

Postprandial Glycemia, Insulinemia, and Satiety Responses in Healthy Subjects after Whole Grain Rye Bread Made from Different Rye Varieties. 2

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ABSTRACT: Rye breads made from commercial rye blends lower the postprandial insulin demand and appear to facilitate glucose regulation. However, differences in metabolic responses may occur between rye varieties. In the present work, five rye varieties (Amilo, Evolo, Kaskelott, Picasso, and Vicello) and a commercial blend of rye grown in Sweden were investigated with regard to their postprandial insulin, glucose, and appetite regulation properties in a randomized crossover study in 20 healthy subjects. The rye flours were baked into whole grain breads, and a white wheat bread (WWB) was used as reference (50 g of available starch). Picasso and Vicello rye bread showed lower glycaemic indices (GIs) compared with WWB (80 and 79, respectively) ($P < .05$). In addition to the GI, two measures of the glycaemic profile (GP and GP²) were calculated by dividing the incremental duration of the plasma glucose curve with the incremental glucose peak and squared incremental glucose peak, respectively. Vicello and Picasso ryes were characterized by a higher GP² than that of the WWB, suggesting a better regulated course of glycaemia. Rye bread made from not only Vicello and Picasso but also Amilo and Kaskelott displayed significantly lower insulin indices (IIs) than WWB (74–82). A high GP and GP² and a low GI were related to a lower II and insulin incremental peak. A high content of insoluble fibers and a high GP² were related to a higher subjective satiety in the early and late postprandial phase (tAUC 0–60 min and tAUC 120–180 min, respectively). The results suggest that there may be differences in the course of glycaemia following different rye varieties, affecting postprandial insulin responses and subjective satiety.

KEYWORDS: rye, whole grain, diabetes, dietary fiber, insulin, glucose

INTRODUCTION

Elevated glucose and insulin excursions may induce oxidative stress and subclinical inflammation¹ as well as insulin resistance.² By counteracting these phenomena the risk of cardiovascular diseases (CVD) and type 2 diabetes (T2D) can be lowered. Indeed, foods with low glycaemic index (GI) and insulin index (II) values have been demonstrated to protect against T2D and CVD in observational studies.^{3–7} Rye (*Secale cereale*) is a common cereal in bread and other food products, especially in northern European countries, and is of interest in this context. Consequently, rye breads made from Swedish and Finnish rye have been demonstrated to produce low insulinemic responses^{8–12} and to facilitate glycaemic regulation.^{11,12} Rye flour is usually made from a blend of different rye varieties, and there is little information about differences in metabolic responses between different common commercial rye varieties.

Rye is also interesting in relation to appetite regulation because rye products have been demonstrated to improve satiety responses both acutely and at a subsequent meal.^{11,12} Suggested mechanisms behind the satiety-inducing properties of rye are their low insulin responses, well-regulated glycaemia, and high content of indigestible carbohydrates, with the ability to induce colonic fermentation. In fact, a lowered insulin response and a low but prolonged course of glycaemia (high GP) have been shown to correlate with a lowered rebound of the hunger-promoting hormone ghrelin in the late postprandial phase after a rye test meal.^{11,12}

We recently showed that the insulin response is not lowered by all rye varieties (accompanying paper). Thus, rye products made from Haute Loire Pop, Dankowskie Zlote, and Nikita generated insulin responses similar to that of a white wheat bread, whereas Amilo and Rekrut displayed the previously observed insulin-saving properties. These differences in metabolic responses could depend on genetic differences between the rye varieties and/or environmental factors, such as cultivation conditions.

In previous studies, Swedish and Finnish commercial rye blends have demonstrated low insulinemic responses,^{8–12} and the purpose of the present work was to elucidate differences in metabolic responses to products made from single rye varieties grown in Sweden. The postprandial blood glucose, insulin, and subjective satiety were measured in healthy subjects after the intake of whole grain bread based on five different rye varieties or a Swedish commercial rye blend, respectively. White wheat bread was used as a reference product, and all bread products were flour-based.

MATERIALS AND METHODS

Reference and Rye Test Breads. Five whole grain breads made from different rye varieties grown in Sweden during 2009 were included

Received: May 18, 2011

Revised: September 30, 2011

Accepted: October 8, 2011

Published: October 08, 2011

in a randomized crossover study together with one rye bread baked from a commercial Swedish whole grain rye blend. A white wheat (endosperm) bread (WWB) was used as a reference bread. The whole grain rye breads were made from Vicello, Picasso, Kaskelott, Amilo, and Evolo rye, respectively. Rye kernels of the commercial blend and Vicello were kindly provided by Lilla Harrie Mills (Kävlinge, Sweden). The other rye varieties were donated by Lantmännen SW Seed AB (Svalöv, Sweden), which also milled all rye kernels in the study. Commercial white wheat flour was obtained from Kungsörnen AB (Järna, Sweden) and dry yeast from Jästbolaget AB (Sollentuna, Sweden).

The WWB was made according to Rosén et al.¹² All six rye breads were made from 3000 g of whole grain rye flour, 1000 g of white wheat flour, 2700 g of water, 50 g of dry yeast, and 40 g of NaCl and baked at Pågen Bakery, Malmö, Sweden, thereby containing 75% of rye on a flour basis. Each dough was mixed for 10 min and proofed at room temperature for 40 min. The dough was then divided into pieces of 1000 g, placed in baking tins, and subjected to a second proofing (37 °C, 77% humidity) for 60 min. Baking was initiated at 250 °C, although the temperature was immediately lowered to 200 °C, and the breads were baked for 35 min.

The WWB was left to cool for 1 h and the rye breads for 22–24 h under cover. Thereafter, the crust was 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) until use.

Chemical Analysis of Reference and Rye Test Breads. Prior to analyses, the bread samples were air-dried and milled to pass through a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden). The available starch content was determined according to the method of Holm et al.¹³ Insoluble and soluble dietary fiber was determined with the gravimetric, enzymatic method described by Asp et al.¹⁴ Protein content was determined using an elemental analyzer (FlashEA 1112, Thermo Fisher Scientific Inc., Waltham, MA). The nutritional compositions of the test breads are presented in Table 1.

Meal Study. *Subjects.* Twenty healthy nonsmoking volunteers (10 men and 10 women) aged 21–37 years (mean ± SEM = 26.7 ± 0.9 years) with normal body mass indices (mean ± SEM = 22.2 ± 0.39 kg/m²) and without drug therapy participated in the study. All subjects had normal fasting plasma glucose concentrations (mean ± SEM = 5.2 ± 0.03 mM). The subjects were recruited in January–September 2010, and the study was performed from April to October 2010. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time they desired. Approval of the study was obtained by the Ethics Committee in Lund, Sweden (Reference 556/2008).

Study Design. The test breads were provided as breakfasts on seven different occasions in random order, with approximately 1 week between each test. The day before the experiment, the bread was taken from the freezer and thawed at ambient temperature, still wrapped in aluminum

foil and in the plastic bag. The subjects were instructed to eat a standardized meal in the evening (9:00–10:00 p.m.) prior to each test, consisting of a few slices of white wheat bread, and to avoid eating and drinking anything but small amounts of water until the start of the test on the following morning. In addition, the subjects were also told to avoid alcohol and excessive physical exercise the day before each test. The subjects reported to the laboratory at 7:45 a.m. on the test day. A peripheral venous catheter (BD Venflon, Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein to be used for plasma sampling, and fasting blood samples were taken prior to the test bread. All breads contributed 50 g of available starch and were served with 250 mL of tap water, and the test subjects were instructed to finish the test breads within 14 min. During the rest of the study day, the subjects were not allowed any food or drink and were kept as still as possible.

Physiological Parameters. Capillary blood samples were taken for analysis of plasma glucose (p-glucose), and venous blood samples were drawn for the analysis of serum insulin (s-insulin) before the test bread (0 min) and at 15, 30, 45, 60, 90, 120, 150, and 180 min after commencing the breakfast. In addition, the subjects were asked to fill in their subjective *feeling of fullness, hunger, and desire to eat*, respectively, using a 100 mm Visual Analogue Scale (VAS) at each time point that glucose and insulin samples were drawn. VAS scores have been shown to be reliable for appetite research by Flint et al.¹⁵ P-glucose concentrations were determined in capillary whole blood using a p-glucose analyzer (Glucose 201+, Hemocue, Ängelholm, Sweden). Serum was left to set for 30 min and then centrifuged for 12 min (1300g, 4 °C). Serum was then immediately frozen at −20 °C until analysis. The s-insulin measurement was performed on an integrated immunoassay analyzer (CODA Open Microplate System; Bio-Rad Laboratories, Hercules, CA) by using an enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden).

Calculations and Statistical Methods. Data are expressed as the mean ± SEM. One subject was excluded from the analysis of the Kaskelott rye bread breakfast due to having a cold on that particular test day. The data for Kaskelott is therefore analyzed with $n = 19$. Four subjects had missing values in the recordings of subjective satiety after the commercial rye bread, causing skewed data. Therefore, these subjects were excluded from all statistical analyses of subjective satiety.

The total and net incremental areas under the glucose, insulin, and appetite curves (tAUC and iAUC) were calculated for each subject and test bread, using the trapezoid model. For the net incremental areas, any areas below the baseline were ignored. The glycemic index (GI) and insulinemic index (II) were calculated using the iAUC (0–120 min) for p-glucose and s-insulin, respectively, using WWB as a reference.¹⁶ Glucose and insulin incremental peaks (iPeak) were calculated as maximum postprandial increase from baseline (fasting). The glycemic profile (GP), defined as the duration of the glucose curve divided by the glucose iPeak, was calculated.¹¹ GP² was calculated in a similar way as GP, but the duration was divided by the squared glucose iPeak, to increase the influence of the highest measured postprandial glucose concentration.

Time × treatment interactions for plasma glucose, serum insulin, and subjective satiety were analyzed using a mixed model (PROC MIXED in SAS release 8, SAS Institute Inc., Cary, NC) with repeated measures and an autoregressive covariance structure. Subjects were modeled as a random variable, and the corresponding baseline (fasting values) was modeled as covariate. The plasma glucose, serum insulin, and subjective satiety data were analyzed using a mixed model analysis of covariance (ANCOVA) with subject as a random variable and corresponding baseline (fasting values) as a covariate. (MINITAB, release 16 (Minitab Inc., State College, PA). Differences between groups were identified using Tukey's multiple-comparison tests. In the cases of unevenly distributed residuals (tested with Anderson–Darling test), Box Cox transformation was performed on the data prior to the analysis.

Correlation analysis was conducted to evaluate the relationship among dependent measures with the use of Spearman's partial

Table 1. Nutritional Composition of the Breads^a

test bread	portion size	g/portion			
		water content	protein	insoluble fiber	soluble fiber
WWB	125.9	61.7	6.7	2.4	0.4
Amilo	154.9	72.8	9.9	9.0	3.6
Evolo	152.6	70.2	9.0	9.3	4.0
Kaskelott	154.1	71.4	8.9	10.0	3.8
Picasso	153.9	72.7	9.0	9.7	3.7
Vicello	148.9	68.6	7.5	10.1	3.0
commercial	157.6	74.2	7.6	10.6	3.3

^a $n = 2$ (available starch and proteins), $n = 3$ (fiber content). All test breads contributed 50 g of available starch.

correlation coefficients controlling for subjects and corresponding baseline values (two-tailed test, SPSS software, version 19; SPSS Inc., Chicago, IL). $p < 0.05$ was considered to be statistically significant.

RESULTS

Glucose Responses. Rye bread made from Vicello and Picasso displayed significantly lower GI values (79 and 80, respectively) than WWB (Table 2). The glucose iPeak was

Table 2. Glucose Responses for Test and Reference Breads^a

test bread	GP (min/mM)	GP ² (min/mM ²)	glucose iPeak (ΔmM)	GI (%)
WWB	41.5 ± 2.8 a	12.5 ± 1.5 c	3.8 ± 0.2 a	100 ± 0.0 a
Amilo	54.2 ± 7.4 a	26.9 ± 10.4 abc	3.1 ± 0.2 b	90 ± 8.4 ab
Evolò	52.8 ± 4.6 a	19.3 ± 2.6 abc	3.2 ± 0.2 b	92 ± 8.1 ab
Kaskelott	47.9 ± 4.1 a	16.6 ± 2.3 abc	3.2 ± 0.2 ab	88.4 ± 8.5 ab
Picasso	52.3 ± 4.7 a	26.7 ± 7.9 ab	2.9 ± 0.2 b	80 ± 8.4 b
Vicello	59.5 ± 9.5 a	31.9 ± 13.4 a	2.9 ± 0.2 b	79 ± 8.0 b
commercial	48.2 ± 6.7 a	20.0 ± 5.2 bc	3.4 ± 0.2 ab	95 ± 8.3 ab

^a Values are the mean ± SEM. $n = 20$ ($n = 19$ for Kaskelott rye). Products not sharing the same letters were significantly different, $p < 0.05$. ANCOVA, followed by Tukey's test.

significantly lower for Vicello, Picasso, Amilo, and Evolo compared to WWB. In addition, Vicello, Picasso, Amilo, and Kaskelott displayed significantly lower early glucose response (tAUC 0–60 min) than WWB (Figures 1 and 2). The GP values were not significantly different between any of the breads, but the GP² was significantly higher for the Vicello and Picasso breads compared to WWB. Furthermore, the GP² of Vicello rye was significantly higher than that of the commercial rye blend. No time × treatment interaction was found (0–180 min, $p = 0.23$).

Insulin Responses. Rye breads made from Vicello, Picasso, Amilo, and Kaskelott had significantly lower IIs than WWB (Table 3). The early insulin response, expressed as tAUC 0–60 min and insulin iPeak, was significantly lower for rye breads made from Vicello, Picasso, Amilo, and Evolo compared to WWB (Figures 1 and 2). No time × treatment interaction was found (0–180 min, $p = 0.24$).

Subjective Satiety. The subjective *feeling of hunger* and *desire to eat* were significantly lower after the commercial rye blend compared to after the WWB. Evolo rye bread induced a significantly lower *feeling of hunger* compared to WWB in the early postprandial phase (0–60 min). Furthermore, Evolo rye induced a higher *feeling of fullness* compared to WWB during 60–120 min after breakfast (tAUC) and also a significantly lower *feeling of hunger* when the entire study period (tAUC 0–180 min) was analyzed. In the early

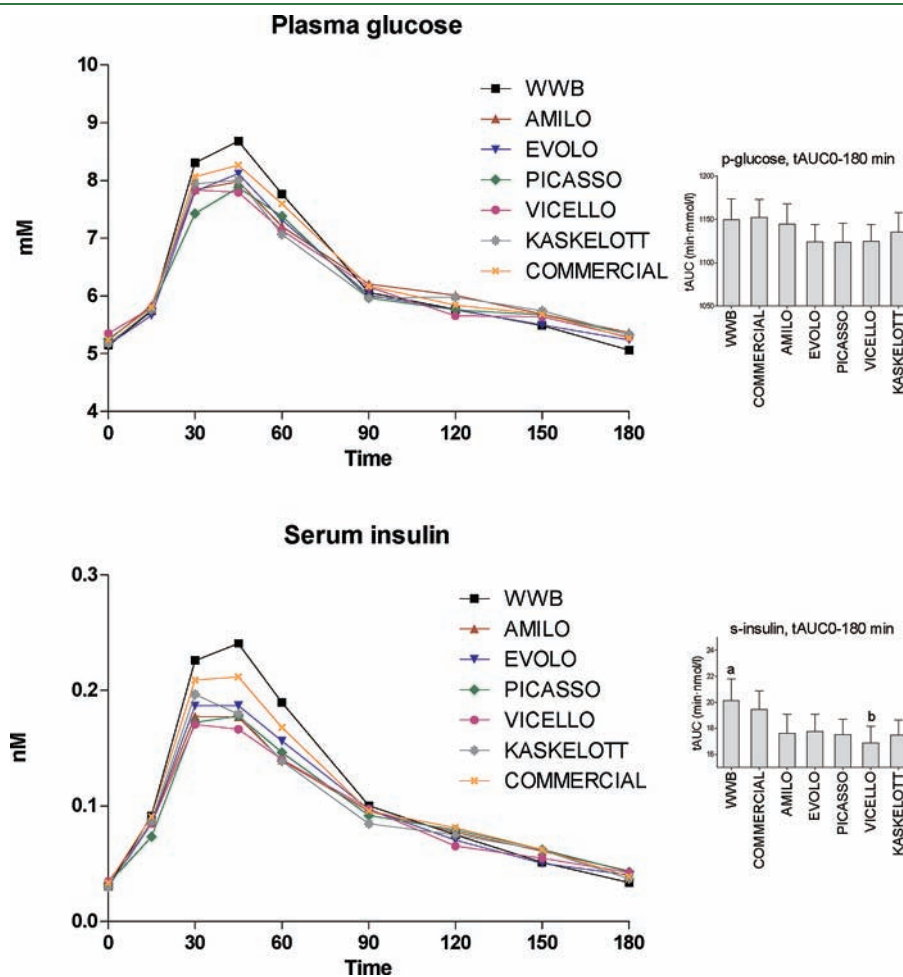


Figure 1. Glucose and insulin responses for the rye test breads and the reference bread. Values are the mean in the graphs and the mean ± SEM in the bars, $n = 20$ ($n = 19$ for Kaskelott). Products not sharing the same letters were significantly different. Products not displaying letters were not different from any other test bread, $p < 0.05$ (ANCOVA, followed by Tukey's test).

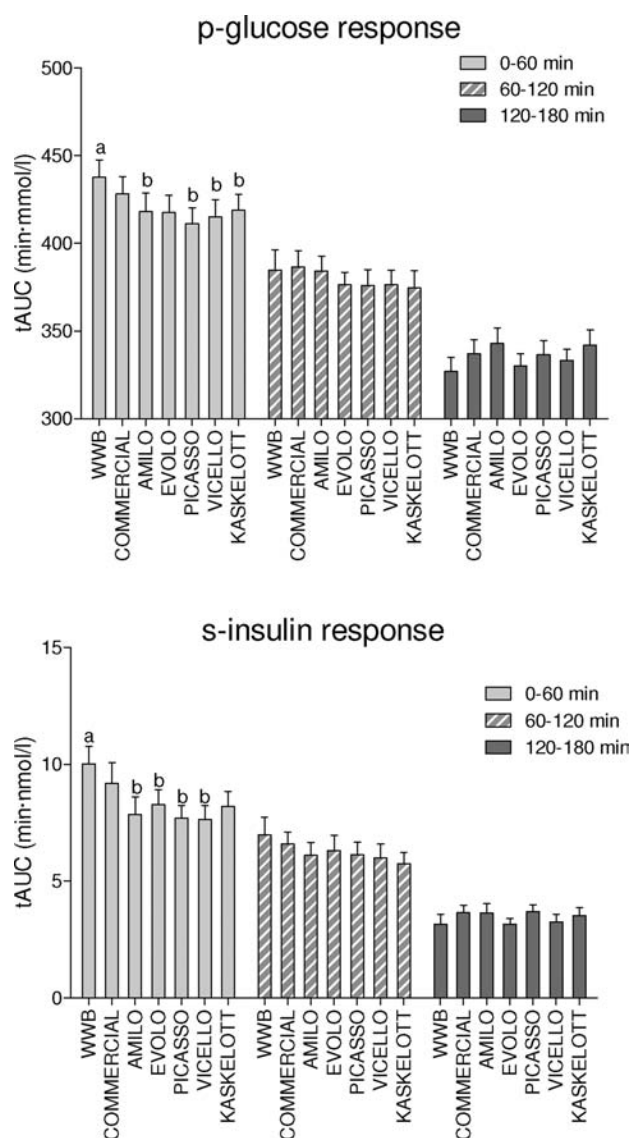


Figure 2. Glucose and insulin responses for rye test breads and reference bread. Values are the mean \pm SEM, $n = 20$ ($n = 19$ for Kaskelott). Products not sharing the same letters were significantly different. Products not displaying letters were not different from any other test bread, $p < 0.05$ (ANCOVA, followed by Tukey's test).

Table 3. Insulin Responses after the Test and Reference Breads^a

test bread	insulin iPeak (Δ nM)	II (%)
WWB	0.237 \pm 0.021 a	100 \pm 0 a
Amilo	0.180 \pm 0.017 b	80.3 \pm 7.2 b
Evolò	0.179 \pm 0.015 b	84.8 \pm 7.4 ab
Kaskelott	0.186 \pm 0.017 ab	81.5 \pm 7.1 b
Picasso	0.175 \pm 0.017 b	81.5 \pm 9.6 b
Vicello	0.165 \pm 0.015 b	73.7 \pm 7.9 b
commercial	0.207 \pm 0.022 ab	92.5 \pm 9.8 ab

^a Values are the mean \pm SEM. $n = 20$ ($n = 19$ for Kaskelott rye). Products not sharing the same letters were significantly different, $p < 0.05$. ANCOVA, followed by Tukey's test.

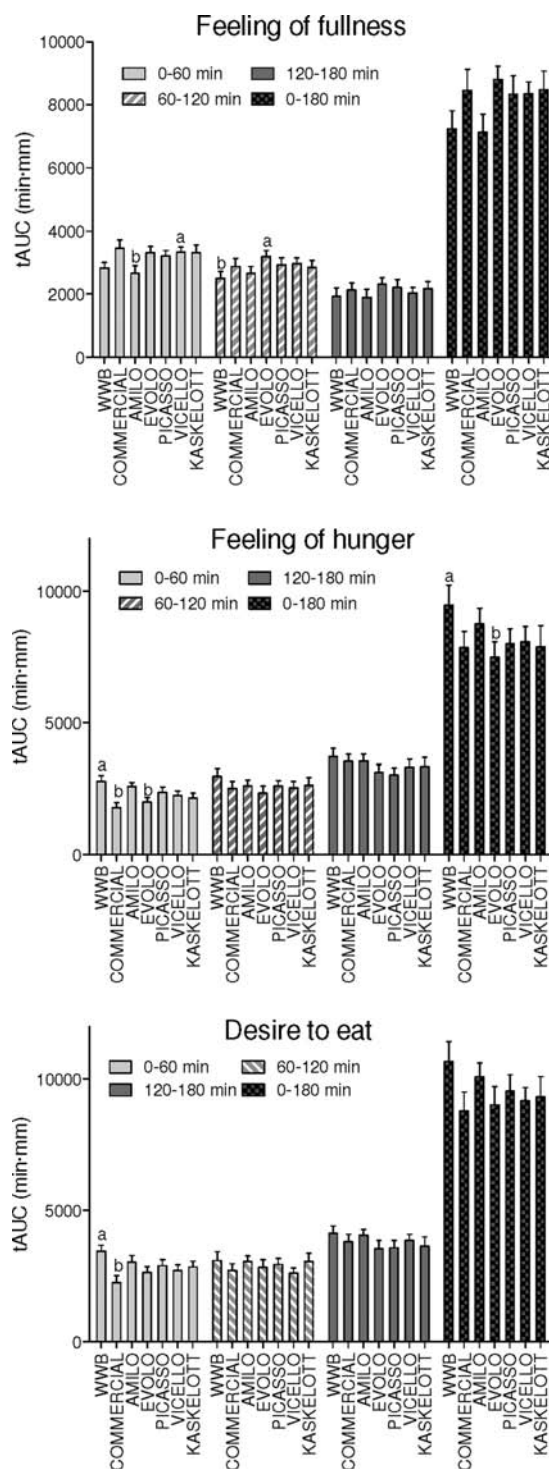


Figure 3. Subjective satiety responses after the rye test breads and the reference bread. Values are the mean \pm SEM, $n = 16$ ($n = 15$ for Kaskelott). Products not sharing the same letters were significantly different. Products not displaying letters were not different from any other test bread, $p < 0.05$ (ANCOVA, followed by Tukey's test).

postprandial phase (tAUC 0–60 min), the subjective *feeling of fullness* was significantly higher following Vicello rye bread compared to Amilo rye bread (Figure 3). No time \times treatment interaction was found for *feeling of fullness*, *hunger*, or *desire to eat* (0–180 min, $p = 0.48$, 0.91 , and 0.75 , respectively).

Table 4. Correlations between Postprandial Glucose, Insulin, and Subjective Satiety^a

		II	insulin iPeak	fullness tAUC 0–60 min	fullness tAUC 120–180 min	hunger 180 min	hunger tAUC 120–180 min	desire to eat 180 min	desire to eat tAUC 120–180 min
glucose iPeak	<i>r</i> =	0.51	0.52	–0.13	–0.21	0.35	0.28	0.34	0.33
	<i>p</i> =	<0.001	<0.001	0.201	0.031	<0.001	0.003	<0.001	0.001
GP	<i>r</i> =	–0.32	–0.43	0.13	0.14	–0.25	–0.14	–0.31	–0.22
	<i>p</i> =	<0.001	<0.001	0.194	0.145	0.010	0.163	0.001	0.021
GP ²	<i>r</i> =	–0.45	–0.54	0.16	0.22	–0.24	–0.16	–0.30	–0.24
	<i>p</i> =	<0.001	<0.001	0.108	0.024	0.015	0.110	0.002	0.012
GI	<i>r</i> =	0.43	0.33	0.04	–0.07	0.23	0.12	0.24	0.17
	<i>p</i> =	<0.001	<0.001	0.715	0.494	0.018	0.237	0.014	0.081
II	<i>r</i> =		0.82	–0.10	–0.02	0.11	0.08	0.22	0.17
	<i>p</i> =		<0.001	0.302	0.808	0.260	0.390	0.022	0.077
insulin iPeak	<i>r</i> =	0.82		–0.20	0.01	0.15	0.03	0.27	0.18
	<i>p</i> =	<0.001		0.039	0.888	0.115	0.725	0.004	0.057

^aSpearman's partial correlation coefficients controlling for subjects and corresponding baseline values (two-tailed test). Significant correlations are shown in bold text. *n* = 20 for glucose and insulin and 16 for subjective satiety. For Kaskelott *n* = *n* – 1.

Correlations. Correlations between postprandial glucose, insulin, and subjective satiety are presented in Table 4. The GI, GP, and GP² were all significantly related to the insulin iPeak and II. However, the GP and GP², respectively, showed stronger relationships to the insulin iPeak than did the GI.

A low glucose iPeak and a high GP² were related to improved feeling of fullness, hunger, and desire to eat in the late postprandial phase (tAUC 120–180 min and/or at 180 min). Also, the late postprandial desire to eat (180 min) was positively correlated to the insulin iPeak and the II. Furthermore, a high insulin iPeak was related to a lower subjective feeling of hunger in the early postprandial phase (tAUC 0–60 min).

A high content of insoluble fibers in the rye breads was related to an improved subjective satiety in the early postprandial phase (tAUC 0–60 min) (fullness, hunger, and desire to eat, *r* = 0.24, –0.28, and –0.43, respectively, *p* < 0.05). A higher insoluble fiber content in the rye breads also lowered the desire to eat in the 60–120 min postprandial phase (*r* = –0.21, *p* < 0.05).

DISCUSSION

In the present study, whole grain breads made from four of five tested rye varieties grown in Sweden induced a significantly lower insulin response (II) compared with a white wheat reference bread (WWB). The exception was Evolo rye, which did not induce lower II than WWB. Interestingly, the bread based on commercial rye blend did not induce a significantly lower II or insulin iPeak compared with WWB. The commercial blend consisted mainly of the rye varieties Evolo, Marcello, Rovrik, Vicello, and Picasso, of which Marcello and Rovrik were not individually tested here. The exact proportions are, however, unknown. The present findings suggest that there are differences in insulin-saving properties between different Swedish rye varieties. The results are in line with previous work (accompanying paper) in which we concluded that pooled samples of three of five rye varieties grown in Hungary, Poland, France, and the

United Kingdom lacked the insulin-saving abilities previously found with breads made from Swedish and Finnish rye blends.^{8–12} The higher GI and II values of the Amilo rye bread measured in the present study compared to data with this rye variety in our previous investigation, (GI = 79, II = 72, accompanying paper) could be explained by the fact that the rye products in the present study contained 75% rye as compared to 100% rye in the previous study. Also, the Amilo rye used in these two studies, respectively, were grown in different locations during different years. This may have affected the levels of amylases and xylanases, which in turn may affect the level of starch and fiber degradation¹⁷ and the proportion of soluble arabinoxylans in the bread products. Furthermore, the groups of test subjects were different between the two studies.

Also, the glycemic responses differed between the test breads. Vicello and Picasso rye had significantly lower GI values compared to WWB, and Vicello, Picasso, Amilo, and Evolo rye induced significantly lowered glucose iPeaks compared to WWB. The GP was not significantly different between the breads here tested. As discussed in the accompanying paper, the GP value of a glycemic curve having a high incremental glucose iPeak, but remaining above fasting for a long time, will be similar to that of a product inducing a low glucose iPeak with short duration. In an attempt to discriminate also between these types of curves, the GP values were divided again by the glucose iPeak, thus giving the highest measured postprandial glucose concentration more weight in the equation. This duration/iPeak² quota is named GP². The Vicello and Picasso ryes were described by a significantly higher GP² than WWB, indicating a more beneficial and well-regulated course of glycemia. A high GP² of the test breads was also well correlated with lowered insulin iPeak and II, and these correlations had higher *r* values than those found between the insulin responses and GP and GI. Taken together, these findings suggest that the GP² may be a better descriptor of both the course of postprandial glycemia and the expected insulin response compared to the GI and GP, respectively.

The possible mechanism behind the facilitated glucose regulation and lowered insulin demand after some rye products could be the comparatively high content of soluble arabinoxylans in rye, particularly those present in the endosperm part of the rye kernel. These soluble fibers may contribute to viscosity in the small intestine and could thereby lower gastric emptying and the rate of carbohydrate uptake.^{18,19} In the present study, no correlation was found between the amount of soluble fibers in the rye test breads and the insulin or glycemic responses. It should be noted, however, that the soluble dietary fiber content is not necessarily directly related to viscosity.

The subjective feeling of satiety (*hunger* and *desire to eat*) in the early postprandial phase was increased after the commercial rye blend bread compared to after the WWB. The commercial rye blend was the rye bread with the highest amount of insoluble fibers and water, both contributing to a bulking effect and thereby positively affecting early satiety.²⁰ This finding is also in line with our previous results with rye products.¹² Amilo rye induced a significantly lower subjective *feeling of fullness* in the early postprandial phase as compared to the Vicello rye bread, and Amilo was also the rye bread with the lowest content of dietary fibers. The lack of significantly higher *feeling of fullness* and reduced *hunger* and *desire to eat* after some of the rye products could be explained by lower contents of rye and, thereby, somewhat less dietary fibers compared to previous studies,¹² (accompanying paper). A high GP and GP² correlated with improved satiety, especially with lowered *desire to eat* in the late postprandial phase (at 180 min). We have previously noted that a beneficial blood glucose profile, indicated by a high GP, is associated with a lower subjective *desire to eat* in the late postprandial phase, which in turn was related to a lowered voluntary energy intake at subsequent meal.¹² In the present study, also the insulin response (iPeak) was positively related to the late postprandial *desire to eat* at 180 min, which is in accordance with our previous findings¹¹ (accompanying paper).

Four of five tested rye varieties grown in Sweden were shown to lower insulin responses, and Vicello and Picasso rye contributed to a better regulated glycemia, with lower glucose iPeaks and longer durations of plasma glucose above fasting level. The commercial blend in the present study did not lower insulin responses, possibly due to an unfortunate mix of rye varieties. Furthermore, rye varieties rich in insoluble fibers and characterized by high GP and GP² and lower insulin iPeaks appear to have enhanced appetite-regulating properties. This knowledge should be considered when choosing rye varieties for commercial blends to optimize health benefits in rye-based products.

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ACKNOWLEDGMENT

We thank Eivor Svensson at Lantmännen SW Seed AB for providing and milling rye kernels and Jörgen Hansson at Lilla Harrie Mills AB for providing rye kernels. For her analytical help we thank Lisbeth Persson. We also thank Patrick Nilsson (Pågen AB) for his help in the manufacturing of the rye breads.

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