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Concentrated Arabinoxylan but Not Concentrated β-Glucan in Wheat Bread Has Similar Effects on Postprandial Insulin as Whole-Grain Rye in Porto-arterial Catheterized Pigs

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ABSTRACT: The acute glycemic effects of concentrated dietary fibers (DF) versus whole-grain rye were studied in porto-arterial catheterized pigs. Two white wheat breads with wheat arabinoxylan (AX) or oat β-glucan (BG), two rye breads with intact rye kernels (RK) or milled rye (GR), and a low DF white wheat bread were fed to six pigs in a randomized crossover design. Blood profiles were collected for 4 h after feeding. Glucose absorption was reduced in pigs fed the AX bread at 60 min postprandial (3.1 mmol/min for AX compared to 9.4 mmol/min for WF, $P = 0.02$) and insulin secretion was lowered at 30 min postprandial for AX and GR (74.4 and 129 pmol/min for AX and GR, respectively, compared to 738 pmol/min for WF, $P < 0.04$). In conclusion, the GR and AX breads were most effective in improving insulin economy, suggesting that arabinoxylan from wheat and rye induces similar outcomes in the metabolic response.

KEYWORDS: *arabinoxylan, β-glucan, whole-grain rye, insulin, glucose*

INTRODUCTION

The Western-style diet is rich in rapidly digestible carbohydrates that cause postprandial elevations of glucose and insulin, which may lead to metabolic disturbances clustered in the metabolic syndrome.[1] Consumption of whole-grains, largely attributed the cereal dietary fibers (DF), has been inversely related to the prevalence of metabolic syndrome, type 2 diabetes, and weight gain.[2−4] The beneficial actions of DF are mainly attributed to their physicochemical properties and the physiological effects they induce. Soluble DF may form viscous solutions or gels in the stomach that delay gastric emptying and physically inhibit the rate of digestion and absorption of macronutrients in the small intestine.[5,6] Intake of soluble DF has been positively correlated with lower postprandial glucose and insulin responses,[7,8] decreased serum cholesterol,[9] and improved gut health.[7] The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), have also been shown to be reduced in response to soluble DF.[10,11]

The main soluble fibers in oats and barley are (1→3)(1→4)-β-D-glucan (referred to as β-glucan), which have approval for health claims by the Food and Drug Administration in the United States[12] and by the European Food Safety Authorities in the European Union.[13] The main soluble fibers in rye and wheat are arabinoxylan; the concentration in rye is substantially higher than in wheat. Whole-grain rye has proven effective in reducing the postprandial insulin response, whereas glucose absorption is often unaffected.[10,14−16] Concentrates of arabinoxylan and β-glucan have, however, been shown to reduce both glucose and insulin responses of normoglycemic subjects, people with impaired glucose tolerance, and people with type 2 diabetes.[17−20] This makes arabinoxylan and β-glucan possible targets for producing healthy functional foods with specifically designed fiber fractions.

Intact whole kernels have also proved to be effective in reducing glucose and insulin responses.[21] This is due to the intact botanical structure that protects the encapsulated starch of the kernel against hydrolysis by enzymes in the gastrointestinal tract. As such, whole kernel rye breads have been shown to produce lower postprandial insulin responses compared to refined white wheat bread in healthy subjects.[14]

In the current study, the porcine porto-arterial catheterization model[22] was used to test the acute postprandial response to two experimental white wheat breads with added wheat arabinoxylan (AX) or oat β-glucan (BG) versus two whole-grain rye breads with intact kernels (RK) or milled rye (GR). A low DF refined white wheat bread (WF) was included as negative control. The intentions were to tailor the carbohydrate fraction to improve glycemic control for human bread consumption and determine the properties of concentrated arabinoxylan, β-glucan, whole-grain rye, and intact kernels. The hypothesis was that all four high DF breads would reduce starch digestibility and as a result decrease the glycemic load and insulinemic response compared to the low DF WF bread. The design allowed for quite different sources of arabinoxylan and β-glucan to be tested against each other. Comparison of concentrated DFs with whole-grain DF has received little attention, which is why this comparison was performed to evaluate the clinical importance of the

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concentrated fibers compared to whole-grain and not only show beneficial effects compared to low-fiber bread. For that reason the acute nutritional and physiological effects of the various fiber sources were evaluated on net glucose flux, secretion of insulin, GIP, GLP-1, and the amount of nonesterified fatty acids (NEFA).

■ MATERIALS AND METHODS

Chemicals. Chemicals used were purchased from Wako Chemicals (Richmond, VA, USA), Phoenix Pharmaceuticals (Inc., Burlingame, CA, USA), Millipore (Billerica, MA, USA), and Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

Experimental Breads and Washout Diet. Soluble wheat arabinoxylan (Manildra Group, Nowra, Australia) was isolated from the soluble fraction after extraction of starch and gluten, concentrated by evaporation, heat treated, further treated with α-amylase and glucoamylase, precipitated with ethanol (1:3 v/v), filtered, and finally dried on a spray-dryer. PromOat (soluble β-glucan, Biovelop AB, Kimstad, Sweden) was obtained from the subaleurone of oat by combining wet-milling and enzymatic hydrolysis. The wheat arabinoxylan fraction had 23.4% arabinoxylan with an arabinose/xylose ratio of 0.94 and a weight-average molecular weight (M_w) of 602 kDa.[23] The content of β-glucan in PromOat, which had a M_w of 1978 kDa, was 35.2%.[23] Vitacel WF 600 (wheat fiber, Rettenmaier & Söhne GmbH, Rosenberg, Germany) produced from wheat straw consisted of 72.7% cellulose and 16.9% arabinoxylan with an arabinose/xylose ratio of 0.09.[23]

Five breads were used in the study: two experimental breads made in-house for this study (AX and BG) and three commercial breads (GR, RK, and WF). AX was made from white wheat flour (67.8%), wheat arabinoxylan concentrate (24.4%, Manildra Group), water, baker's yeast (3.5%), sugar (0.9%), salt (1.7%), and shortening (1.8%). BG was made from white wheat flour (71.0%), PromOat (13.3%, Biovelop AB), Vitacel WF 600 (6.9%, Rettenmaier & Söhne GmbH), water, wheat gluten (1.0%), baker's yeast (3.5%), sugar (0.9%), salt (1.7%), and shortening (1.8%). Because the β-glucan in PromOat caused the dough to be very sticky, Vitacel was used to balance the DF content in the BG bread relative to the other high DF breads. The commercial breads were produced at Lantmännen Schulstad A/S (Hammerholmen, Hvidovre, Denmark). Commercial names are Sundbrød Hvede Toast (WF), Mørkt Rugbrød (GR), and Levebrød Multikernerugbrød (RK). The chemical compositions of the breads are shown in Table 1.

A semisynthetic wheat flour based diet low in DF was used for washout between the dietary interventions. This diet provided all the nutrients, vitamins, and minerals required for pigs of this size and consisted of wheat flour (807 g/kg), Lacprodan-87 (73 g/kg; Arla Foods Ingredients amba, Viby, Denmark), canola oil (30 g/kg), Vitacel WF 600 (Rettenmaier & Söhne GmbH), and a vitamin/mineral mixture containing synthetic amino acids. The chemical composition is shown in Table 1.

Table 1. Chemical Compositions of the Experimental Test Breads and Washout Diet[a]

chemical composition, % of dry matter	WF	GR	RK	AX	BG	washout diet
dry matter, %	63.4	52.0	54.3	70.8	61.5	88.4
gross energy, MJ/kg DM	18.3	17.9	17.9	18.5	18.3	18.8
ash	2.4	3.6	3.2	4.2	2.9	3.8
protein (N × 6.25)	13.2	9.5	9.1	21.0	12.2	17.7
fat	3.4	2.2	2.3	2.8	3.1	4.8
starch	71.1	58.8	60.8	51.4	61.2	62.4
total sugars	4.7	3.2	2.3	3.9	5.8	0.1
glucose	0.7	0.7	0.3	1.5	0.3	
fructose	1.6	1.2	1.1	1.2	1.0	
sucrose	0.1	0.2	0.5	0.3	0.1	
maltose	2.3	1.1	0.4	0.9	4.4	0.1
RS[b]	0.4	0.9	1.4	0.7	1.8	0.3
cellulose	0.6	1.9	1.8	0.6	5.3	3.9
β-glucan	0.3	2.1	1.9	0.3	5.2	0.3
soluble	0.2	0.7	0.4	0.2	4.0	0.07
insoluble	0.1	1.5	1.5	0.2	1.2	0.2
arabinoxylan	1.7	7.6	7.7	7.8	3.2	2.6
soluble	1.3	3.6	3.7	6.6	0.9	0.5
insoluble	0.3	3.9	4.0	1.2	2.3	2.0
total NSP	3.5	13.4	13.9	11.6	16.3	7.7
soluble NSP	1.7	5.3	5.0	8.6	5.4	0.9
insoluble NSP	1.7	8.1	8.9	3.0	10.9	6.8
LMW NDC[b]	3.0	5.2	5.3	8.1	1.0	2.4
total NDC	6.9	19.5	20.6	20.4	19.0	10.4
Klason lignin	0.8	1.4	1.4	0.8	0.9	0.1
dietary fiber (NDC + lignin)	7.7	20.9	22.0	21.2	19.9	10.5
available CHO[b], g/meal	210	210	211	225	229	

[a]WF, white wheat bread; GR, dark ground rye bread; RK, rye bread with kernels; AX, wheat bread with arabinoxylan concentrate; BG, wheat bread with β-glucan concentrate; DM, dry matter; RS, resistant starch; NSP, nonstarch polysaccharides; LMW NDC, low molecular weight nondigestible carbohydrates; CHO, carbohydrate. [b]Calculated.

Animal Model. Pigs (crossbreeds of Duroc × Danish Landrace × Yorkshire from the swineherd at Aarhus University, Foulum, Tjele, Denmark) were selected for surgery and included in the study if they had a plasma glucose level below 5.8 mmol/L 1 h after ingesting a traditional swine diet. Additionally, due to the bitterness of rye, not all pigs will eat it. Consequently, only pigs that during testing would eat rye bread were selected for surgery. Six healthy female pigs with an initial normal body weight of 60.2 (SEM 3.1) kg were catheterized in the mesenteric artery and the portal vein and fitted with an ultrasonic blood flow probe around the portal vein using the equipment and procedures described by Jørgensen et al.[24] Sterile saline (0.5 L iv) was infused into the portal vein catheter the first postoperative day and the pigs were exercised for 3 days postoperative. In total 7−9 days were spent to recover. Catheters were flushed aseptically with 1000 IU/mL heparin solution every 3−4 days and otherwise if needed to maintain patency. Catheters were secured using pouches attached to the side of the pig using Leucoplast/Tensoplast (BSN medical, Hull, UK) and an elastic tubular net. The pigs were kept individually in pens, and an elevated plastic grid covering half of the pen allowed the pigs to rest and stay dry. No straw was supplied.

The animal experiment was conducted according to the license obtained by the Danish Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food Administration.

Experimental Design. Pigs were fed each of the five experimental breads on separate days in a randomized 5 × 6 incomplete crossover design with washout periods between to have six observations per diet. Pigs were fed three times a day at 9:00 a.m., 2:00 p.m., and 7:00 p.m. with 635 g of washout diet per meal until 70 kg body weight. Thereafter they received 750 g at each meal. On sampling days (Mondays and Thursdays) the morning meal was replaced with a pulse dose of one of the experimental breads. Breads were portioned to give approximately 200 g of available carbohydrate (Table 1), and the amounts provided per meal were 439 g WF, 642 g GR, 603 g RK, 577 g AX, and 573 g BG. All of the breads were eaten within 15 min. Once a week, the pigs were weighed and received an im supplement of 400 mg Fe^{3+} (Uniferon, Pharmacosmos A/S, Holbæk, Denmark). The pigs had free access to water throughout the entire study period.

Sampling Protocol. At each sampling day blood was collected from the mesenteric artery and portal vein at −15 (fasting value, t_0), 30, 60, 90, 120, 180, and 240 min after feeding with a pulse dose of experimental bread. Heparinized vacutainers (Greiner Bio-One, Kremsmuenster, Austria) were used for glucose, insulin, and NEFA analysis. EDTA vacutainers (Greiner Bio-One) containing dipeptidyl peptidase-IV inhibitor (final concentration = 50 μM) (Merck Millipore, Billerica, MA, USA) were used for GIP analysis, and EDTA vacutainers (Greiner Bio-One) containing aprotinin (0.6 TIU/mL blood) (Sigma-Aldrich, St. Louis, MO, USA) were used for GLP-1 analysis. Blood flow was measured continuously for 2 min prior to each blood sampling (T201D flowmeter with P-option; Transonic Systems) and recorded using PowerLab (ADInstruments, Sydney, Australia). After blood collection, catheters were flushed with 5 mL of sterile saline and filled with 100 IU/mL heparin solution to replace fluid loss and prevent clotting of the catheters. Hematocrit values were measured at −15 and 240 min postprandially. Blood was centrifuged at 2000g for 12 min at 4 °C, and plasma was kept frozen at −20 °C (glucose, insulin, NEFA) or −80 °C (GIP, GLP-1) until further analysis.

Chemical Analysis. Test breads and the washout diet were freeze-dried and ground to a particle size of <0.5 mm for chemical analyses. All analyses were performed in duplicate. Dry matter (DM) was determined by drying to constant weight at 103 °C for 20 h, ash was analyzed according to an AOAC method,[25] protein (N × 6.25) was measured by DUMAS,[26] and fat was extracted with diethyl ether after HCl hydrolysis according to the Stoldt procedure.[27] Gross energy was analyzed by use of an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). β-Glucan was analyzed by using the enzymatic–colorimetric method of McCleary and Glennie-Holmes.[28] Klason lignin was measured gravimetrically as the sulfuric acid-insoluble residue as described by Theander and Åman.[29] Content of available (digestible) low molecular weight sugars was analyzed as described by Kasprzak et al.[30] Starch and nonstarch polysaccharides (NSP) were analyzed essentially as described by Bach Knudsen;[31] 2 M H_2SO_4 for 1 h was used instead of 1 M H_2SO_4 for 2 h for the NSP analysis. The content of NDC was determined by direct acid hydrolysis without starch removal and alcohol precipitation. The total NDC was calculated by subtraction of the starch content. The content of low molecular weight nondigestible carbohydrates (LMW NDC) was calculated as[30]

$$\text{LMW NDC} = \text{total carbohydrates} - \text{NSP} - \text{starch}$$

The content of resistant starch (RS) in the breads was calculated as[30]

$$\text{RS} = \text{NSP}_{\text{glucose}} - \left(\text{cellulose} + \beta\text{-glucan}\right)$$

The content of available carbohydrates was calculated as

$$\text{available carbohydrates} = \text{starch} + \text{fructose} + \text{sucrose}$$

Plasma samples were analyzed for glucose according to a standard procedure using glucose hexokinase II and enzymatic colorimetric determination (Siemens Diagnostics Clinical Methods for ADVIA 1650, Tarrytown, NY, USA). NEFA were determined using the Wako NEFA C ACS-ACOD assay method (Wako Chemicals, Richmond, VA, USA). Glucose and NEFA analyses were performed on an ADVIA 1650 autoanalyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Plasma insulin was analyzed by time-resolved fluoroimmunoassay as described by Løvendahl and Purup.[32] The GIP hormone was analyzed by competitive RIA using a human GIP kit (Phoenix Pharmaceuticals, Inc.). GLP-1 was analyzed using a Glucagon-Like Peptide-1 (Active) ELISA kit (Millipore, Billerica, MA, USA) with intra-assay variation of 7.4 ± 1.1% and interassay variation of 8 ± 4.8%.

Calculations. Net nutrient flux and apparent hormone secretion were calculated from plasma portal-arterial differences and portal flow measurements as described by Rérat et al.[22] using the equation

$$q = (C_{\rm p} - C_{\rm a})F(\mathrm{d}t)$$

where q is the net flux of nutrient or hormone within time period dt. C_p and C_a are the concentrations of nutrient or hormone in portal and arterial plasma, and F is the blood flow in the portal vein. The total nutrient absorption or hormone secretion (Q) from t_0 to t_1 can be calculated using the formula

$$Q = \sum_{t_1}^{t_0} q$$

The calculated insulin secretion can be described only as apparent in the present study due to a pulsatile secretion, a variable half-life (10−30 min), and breakdown by liver and kidney. Glycemic and insulinemic indices were calculated from Q values using WF as a reference (GI and II = 100). Incremental areas under the curve (iAUC 0−120 min) were calculated using the trapezoid model.[33]

Statistical Analysis. Effects of breads, time, and their interactions were analyzed as repeated measurements using the MIXED procedure of Statistical Analysis Software (version, 9.3, SAS Institute Inc., Cary, NC, USA). Plasma variables were analyzed as a linear mixed model

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \gamma_k + \delta_{ikl} + \rho_{ijkl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable; μ is the overall mean; α_i is the effect of bread (i = WF, GR, RK, BG, AX); β_j is the time after feeding (j = −15 (0), 15, 30, 45, 60, 90, 120, 180, and 240 min); and $\alpha\beta_{ij}$ is the interaction term. The three terms y_k (k = pig 1, ...), δ_{ikl} (l = period 1, ..., 5), and ρ_{ijkl} accounted for repeated measurements being performed on the same pig (y_k) among periods in the crossover design (δ_{ikl}) and on the same pig within a sampling day (ρ_{ijkl}). ε_{ijkl} is the residual error component. The covariance structure of ρ_{ijkl} was modeled using the spatial power option, which takes into account the different intervals between repeated measurements. Arterial and portal hormone concentrations, NEFA concentrations, and net fluxes were transformed to ln(x) before the statistical analysis to obtain variance homogeneity. Glycemic and insulinemic indices and iAUCs were analyzed using the same linear mixed model but without the time effect. Statistical significance was set as $P < 0.05$ and $0.05 \leq P < 0.10$ as trends.

RESULTS

The pigs were well throughout the experiment, and the hematocrit values revealed that there were no problems taking the amounts of blood used for analysis.

Breads. The five breads used for intervention were attempted to be portioned to provide equal amounts of available/digestible carbohydrate (∼200 g). The commercial breads, WF, GR, and RK, provided similar amounts (210, 210, and 211 g, respectively), whereas the experimental breads, AX and BG, resulted in slightly higher intakes (225 and 229 g, respectively). Two hundred grams of available carbohydrate was chosen as pigs eat ∼4 times more carbohydrate per day than humans, and a standard human test meal usually contains 50 g of available carbohydrate. The low DF WF control bread contained 3.5% of DM of total NSP and 7.7% of DM of DF, whereas the high DF breads contained 11.6−16.3% of DM of NSP and 19.9−21.2% of DM of DF (Table 1). GR and RK were very similar in chemical composition and were characterized by high arabinoxylan contents (7.6−7.7% of DM) similar to the AX bread (7.8% of DM) and a smaller amount of β-glucan (1.9−2.1% of DM). The β-glucan content was, however, lowest in the WF and AX breads (0.3% of DM). The AX bread had a very high protein content (21% of DM compared to 9.1−13.2% of DM for the four other breads) that originated from the fraction of refined wheat arabinoxylan. The refined arabinoxylan fraction also had a high content of LMW NDC (8.1% of DM in AX), which was not present in the β-glucan concentrate (1.0% of DM in BG). The AX bread was shown to have 37−42% more soluble NSP and 2−3 times less insoluble NSP compared to the other three high-fiber breads. The GR, RK, and BG breads all showed a higher ratio of insoluble to soluble total NSP, which was opposite that found in the AX bread. The dietary content of soluble β-glucan was, however, higher than the insoluble β-glucan content in the BG

Table 2. Incremental Areas under the Curve (iAUC 0−120 min) for Glucose, Insulin, GLP-1, and GIP in Arterial (A) and Portal (V) Plasma from Pigs Fed the Experimental Test Breads[a]

	WF	GR	RK	AX	BG	*P* value
glucose, mmol/L·120 min						
V	477 ± 69.4	220 ± 69.4	304 ± 69.4	255 ± 69.4	287 ± 69.4	0.11
A	137 ± 21.8	63.8 ± 21.8	97.9 ± 21.8	87.5 ± 21.8	71.8 ± 21.8	0.17
insulin, nmol/L·120 min						
V	35.8 ± 4.7a	10.5 ± 4.7b	17.9 ± 4.7b	17.4 ± 5.1b	15.8 ± 4.7b	0.005
A	13.0 ± 2.6	7.9 ± 2.4	9.2 ± 2.4	11.4 ± 2.4	10.0 ± 2.4	0.62
GLP-1, nmol/L·120 min						
V	0.99 ± 0.23	0.46 ± 0.26	0.78 ± 0.21	0.65 ± 0.21	0.38 ± 0.23	0.38
A	0.34 ± 0.11	0.25 ± 0.12	0.39 ± 0.12	0.30 ± 0.11	0.22 ± 0.12	0.85
GIP, nmol/L·120 min						
V	58.1 ± 10.3a	25.2 ± 10.6b	15.7 ± 10.6b	39.8 ± 10.3ab	22.9 ± 10.3b	<0.001
A	43.8 ± 11.0a	16.1 ± 12.6b	13.6 ± 12.1b	30.2 ± 11.0ab	11.7 ± 12.6b	0.02

[a]Values are means ± SEM (n = 6). Means in a row with the same letter are not significantly different. WF, white wheat bread; GR, dark ground rye bread; RK, rye bread with kernels; AX, wheat bread with arabinoxylan concentrate; BG, wheat bread with β-glucan concentrate.

bread (4.0% of DM). The BG bread also had a high content of cellulose (5.3% of DM) originating from the Vitacel fiber, which was also applicable for the washout diet.

Blood Flow. Portal blood flow was not influenced by the dietary interventions, but varied in relation to the time after feeding (P < 0.001). The flow was 2.8 L/min before feeding corresponding to 34.8 mL/(kg BW·min) and increased to a maximum of 3.3 L/min at 45 min postprandial, corresponding to 40.7 mL/(kg BW·min). The flow had almost returned to baseline at 240 min postprandial (data not shown).

Glucose Kinetics. Plasma glucose concentrations in the mesenteric artery showed a significant bread × time interaction (P = 0.03), whereas a trend was observed in the portal glucose concentrations (P = 0.07) (data not shown). The concentration of glucose in the artery and vein was ∼5 mmol/L at feeding, after which it increased and peaked between 30 and 60 min at 8−10 mmol/L in portal and 6−7 mmol/L in arterial plasma. Glucose concentrations were reduced in the artery at 15 and 30 min postprandially in response to all four high DF breads (P < 0.08) compared to the WF bread (data not shown). The incremental areas under the glucose curve (iAUC 0−120 min, Table 2) for the portal vein and mesenteric artery did not show any significant differences between the breads; however, the WF bread showed numerically higher values than the four other breads.

Net glucose flux (Figure 1A) increased immediately after feeding and peaked between 45 and 60 min postprandially at 9.4 mmol/min for pigs fed the WF bread and at 6−7 mmol/min for pigs fed the four other breads. The RK bread gave a significantly lower glucose flux than the WF bread at 15 min (P = 0.05) and AX at 60 min (P = 0.02), respectively. A gradual decrease in glucose absorption back to the prefeeding level was observed after peaking. Total glucose absorption showed a significant bread × time interaction (P = 0.04) (data not shown), and pigs tended to take up less glucose after 15 and 30 min (P < 0.05) when fed the RK bread compared to the other AX, BG, and WF breads. However, adjustment for available starch reduced the bread × time interaction to a trend (P = 0.09) (Figure 1B), and no significant differences in the glycemic index between breads were observed (Table 3). The total recovery of glucose was between 58 and 68% of the ingested starch and sugars.

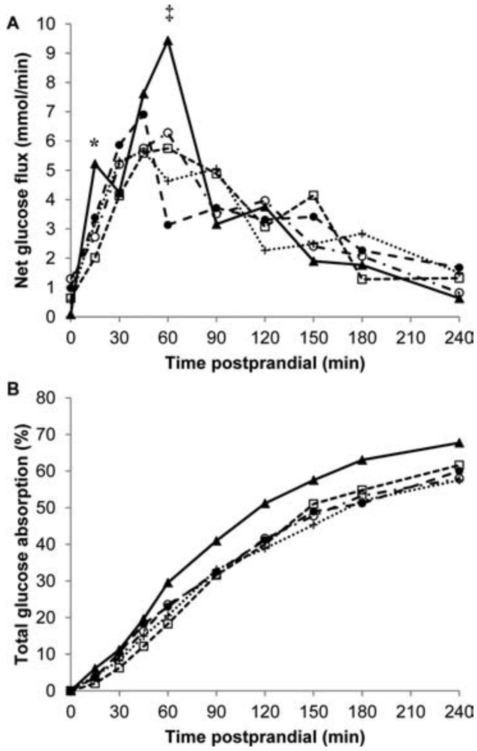

Figure 1. In pigs fed breads with contrasting content and composition of arabinoxylan and β-glucan (n = 6): (A) net glucose flux (bread P = 0.69, time P < 0.001, bread × time P = 0.04); (B) total glucose absorption in percent of available starch (bread P = 0.35, time P < 0.001, bread × time P = 0.09). ▲, white wheat bread (WF); +, dark ground rye bread (GR); □, rye bread with kernels (RK); ●, wheat bread with arabinoxylan concentrate (AX); ○, wheat bread with β-glucan concentrate (BG). Symbols indicate that means at the same time point differ: *, WF > RK (P = 0.05); ‡, WF > AX (P = 0.02).

Insulin Responses. Insulin concentrations in the mesenteric artery and the portal vein showed significant bread × time interactions (P < 0.013). Portal insulin increased and peaked between 15 and 30 min after feeding with 43 and 35% lower concentrations for the AX and GR breads (P < 0.10) compared to the WF bread. After the peak, concentrations followed a descending pattern reflecting the decline in glucose absorption. The insulin level in the mesenteric artery mimicked that in the portal vein but was lower overall (data not shown). The iAUC for insulin in the portal vein was furthermore significantly decreased for all four high DF breads compared to the WF

Table 3. Glycemic Index (GI) and Insulinemic Index (II) at 120 min Postprandial for Pigs Fed the Experimental Test Breads Relative to the WF Bread[a]

	WF	GR	RK	AX	BG	*P* value
GI, %	100	70 ± 12	83 ± 12	74 ± 12	89 ± 12	0.10
II, %	100	61 ± 17	78 ± 17	93 ± 17	79 ± 17	0.17

[a]Values are means ± SEM (n = 6). WF, white wheat bread; GR, dark ground rye bread; RK, rye bread with kernels; AX, wheat bread with arabinoxylan concentrate; BG, wheat bread with β-glucan concentrate.

bread, whereas no differences were found in the mesenteric artery (Table 2).

Similar to portal and arterial concentrations, a rapid increase in the apparent insulin secretion (Figure 2A) after feeding was observed. Insulin secretion peaked at 15–30 min after feeding and was 48 and 27% lower for pigs fed the AX and GR breads, respectively, compared to the WF bread. The WF bread also caused a very rapid decrease back to the prefeeding level at 30–60 min postprandial. Total insulin secretion (Figure 2B) was higher after consumption of the WF bread compared to the GR bread from 30 to 150 min postprandial. Insulin secretion was 55–76% lower in pigs fed the GR bread at 240 min than in pigs fed the four other kinds of breads. Pigs fed the AX bread furthermore had increased secretion of insulin from 120 to 240 min postprandially, but without any high excursions in the net insulin flux. No significant differences in the insulinemic index were, however, observed between the WF bread and the other four high DF breads (Table 3).

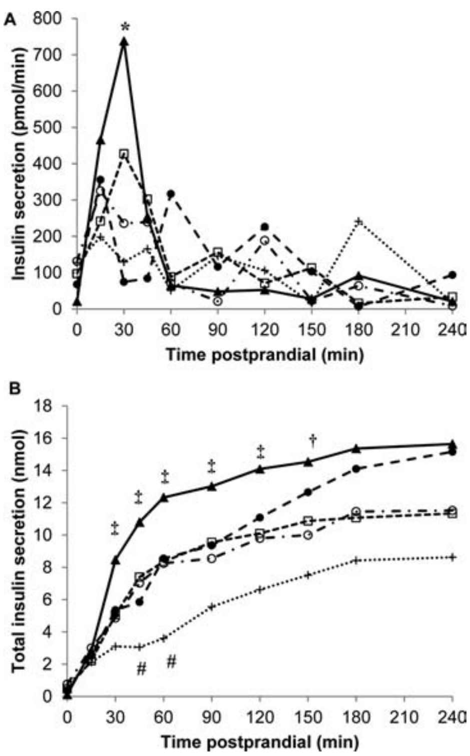

Figure 2. In pigs fed breads with contrasting content and composition of arabinoxylan and β-glucan (n = 6): (A) apparent secretion of insulin (bread P = 0.81, time P < 0.001, bread × time P = 0.001); (B) total apparent secretion of insulin (bread P = 0.40, time P < 0.001, bread × time P < 0.001). ▲, white wheat bread (WF); +, dark ground rye bread (GR); □, rye bread with kernels (RK); ●, wheat bread with arabinoxylan concentrate (AX); ○, wheat bread with β-glucan concentrate (BG). Symbols indicate that means at the same time point differ: *, WF > AX, GR (P < 0.04); ‡, WF > GR (P < 0.05); †, WF > GR (P = 0.08); #, AX, BG, RK > GR (P < 0.09).

Incretins. Portal GIP increased during the first 60 min, after which it gradually declined to the prefeeding levels (Figure 3A).

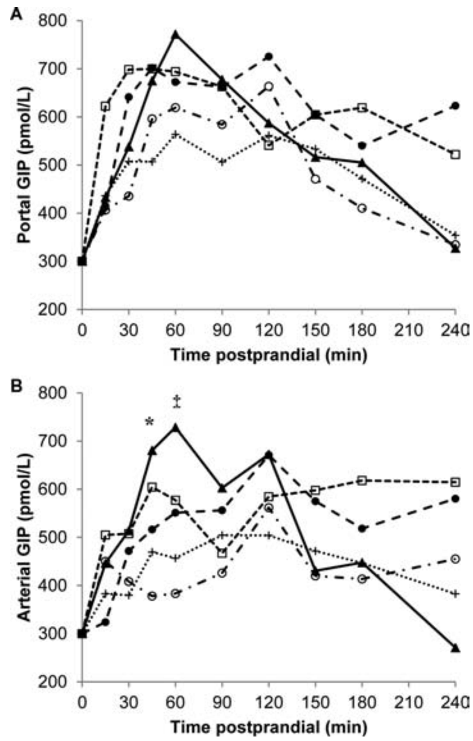

Figure 3. In pigs fed breads with contrasting content and composition of arabinoxylan and β-glucan (n = 6): (A) portal plasma GIP (bread P = 0.42, time P < 0.001, bread × time P = 0.11); (B) arterial plasma GIP (bread P = 0.48, time P = 0.005, bread × time P = 0.016). ▲, white wheat bread (WF); +, dark ground rye bread (GR); □, rye bread with kernels (RK); ●, wheat bread with arabinoxylan concentrate (AX); ○, wheat bread with β-glucan concentrate (BG). Symbols indicate that means at the same time point differ: *, WF > BG (P = 0.01); ‡, WF > BG, GR (P < 0.05). An average of fasting t_0 samples is presented due to missing values at this time point.

The same applied to the mesenteric artery (Figure 3B), which furthermore showed similar concentration levels as in the portal vein. Arterial GIP concentrations showed significant bread × time interactions (P < 0.016) and were significantly lower in response to the BG and GR breads compared to the WF bread at 60 min postprandial. The WF bread furthermore induced significantly higher iAUC (Table 2) than the BG, GR, and RK breads in both arterial and portal blood.

Portal GLP-1 increased within the first 30–90 min, after which the concentrations gradually declined back to the prefeeding level (Figure 4A). Arterial GLP-1 concentrations (Figure 4B) followed the same development as in the portal vein, but concentrations were lower overall. Portal and arterial GLP-1 were, however, not significantly affected by the different kinds of breads. Nor were there any differences between breads from the iAUC (Table 2).

Nonesterified Fatty Acids (NEFA). NEFA concentrations were analyzed only in portal vein samples, and no differences were observed due to dietary interventions (Figure 5). NEFA concentrations were reduced the first 30 min postprandial in

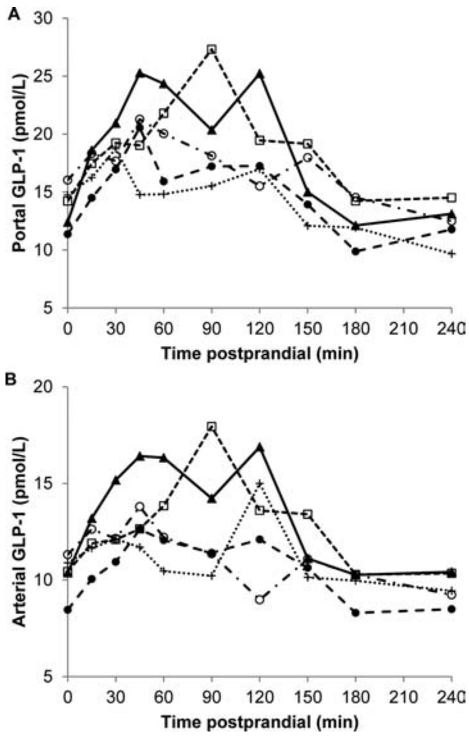

Figure 4. In pigs fed breads with contrasting content and composition of arabinoxylan and β-glucan ($n = 6$): (A) portal plasma GLP-1 (bread $P = 0.90$, time $P < 0.001$, bread × time $P = 0.63$): (B) arterial plasma GLP-1 (bread $P = 0.94$, time $P < 0.001$, bread × time $P = 0.34$). ▲, white wheat bread (WF); +, dark ground rye bread (GR); □, rye bread with kernels (RK); ●, wheat bread with arabinoxylan concentrate (AX); ○, wheat bread with β-glucan concentrate (BG).

response to the intake of all test breads and did not increase again within the 240 min.

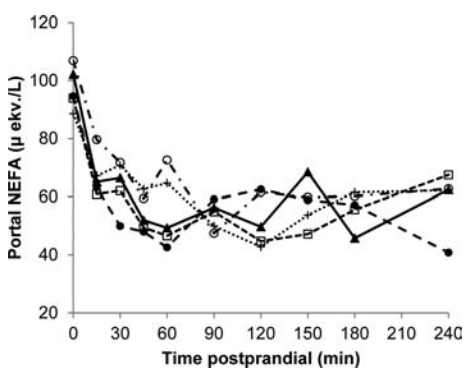

Figure 5. In pigs fed breads with contrasting content and composition of arabinoxylan and β-glucan ($n = 6$): portal plasma nonesterified fatty acids (NEFA) (bread $P = 0.68$, time $P < 0.001$, bread × time $P = 0.82$). ▲, white wheat bread (WF); +, dark ground rye bread (GR); □, rye bread with kernels (RK); ●, wheat bread with arabinoxylan concentrate (AX); ○, wheat bread with β-glucan concentrate (BG).

■ DISCUSSION

The porcine porto-arterial catheterization model is useful for studying the kinetics of nutrient uptake and apparent hormone secretion across the small intestine during a dietary intervention. The portal blood allows quantitative measurements of absorbed nutrients, insulin, and incretins, whereas arterial blood represents the circulating concentrations.[11,34] The difference between portal and arterial concentrations is the enrichment of nutrients and hormones, and simultaneous blood flow measurements allow quantification of the flux.[34] In the current study, this animal model was used to assess the acute metabolic response to test breads with arabinoxylan, β-glucan, dark ground rye, intact rye kernels, and a control white wheat bread on glucose, insulin, incretins, and NEFA.

AX and RK were the only breads that significantly decreased the immediate glucose flux compared to WF, although it seemed evident that glucose absorption was numerically lower in pigs fed all of the high DF breads at 60 min postprandial. Significant differences in total glucose absorption were furthermore not found when adjusted for available carbohydrate. This was despite differences in amounts of soluble and insoluble NSP. Reduction in net glucose flux, with respect to the AX bread, was most likely due to the high content of soluble arabinoxylan causing delayed gastric emptying and an increased viscosity of digesta. In a parallel study with ileal fistulated pigs we found that the ileal viscosity was significantly higher after the consumption of AX bread compared to RK and GR despite similar dietary concentrations of arabinoxylan.[30] The increased viscosity may alter mixing of the food bolus with intestinal contents, decrease the availability of digestive enzymes, and thereby inhibit the absorption of nutrients,[7,35] resulting in attenuated glucose plasma peaks. Other studies with wheat arabinoxylan concentrates have also shown plasma glucose to be attenuated.[18−20] In contrast, studies with arabinoxylan-rich rye foods (including whole rye kernels) found only the insulin response to be decreased in healthy subjects[10,14,15] and catheterized pigs,[16] whereas glucose was unaffected. The most likely reason for the difference in glucose absorption between concentrated arabinoxylan from wheat and the arabinoxylan from whole-grain rye is that the proportion of solubilized arabinoxylan is higher when provided in the concentrated form from wheat compared to arabinoxylan present in the cell wall matrix of whole grain rye.[23,30] The study of Kasprzak et al.[23] further indicated that the higher branching of the concentrated arabinoxylan from wheat than of arabinoxylan found in the two rye breads gave rise to a higher protection against microbial degradation in the small intestine and thereby allowed a higher concentration of arabinoxylan in the ileal supernatant, which also resulted in a higher viscosity.

A study by Hooda et al.[11] found a decreased glucose absorption in catheterized pig within the first hour after feeding with a diet containing 6% β-glucan compared to 0% β-glucan. The total DF contents in the diets were, however, higher in the study of Hooda et al. (22−33% of dry matter). The absent effect of the BG bread on glucose absorption and insulin secretion could be caused by degradation of the β-glucan content by microbes in the small intestine, which is higher in pigs[36] than in humans.[37] In the parallel study with ileal fistulated pigs it was found that the oat β-glucan was substantially degraded during passage through the small intestine, resulting in the lowest ileal viscosity among the studied breads.[23,30] Arabinoxylan has a more complex and firm structure, making it less prone to degradation than β-glucan,[38] which was also found in the study by Kasprzak et al.[23] The BG bread furthermore had a lower content of β-glucan than the arabinoxylan content in the AX and rye breads, which could also influence the results despite the higher content of RS and cellulose.

The total glucose absorption after 4 h (58 and 68% of available carbohydrate) was similar to that of other studies with catheterized pigs fed breads. Here the quantitative recovery of starch and sugars has been between 55 and 66% within a 4 h period for wheat- and oat-based products.[39,40]

Despite the fact that RK lowered glucose absorption in the beginning, only the AX and GR breads lowered the immediate insulin response compared to the WF bread, whereas BG and RK did not significantly affect the insulin secretion, even though their iAUCs for insulin in the portal vein were decreased compared to WF. Structural and compositional properties have been suggested to be the main contributors to the insulin response as opposed to the amount or type of DF.[10,15] It has furthermore been suggested that intact whole rye kernels might be better in lowering both glucose and insulin responses due to the encapsulation of starch, making it less accessible for hydrolysis.[10,14] This, however, seemed not to be evident in the present study as the GR bread was better than RK in attenuating the insulin response. The rye kernels in the RK bread might have been disrupted during the dough and baking processes as indicated by in vitro digestion data[41] as well as the in vivo digestion data from ileal fistulated pigs,[30] making them more prone to enzymatic starch degradation. It has, on the other hand, been speculated that unknown bioactive components, such as antioxidants and plant sterols, in milled rye are able to improve insulin economy.[42,43] Differences in the bioavailability of these bioactive components as well as the arabinoxylan content might explain the observed differentiation between GR and RK despite very similar chemical compositions of the two rye breads. The data presented in this study, however, cannot give any clue on possible bioactive components, but it is striking that similar effects of diets on the insulin response are obtained with refined arabinoxylan from wheat (low levels of bioactive components) and arabinoxylan from milled whole-grain rye (high in bioactive components). This suggests soluble arabinoxylan to be the primary factor for a reduced insulin response.

The lowered insulin secretion in response to the AX bread could be a consequence of a reduced glucose flux. The AX bread, however, showed a marked increase in total insulin secretion after 90 min that reached the negative control at 240 min. The AX bread had the highest proportion of soluble NSP from the wheat arabinoxylan but also a higher protein concentration. Amino acids are able to stimulate insulin secretion,[44,45] whereas the high amount of soluble NSP could potentially delay protein degradation and absorption through its gel-forming properties, causing this delayed increase.

GIP and GLP-1 are secreted by enteroendocrine K and L cells in the upper and lower small intestine, respectively, and increase insulin secretion in response to luminal glucose and other nutrients.[46] The GR, RK, and BG breads significantly decreased the iAUC for both GIP and insulin in the portal vein, which suggest a regulation of postprandial insulin by the GIP hormone. Similar concentration levels of GIP in the portal vein and mesenteric artery could suggest that GIP was less degradable than GLP-1 and insulin, which showed higher concentrations in the portal vein compared to the artery. GLP-1 was not reduced in response to any of the high DF breads. GLP-1 is released by carbohydrates, fat, and protein, whereas the effect of fiber is unclear.[47] Its secretion can be modulated both neurally and hormonally[48] and has a direct effect on L cells. GIP and GLP-1 secretions are closely correlated, for which reason it is unknown why GIP was affected but GLP-1 was not. Protein stimulates GLP-1 release even more than carbohydrates,[47] and the variable protein concentration in the breads could potentially influence the results.

In a study with healthy subjects by Juntunen et al.[10] they also found a reduced GIP response to whole kernel rye bread and to a lesser extent to rye bread with added β-glucan. GLP-1 was furthermore reduced in response to the whole kernel rye bread. Different processing and baking methods might cause the dissimilar results. In the study by Hooda et al.,[11] reduced GIP and GLP-1 concentrations, together with reduced insulin secretion, were also observed in response to the 6% β-glucan diet compared to the 0% β-glucan diet. However, the higher DF content of the 6% β-glucan diet compared to the breads in the present study or the different amounts of available carbohydrate between diets could cause different results.

NEFA concentrations (lipolysis) decreased as a response to glucose uptake and the switch in the cellular utilization of energy, but no differences in the portal plasma concentrations of NEFA were observed among the five breads. It was expected that NEFA would rebound following removal of the raised insulin concentrations. The rebound of free fatty acids has been related to glycemic responses.[49] However, the antilipolytic effects of insulin might first have disappeared after the 4 h study period.

This study demonstrates that the GR and AX breads were better in attenuating the immediate insulin response in catheterized pigs compared to the WF, BG, and RK breads. This was associated with a reduced net glucose flux with regard to the AX bread, which was not present for the GR bread. However, GR, RK, and BG reduced GIP secretion. These results demonstrate that concentrated arabinoxylan from wheat and arabinoxylan from whole-grain rye seem to possess similar effects in regard to insulin secretion. Consequently, concentrated arabinoxylan might be a useful additive in meals for the prevention and treatment of type 2 diabetes.

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Notes

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ABBREVIATIONS USED

AX wheat bread with arabinoxylan concentrate; BG wheat bread with β-glucan concentrate; DF dietary fiber; DM dry matter; GI glycemic index; GIP glucose-dependent insulinotropic polypeptide; GLP-1 glucagon-like peptide 1; II insulinemic index; LMW NDC low molecular weight nondigestible carbohydrates; GR dark ground rye bread; NEFA nonesterified fatty acids; NSP nonstarch polysaccharides; RK rye bread with kernels; RS resistant starch; WF white wheat bread

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