

# Structural differences between rye and wheat breads but not total fiber content may explain the lower postprandial insulin response to rye bread<sup>1–3</sup>

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## ABSTRACT

**Background:** Rye bread has a beneficial effect on the postprandial insulin response in healthy subjects. The role of rye fiber in insulin and glucose metabolism is not known.

**Objective:** The aim of the study was to determine the effect of the content of rye fiber in rye breads on postprandial insulin and glucose responses.

**Design:** Nineteen healthy postmenopausal women aged  $61 \pm 1$  y, with a body mass index (in  $\text{kg}/\text{m}^2$ ) of  $26.0 \pm 0.6$ , and with normal glucose tolerance participated in the study. The test products were refined wheat bread (control), endosperm rye bread, traditional rye bread, and high-fiber rye bread; each bread provided 50 g available carbohydrate and was served with breakfast. Plasma glucose, insulin, glucose-dependent insulintropic polypeptide, glucagon-like peptide 1, and serum C-peptide were measured in fasting and 8 postprandial blood samples. In vitro starch hydrolysis and the microscopic structure of the breads were also determined.

**Results:** Postprandial insulin, glucose-dependent insulintropic polypeptide, and C-peptide responses to the rye breads were significantly lower than the response to the control; no significant differences in insulin and C-peptide responses to the rye breads were found. Glucose and glucagon-like peptide 1 responses to the rye breads were not significantly different from those to the control, except at 150 and 180 min. In vitro starch hydrolysis was slower in all rye breads than in the control, and the structure of continuous matrix and starch granules differed between the rye and control breads.

**Conclusion:** Total fiber content does not explain the lower postprandial insulin response to rye bread than to wheat bread, but structural differences between rye and wheat breads might. *Am J Clin Nutr* 2003;78:957–64.

**KEY WORDS** Rye, wheat, cereal fiber, postprandial, blood glucose, insulin, C-peptide, glucose-dependent insulintropic polypeptide, glucagon-like peptide 1, postmenopausal women

## INTRODUCTION

The Western-type, carbohydrate-rich diet—which involves frequent snacking—leads to continuous pancreatic stimulation and repeated postprandial excursions of insulin. This type of diet has been hypothesized to predispose to insulin resistance,

$\beta$ -cell dysfunction, and ultimately type 2 diabetes (1). A low-glycemic index diet that is high in fiber and whole-grain cereal products results in decreases in postprandial insulin and glucose responses, which is thought to be beneficial to insulin and glucose metabolism (2).

We showed previously that whole-meal rye bread and rye bread baked with whole kernels produce a lower insulin response than does refined wheat bread but no differences in glucose responses in healthy subjects (3, 4). These findings suggest that less insulin is required for the regulation of postprandial glucose excursions after the consumption of rye breads. The differences in the fiber content and structural characteristics between the rye and wheat breads may explain this finding. In most earlier studies in healthy (5) and diabetic (6–9) persons, with breads baked with milled flour, the postprandial glucose response was not affected by the amount of cereal fiber. However, in that study, the insulin response was not determined in healthy persons (5).

Preservation of the intact botanical structure of cereal grains has also been shown to lower the insulin response (10). Furthermore, food processing, such as baking, has been shown to reduce the digestibility of starch (11), which indicates the importance of preserved food structure and resistant starch for reduced hydrolysis.

In the present study we aimed to clarify the effect of rye fiber on the postprandial insulin response by changing the fiber content in rye breads. We also sought to clarify the role that differences in the structural properties of starch granules and the bread matrix may play in determining the postprandial insulin responses to rye and wheat breads.

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**TABLE 1**  
Characteristics of the subjects at the time of entry into the study<sup>1</sup>

	Women
Age (y)	61 ± 4.8 (51–69)
BMI (kg/m <sup>2</sup> )	26.0 ± 2.5 (22.5–30.2)
Systolic blood pressure (mm Hg)	125 ± 18 (96–151)
Diastolic blood pressure (mm Hg)	77 ± 9 (63–97)
Serum total cholesterol (mmol/L)	6.1 ± 0.8 (4.7–7.4)
Serum HDL cholesterol (mmol/L)	1.7 ± 0.3 (1.3–2.3)
Serum triacylglycerol (mmol/L)	1.1 ± 0.4 (0.7–2.2)
Oral-glucose-tolerance test	
Plasma glucose, 0 min (mmol/L)	5.7 ± 0.4 (5.2–6.9)
Plasma glucose, 120 min (mmol/L)	5.6 ± 1.0 (4.3–7.5)
Plasma insulin, 0 min (pmol/L)	44.4 ± 13.8 (25.3–74.5)
Plasma insulin, 120 min (pmol/L)	279.6 ± 175.8 (27.6–795.7)

<sup>1</sup> $\bar{x} \pm$  SD; minimum and maximum values in parentheses.  $n = 19$ .

## SUBJECTS AND METHODS

### Subjects

Twenty healthy, nondiabetic, postmenopausal women were recruited for the study. One woman discontinued the study because of heart problems after the first visit. The basic characteristics of the remaining 19 subjects are shown in **Table 1**. All but one woman had normal glucose tolerance at the time of entry to the study, as determined by a 2-h oral-glucose-tolerance test according to World Health Organization criteria (12). One woman had impaired fasting glucose. The subjects' mean energy intake, calculated from a 4-d food record kept before the first study visit, was 7213 kJ/d (1723 kcal/d). Their body weight and intakes of energy, carbohydrates, and fiber before each test day remained unchanged throughout the study. The MICRO-NUTRICA software package (version 2.5; Finnish Social Insurance Institution, Turku, Finland) was used to calculate energy and nutrient intakes. The protocol for the study was approved by the Ethics Committee of the Kuopio University and University Hospital.

### Postprandial study

The subjects fasted 12–15 h before the tests. On the test morning, body weight was measured and an intravenous catheter was inserted in the antecubital vein of the arm. After the fasting blood sample was taken, the subjects received the test meal, which contained the test bread (50 g available carbohydrates), 40 g cucumber, and 3 dL of a noncaloric orange drink. Eight blood samples were taken after the start of eating (15, 30, 45, 60, 90, 120, 150, and 180 min) for the measurement of plasma glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1), and 3 blood samples were taken after the start of eating (30, 60, and 120 min) for the measurement of serum C-peptide concentrations.

The test bread portions were served in random order at intervals of 1–2 wk. Wheat bread (control) was served twice to reduce the intraindividual variation, and the mean of these 2 determinations was used in the statistical analysis. Eating of traditional and high-fiber rye breads took longer than that of

wheat bread ( $P = 0.006$  and  $P = 0.0001$ , respectively). On average, refined wheat bread was eaten in 7 min and 46 s, endosperm rye bread in 8 min and 24 s, traditional rye bread in 9 min and 26 s, and high-fiber rye bread in 12 min and 42 s.

The subjects were advised to maintain their diet, body weight, and other living habits. Body weight was measured at each visit, and energy intake was quantified by recording the foods eaten before each test day. Heavy exercise and unusually large portions of food were forbidden on the day before each test, as was the consumption of alcohol for 2 d before the tests. Smoking was also forbidden on the morning of each test. The subjects were asked to arrive for all 5 study visits at the laboratory by car or by bus, if possible, to avoid extra stress.

### Test products

The rye breads chosen as test products were endosperm rye bread, traditional whole-meal rye bread, and whole-meal rye bread enriched with rye bran (high-fiber rye bread). The test products for the postprandial study were chosen by using the in vitro starch hydrolysis method (*see* below). Commercial refined wheat bread (EloPakari; Vaasan & Vaasan Oy, Kuopio, Finland) was used as the reference (control) bread.

Sourdough containing both yeast and lactobacilli was used in all rye breads. The endosperm rye bread formula comprised commercial rye endosperm flour (900 g), sourdough (731 g), water (430 g), fresh yeast (19 g), and salt (12.9 g). Sourdough was prepared from commercial endosperm flour (380 g), L62 (0.4 g *Lactobacillus brevis*) L73 (0.4 g *L. plantarum*), fresh yeast (3.8 g), and water (632 g). Traditional rye bread formula comprised commercial whole-meal rye flour (900 g), sourdough (731 g), water (450 g), fresh yeast (19 g), and salt (12.9 g). Sourdough was prepared from whole-meal rye flour (380 g), L62 (0.4 g *L. brevis*), L73 (0.4 g *L. plantarum*), fresh yeast (3.8 g), and water (632 g). High-fiber rye bread comprised whole-meal rye flour (540 g), rye bran (390 g), sourdough (731 g), water (450 g), fresh yeast (19 g), and salt (12.9 g). The sourdough was prepared similarly to whole-meal rye bread.

The dietary fiber content of the breads was determined according to Asp et al (13), the protein content by the Kjeldahl method (nitrogen  $\times$  5.7), and the fat content gravimetrically by extraction in diethyl ether and petroleum ether after hydrolysis with acid (Association of Official Analytical Chemists method 922.06, 1995). The moisture content 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 following formula:

$$\text{Energy} = [17 \times \text{protein} (\%)] + [37 \times \text{fat} (\%)] + [17 \times \text{available carbohydrate} (\%)] \quad (1)$$

The nutrient composition of the test bread portions is shown in **Table 2**. The rye-bread portions differed from each other and from the refined wheat bread, especially in the amount of total and insoluble dietary fiber. The variation in the amount of soluble dietary fiber, however, was much smaller.

### In vitro starch hydrolysis

The rate of in vitro starch hydrolysis of the test products was determined by the enzymatic hydrolysis method (14). The



**TABLE 2**  
Nutrient composition of the test bread portions

	Refined		Traditional rye bread	High-fiber rye bread
	wheat bread	Endosperm rye bread		
Portion size (g)	105.5	111.9	142.7	199.4
Available carbohydrate (g)	50	50	50	50
Total dietary fiber (g)	2.7	6.1	15.2	29.0
Insoluble dietary fiber (g)	1.5	3.1	10.9	24.1
Soluble dietary fiber (g)	1.2	3.0	4.3	4.8
Protein (g)	9.0	4.9	11.1	16.7
Fat (g)	5.2	3.4	7.8	10.2
Moisture (g)	38.2	46.7	58.2	90.2
Energy content (kJ)	1177	1056	1295	1486

amount of available starch was determined with the use of a specific enzymatic kit (Megazyme, Bray, Ireland). An equivalent amount of available starch (1 g based on the analyzed data) from each test product was chewed for 15 s by 3 subjects. The sample sizes were 2.11 g refined wheat bread, 2.24 g endosperm rye bread, 2.85 g traditional rye bread, and 3.99 g high-fiber rye bread. After the bread was chewed and incubated with pepsin, pancreatic  $\alpha$ -amylase was used to hydrolyze the starch in a dialysis tube. Aliquots of the dialysate were removed for analysis of the reducing sugar content by the 3,5-dinitro salicylic method (15). 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. The hydrolysis index was calculated as the area under the curve (AUC) for hydrolysis (0–180 min), with the product as a percentage of the corresponding AUC with refined wheat bread.

### Microscopy of the breads

To determine the microscopic structure of the breads, several pieces were taken from the center of the bread crumb. Samples were embedded in agar gel and were chemically fixed in 1% glutaraldehyde, dehydrated, and embedded in Histo-resin (Jung, Heidelberg, Germany) as recommended by the manufacturer. Sections (4- $\mu$ m thick) were cut with a microtome (RM2055; Leica Jung, Nussloch, Germany) and stained with 0.1% Light Green (Gurr, BDH Ltd, Poole, United Kingdom), Lugol iodine solution [0.33% I<sub>2</sub> (wt:vol) and 0.67% KI (wt:vol)], and 0.01% Calcofluor White M2R New (Fluorescent Brightener 28; Aldrich, Germany), and 0.1% Acid Fuchsin (Gurr, BDH Ltd) (16) and examined with a microscope (BH-2 microscope; Olympus, Tokyo). In light micrographs of bread, protein appears green, amylopectin starch appears brown to gray, and amylose starch appears blue.

### Biochemical analyses

The serum samples were collected in prechilled tubes for measurement of C-peptide. Plasma samples were collected in prechilled EDTA-containing tubes for measurement of insulin, GIP, and GLP-1, and in prechilled fluoride citrate-containing tubes for glucose. The samples were centrifuged for 10 min at 2100  $\times$  g at  $-4^{\circ}$ C to separate serum or plasma. The samples were stored at  $-70^{\circ}$ C until analyzed. Plasma glucose was analyzed by the enzymatic photometric method (Granustest 250; Merck, Darmstadt, Germany) with the use of Kone Pro Clinical

Analyser (Kone Ltd, Espoo, Finland) and plasma insulin by radioimmunoassay (Phadaseph Insulin RIA 100; Pharmacia Diagnostica, Uppsala, Sweden). The interassay CVs in glucose measurements were 3.9% ( $n = 149$ ) and 3.7% ( $n = 149$ ) for the lowest and highest glucose controls, respectively, and for 3 different concentrations of insulin were 3.8% ( $n = 37$ ), 3.2% ( $n = 37$ ), and 4.7% ( $n = 37$ ) for the lowest, middle, and highest controls, respectively.

The AutoDELFI C-peptide, time-resolved fluoroimmunoassay method (TR-FIA; Perkin-Elmer Wallac, Turku, Finland) was used for the measurement of serum C-peptide. The intra- and interassay CVs varied between 3.1–5.0% and 1.9–3.0%, respectively, in the concentration range 0.5–2.9 nmol/L ( $n = 27$ ).

GIP and GLP-1 concentrations in plasma were measured by radioimmunoassay after extraction of plasma with 70% ethanol. For the GIP radioimmunoassay, the carboxyl-terminal directed antiserum R 65 was used, which cross-reacts fully with human GIP but not with the so-called GIP 8000 (17). Human GIP and <sup>125</sup>I human GIP (70 MBq/nmol) were used for standards and tracer. Plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1 7–36 amide with the use of antiserum (code no. 89390) that is specific for the amidated carboxyl terminal of GLP-1 and, therefore, mainly reacts with GLP-1 of intestinal origin (18). For these assays, the detection limit was < 1 pmol/L, and the intraassay CV was < 6% at 20 pmol/L. All samples from the same subject were assayed in the same assay run.

Maximal glucose, insulin, C-peptide, GIP, and GLP-1 responses were calculated by subtracting the highest value from the corresponding fasting value. The plasma AUC at 180 min for glucose, insulin, GIP, and GLP-1 and the AUC at 120 min for C-peptide were calculated from the postprandial curve above the fasting concentration (19).

### Statistics

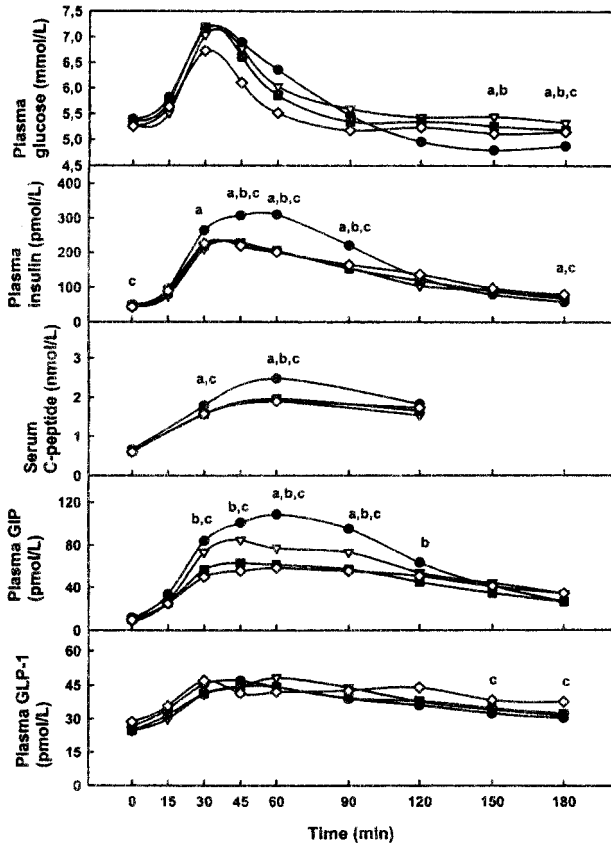
The statistical analyses of the clinical data were done by comparing the rye breads with the refined wheat bread and the rye breads with each other. The statistical significance of the differences was assessed by using the nonparametric Friedman's test followed by Wilcoxon's test for pairwise comparisons. To control the overall level of significance, the Bonferroni adjustment was used. In all analyses,  $P$  values < 0.05 were considered to be statistically significant. The results are expressed as means  $\pm$  SDs or SEMs. Data were analyzed with SPSS for WINDOWS 8.0 program (SPSS, Chicago) (20).

## RESULTS

### Postprandial plasma glucose and insulin responses

No significant differences in glucose responses to the test breads were observed during the first 2 h after the breads were eaten (**Figure 1**). The maximal glucose responses, the times to reach the maximal response, and the AUCs did not differ significantly among the breads (**Table 3**). However, glucose concentrations in response to refined wheat bread had fallen below baseline fasting concentrations and were lower than corresponding concentrations in response to endosperm rye





**FIGURE 1.** Mean fasting and postprandial glucose, insulin, C-peptide, glucose-dependent insulintropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1) responses to endosperm rye ( $\nabla$ ), traditional rye ( $\blacksquare$ ), high-fiber rye ( $\diamond$ ), and refined wheat ( $\bullet$ ) breads over 180 min. For each time point, means significantly different from the mean for refined wheat bread are indicated by letters (a, endosperm rye bread; b, traditional rye bread; c, high-fiber rye bread;  $P < 0.05$ , Wilcoxon's test with Bonferroni adjustment). The pooled SEM of glucose was 0.8 for endosperm rye bread, 0.7 for traditional rye bread, 1.0 for high-fiber rye bread, and 1.0 for refined wheat bread. The corresponding pooled SEMs for insulin were 59.6, 58.3, 74.1, and 92.3 mmol/L; those for C-peptide were 0.5, 0.4, 0.5, and 0.6 nmol/L; those for GIP were 27.5, 17.6, 18.4, and 23.7 pmol/L; and those for GLP-1 were 16.6, 11.6, 14.1, and 13.3 pmol/L.  $n = 19$ .

**TABLE 3**

Maximal glucose, insulin, C-peptide, glucose-dependent insulintropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1) concentrations and areas under the curve in response to the consumption of the test breads<sup>1</sup>

	Refined wheat bread	Endosperm rye bread	Traditional rye bread	High-fiber rye bread
Maximal response				
Glucose (mmol/L)	2.1 $\pm$ 0.2	2.0 $\pm$ 0.2	2.0 $\pm$ 0.1	1.7 $\pm$ 0.2
Insulin (pmol/L)	299.2 $\pm$ 28.1	206.1 $\pm$ 18.8 <sup>2</sup>	220.5 $\pm$ 20.8 <sup>2</sup>	222.2 $\pm$ 29.1 <sup>2</sup>
C-peptide (nmol/L)	1.9 $\pm$ 0.1	1.4 $\pm$ 0.1 <sup>2</sup>	1.4 $\pm$ 0.1 <sup>2</sup>	1.5 $\pm$ 0.1 <sup>2</sup>
GIP (pmol/L)	107.2 $\pm$ 7.4	87.7 $\pm$ 10.3	59.1 $\pm$ 4.6 <sup>2,3</sup>	60.5 $\pm$ 5.0 <sup>2,3</sup>
GLP-1 (pmol/L)	28.3 $\pm$ 4.7	30.6 $\pm$ 6.3	25.9 $\pm$ 3.3	26.4 $\pm$ 5.4
Area under the curve				
Glucose (mmol $\cdot$ min/L)	99.6 $\pm$ 15.1	99.4 $\pm$ 16.0	77.8 $\pm$ 11.6	83.3 $\pm$ 23.3
Insulin (pmol $\cdot$ min/L)	22151 $\pm$ 2288	15831 $\pm$ 1276 <sup>2</sup>	16389 $\pm$ 1374 <sup>2</sup>	18270 $\pm$ 1755
C-peptide (nmol/L)	151.4 $\pm$ 9.6	115.9 $\pm$ 7.6 <sup>2</sup>	119.7 $\pm$ 6.8 <sup>2</sup>	122.2 $\pm$ 7.8 <sup>2</sup>
GIP (pmol $\cdot$ min/L)	10496 $\pm$ 667	8347 $\pm$ 701 <sup>2</sup>	6357 $\pm$ 559 <sup>2,3</sup>	6506 $\pm$ 531 <sup>2</sup>
GLP-1 (pmol $\cdot$ min/L)	2089 $\pm$ 308	2557 $\pm$ 430	2309 $\pm$ 315	2141 $\pm$ 402

<sup>1</sup> $\bar{x} \pm$  SEM;  $n = 19$ .

<sup>2</sup>Significantly different from refined wheat bread,  $P < 0.05$  (Wilcoxon's test with Bonferroni adjustment).

<sup>3</sup>Significantly different from endosperm rye bread,  $P < 0.05$  (Wilcoxon's test with Bonferroni adjustment).

bread and to traditional rye bread at 150 and 180 min ( $P = 0.012$ – $0.036$ ) and to high-fiber rye bread at 180 min ( $P = 0.048$ ) (Figure 1).

Insulin responses at several time points after the consumption of all rye breads were significantly different from those after the refined wheat bread, but there were no significant differences between the responses of the rye breads (Figure 1). Compared with the refined wheat bread, significantly lower insulin responses were observed to endosperm rye bread at 30, 45, 60, and 90 min, and significantly higher responses were observed at 180 min ( $P = 0.0001$ – $0.006$ ). Similarly, the responses to traditional rye bread at 45, 60, and 90 min ( $P = 0.0001$ – $0.024$ ) and to high-fiber rye bread at 0, 45, 60, and 90 min were lower and the response to the latter was higher at 180 min ( $P = 0.0001$ – $0.030$ ). Furthermore, the maximal insulin responses to rye breads were lower than those to refined wheat bread ( $P = 0.0001$ – $0.006$ ) (Table 3). Also, the postprandial insulin AUCs were significantly smaller for endosperm and traditional rye breads than for refined wheat bread ( $P = 0.0001$  and  $P = 0.006$ , respectively) and the AUC was nearly significantly smaller for high-fiber rye bread ( $P = 0.06$ ). The time intervals to reach the maximal insulin response did not differ significantly in any comparison among the test breads.

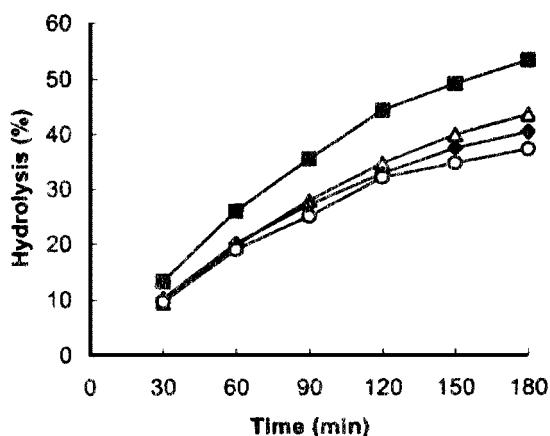
#### Postprandial serum C-peptide responses

The serum C-peptide responses mirrored the insulin responses (Figure 1). Responses to endosperm rye and high-fiber rye breads at 30 and 60 min ( $P = 0.0001$ – $0.036$ ) and to traditional rye bread at 60 min ( $P = 0.0001$ ) were lower than those to refined wheat bread. In addition, the maximal C-peptide responses ( $P = 0.0001$ – $0.006$ ) and the 120-min AUC to all rye breads ( $P = 0.006$  for all breads) were smaller than those to wheat bread (Table 3). No significant differences among the breads in the time interval to reach the maximal C-peptide responses were seen.

#### Postprandial plasma GIP and GLP-1 responses

The plasma GIP responses to rye breads were significantly lower than those to wheat bread at several time points: for





**FIGURE 2.** Rate of starch hydrolysis after endosperm rye bread ( $\Delta$ ), traditional rye bread ( $\blacklozenge$ ), high-fiber rye bread ( $\circ$ ), and refined wheat bread ( $\blacksquare$ ) were chewed, incubated with pepsin, and subsequently incubated with pancreatic  $\alpha$ -amylase in a dialysis tube.

endosperm rye bread at 60 and 90 min ( $P = 0.0001$  and  $P = 0.018$ , respectively); for traditional rye bread at 30, 45, 60, 90, and 120 min ( $P = 0.0001$ – $0.006$ ); and for high-fiber rye bread at 30, 45, 60, and 90 min ( $P = 0.0001$ – $0.006$ ) (Figure 1). In addition, the GIP responses to endosperm rye bread were higher than those to traditional rye bread at 30 and 60 min and to high-fiber rye bread at 30, 45, and 60 min ( $P = 0.006$ – $0.042$ ).

Furthermore, the maximal GIP increases after traditional and high-fiber rye breads were significantly smaller than the increase after wheat bread ( $P = 0.0001$  and  $P = 0.0001$ , respectively), as were the AUCs for all rye breads ( $P = 0.0001$ – $0.036$ ) (Table 3). Also, the maximal responses to traditional rye and high-fiber rye breads ( $P = 0.012$  and  $P = 0.030$ , respectively) and the AUC for traditional rye bread ( $P = 0.024$ ) were smaller than the corresponding values for endosperm rye bread (Table 3).

No significant differences were found in the GLP-1 responses among the test breads, except at the end of the study between the high-fiber rye bread and wheat bread ( $P = 0.012$  at 150 min and  $P = 0.012$  at 180 min) (Figure 1). The maximal increases, maximal times, and the AUCs for GLP-1 also did not differ significantly among the test products (Table 3).

In one of the subjects the GIP and GLP-1 responses did not rise above the fasting concentration during the first postprandial test for refined wheat bread. The AUCs for GIP and GLP-1 calculated from the second postprandial test with refined wheat bread were therefore used as the mean values of the responses to wheat bread in this subject. The statistical analyses were also repeated after this subject's data were eliminated from the analyses, but the results remained essentially unchanged.

### In vitro starch hydrolysis

In vitro starch hydrolysis differed among the test breads ( $P = 0.029$ ; Figure 2). Hydrolysis indexes of  $82 \pm 3$ ,  $76 \pm 2$ , and  $71 \pm 4$  were obtained for the endosperm, traditional, and high-fiber rye breads, respectively.

### Microscopy of the breads

The structure of starch granules and the continuous matrix between the starch granules were very different between the

wheat and rye breads. In wheat bread, green-stained protein (gluten) formed a continuous matrix in which starch granules were dispersed (Figure 3A). In rye breads, starch granules were swollen, and amylose had partly leached out (Figure 3, B–D). The starch granules were also closely packed and formed a continuous matrix. The softness and porousness of refined wheat bread and the hardness of rye breads are based on these structural differences between rye and wheat breads.

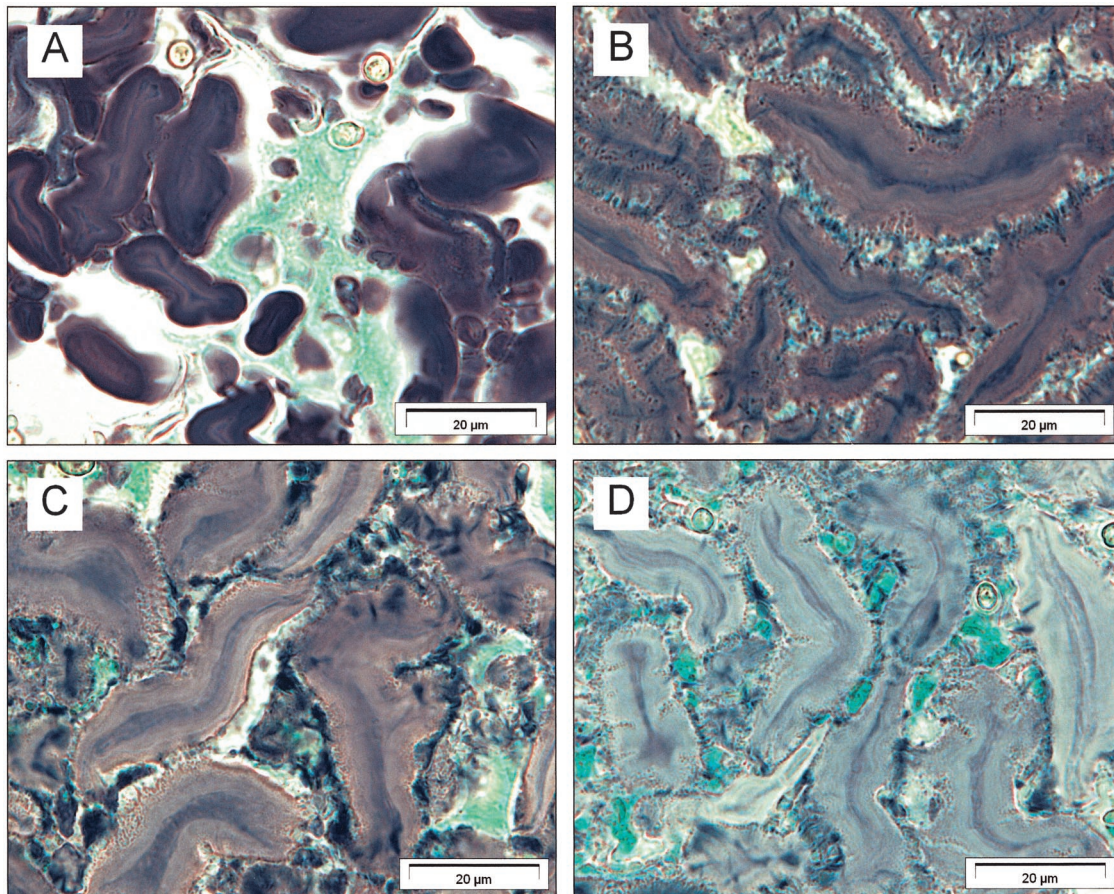
### DISCUSSION

The present study confirmed our previous findings that, in healthy subjects, less insulin is needed for the control of postprandial glucose excursions after the ingestion of rye bread than after the ingestion of refined wheat bread and that a lower insulin response is associated with parallel changes in plasma GIP (3, 4). Furthermore, we also showed that the lower insulin response after rye bread is not explained by the amount of fiber in rye bread. The lower C-peptide response after ingestion of rye bread indicates that the diminished pancreatic secretion of insulin rather than enhanced liver extraction contributes to the lower insulin response. Our study was tightly controlled by design and dietary and lifestyle factors to minimize the possible confounding effects; therefore, our study offers convincing evidence of the beneficial effect of rye bread on insulin metabolism, at least acutely. Importantly, the intake of energy during the day preceding each postprandial experiment and the body weights of the study subjects did not change during the study.

In agreement with previous findings (5–9), the postprandial glucose response in the present study was unaffected by the content of fiber in the breads. Even though the glucose responses did not initially differ quantitatively, wheat bread was characterized by a decrease in glucose below fasting concentrations at the tail of the curve between 2 and 3 h. These decreased circulating glucose concentrations postprandially may increase hunger, increase the drive to eat, and stimulate the release of counterregulatory hormones (21). We also found smaller hydrolysis indexes for the rye breads (index: 71–82) than for wheat bread (index: 100) in vitro, which indicates a slower hydrolysis of starch in rye products. The findings of our postprandial study suggest that plasma glucose is tightly regulated in healthy persons, and possible differences in the release of glucose from different cereals are observed only as parallel changes in the demand of insulin. However, the fact that peripheral blood glucose represents the net effect of many postabsorptive processes in addition to the rate of glucose absorption should not be overlooked (22).

The insoluble components of cereal fibers are known to be ineffective in the regulation of postprandial glycemia and insulinemia when ingested with a glucose load (23, 24), although the role of insoluble cereal fiber in bread has not been well studied. Rye fiber also contains soluble fiber in the form of arabinoxylan (9%) and  $\beta$ -glucan (2–3%) (25, 26). In the present study, however, the amount of soluble fiber in rye bread portions was small (3.0–4.8 g/portion), although it was greater than in refined wheat bread (1.2 g/portion). Furthermore, the absolute difference between the wheat and endosperm rye breads was as great as was the difference between endosperm and high-fiber rye bread. In earlier studies that showed a decrease in both the insulin and the glucose responses





**FIGURE 3.** Light micrographs of refined wheat bread (A), endosperm rye bread (B), high-fiber rye bread (C), and traditional rye bread (D). Protein appears green, amylopectin starch appears brown to gray, and amylose starch appears blue. Protein (gluten, stained green) makes the refined wheat bread soft, whereas the highly swollen starch granules and outleached amylose starch make the rye bread hard.

with arabinoxylan-enriched wheat breads and  $\beta$ -glucan-rich barley breads, the content of soluble fiber was considerably larger, ranging from 3.7 to 14.1 g/portion (27, 28).


The reduced insulin response after the rye breads may also have been due to larger portions and longer ingestion times after traditional rye bread and high-fiber rye bread than after ingestion of wheat bread. This is not likely, however, because the portions and eating times for wheat and endosperm rye breads were almost identical, and the insulin responses between the endosperm and traditional, or the endosperm and high-fiber rye breads, did not differ significantly. There were also small differences in the fat and protein contents of the test bread portions. Although both nutrients are known to affect postprandial glucose and insulin responses (29, 30), findings with starchy foods (bread, spaghetti, and rice) (5) and with mixed meals (31) have shown that small differences in the intake of these nutrients have negligible effects on the overall postprandial glucose and insulin responses.

Incretins are hormones that are secreted during meals and that potentiate the insulin response to levels above those observed when the corresponding stimulus (usually glucose) is administered intravenously (32). The most important insulinotropic incretins are GLP-1 and GIP. It is possible that the lower insulin response after rye bread in the present study was in part mediated by GIP. However, our previous intervention study showed that the first-phase insulin response to intrave-

nous glucose was enhanced by 8 wk of rye bread ingestion as compared with ingestion of refined wheat bread (33). Because the response to intravenous glucose bypasses the gut incretin effect, the lowered postprandial insulin response to rye bread may not be solely explained by the reduced GIP response. On the other hand, different mechanisms may explain the decreased response of GIP to rye bread. Soluble fibers, such as guar gum, have been shown to decrease postprandial insulin and GIP responses in healthy persons (34–36) and in persons with type 2 diabetes (36, 37)—with one exception (38)—whereas insoluble fiber in the form of wheat bran (34) or cellulose (39) showed no effect. Also, the food structure may have an influence on GIP, but no studies regarding the role of mechanical food structure in the release of incretin have been published.

Because the present results on postprandial insulin responses are unlikely to be explained by the amount of dietary insoluble or soluble fiber, there may be other relevant differences between the wheat and rye breads. The structures of the continuous matrix and starch granules differed between rye and wheat breads after baking. In rye bread, a continuous phase was formed by closely packed starch granules, whereas in wheat bread the starch granules were entrapped in an extensible gluten network that formed the continuous phase. This caused a less porous and mechanically firmer structure in rye breads (40). Therefore, particle size before swallowing was probably

much higher for rye than for wheat breads and could explain the slower rate of hydrolysis found in this study and previously (41). Furthermore, in wheat bread, starch remained inside the granule, became gelatinized, and was more accessible to hydrolysis by amylolytic enzymes. In contrast, in rye breads, amylose leached out and coated the starch granules, which made the starch resistant to hydrolysis after cooling. This phenomenon has been reported previously in rye bread (42). The coating of amylose on the surface of starch granules has also been suggested to retard the hydrolysis of amylopectin, the other main constituent of starch (43). In addition, the endogenous arabinoxylan-degrading enzyme xylanase in rye flour contributes to the release of amylose from starch granules (16).

The present study showed that a lower insulin secretion after the ingestion of rye bread than after the ingestion of wheat bread is not explained by the quantity of rye fiber in the bread but may be explained by differences in the structural properties of the 2 breads. 

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