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Postprandial Glycemia, Insulinemia, and Satiety Responses in Healthy Subjects after Whole Grain Rye Bread Made from Different Rye Varieties. 1

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ABSTRACT: Rye products typically induce low insulin responses and appear to facilitate glucose regulation. The objective of this study was to investigate differences in postprandial glucose, insulin, and satiety responses between breads made from five rye varieties. Breads made from whole grain rye (Amilo, Rekrut, Dankowski Zlote, Nikita, and Haute Loire Pop) or a white wheat bread (WWB) were tested in a randomized cross-over design in 14 healthy subjects (50 g available starch). Metabolic responses were also related to the composition of dietary fiber and bioactive compounds in the breads and to the rate of in vitro starch hydrolysis. The Amilo and Rekrut rye breads induced significantly lower insulin indices (II) than WWB. Low early postprandial glucose and insulin responses (tAUC 0−60 min) were related to higher amounts of caffeic, ferulic, sinapic, and vanillic acids in the rye breads, indicating that the phenolic acids in rye may influence glycemic regulation. All rye breads induced significantly higher subjective *feelings of fullness* compared to WWB. A low II was related to a higher *feeling of fullness* and a lower *desire to eat* in the late postprandial phase (180 min). The data indicate that some rye varieties may be more insulin-saving than others, possibly due to differences in dietary fiber, rate of starch hydrolysis, and bioactive components such as phenolic acids.

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

INTRODUCTION

Acute studies in healthy subjects have shown that whole grain rye products produce low insulinemic responses.[1−5] Furthermore, rye products appear to facilitate glycemic regulation, with lower maximum blood glucose increment and avoidance of hypoglycemia in the later postprandial phase.[2,3] A diet rich in rye products might therefore contribute to a lowered risk of insulin resistance[6] and oxidative stress.[7] It can be hypothesized that the specific postprandial glycemic pattern observed with rye products may be responsible for the lowered insulin demand seen in this product group. Consequently, the metabolic relevance of the course of glycemia has been emphasized, and it has been suggested that calculation of the glycemic profile (GP) may be useful for this purpose. Hence, the GP, defined as the duration for the incremental postprandial glycemic response divided by the incremental glucose peak (min/mM), was a better predictor than the glycemic index (GI) of acute postprandial insulin demand,[2,3] subjective rating of satiety in the late postprandial phase,[2] and second meal voluntary food intake,[3] in a series of cereal products dominated by rye.

The cause for the lower insulin demand and higher GP observed with rye products is not known, but may be related to soluble dietary fiber, for example, arabinoxylans, resulting in high viscosity, thereby lowering gastric emptying and the rate of carbohydrate uptake from the small intestine.[8,9] As judged from the absence of an insulin-lowering effect following enrichment of white wheat bread (WWB) with rye bran (35%), the improved insulin economy appears to be associated with the endosperm fraction of rye.[2] Cyran et al.[10] recently demonstrated that bread made from rye endosperm has higher viscosity than whole grain rye bread, possibly due to the higher arabinose/xylose ratio associated with the dietary fiber fraction in the endosperm. Another plausible explanation for the improved insulin economy seen with rye products is the presence of bioactive components. Rye grain is a rich source of such components including antioxidants, for example, tocotrienols, tocopherols, alkylresorcinols, and phenolic acids. Furthermore, rye contains plant sterols, known for their cholesterol-lowering effect and, additionally, folate, choline, and betaine, which function as methyl donors in the remethylation of homocysteine.[11] Little is known about the extent to which these components may modulate acute postprandial insulin and glucose

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responses, but phenolic acids such as caffeic and ferulic acids have been reported to decrease blood glucose concentration[12,13] and to enhance insulin secretion in diabetic rats.[12] These bioactive components are usually concentrated in the aleurone layer and other parts of the bran. However, due to the difficulty in separating the outer layers from endosperm, rye bread made with "endosperm flour" may contain as much as 80% of the total phenolic compounds and 25% of the total tocotrienols present in whole grain.[14]

Rye products are also of interest in relation to appetite regulation. Products made from rye have thus been demonstrated to improve satiety responses both acutely and at a subsequent meal.[2,3] The cause is probably multifactorial and relates, for example, to these products' high content of indigestible carbohydrates, their ability to induce colonic fermentation, and their low insulin responses. Thus, the lowered insulinemic response and benefits on well-regulated glycemia coincide with attenuated late levels of the hunger-promoting hormone ghrelin.[2,3]

The purpose of the present study in healthy subjects was to evaluate potential differences in glycemic response and acute insulin demand depending on the rye variety selected. Five rye varieties varying in contents of dietary fiber and in composition of potentially bioactive components were selected. The rye varieties were baked into flour-based whole grain rye bread, and a white wheat bread was used as a reference product. Attempts were made to elucidate whether postprandial plasma glucose and serum insulin, respectively, could be related to specific bioactive components and to what extent the rate of starch digestion was involved, as estimated in vitro. Additionally, subjective appetite ratings were performed following the bread meals.

MATERIALS AND METHODS

Test Breads. Five whole grain rye test breads and a white wheat (endosperm) reference bread (WWB) were included. The whole grain rye breads were made from five different rye varieties: Amilo, Nikita, Dankowskie-Zlote (D. Zlote), Haute Loire Pop (H. Loire), and Rekrut. The rye flours were obtained within the HEALTHGRAIN project. The rye varieties were blends of harvests from four different sites in Europe (Martonvásár, Hungary; Woolpit, U.K.; Choryn, Poland; and Clermont Ferrand, France (see Shewry et al., ref 15, for details).The commercial white wheat flour was obtained from Kungsörnen AB (Järna, Sweden). Dry yeast was acquired from Jästbolaget AB (Sollentuna, Sweden).

The WWB was made from 540 g of white wheat flour, 360 g of water, 4.8 g of dry yeast, 4.8 g of NaCl, and 12 g of monoglycerides. WWB was baked in a bread-baking machine (BM 3983, Severin, Sundern, Germany) using a program for white bread. The rye breads were made from 6000 g of whole grain rye flour, 4500 g of water, 101 g of dry yeast, and 52 g of NaCl and baked at Pågen bakery, Malmö, Sweden. The doughs were mixed in a mixing bowl for 8 min and proofed at room temperature for 30 min. They were then divided into pieces of 750 g each, placed in baking tins, and subjected to a second proofing (38 °C, 85% humidity) for 30−52 min (until reaching a standardized volume, similar among the rye breads). Baking was initiated at 250 °C with 3 s of steam, and then the temperature was immediately lowered to 200 °C, and the bread was kept in the oven until the center of the bread was just above 96 °C (35−38 min).

The WWB was left to cool for 1 h and the rye breads were left for 22 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. The day before the experiment, the breads were taken from the freezer and were thawed overnight at ambient temperature, still wrapped in aluminum foil and in the plastic bag.

Chemical Analysis of the Test Breads. Prior to analysis of available starch, free sugars, dietary fiber, and crude protein, the samples were air-dried and milled to pass a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden). Measurements of resistant starch (RS) and in vitro rate of starch hydrolysis (HI) were performed on the fresh products. Analyses of alkylresorcinols (AR), folate, tocols, sterols, betaine, choline, and phenolic compounds were made on freeze-dried and milled bread.

The available starch content was determined according to the method of Holm et al.[16] Available starch was used to calculate portion sizes providing 50 g of available starch. RS was analyzed according to the method of Åkerberg et al.[17] Insoluble and soluble dietary fibers were determined with a gravimetric, enzymatic method described by Asp et al.[18] Protein content was determined using an elemental analyzer (FlashEA 1112, Thermo Fisher Scientific Inc., Waltham, MA). The HI was determined using an in vitro procedure based on chewing[19] with WWB as a reference. Portion sizes and contents of dietary fiber, RS, and protein are displayed in Table 1. ARs were analyzed according to the method of Andersson et al.[20] The coefficient of variation of the AR analysis has been shown to be <10%.[21] An in-house reference sample (whole grain rye flour) was used to ensure the quality of the analysis. Folate and sterols was analyzed according to the method of Piironen et al.[22,23] Tocols were analyzed according to the method of Lampi et al.[24] The coefficient of variation of the folate analysis was <10%, whereas the coefficients of variation of the tocol and sterol analyses were <5%. Free phenolic compounds were analyzed according to the method of Li et al.[25] Betaine and choline were analyzed according to the method of Howarth et al.[26]

Meal Study. *Test Subjects.* Fourteen healthy nonsmoking volunteers (7 men and 7 women) aged 21−28 years (mean ± SEM = 23.6 ± 0.5 years) with normal body mass indices (mean ± SEM = 22.0 ± 0.5 kg/m$^{2)}$ and without drug therapy participated in the study. All subjects had normal fasting plasma glucose concentrations (mean ± SEM = 5.3 ± 0.04 mM). The subjects were recruited in March 2008, and the study was performed between March and June 2008. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time. Approval of the study was obtained from the Regional Ethical Review Board in Lund, Sweden (Reference 477/2007).

Study Design. The test breads were provided as breakfasts on six different occasions in random order, separated by approximately 1 week. The subjects were instructed to eat a standardized evening meal (9:00−10:00 p.m.) prior to the test, consisting of a few slices of white wheat bread. No eating or drinking except for small amounts of water was then allowed until the start of the test. The subjects were also told to avoid alcohol and excessive physical exercise the day before each test, and otherwise as far as possible to maintain their regular lifestyle throughout the entire study. The subjects arrived at 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. Fasting blood samples were taken prior to the breakfast meal at time 0. Thereafter, the test breads were served with 250 mL of tap water. The subjects finished the bread meals within 14 min. During the rest of the experimental 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 the analysis of plasma glucose (p-glucose), and venous blood samples were drawn for the analysis of serum insulin (s-insulin) before the meal (0 min) and at 15, 30, 45, 60, 90, 120, and 180 min after commencing breakfast. In addition, the subjects were asked to fill in their subjective *feelings of fullness*, and *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. P-glucose concentrations were determined in capillary whole blood using a glucose analyzer (Glucose 201+, Hemocue, Ängelholm, Sweden). Serum was kept cool for 30 min (4 °C) until centrifuged for 11 min (1800g, 4 °C). Serum was then immediately frozen

Table 1. Portion Size and Contents of Available and Resistant Starch, Dietary Fiber, Protein, Betaine, Choline, and Folate in the White Wheat Bread (WWB) and Rye Test Breads, Expressed in Dry Weights and in Amounts per Portion[a]

	WWB	D. Zlote	H. Loire	Nikita	Rekrut	Amilo
			per 100 g DW (per portion)			
portion size (g)	(122.7)	(165.3)	(175.8)	(168.8)	(171.0)	(171.9)
available starch (g)	76.0 (50.0)	59.3 (50.0)	54.7(50.0)	58.4 (50.0)	56.7 (50.0)	57.6 (50.0)
insoluble fiber (g)	2.4 (1.6	11.2 (9.4)	12.0 (11.0)	12.0 (10.3)	12.5 (11.0)	13.1 (11.4)
soluble fiber (g)	1.6 (1.1)	3.5 (3.0)	4.5 (4.1)	4.8 (4.1)	5.9 (5.2)	4.8 (4.2)
resistant starch (g)	1.11 (0.73)	0.63 (0.53)	0.48 (0.44)	0.66 (0.560	1.46 (1.28)	0.54 (0.46)
protein (g)	11.5 (7.6)	13.2 (11.2)	15.2 (13.9)	12.7 (10.9)	13.2 (11.6)	13.2 (11.5)
betaine (mg)	61.9 ± 0.5 (40.8 ± 0.3)	272.8 ± 5.0 (230.1 ± 4.2)	249.6 ± 4.1 (228.3 ± 3.8)	270.2 ± 7.6 (231.3 ± 6.5)	291.5 ± 2.4 (257.2 ± 2.1)	251.8 ± 5.9 (218.4 ± 5.1)
choline (mg)	9.5 ± 0.1 (6.2 ± 0.0)	34.8 ± 0.80 (29.4 ± 0.6)	39.0 ± 1.0) (35.6 ± 0.9)	34.9 ± 0.9) (29.9 ± 0.8)	37.6 ± 0.3) (33.2 ± 0.3)	36.0 ± 1.1) (31.2 ± 1.0)
folate (μg)	44.4 (29.2)	82.1 (69.2)	88.0 (80.5)	99.1 (84.8)	85.0 (75.0)	97.2 (84.4)

[a] $n = 2$ (available starch, proteins, folate, betaine, and choline), $n = 3$ (fiber content), $n = 6$ (resistant starch). Values are the mean ± SEM for betaine and choline.

Table 2. Tocols and Sterols in the White Wheat Bread (WWB) and Rye Test Breads, Expressed in Dry Weights and in Amounts per Portion[a]

	WWB	D. Zlote	H. Loire	Nikita	Rekrut	Amilo
			per 100 g DW (per portion)			
α-tocopherol (μg)	121.3 (79.8)	779.4 (657.4)	734.3 (671.5)	691.5 (591.6)	681.5 (601.2)	722.6 (626.8)
β-tocopherol (μg)	0.0 (0.0)	280.6 (236.6)	283.2 (259.0)	258.0 (220.8)	241.1 (212.7)	278.7 (241.8)
α-tocotrienol (μg)	0.0 (0.0)	966.5 (815.1)	797.3 (729.1)	815.3 (697.6)	807.3 (712.2)	774.3 (671.6)
β-tocotrienol (μg)	212.3 (139.7)	1122.4 (946.6)	849.7 (777.1)	918.5 (785.9)	954.1 (841.7)	825.9 (716.4)
brassicasterol (mg)	0.2 (0.1)	0.7 (0.6)	0.7 (0.7)	0.7 (0.6)	0.3 (0.3)	0.7 (0.6)
campesterol (mg)	9.5 (6.3)	18.8 (15.9)	20.7 (18.9)	17.9 (15.3)	18.2 (16.1)	17.3 (15.0)
campestanol (mg)	2.1 (1.4)	9.5 (8.0)	6.9 (6.3)	7.8 (6.7)	8.3 (7.3)	7.9 (6.9)
stigmasterol (mg)	0.4 (0.3)	3.4 (3.0)	3.3 (3.0)	3.5 (3.0)	3.5 (3.1)	3.5 (3.0)
sitosterol (mg)	28.6 (18.8)	49.4 (41.6)	57.6 (52.7)	47.6 (40.7)	48.5 (42.8)	48.0 (41.6)
sitostanol (mg)	2.5 (1.7)	11.0 (9.3)	10.1 (9.2)	9.4 (8.0)	9.9 (8.7)	10.3 (9.0)
other sterols/stanols (mg)	2.0 (1.3)	15.5 (13.1)	16.8 (15.4)	13.6 (11.7)	14.3 (12.6)	15.2 (13.2)

[a] $n = 2$.

at −20 °C until analysis. The serum 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. The total and net incremental areas under the curves (tAUC and iAUC) were calculated for each subject and test meal, using the trapezoid model. The glycemic index (GI) and insulinemic index (II) were calculated using the iAUC (0−120 min) for p-glucose and s-insulin, respectively, with WWB as a reference.[27] Glucose and insulin 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.[2] Hydrolysis indices (HI) were calculated from the 180 min AUC for in vitro starch hydrolysis, in a similar way to the calculation of GI and II values, using WWB as a reference for chewing.[19] Time × treatment interactions for p-glucose, s-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 corresponding baseline (fasting values) values were modeled as covariate. The 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. For HI, a mixed model analysis of variance (ANOVA) was used with subject as a random variable. Differences between groups were identified using Tukey's multiple-comparison test (MINITAB, release 16, Minitab Inc., State College, PA). In the cases of unevenly distributed residuals (tested with the Anderson−Darling test), Box Cox transformations were performed on the data prior to the ANCOVA and ANOVA. Correlation analysis was conducted to evaluate the relationship among dependent measures with the use of Spearman's partial 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. Correlations between bioactive components and fiber content were performed using Pearson's correlation.

RESULTS

Content of Dietary Fiber and Bioactive Components in the Test Breads. The contents of dietary fibers, betaine, choline, folate, tocols, sterols, AR, and free phenolic acids expressed on a dry weight basis and in amounts per portion are presented in Tables 1− 3.

Table 3. Free Phenolic Acids and Alkylresorcinols (AR) in the White Wheat Bread (WWB) and Rye Test Breads, Expressed in Dry Weights and in Amounts per Portion[a]

	WWB	D. Zlote	H. Loire	Nikita	Rekrut	Amilo
			per 100 g DW (per portion)			
4-hydroxybenzoic acid (μg)	276.3 (181.8)	309.0 (260.6)	390.1 (356.7)	314.2 (268.8)	340.7 (300.5)	313.8 (272.2)
vanillic acid (μg)	250.0 (164.5)	835.8 (704.9)	1098.6 (1004.6)	884.8 (757.0)	960.7 (847.5)	1004.1 (871.0)
syringic acid (μg)	0.0 (0.0)	105.3 (88.9)	117.9 (107.8)	105.9 (90.6)	119.6 (105.5)	119.4 (103.5)
syringic aldehyde (μg)	329.7 (217.0)	414.6 (349.6)	426.6 (390.2)	408.0 (349.1)	428.6 (378.1)	411.9 (357.3)
caffeic acid (μg)	0.0 (0.0)	239 (201.6)	214.4 (196.0)	244.1 (208.9)	262.9 (231.9)	338.3 (293.4)
2,4-dihydroxybenzoic acid (μg)	74.1 (48.8)	67 (56.5)	106.8 (97.7)	62.7 (53.7)	88.3 (77.9)	62.3 (54.1)
sinapic acid (μg)	0.0 (0.0)	858.7 (724.2)	953.5 (872.0)	867.0 (741.9)	898.0 (792.2)	1144.6 (992.9)
ferulic acid (μg)	372.8 (245.4)	2641.4 (2227.8)	3227.7 (2951.7)	2810.5 (2404.7)	2920.5 (2576.4)	2992.2 (2595.5)
AR C 17:0 (mg)	0.00 (0.00)	17.79 (15.01)	16.62 (15.20)	17.95 (15.36)	16.34 (14.41)	18.39 (15.95)
AR C 19:0 (mg)	1.62 (1.06)	25.67 (21.65)	25.45 (23.27)	27.41 (23.46)	24.76 (21.84)	28.60 (24.81)
AR C 21:0 (mg)	1.00 (0.66)	15.90 (3.41)	17.37 (15.88)	19.41 (16.61)	16.48 (14.54)	18.47 (16.02)
AR C 23:0 (mg)	0.27 (0.18)	6.93 (5.84)	8.08 (7.39)	8.73 (7.47)	7.41 (6.54)	8.74 (7.58)
AR C 25:0 (mg)	0.12 (0.08)	6.71 (5.66)	7.34 (6.71)	7.36 (6.30)	6.98 (6.16)	7.60 (6.59)

[a] $n = 2$.

Table 4. Hydrolysis Index (HI) and P-Glucose and Insulin Responses after the Test Breads[a]

	HI (%)	GI (%)	glucose iPeak (Δ mM)	GP (min/mM)	II (%)	insulin iPeak (Δ nM)
WWB	100 ± 0 bc	100 ± 0.0 a	3.7 ± 0.3 a	47.1 ± 3.4 a	100 ± 0.00 a	0.287 ± 0.028 a
D. Zlote	117 ± 2.8 a	96 ± 9.0 a	3.7 ± 0.3 a	40.4 ± 2.7 a	89 ± 6.69 abc	0.267 ± 0.030 a
H. Loire	113 ± 4.5 a	96 ± 9.7 a	3.5 ± 0.2 a	45.7 ± 3.5 a	103 ± 12.07 ab	0.286 ± 0.045 a
Nikita	115 ± 3.5 a	91 ± 11.1 a	3.5 ± 0.3 a	42.0 ± 4.5 a	93 ± 9.14 abc	0.262 ± 0.027 a
Rekrut	109 ± 4.3 ab	84 ± 6.9 a	3.5 ± 0.2 a	41.1 ± 3.4 a	78 ± 7.54 bc	0.278 ± 0.058 ab
Amilo	92 ± 1.6 c	79 ± 5.2 a	3.1 ± 0.3 a	49.5 ± 6.2 a	72 ± 6.27 c	0.213 ± 0.031 b

[a] Values are the mean ± SEM. $n = 6$ for HI; $n = 14$ for p-glucose and s-insulin responses. Breads not sharing the same letters were significantly different, $p < 0.05$ (ANOVA for HI, ANCOVA for p-glucose and s-insulin, followed by Tukey's test).

Among the rye breads, Rekrut contained the highest amount of soluble dietary fiber, whereas D. Zlote contained the lowest (5.21 and 2.95 g/portion, respectively). Amilo contained the highest amount of insoluble dietary fiber, and D. Zlote contained the least (11.38 and 9.42 g/portion). Rekrut contained the highest amount of betaine per portion, whereas Amilo contained the lowest (257.2 and 218.4 mg/portion, respectively). H. Loire contained the highest amount of choline, and D. Zlote contained the lowest (35.62 and 29.38 mg/portion, respectively). D. Zlote also contained the lowest amount of folate, whereas Nikita had the highest (69.2 and 84.8 μg/portion). D. Zlote contained the highest amount of tocotrienols, whereas Amilo had the lowest (1761.7 and 1388.0 μg/portion, respectively). H. Loire contained the highest amount of tocopherols (671.5 and 259.0 μg/portion for α- and β-tocopherols, respectively). Nikita contained the lowest amount of α-tocopherol (591.6 μg/portion) and Rekrut the lowest amount of β-tocopherols (212.7 μg/portion). H. Loire contained the highest amounts of brassicasterol, campesterol, sitosterol, and "other" sterols (0.67, 18.90, 52.67, and 15.35 mg/portion, respectively), whereas it had the lowest amount of campestanol (6.33 mg). Nikita contained the lowest contents of sitosterols, sitostanols, and "other" sterols (40.71, 8.04, and 11.66 mg, respectively). Rekrut had the lowest amounts of brassicasterol (0.28 mg/portion), and Amilo contained the lowest amount of campestanol (15.04 mg/portion). Amilo contained the highest amount of AR per portion, whereas Rekrut contained the lowest (total AR = 70.96 and 61.56 mg/portion). The C:19 AR homologue was most the abundant in all rye breads. Amilo rye bread contained the highest amount of caffeic acid, whereas H. Loire contained the least (293.4 and 196.0 mg/portion, respectively. Amilo was also characterized by the highest amount of sinapic acid (992.9 μg/portion), whereas D. Zlote contained the lowest amount (742.2 μg/portion). H. Loire contained the highest amounts of 4-hydroxybenzoic, vanillic, syringic, and ferulic acids, whereas D. Zlote contained the lowest amounts of these acids (356.7, 1004.6, 107.8, and 2951.7 μg/portion, respectively, for H Loire and 260.6, 704.9, 88.9, and 2227.8 μg/portion, respectively, for D. Zlote). H. Loire also contained the highest amounts of syringic aldehyde and 2,4-dihydroxybenzoic acid, whereas Nikita contained the lowest amounts (390.2 and 97.7 μg/portion, respectively, for H. Loire and 349.1 and 53.7 μg/portion, respectively, for Nikita).

In Vitro Hydrolysis Index. The rye bread made from Amilo displayed significantly lower HI compared to all other rye breads (Table 4). All rye breads with the exception of those from the Amilo and Rekrut varieties induced significantly higher HI than WWB.

Glucose Responses. P-glucose responses after the test and reference breads are presented in Figures 1 and 2 and Table 4. There were no significant differences in GI between the test breads. Neither were the glucose iPeaks significantly different between the test breads. The tAUC 60−120 min was significantly larger after WWB compared to after the Rekrut bread. The

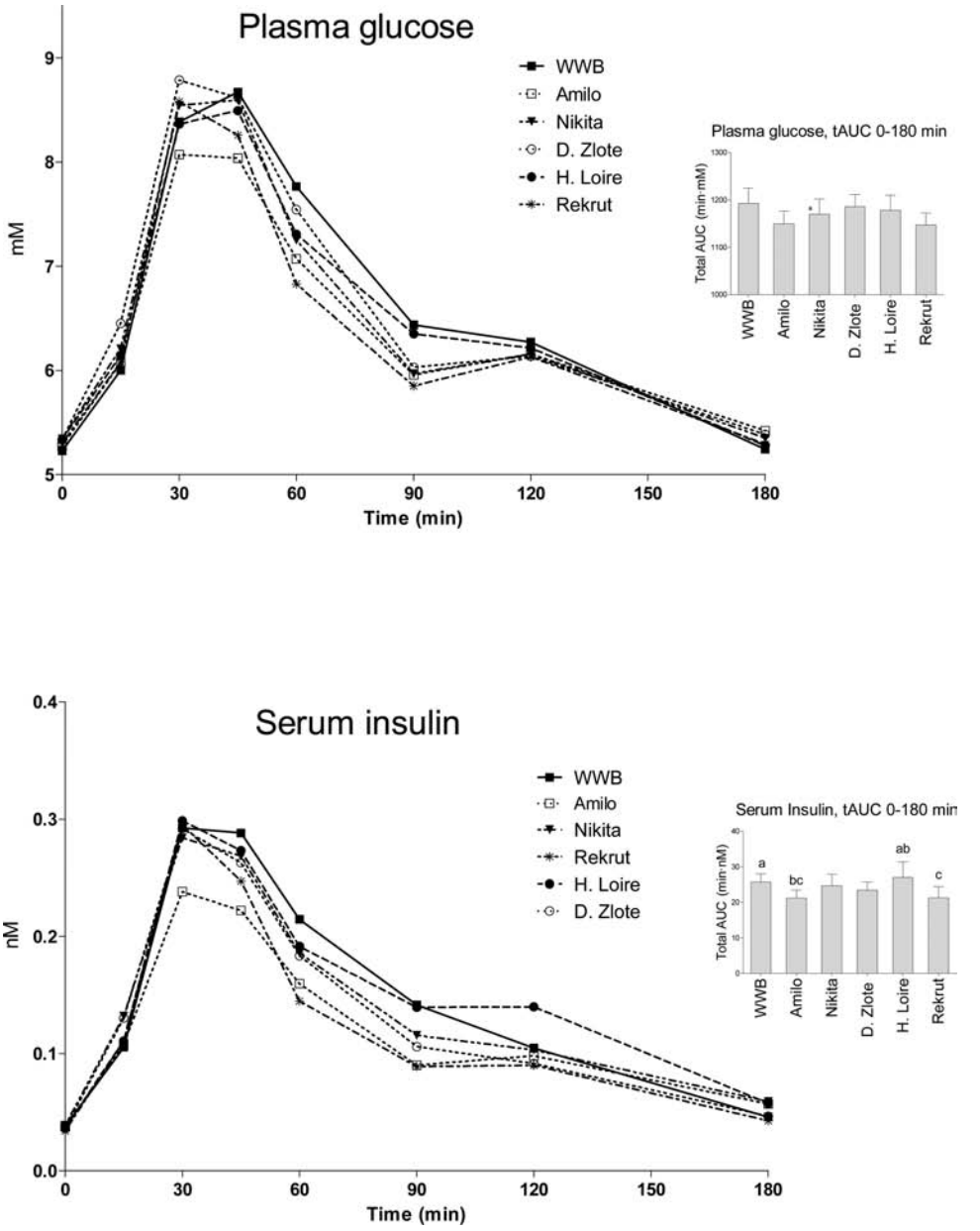

Figure 1. Glucose and insulin responses after the rye test breads and the reference bread. Values are the mean in the graphs and the mean ± SEM in the bars, $n = 14$. Breads not sharing the same letters were significantly different. Breads not displaying letters were not different from any other test bread or the reference bread, $p < 0.05$ (ANCOVA, followed by Tukey's test).

GP did not differ between test breads or between the reference and rye test breads. No time × treatment interaction was found (0−180 min).

Insulin Responses. Serum insulin responses after the test and reference breads are presented in Figures 1 and 2 and Table 4. Rye bread from Amilo and Rekrut displayed significantly lower IIs (72 and 78, respectively) than WWB. In contrast, bread from the H. Loire rye showed a II of 103, which was significantly higher than the corresponding response to Amilo bread. Amilo rye bread induced significantly lower insulin responses compared to WWB and D. Zlote and Nikita rye breads in the early postprandial phase (tAUC 0−60 min). In the 60−120 min phase, Amilo and Rekrut rye bread induced lower insulin responses compared with H. Loire and WWB. Rekrut also induced lower insulin responses than H. Loire in the 120−180 min phase. The insulin iPeak was significantly lower for Amilo bread compared with all other rye breads, except Rekrut. No time × treatment interaction was found for insulin responses (0−180 min).

Subjective Satiety. Subjective satiety responses after the test and the reference breads are presented in Figure 3. All rye breads induced a higher *feeling of fullness* than WWB (tAUC 0−180 min). The rye breads made from D. Zlote and Rekrut induced a higher *feeling of fullness* and a lower *feeling of hunger* than the WWB in the early postprandial phase (tAUC 0−60 min). In the 60−120 min phase, Nikita, D. Zlote, and Rekrut induced a higher *feeling of fullness* than WWB. D. Zlote and Rekrut also induced a lower *feeling of hunger* than WWB in the same interval. All rye breads except H. Loire caused a higher *feeling of fullness* in the

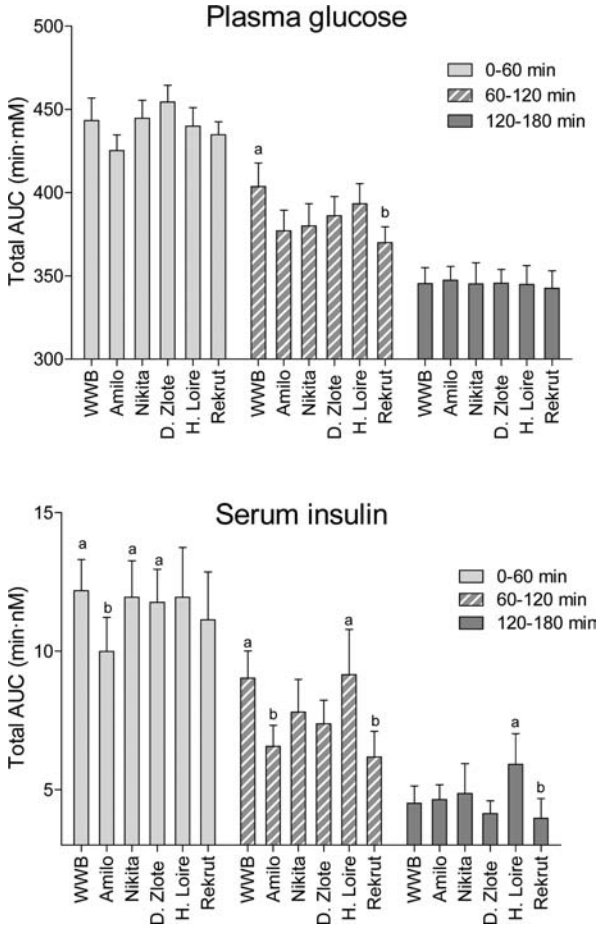

Figure 2. Glucose and insulin responses after rye test breads and reference bread. Values are the mean ± SEM, $n = 14$. Breads not sharing the same letters were significantly different. Breads not displaying letters were not different from any other test bread or the reference bread, $p < 0.05$ (ANCOVA, followed by Tukey's test).

later postprandial phase (tAUC 120−180 min), whereas D. Zlote rye bread induced a lower *feeling of hunger*, compared to WWB. No differences were found in the *desire to eat*. No time × treatment interactions were found for *feeling of fullness*, *feeling hunger*, or *desire to eat* (0−180 min).

Correlations. Correlations between glycemic, insulinemic, and satiety responses were performed using data from all breads, including the WWB. The GI and II were correlated ($r = 0.54$, $p < 0.001$). The insulin iPeak was not related to the GP or the GI ($r = -0.22$, $p = 0.052$, and $r = 0.15$, $p = 0.17$, respectively). A higher II following the breads was related to a lower *feeling of fullness* and a higher *desire to eat* in the late postprandial phase (180 min, $r = -0.32$ and 0.23, $p < 0.05$, respectively). A higher insulin iPeak was related to a higher *feeling of hunger* between 60 and 120 min (tAUC) and to a lower *feeling of fullness* at 180 min ($r = 0.24$ and -0.29, $p < 0.05$, respectively). The GI value of the bread was also correlated with a higher *desire to eat* in the late postprandial phase (180 min, $r = 0.39$, $p < 0.001$).

Correlations between the p-glucose response and the contents of bioactive components in the rye test breads are shown in Table 5. A high early glucose response (tAUC 0−60 min) following the rye test breads was related to a high HI as well as a low dietary fiber (insoluble and soluble fiber) content. A high early glucose response (tAUC 0−60 min) was also related to a

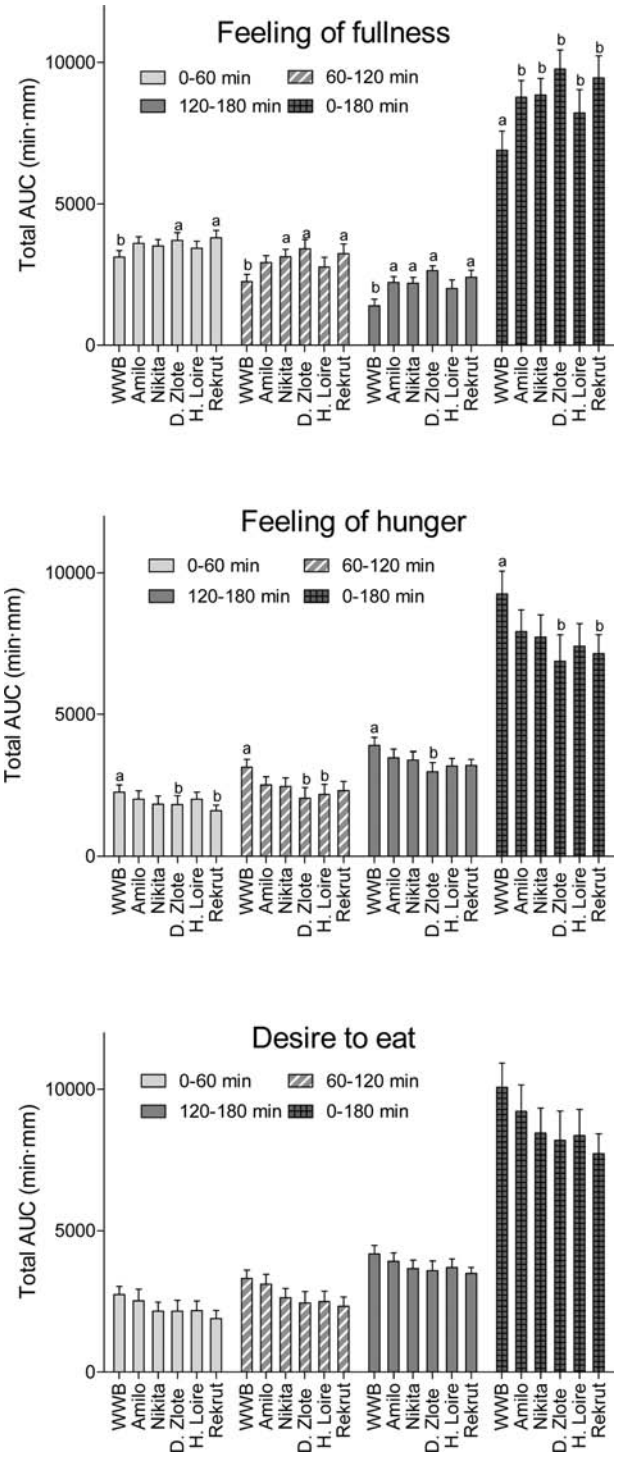

Figure 3. Subjective satiety responses after rye test breads and reference bread. Values are the mean ± SEM, $n = 14$. Breads not sharing the same letters were significantly different. Breads not displaying letters were not different from any other test bread or the reference bread, $p < 0.05$ (ANCOVA, followed by Tukey's test).

low content of free 4-hydroxybenzoic, vanillic, syringic, caffeic, sinapic, and ferulic acids in the rye breads. A high content of total AR was related to a lower early glycemic response (tAUC 0−60 min). The combined content of phenolic compounds (free phenolic acids, aldehyde, and AR) was also negatively related to

Table 5. Correlations between Postprandial Glucose and Insulin Response Following the Rye Test Breads and Their Contents of Indigestible Carbohydrates, Free Phenolic Acids, Alkylresorcinols (AR), Tocols, Sterols, Choline, and Betaine[a]

		glucose (tAUC 0−60 min)	insulin iPeak	insulin (tAUC 0−60 min)	II
HI	*r* =	**0.47**	**0.43**	**0.47**	**0.40**
	p =	**>0.001**	**>0.001**	**>0.001**	**0.001**
insoluble fiber	*r* =	**−0.47**	**−0.43**	**−0.47**	**−0.40**
	p =	**>0.001**	**>0.001**	**>0.001**	**0.001**
soluble fiber	*r* =	**−0.41**	**−0.31**	**−0.40**	**−0.34**
	p =	**0.001**	**0.011**	**0.001**	**0.005**
4-hydroxybenzoic acid	*r* =	**−0.25**	−0.05	−0.20	−0.07
	p =	**0.037**	0.686	0.099	0.566
vanillic acid	*r* =	**-0.31**	−0.17	−0.27	−0.13
	p =	**0.01**	0.169	0.029	0.301
syringic acid	*r* =	**−0.25**	−0.05	−0.22	−0.11
	p =	**0.037**	0.659	0.071	0.380
caffeic acid	*r* =	**−0.36**	**−0.44**	**−0.37**	**−0.40**
	p =	**0.003**	**0.000**	**0.002**	**0.001**
sinapic acid	*r* =	**−0.42**	**−0.36**	**−0.40**	**−0.29**
	p =	**>0.001**	**0.002**	**0.001**	**0.016**
ferulic acid	*r* =	**−0.31**	−0.17	−0.027	−0.13
	p =	**0.01**	0.169	0.029	0.301
total AR	*r* =	**−0.33**	−0.30	−0.21	−0.16
	p =	**0.006**	0.013	0.082	0.201
total phenolic compounds	*r* =	**−0.35**	**−0.30**	**−0.28**	−0.17
	p =	**0.004**	**0.014**	**0.024**	0.161
α-tocotrienol	*r* =	**0.38**	**0.37**	**0.28**	**0.26**
	p =	**0.001**	**0.002**	**0.020**	**0.031**
β-tocotrienol	*r* =	**0.35**	**0.30**	**0.28**	0.17
	p =	**0.004**	**0.014**	**0.024**	0.161
stigmasterol	*r* =	**−0.39**	**−0.31**	**−0.34**	−0.32
	p =	**0.001**	**0.010**	**0.004**	0.008
campesterol	*r* =	0.14	**0.31**	0.13	0.20
	p =	0.241	**0.009**	0.288	0.103
choline	*r* =	**−0.25**	−0.05	−0.20	−0.07
	p =	**0.037**	0.686	0.099	0.566

[a] Total phenolic compounds = total free phenolic acids and aldehydes + AR. Spearman's partial correlation coefficients controlling for subjects and corresponding baseline values (two-tailed test). Significant correlations are shown in boldface type.

the early glucose response. By contrast, a high content of α- and β-tocotrienols in the rye breads was related to a high early glucose response (tAUC 0−60 min). A high content of stigmasterol in the rye breads was correlated with a lower p-glucose response (tAUC 0−60 min), whereas a high content of choline was correlated with a lower early glucose response (tAUC 0−60 min).

Correlations between insulin response and bioactive components in the rye test breads are given in Table 5. A high HI as well as a low fiber (insoluble and soluble fibers) content of the rye breads was related to a high insulin response (tAUC0−60 min, II and insulin iPeak) following consumption. A high content of free vanillic, caffeic, sinapic, and ferulic acids was related to a lower early postprandial insulin response. Free caffeic and sinapic acids were also negatively related to the II. A high content of total alkylresorcinols was related to a lower insulin iPeak. High contents of α- and β-tocotrienol were related to a higher early insulin response (tAUC 0−60 min and insulin iPeak). A high content of stigmasterol was correlated with a lower insulin response (tAUC0−60 min, II and insulin iPeak). A high content of campesterol was related to a higher insulin iPeak.

The contents of total and soluble fibers in the rye breads were positively related to the amount of stigmasterol (r = 0.94 and 0.90, $p < 0.05$), and the insoluble fiber content was positively correlated with available syringic aldehyde (r = 0.90, $p < 0.05$).

DISCUSSION

The present study indicates differences in insulin-saving properties between breads made from different rye varieties. Equicarbohydrate portions of rye bread baked from Amilo and Rekrut rye, respectively, induced a significantly lower insulin response compared to WWB in healthy subjects, whereas bread made from the other rye varieties induced insulin responses similar to that of WWB. Postprandial glycemia responses following the rye breads were similar to that of WWB. However, a tendency toward a lowered glycemic response was seen following the Amilo and Rekrut rye breads. Previous studies of rye products have demonstrated a lowered postprandial insulin response as a general feature, both with and without a simultaneous lowering of the glycaemic response in comparison with WWB.[1−5] Consequently, the relatively high insulin responses following consumption of Nikita, D. Zlote, and H. Loire rye breads are surprising and reveal differences in acute metabolic responses depending on the rye variety. We have previously reported favorable postprandial glucose profiles following several rye products, with a low but prolonged course of glycemia (high glycemic profile, GP).[2,3] In the present work, however, all rye breads had GP values similar to that of WWB. The GP also showed a poor relationship to the postprandial insulin response, in contrast with previous findings. The rye breads tested in the present study were made from single varieties pooled from four different growing locations (France, Poland, Germany, and Hungary). Instead, the rye products in previous studies were made from Swedish or Finnish commercial rye blends, respectively. Another difference between the studies was that acceptable breads could be made from 100% commercial Swedish rye blend,[3] whereas the breads in the present study more resembled porridges. Indeed, the glucose and insulin responses to the breads in the present work are more similar to the postprandial responses previously reported for whole grain rye porridge.[2]

Higher amounts of both soluble and insoluble fibers in the rye test breads were related to lower early insulin and glucose responses (tAUC 0−60 min) and to a lower II. We have previously demonstrated that endosperm rye breads displayed low insulin responses, whereas a white wheat bread enriched with rye bran did not.[2] The endosperm part of the rye grain contains a substantial amount of soluble fiber, which is likely to contribute to this low insulin response, possibly by inducing a higher viscosity, leading to a slower gastric emptying rate and a lowered rate of small intestinal carbohydrate uptake.[8,9] Endosperm rye flour obtained through conventional milling may also contain a substantial amount of bioactive components, as the bran is difficult to separate during milling.

We also investigated the rate of starch hydrolysis in vitro and found that the early glucose response (tAUC 0−60 min) as well as the insulin response (II, tAUC 0−60 min, and insulin iPeak) was positively related to the HI of the rye breads. Although a tendency toward lower HI of rye breads has been reported in comparison with white wheat bread, no significant correlations between the glucose or insulin response and the HI of rye breads have previously been found in our laboratory.[2,3] In the present study, all rye breads were made of whole grain, and thus the products were similar compared with one another. In contrast, in our previous studies the rye products differed in extraction rate and processing. Possibly, the rate of starch digestion plays a larger role in discriminating breads of similar extraction rate, whereas other factors play a larger role between rye products differing in extraction rate.

Interestingly, although commonly referred to as dietary fiber copassengers, the levels of different bioactive components showed little relationship to the amount of dietary fiber in the rye test breads. Among the investigated bioactive components, only the amount of stigmasterol and syringic aldehyde was correlated to the amount of dietary fibres. The most interesting bioactive components in this context were the phenolic acids. Ferulic and caffeic acids have previously been reported to lower glycemia by obstructing carbohydrate digestion and uptake, to inhibit intestinal sucrase, and to reduce Na^+-dependent glucose uptake in rat intestinal brush border membrane vesicles.[28,29] Adisakwattana et al.[30] demonstrated an inhibition of intestinal sucrase by ferulic acid in rats and inhibition of intestinal maltase by ferulic and caffeic acids. We chose to study potential relations between postprandial glucose and insulin response, respectively, and free phenolic acids in the rye breads, because these acids have been reported to peak in postprandial blood between 1 and 3 h after ingestion.[31] High contents of several of the free phenolic acids analyzed here, for example, caffeic and ferulic acids, were negatively related to the early glucose and insulin responses (tAUC 0−60 min). Amilo rye bread contained the highest amount of free caffeic acid, whereas H. Loire contained the least. In contrast, H. Loire, a rye bread inducing a high insulin response, was rich in most of the other analyzed phenolic acids. Ferulic acid has been shown to stimulate insulin secretion from pancreatic β-cells in normal rats,[32] and it may have contributed to the high insulin response seen after H. Loire. However, this increase in insulin was not related to a corresponding lowering of the postprandial blood glucose and would suggest lowered insulin sensitivity after H. Loire. The concentrations of phenolic acids in the rye test breads consumed in the present study were generally low, and the postprandial plasma levels of the phenolic acids were not analyzed. However, the observed correlations between free phenolic acids and improved postprandial glycemia suggest the need to study the bioavailability of phenolic acids in rye products and their potential short-term effects on glucose metabolism.

In the present work, the Amilo rye bread induced a low glycemic profile of short duration, thereby yielding a GP value similar to those of H. Loire and WWB, which were characterized by glycemic curves with high iPeaks, but remaining above fasting for a long time. In an attempt to better reveal the potential impact of differences in the glucose iPeak, the duration of the p-glucose

curve was divided by the squared glucose iPeak, instead of just the iPeak. There were no significant differences between the test breads when the glucose profile was expressed this way, but the values fell within a wider range than the GP values. For example, Amilo had the highest value of 19.4 ± 4.1 min/mM^2, and D. Zlote the lowest (11.8 ± 1.4 min/mM^2), among the rye breads. In the case of the WWB reference, the value was 14.7 ± 1.9 min/mM^2. An inverse relationship was found between the duration/iPeak^2 calculation and the insulin response (insulin iPeak and II, $r = -0.27$ and -0.26, $p < 0.05$, respectively). Also, there was a positive correlation between the content of free sinapic acid and the profile assessment obtained from the duration/iPeak^2 calculation ($r = 0.26$, $p = 0.031$), suggesting a more beneficial glucose profile following rye breads rich in sinapic acid.

In the present study, we determined appetite ratings after intake of the different rye breads. All rye breads induced a higher *feeling of fullness*, and D. Zlote and Rekrut also induced a lower *feeling of hunger* compared to WWB (tAUC 0−180 min). Interestingly, there was also a relationship between a higher insulin response and decreased late satiety. We have previously noted that a low insulin response together with a large portion size and a high content of indigestible carbohydrates beneficially affect satiety after rye products, possibly mediated by colonic fermentation.[3] In the present work, the portion sizes and dietary fiber contents of the rye breads were similar and larger than that of WWB.

The studies reported here indicate that some rye varieties are more insulin saving than others. The low insulin demand previously seen with whole grain rye breads was confirmed with bread made from Amilo and Rekrut, but not with H. Loire, Nikita, or D. Zlote. This is new knowledge that needs further investigation and should possibly be considered when selecting rye varieties for the manufacture of commercial rye blends. The mechanism is probably multifactorial, including the effects of dietary fiber and a lowered rate of starch hydrolysis. The results also indicate that bioactive components such as caffeic, ferulic, and sinapic acids may play a role in the beneficial glucose- and insulin-regulating properties seen with some rye products.

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ABBREVIATIONS USED

AR, alkylrescorcinols; BMI, body mass index; D. Zlote, Dankowskie-Zlote; GI, glycemic index; GP, glycemic profile; HI, hydrolysis index; H. Loire, Haute Loire Pop; II, insulinemic index; iPeak, incremental peak; iAUC, incremental area under the curve; tAUC, total area under the curve; WWB, white wheat bread.

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