The sample sizes were 2.25 g white wheat bread, 2.70 g whole-kernel rye bread, 3.38 g β-glucan rye bread, and 3.58 g boiled pasta. After the subjects had chewed a sample, it was incubated with pepsin and hydrolyzed by pancreatic amylase in a dialysis tube. Aliquots of the dialysate were removed for analysis of reducing sugar content by using the 3,5-dinitro salicylic method (33). A standard curve was prepared by using maltose. The degree of hydrolysis was calculated as the proportion of the potentially available starch degraded to maltose. A hydrolysis index was calculated as the area under the hydrolysis curve (0–180 min) with the product as a percentage of the corresponding area with white wheat bread.

The β-glucan content of the breads was determined by a specific enzymatic kit (Megazyme). The dietary fiber content of the breads was determined according to Asp et al (34), the pentosan content was determined by the colorimetric method
of Douglas (35), and the protein content was determined by the Kjeldahl method (nitrogen × 5.7). The fat content of the breads was determined gravimetrically by extraction in diethyl ether after hydrolysis with acid according to the Association of Official Analytical Chemists (36). The moisture content of the breads was determined by oven drying at 130 °C for 1 h. The energy value (kJ) per test portion was calculated by using the weight of the portion (g) and the formula [17 × protein (%) + [37 × fat (%)] + [17 × available carbohydrate (%)].

To study the test breads by microscopy, pieces of bread crumb (0.5 cm) were taken from the middle of the loaf, embedded in 2% agar, fixed in 1% glutaraldehyde in 0.1 mol phosphate buffer/L (pH 7.0), dehydrated with ethanol, embedded in Historesin (Leica, Heidelberg, Germany), and sectioned with a microtome (Leica). The bread sections (2 µm) were stained for light microscopic examination. Protein was stained with aqueous 0.1% (wt:vol) Light Green (Gurr, BDH Ltd, Poole, United Kingdom) for 1 min and with starch with 1:10 diluted Lugol’s iodine solution (wt:vol, 0.33% I2, and 0.67% KI). Light Green stains protein green, and iodine stains the amylose component of starch blue and amylopectin brown. The samples were examined with an Olympus BX-50 microscope (Olympus, Tokyo). Micrographs were obtained with a SenSiCam PCO CCD camera (Hamamatsu Photonics KK, Hamamatsu-City, Japan) and the AnalySIS 3.0 image analysis program (Soft Imaging System, Münster, Germany).

The molecular weight distribution of β-glucan was analyzed with size-exclusion HPLC by using µhydrogel columns, 0.05 mol NaOH eluent/L, postcolumn calcofluor staining, and fluorometric detection according to Suortti (37). The samples were dissolved in 0.1 mol NaOH/L containing 0.1% NaBH4 to achieve a β-glucan concentration of 20–100 mg/L. Previously constructed size-exclusion calibration curves based on isolated high-molecular-weight β-glucan fractions were used for the calculations.

### Test meals

All test meals contained 50 g available carbohydrate from the test products (Table 1). The test portion contained test bread, 40 g cucumber, and a 0.3 L-nonenergy-containing orange drink. The pasta portion was served with 19 g crushed tomatoes and the nonenergy-containing orange drink. The intake of energy and carbohydrates from the portion of cucumber and crushed tomatoes were equal.

### Study protocol

The subjects were told to maintain their diet and other living habits, and the use of analgesic drugs that contained paracetamol was forbidden throughout the study. The subjects were weighed at each visit and their energy intake were quantitated from records of their food intakes before each test day. Heavy exercise and the consumption of unusually large portions of food were forbidden on the day before each test, as was the consumption of alcohol for 2 d before and smoking on the morning of the test. Subjects were asked to arrive at the laboratory by car or by bus if possible to avoid extra physical stress.

The test products (pasta, whole-kernel rye bread and white wheat bread) were served in random order during intervals of 1–2 wk. To eliminate the possible effects of freezing on the properties of the β-glucan in β-glucan rye bread, the product was served fresh-baked during the last visit. The white wheat bread was served twice to reduce the intra-individual variation, and the mean of these 2 determinations was used in the statistical analysis.

The subjects fasted 12–15 h before the tests. On the morning of the test, the subjects’ body weights were measured and an intravenous catheter was inserted in the antecubital vein of their arms. The subjects received their test meal. Eating the rye bread took longer than eating the white wheat bread and pasta. It took an average of 8 min and 51 s to eat the white wheat bread and pasta. It took an average of 8 min and 51 s to eat the whole-kernel rye bread, 8 min and 23 s to eat the β-glucan rye bread, 6 min and 43 s to eat the pasta, and 7 min and 3 s to eat the white wheat bread. After the fasting blood sample was drawn, 8 additional samples were drawn (15, 30, 45, 60, 90, 120, 150, and 180 min after consumption of the test meal) for the measurement of plasma glucose, GIP, and GLP-1 and serum insulin and paracetamol concentrations. The protocol for the postprandial study was approved by the Ethics Committee of the Kuopio University and University Hospital.

### Biochemical and dietary analysis

Plasma glucose was analyzed with the enzymatic photometric method (Granatest 100; Merck, Damstadt, Germany) with use of a Kone Specific Clinical Analyzer (Kone Ltd, Espoo, Finland), and serum insulin was analyzed by radioimmunoassay (Phadaseph Insulin RIA 100; Pharmacia Diagnostica, Uppsala, Sweden). Serum paracetamol and paracetamol concentrations in the test breads were measured by enzyme immunoassay ( Emit Acetaminophen Assay; Behring Diagnostics Inc, Cupertino, Canada).

### Table 1

<table>
<thead>
<tr>
<th>Nutrient composition of the test product portions</th>
<th>White wheat bread</th>
<th>Whole-kernel rye bread</th>
<th>β-Glucan rye bread</th>
<th>Whole-meal pasta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portion size (g)</td>
<td>112.4</td>
<td>135</td>
<td>169</td>
<td>179.1</td>
</tr>
<tr>
<td>Available carbohydrate (g)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Total dietary fiber (g)</td>
<td>3.1</td>
<td>12.8</td>
<td>17.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Soluble dietary fiber (g)</td>
<td>0.9</td>
<td>3.8</td>
<td>6.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Pentosan (g)</td>
<td>2.0</td>
<td>8.7</td>
<td>10.3</td>
<td>ND</td>
</tr>
<tr>
<td>β-Glucan (g)</td>
<td>0.2</td>
<td>1.3</td>
<td>5.4</td>
<td>ND</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>8.4</td>
<td>7.4</td>
<td>10.5</td>
<td>12.1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.0</td>
<td>2.6</td>
<td>2.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Moisture (g)</td>
<td>43.2</td>
<td>53.1</td>
<td>80.6</td>
<td>102.4</td>
</tr>
<tr>
<td>Energy content (kJ)</td>
<td>1117</td>
<td>1084</td>
<td>1134</td>
<td>1250</td>
</tr>
</tbody>
</table>

ND, not determined.
GIP and GLP-1 concentrations in plasma were measured by radioimmunoassay after the extraction of plasma with 70% ethanol. For the GIP radioimmunoassay, the C-terminally directed antiserum R65 was used, which cross-reacts fully with human GIP but not with the so-called GIP 8000 (38). Human GIP and 125I human GIP (70 MBq/nmol) were used as standards and tracer. The plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1 [7-36] amide by using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 of intestinal origin (39). For these assays, the sensitivity was <1 pmol/L, the intraassay CV was <6% at 20 pmol/L, and the recovery of standard (added to plasma before extraction) was ~100% when corrected for losses inherent in the plasma extraction procedure.

Maximal glucose, insulin, GIP, and GLP-1 responses were calculated by subtracting the highest value of each from the corresponding fasting value. The areas for glucose, insulin, GIP, and GLP-1 were calculated from the initial rise above baseline (40). Nutrient intakes were calculated by using the MICRO-NUTRICA software package (version 2.0; Finnish Social Insurance Institution, Turku, Finland) (41).

**Statistical analysis**

The statistical analysis of the clinical data was done by comparing the glycemic and insulinenic responses after consumption of rye breads and dark durum wheat pasta with those after consumption of white wheat bread. Statistical significance was assessed using the nonparametric Friedman’s test followed by Wilcoxon’s test for pairwise comparisons. The Bonferroni’s adjustment was used to control the overall level of significance. The nonparametric Mann-Whitney U test was used to study the differences between men and women. In all analyses, P values < 0.05 were considered to be statistically significant. The results are expressed as means ± SEMs. Data were analyzed with SPSS for WINDOWS 8.0 (SPSS, Chicago) (42).

**RESULTS**

**Characterization of the test products**

The difference in the hydrolysis indexes of the 3 test products was significant (P = 0.017). The dark durum wheat pasta had the lowest hydrolysis index (56 ± 2), followed by the whole-kernel (72 ± 3) and β-glucan (97 ± 3) rye breads. Light microscopy showed that starch was highly gelatinized in all bread samples (Figure 1). In the reference white wheat bread (Figure 1A), a gluten network (green) surrounded the swollen starch granules. Amylose had partially diffused in the center of the granules and cavities created by α-amylolysis during baking (Figure 1A, arrow). In the whole-kernel rye bread (Figure 1B), the starch was more swollen and gelatinized than in the reference white wheat bread. Amylose had partially diffused in the center and out of the granules and had formed a continuous starch phase with the swollen granules. Separate patches of protein (green) were seen embedded in the starch matrix. As a result of the autoclaving, the starch granules in the rye kernels in the whole-kernel rye bread (Figure 1C) appeared more swollen and distorted than did the starch originating from the rye flour (Figure 1B). However, the grain structure appeared to be intact, and the gelatinized starch granules were enclosed in starchy endosperm cells surrounded by a sturdy aleurone layer. In the β-glucan rye bread baked with oat β-glucan concentrate, the starch granules stained light brown, indicating phase separation of amylose and amylopectin and that most of the amylose had leached out (Figure 1D). The water content of the dough was very high because of the high β-glucan concentration. The high water content probably caused the total leakage of amylose out of the swollen starch granules.

Although the average molecular weight of β-glucan in the oat β-glucan concentrate was >1000000, it was <250000 in the β-glucan rye bread (Table 2). This was probably due to the action of endogenous β-glucanases of rye flour during baking.

**Postprandial glucose and insulin responses**

There were no consistent differences in glucose, insulin, GIP, GLP-1, or paracetamol responses between the men and women; therefore, the results are presented including the data of both men and women. The differences in fasting plasma glucose and serum insulin concentrations were not significant. The differences in glucose responses during the first 90 min postprandially were not significant (Figure 2). However, glucose responses to pasta 120, 150, and 180 min postprandially (P = 0.030, P = 0.021, and P = 0.006, respectively) and to β-glucan rye bread 120 min postprandially (P = 0.012) were greater than the response to white wheat bread (Figure 2). At these time points, glucose concentrations after the consumption of pasta and the β-glucan rye bread were greater than the respective fasting concentrations. Maximal glucose responses to pasta consumption were significantly lower than those to white wheat bread consumption (P = 0.006; Table 3), but postprandial glucose areas above the fasting concentration did not differ significantly between the test products and the white wheat bread.

Insulin responses at several time points after the consumption of the 2 rye breads and the pasta were significantly different from the responses to the white wheat bread (Figure 3). The insulin responses to whole-kernel rye bread were significantly lower than those to white wheat bread at 30, 45, 60, 90, 120, and 150 min postprandially (P = 0.003, 0.0001, 0.006, 0.0001, 0.021, and 0.036, respectively) and to β-glucan rye bread at 45, 60, 120, 150, and 180 min postprandially (P = 0.006, 0.015, 0.027, 0.045, and 0.033, respectively). Insulin responses to pasta consumption were significantly lower than those to white wheat bread at 30, 45, 60, 90, and 180 min postprandially (P = 0.0001, 0.0001, 0.0001, 0.018, and 0.006, respectively). Maximal insulin responses to the consumption of the rye breads and pasta were significantly different from these to white wheat bread (P = 0.0001 for whole-kernel rye bread, P = 0.009 for β-glucan rye bread, and P = 0.0001 for pasta; Table 3). Postprandial insulin areas of whole-kernel rye bread and pasta above the fasting concentration were significantly smaller than the insulin area after the consumption of white wheat bread (P = 0.0001 and P = 0.0001, respectively).

**Postprandial GIP, GLP-1, and paracetamol responses**

Fasting plasma GIP concentrations were not significantly different between test groups, whereas fasting GLP-1 concentrations were (P = 0.016). The postprandial GIP and GLP-1 responses (Figures 4 and 5) followed closely the pattern of the respective insulin responses to test product consumption (Figure 3) except for GLP-1 responses to β-glucan rye bread (Figure 5). However, the insulin responses were more distinct than were corresponding GIP and GLP-1 responses. GIP responses were significantly lower after whole-kernel rye bread consumption than...
FIGURE 1. Light microscopy of the test breads: A, reference bread made from white wheat flour (amylose is partially diffused in the center of the starch granules as indicated by arrow); B and C, rye bread baked with whole rye kernels; and D, rye bread baked with oat β-glucan concentrate. The embedded sections were stained to show protein (green), the amylose component of starch (blue) and amylpectin (brown), and lignified cell walls and yeast cells (yellow).
TABLE 2
Molecular weight distribution of β-glucan in the oat β-glucan concentrate and the rye bread with 20% β-glucan concentrate

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>Oat β-glucan concentrate</th>
<th>β-Glucan rye bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1000000</td>
<td>56</td>
<td>7</td>
</tr>
<tr>
<td>250000–1000000</td>
<td>29</td>
<td>18</td>
</tr>
<tr>
<td>&lt; 250000</td>
<td>14</td>
<td>75</td>
</tr>
</tbody>
</table>

after white wheat bread consumption at all postprandial measuring points (at 15 min, \( P = 0.045 \); at 30, 45, 60, 90, 120, and 150 min, \( P = 0.0001 \); and at 180 min, \( P = 0.015 \) and 30, 45, 60, 90, and 120 min after β-glucan rye bread consumption \( P = 0.003, 0.0001, 0.0001, 0.0001, \) and 0.048, respectively; Figure 4). GIP responses to pasta were lower 15, 30, 45, 60, and 90, and 120 min \( P = 0.003, 0.0001, 0.0001, 0.0001, \) and 0.0003, respectively. Maximal GIP responses after consumption of the whole-kernel rye bread, β-glucan rye bread, and pasta were significantly different from the responses to the white wheat bread \( P = 0.0001, 0.0001, \) and 0.0001, respectively; Table 3). GIP areas of the rye breads and pasta above the fasting concentrations were significantly smaller than was the GIP area of the white wheat bread \( P = 0.0001 \) for whole-kernel rye bread, \( P = 0.0001 \) for β-glucan rye bread, and \( P = 0.0001 \) for pasta).

GLP-1 responses to β-glucan rye bread consumption were not the same as the corresponding insulin and GIP responses (Figures 3, 4, and 5). The GLP-1 responses tended to be higher 15 min after consumption of β-glucan rye bread than after white wheat bread \( P = 0.09 \); Figure 5). Thereafter, the GLP-1 response to β-glucan rye bread was similar to that to the white wheat bread but remained higher than the response to the white wheat bread at 120 and 150 min postprandially \( P = 0.045 \) and 0.003, respectively. The GLP-1 responses were significantly lower after consumption of the whole-kernel rye bread and pasta than after white wheat bread consumption at several time points: whole-kernel rye bread 30, 45, and 60 min postprandially \( P = 0.012, 0.015, \) and 0.003, respectively) and pasta 30, 45, 60, 90, and 120 min \( P = 0.0001, 0.006, 0.009, 0.033, \) and 0.027, respectively). The maximal GLP-1 responses were significantly lower after consumption of the whole-kernel rye bread \( P = 0.042 \) and pasta \( P = 0.045 \) than after white wheat bread consumption (Table 3), but the GLP-1 areas of the rye breads and pasta above the fasting concentrations did not differ significantly from the area of the white wheat bread. The GLP-1 areas were calculated for only 17 of the 20 subjects because the measured concentration did not rise above the fasting concentration for some of the test products in 3 of the subjects. Data for each subject was included in the analysis only if the GLP-1 areas could be calculated for all test products consumed.

Paracetamol concentrations after consumption of the rye breads were not significantly different from those after consumption of white wheat bread during the first half of the postprandial period (Figure 6). The paracetamol concentrations were significantly lower 120 min after consumption of the whole-kernel rye bread than after the white wheat bread \( P = 0.004 \).

DISCUSSION

The present study showed no significant differences between glucose responses to rye breads and pasta relative to those white wheat bread or in the rate of gastric emptying after the consumption of these grain products in healthy men and women. However, it confirmed our earlier finding of a diminished need of postprandial insulin to regulate plasma glucose after the ingestion of rye bread (6). The present study also showed that food form and structure have an ability to alter the secretion of incretin hormones. The possible confounding effects of these variables were minimized by the study design (a randomized study, except for the β-glucan–containing rye bread) and by controlling the dietary and living habits of the subjects.

**Food form compared with fiber content**

The factors responsible for the lowered insulin response after the consumption of various carbohydrates are not evident. Both the enrichment of soluble fiber (β-glucan) and the addition of whole kernels to rye bread resulted in a reduction in the insulin response. The data suggest that the structural and compositional properties of fiber play more of a role in the regulation of the insulin response than does the amount of fiber consumed because the rye bread with β-glucan contained more fiber than did the whole-kernel rye bread. The high molecular weight of β-glucan was broken down to a lower molecular weight during baking, probably thereby lessening the expected viscosity effect of the bread. It was shown in earlier studies with oats that viscosity clearly decreases when β-glucan is partially hydrolyzed (43). In addition, the present study showed that the lowered insulin response was not dependent on the type of cereal consumed because pasta made from wheat and bread made from rye lowered the insulin response as well.

The other rye bread used in this study contained a considerable proportion of whole kernels, supporting the suggestion that the intact botanical structure of cereal might be important for glucose and insulin metabolism (11, 12, 19, 32). As the micrographs of the test breads showed, the rye kernels in whole-kernel rye bread seemed unbroken, indicating that autoclaving and baking do not disrupt the kernels. Hence, it could be assumed that the outer layer of the grain kernel protects the starch from enzymatic

**FIGURE 2.** Mean fasting and postprandial glucose responses to rye and wheat products over 180 min \( n = 20 \). Postprandial glucose responses to β-glucan rye bread \( b \) and pasta \( c \) were significantly different from those to white bread, \( P < 0.05 \). The pooled SEM was 0.9 for the whole-kernel rye bread, 0.9 for the β-glucan rye bread, 0.6 for the pasta, and 0.8 for the white wheat bread.
hydrolysis. Furthermore, pasta, which gave the lowest insulin response, contained less fiber than the rye breads, further emphasizing the importance of food structure. On the basis of hydrolysis indexes, the starch in pasta and whole-kernel rye bread was less accessible for starch hydrolysis than was the starch in white wheat bread. In β-glucan–enriched rye bread, the rate of starch hydrolysis was not significantly different from that of white wheat bread, despite the high content of soluble fiber in the rye bread. Our findings agree with those of Järvi et al (13, 15), who compared diets with identical nutrient compositions and type and amount of fiber and produced differences in the glycemic index mainly by altering the structure of the starchy foods. They found that consumption of a diet with a low glycemic index and a preserved food structure improved glucose and insulin responses.

**Role of gastric emptying rate**

The test breads did not produce different plasma paracetamol responses, indicating that the rate of gastric emptying is not a critical factor in the regulation of glucose metabolism in this setting. Our results disagree with those of an earlier study by Benini et al (44), who noticed that a high-fiber meal (fiber sources naturally present in food: 4.8 g fiber/MJ) delayed gastric emptying compared with a very low fiber meal (0.9 g fiber/MJ). On the other hand, Hagander et al (26) showed that the gastric emptying rate of a high-fiber meal with whole-grain wheat and rye bread was similar to that of a low-fiber meal. Many studies have, however, pointed out that soluble fiber can slow the rate of gastric emptying (45–47) by producing viscous solutions in water (22,48), whereas the cellulose type of insoluble fiber has no influence (47). However, Rigaud et al (49) showed that a single dose of 7.4 g pectin administered before the meal did not alter the rate of gastric emptying relative to the effect of a placebo. This agrees with our findings because the ingested bread portions contained 0.9–6.8 g soluble fiber. It was also suggested that the form of food is more essential for the regulation of gastric emptying than is the fiber content (31). Neither starch nor organic acids and their salts in the rye breads modified the gastric emptying rate in the present study.

**GIP and GLP-1 responses**

Similar to the insulin responses, the GIP and GLP-1 responses were lower after rye bread and pasta consumption than after white wheat bread consumption, indicating the regulation of postprandial insulin by these incretin hormones. Our results

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**TABLE 3**

Maximal glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1) responses to the consumption of the test breads and pasta

<table>
<thead>
<tr>
<th>Maximal response</th>
<th>White wheat bread</th>
<th>Whole-kernel rye bread</th>
<th>β-Glucan rye bread</th>
<th>Whole-meal pasta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>1.8 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>231.6 ± 25.2</td>
<td>135 ± 13.2</td>
<td>181.8 ± 17.4</td>
<td>94.8 ± 9.0</td>
</tr>
<tr>
<td>GIP (pmol/L)</td>
<td>52.3 ± 3.4</td>
<td>25.3 ± 2.2</td>
<td>36.7 ± 3.7</td>
<td>24.4 ± 2.3</td>
</tr>
<tr>
<td>GLP-1 (pmol/L)</td>
<td>14.4 ± 2.1</td>
<td>9.6 ± 1.6</td>
<td>12.5 ± 1.7</td>
<td>9.1 ± 1.8</td>
</tr>
</tbody>
</table>

1 x ± SEM; n = 20.
2 Significantly different from white wheat bread, P < 0.05.

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**FIGURE 3.** Mean fasting and postprandial insulin responses to rye and wheat products over 180 min (n = 20). Postprandial insulin responses to whole-kernel rye bread (a), β-glucan rye bread (b), and pasta (c) were significantly different from those to white wheat bread, P < 0.05. The pooled SEM was 40.5 for the whole-kernel rye bread, 56.3 for the β-glucan rye bread, 36.3 for the pasta, and 73.8 for the white wheat bread.

**FIGURE 4.** Mean fasting and postprandial glucose-dependent insulinotropic polypeptide (GIP) responses to rye and wheat products over 180 min (n = 20). Postprandial GIP responses to whole-kernel rye bread (a), β-glucan rye bread (b), and pasta (c) were significantly different from those to white wheat bread, P < 0.05. The pooled SEM was 7.6 for the whole-kernel rye bread, 11.1 for the β-glucan rye bread, 8.9 for the pasta, and 12.1 for the white wheat bread.
The postprandial paracetamol response to whole-kernel rye bread (a) agrees with the findings of Ørskov et al (50), who noticed that plasma insulin responses correlate significantly with GIP and GLP-1 responses in healthy volunteers. Earlier studies showed that the consumption of breads containing guar gum (27), whole-grain wheat and rye, oat and barley flour, wheat bran (26), and rye flakes (8) can reduce GIP responses more considerably than can white wheat bread. However, Levitt et al (28) did not find any effect of fiber in a mixed meal on postprandial insulin and GIP responses, even though fiber improved glucose tolerance in the same study. The GLP-1 responses to the β-glucan rye bread were different from the responses of insulin and GIP. The high fasting concentration and postprandial response of GLP-1 to the β-glucan rye bread remains unexplained.

To conclude, we studied in detail the in vitro properties of starch from various sources; the effects of botanical structure, food form, and fiber content of these grain products on postprandial glucose, insulin, and incretin responses; and the rate of gastric emptying in response to the starches from different foods. The results indicate that the lower insulinemic response to the rye breads and pasta than to the white bread is not explained by the fiber content, type of cereal, or rate of gastric emptying, but by the structural properties of the food. These properties should be studied further to produce foods that could be useful in preventing the deterioration of glucose tolerance in Western persons.

We thank Kati Katina for baking the test breads, Marjatta Salmenkallio Marttila for assisting with the microscopy, Karin Autio for interpreting the microscopy graphs, Tapani Suortti for analyzing the molecular weight of β-glucan, Erja Kinnunen and Siv Matomaa for assisting with the technical aspects of the study, technical assistance, and Pirjo Halonen for helping with the statistical analysis.

REFERENCES


![FIGURE 5](image1.png)

**FIGURE 5.** Mean fasting and postprandial glucagon-like peptide 1 (GLP-1) responses to rye and wheat products over 180 min (n = 20). Postprandial GLP-1 responses to whole-kernel rye bread (a), β-glucan rye bread (b), and pasta (c) were significantly different from those to white wheat bread, P < 0.05. The pooled SEM was 7.6 for the whole-kernel rye bread, 8.4 for the β-glucan rye bread, 7.4 for the pasta, and 7.6 for the white wheat bread.

![FIGURE 6](image2.png)

**FIGURE 6.** Mean fasting and postprandial paracetamol responses to test breads containing either rye or wheat over 180 min (n = 20). The postprandial paracetamol response to whole-kernel rye bread (a) was significantly different from that white wheat bread, P < 0.05. The pooled SEM was 10.6 for the whole-kernel rye bread, 9.1 for the β-glucan rye bread, and 12.6 for the white wheat bread.


41. Juntunen ET AL. 262 Juntunen ET AL.


43. Juntunen ET AL. 262 Juntunen ET AL.


