**Dietary Oat β-Glucan Reduces Peak Net Glucose Flux and Insulin Production and Modulates Plasma Incretin in Portal-Vein Catheterized Grower Pigs**

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Abstract

Net glucose and SCFA flux and insulin secretion into the portal vein might be associated with the incretins glucose-dependent insulinoactive polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Our objectives were to clarify this association and study the impact of 2 doses of dietary oat β-glucan on the variables. Three 35-kg portal vein-catheterized pigs were fed 3 diets containing 0, 3, or 6% oat β-glucan concentrate (BG0, BG3, and BG6) for 7 d in a repeated 3 × 3 Latin square. On d 7, blood was sampled for 12 h postprandially. Net glucose flux and apparent hormone production were calculated from plasma portal-arterial differences × flow. Postprandially, pigs fed BG6 had lower (P < 0.05) portal glucose at 15, 30, and 45 min and a lower (P < 0.05) net glucose flux during the first hour. Pigs fed BG6 tended to have lower (P < 0.10) portal C-peptide without lowering insulin, indicating that pigs fed BG6 had lower actual insulin release combined with a higher prehepatic retention of insulin. Pigs fed BG6 had lower (P < 0.05) portal GIP and GLP-1, which in turn were correlated (R² = 0.81 and 0.88, respectively; P < 0.01) with portal glucose. Pigs fed BG3 and BG6 had a higher (P < 0.05) net SCFA flux than pigs fed BG0, indicating increased fermentation. In conclusion, dietary supplementation of 6% oat β-glucan concentrate decreased net glucose flux, increased net SCFA flux, and decreased peak apparent insulin production, changes that were associated with GIP and GLP-1 mediation.


Introduction

The kinetics of glucose absorption affects glucose metabolism and insulin responses, 2 major factors for control and prevention of type-II diabetes in humans (1). Among soluble nonstarch polysaccharides (NSP), the effects of β-glucan concentrate derived from oat and barley have been studied extensively in humans in relation to glucose metabolism and insulin responses using glucose measurements in peripheral blood (2–4). Soluble NSP may decrease peak glucose flux by increasing viscosity and water-binding capacity of gastrointestinal contents (5) and interfering with enzymatic digestion and mucosal absorption (6). To study the kinetics of nutrient uptake and hormone production and the control of glucose homeostasis by insulin, advanced surgical models or isotope techniques are ideal, but their use is obviously limited in normal human participants. Instead, the porcine porto-arterial catheterization model allows studying the effects of changes in dietary composition on net nutrient flux and apparent production of gastrointestinal and pancreatic hormones (7).

The incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoactive polypeptide (GIP) are released from L and K cells (enteroendocrine cells) of the large and small intestine, respectively, in response to luminal glucose (8) and might thus be reduced by viscous NSP (9). Both GLP-1 and GIP enhance glucose-dependent insulin secretion, promote β-cell proliferation, and inhibit β-cell apoptosis and thus increase pancreatic β-cell mass (8). Recently, incretins have been studied as potential therapy for type-II diabetes (10). Undigested nutrients and NSP are fermented in the intestine, producing SCFA that serve as nutrients systemically and for the colonic epithelium regulate proliferation, differentiation, and gene expression, and promote growth of Bifidobacterium and Lactobacillus species (11). The SCFA increase the expression of the proglucagon gene in L cells of the intestine and thus may increase GLP-1 secretion (12). However, the knowledge about incretin modulation by dietary viscous and fermentable NSP

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**Abbreviations used:** BG0, BG3, and BG6, diets containing 0, 3, and 6% oat β-glucan; GIP, glucose-dependent insulinoactive polypeptide; GLP-1, glucagon-like peptide-1; NSP, nonstarch polysaccharide; PC, principal component.

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such as oat β-glucan is limited (9). Moreover, the complex network of glucose kinetics, insulin and incretin responses, and SCFA flux in response to feeding oat β-glucan is poorly understood.

Thus, a porcine porto-arterial catheterization model was used to test the hypotheses that β-glucan as a soluble NSP decreases the peak net glucose flux and insulin secretion and increases net SCFA flux and that these changes are associated with incretins. The objectives were to clarify this association and to study the impact of 2 doses of dietary oat β-glucans on the variables.

Materials and Methods

Animal care and experimental design

Surgical model. The animal protocol was approved by the Animal Care and Use Committee for Livestock at the University of Alberta and was conducted at the Swine Research and Technology Centre. Three female pigs (35–40 kg body weight) were catheterized in the portal vein and carotid artery using modified polyvinyl tube catheters; a 14-mm blood flow probe (Transonic Systems) was implanted around the portal vein (13). Postoperative management included antibiotics, analgesics, gut motility drugs, and i.v. fluids for 3 d. Catheters were flushed aseptically daily with 200 mL of heparinized normal saline to maintain their patency and were secured using pouches. Only 3 pigs were used due to the complexity of surgery and maintenance requirements of the animals.

Diets. The diets (Table 1) were based on wheat, soybean meal, canola oil, and vitamin and mineral premix. The control diet (BG0) did not contain supplemental oat β-glucan. The 50% oat β-glucan concentrate (Viscofiber, Cevena Bioproducts) was included in 2 diets to contain 3 and 6% of supplemental β-glucan (BG3 and BG6, respectively). Diets were formulated to be isocaloric and had an equal content of total carbohydrates and vitamin and mineral premix. Pigs were fed 1 of 3 diets for 7 d in a double 3 × 3 Latin square repeated over time to have 6 observations.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Ingredient and chemical composition of diets varying in β-glucan concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Diets</td>
</tr>
<tr>
<td></td>
<td>BG0</td>
</tr>
<tr>
<td>Ingredient, g/kg diet</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>722.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>220.0</td>
</tr>
<tr>
<td>Canola oil</td>
<td>18.0</td>
</tr>
<tr>
<td>Premix1</td>
<td>40.0</td>
</tr>
<tr>
<td>Oat BG2</td>
<td>—</td>
</tr>
<tr>
<td>Chemical composition, g/kg dry matter</td>
<td></td>
</tr>
<tr>
<td>β-glucan</td>
<td>6.2</td>
</tr>
<tr>
<td>Gross energy, MJ/kg</td>
<td>18.5</td>
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<tr>
<td>Starch</td>
<td>442.4</td>
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<tr>
<td>Soluble DF</td>
<td>42.0</td>
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<tr>
<td>Insoluble DF</td>
<td>176.9</td>
</tr>
<tr>
<td>Total DF</td>
<td>218.9</td>
</tr>
<tr>
<td>Carbohydrates (starch + total DF)</td>
<td>661.3</td>
</tr>
</tbody>
</table>

1 Provided the following per kg of diet: Na, 3.18 g as salt; Cl, 4.90 g as salt; Fe, 214 mg as ferrous sulfate; Zn, 107 mg as ZnCO3; Mn, 48.6 mg as MnSO4; Cu, 124 mg as CuSO4; K, 0.36 mg as KI; Se, 0.06 mg as Na2SeO3; vitamin B1, 0.002 mg; riboflavin, 5 mg; niacin, 22 mg; B-panthothenic acid, 15 mg; biotin, 0.09 mg; and choline, 260 mg.

2 50% oat β-glucan concentrate containing (1%): moisture, 6.58%; total carbohydrate, 80.70%; total dietary fiber, 70.64%; soluble dietary fiber, 57.27%; insoluble dietary fiber, 13.37%; β-glucan, 50.45%; starch, 7.17%; protein, 4.69%; lipids, 3.98; and ash, 4.06.

Sampling protocol. Blood was collected on d 7 of each period in heparinized and EDTA tubes from the carotid artery and portal vein. Blood was collected every 15 min from 15 to 60 min, then every 30 min up to 240 min, then every 60 min up to 480 min, and 600 min and 720 min postprandially. Blood flow was measured simultaneously (model TS 206; Transonic Systems) and was recorded continuously at each collection for 10 min using Windaq software (Dataq Instruments). After collection, catheters were flushed with 10 mL of 10 u/mL heparinized saline to prevent clotting and replace the fluid loss. Hematocrit values were measured immediately using the standard method. Blood was centrifuged at 1500 × g for 10 min and plasma was frozen at −20°C in heparinized tubes for glucose, insulin, SCFA, and C-peptide analyses and in EDTA tubes at −80°C for GLP-1 and GIP analyses.

Analytical procedures

Chemical analyses. Diets were analyzed in duplicate for dry matter by drying at 135°C in an airflow-type oven for 2 h [method 930.15 (14)] and gross energy by adiabatic bomb calorimetry (Model 5003, Ika-Werke GMBH & Co. KG). The NSP were analyzed by the modified procedure of Englyst et al. (15). Total starch, mixed linked β-glucan, and total dietary fiber were analyzed using a kit (Megazyme Microbial International Ireland) based on enzymatic analysis [methods 996.11, 995.16, and 985.29, respectively (14)].

Glucose and SCFA were analyzed in all plasma samples; however, insulin, C-peptide, GLP-1, and GIP were analyzed only in plasma collected until 480 min postprandially. Plasma was analyzed for glucose using a glucose oxidase kit (16) (Diagnostics Chemicals) and for SCFA (acetate, propionate, butyrate, valerate, caproate, isovalerate, and iso-butyrare) using the procedure described by Brehmert (17) using isocapronate as internal standard. Briefly, 400 μL of plasma was mixed with 50 μL internal standard and then deproteinized by adding 32 μL of 25% phosphoric acid, followed by incubating at 60°C for 30 min. The solution was centrifuged at 8000 × g for 30 min to remove the proteins and the supernatant was analyzed for SCFA using GC.

Insulin was analyzed by RIA using a porcine insulin kit (Linco Research; intra-assay CV = 6.4 and inter-assay CV = 9.9). The C-peptide was analyzed by RIA using a porcine C-peptide kit (Linco Research; intra-assay CV = 9.7 and inter-assay CV = 5.8). The GLP-1 was quantified using a double antibody RIA after extraction with alcohol (Linco Research; intra-assay CV = 11.9 and inter-assay CV = 4.4) and combined extraction and assay recoveries of cold spiked kit quality control was 70.2 ± 5.9%. The GIP was analyzed by RIA (Bachem Americas; intra-assay CV = 7.7 and inter-assay CV = 15.2). For GIP analysis, plasma was thawed at 4°C and 85 μg of aprotinin (Roche Diagnostics) in 25 μL deionized water was added to each mL of plasma during thawing.

Calculations. Net nutrient flux and apparent hormone production were calculated from plasma portal-arterial differences and plasma flow measurements using the formula

\[
q = \frac{(C_p - C_a) F (dt)}{Q}
\]

where \(q\) is the amount of nutrient absorbed or hormone produced within time period \(dt\), \(C_p\) and \(C_a\) are the concentration of nutrient or hormone in portal and arterial plasma, respectively, and \(F\) is the plasma flow in the portal vein. Plasma flow rate was calculated from blood flow rate using the following equation: plasma flow = blood flow × (1 − (hematocrit/100)). Cumulative glucose flux can be calculated subsequently using the formula

\[
Q = \sum_{i=0}^{n} q_i
\]

where \(Q\) is the amount of nutrient absorbed or hormone produced from time \(t_0\) to \(t_1\). The term net glucose and SCFA flux is used for net portal
and diet included period within square and pig as random effects and diet, time, production were analyzed as repeated measures. The statistical model and hormone data and net nutrient flux and apparent hormone adjusted for period, pig, and diet effects (20).

REG procedure of SAS with predicted values of the dependent variable at 12 time points using the weighted linear regression analysis using the glucose and portal concentrations of insulin and incretins were analyzed i.e. PC 1 and PC 2. Subsequently, specific relationships between portal glucose and portal concentrations of insulin and incretins were analyzed at 12 time points using the weighted linear regression analysis using the REG procedure of SAS with predicted values of the dependent variable adjusted for period, pig, and diet effects (20).

Results

Diet. Gross energy was similar for the 3 diets (Table 1). The NSF replaced starch in the BG-supplemented diets, similar to the approach reported by other groups (21,22). Total dietary fiber (DF) and NSF were higher in BG6 and BG3 than BG0, with mainly an increase in soluble DF and NSF (Table 1 and Supplemental Table 1, respectively), whereas starch was higher in BG0 than in BG3 and BG6. The dietary β-glucan content was slightly higher than expected, because the rest of the diet contained some β-glucan, likely from wheat.

Blood flow. Portal blood flow was 0.86 L/min before feeding and increased (P < 0.001) to a maximum of 1.33 L/min at 90 min postprandial; portal flow returned to the basal prefeeding value at 300 min (data not shown). Mean blood flow was 1.17 L/min, which converts to 28.3 mL/(kg body weight-min), and was not influenced by diet.

Glucose kinetics. Portal glucose at the time of feeding tended to be higher (P < 0.10) for pigs fed BG0 compared with pigs fed BG3 and BG6 and increased (P < 0.05) to a maximum at 45 min postprandial (Supplemental Fig. 1A). Portal glucose was lower (P < 0.05) postprandially in pigs fed BG3 and BG6 than BG0 by 19 and 28%, 7 and 17%, and 8 and 14% at 15, 30, and 45 min, respectively. Net glucose flux increased (P < 0.05) immediately postprandially and peaked at 45 min at a concentration that was less (P < 0.05) in pigs fed BG6 than pigs fed BG0 and BG3 (Fig. 1). Total glucose flux during the first hour was 22 and 51% lower (P < 0.05), respectively, for pigs fed BG3 and BG6 than for pigs fed BG0 (Fig. 2A). After adjustments for the different starch content among diets (Fig. 2B), treatment differences in total glucose flux in the first hour after feeding β-glucan diets were maintained, indicating that differences in the dietary starch content of diets were not responsible for the observed glucose flux effects.

Insulin responses. At 30 min postprandial, portal insulin peaked for all pigs and did not differ among diets. At 90 min postprandial, portal insulin was 60% lower (P < 0.05) for pigs fed BG6 than for pigs fed BG0 (Supplemental Fig. 2A). Portal C-peptide followed the same trend as insulin and was lowest (P < 0.05) for pigs fed BG6 at 90 min postprandial (Supplemental Fig. 2B). Similar to portal concentrations, the apparent production of insulin was less (P < 0.05) at 90 and 120 min postprandial in pigs fed BG6 than in pigs fed BG0 (Fig. 3A). Similarly, the apparent production of C-peptide was less (P < 0.05) in pigs fed BG6 and BG3 than in pigs fed BG0 at 90 min and was less (P < 0.05) only in pigs fed BG6 at 120 min postprandial (Fig. 3B). Mean arterial insulin and C-peptide from 0 to 480 min was not affected by diet. Pigs fed BG6 tended to have lower (P < 0.10) mean portal C-peptide than pigs fed BG0 and BG3. Mean apparent production of C-peptide tended to be lower (P < 0.10) in pigs fed BG6 than BG0 without affecting the apparent production of insulin (Supplemental Table 2).

Incretins. Both arterial and portal GIP were affected by time (P < 0.001), increased postprandially, and reached a maximum that was lower (P < 0.05) for pigs fed BG3 and BG6 than for pigs fed BG0 during the early postprandial phase (Fig. 4A).
Portal GLP-1 was affected by time \((P < 0.05)\); pigs fed BG6 had a lower \((P < 0.05)\) portal GLP-1 at 90, 120, and 180 min postprandial than pigs fed BG0 and BG3 (Fig. 4B). Pigs fed BG3 and BG6 had a lower \((P < 0.05)\) mean arterial GIP, whereas pigs fed BG6 tended to have lower \((P < 0.10)\) mean portal GIP and apparent production of GIP (Supplemental Table 2). Similarly, mean portal GLP-1 was lower in pigs fed BG6 than in pigs fed BG0 and BG3 without an effect on arterial GLP-1 and apparent production of GLP-1 (Supplemental Table 2).

**SCFA.** Pigs fed BG6 had higher \((P < 0.05)\) portal propionate and butyrate and tended to have higher \((P < 0.10)\) total SCFA and acetate than pigs fed BG0 (Supplemental Table 2 and Supplemental Fig. 1B). Pigs fed BG6 had a higher \((P < 0.05)\) net flux of propionate and butyrate than pigs fed BG0 and BG3 (Supplemental Table 1).

**PC and regression analyses.** The loading plot (Fig. 5) showed portal glucose, insulin, C-peptide, GLP-1, GIP, and SCFA as affected by PC 1 and PC 2 and correlations among these variables. This loading plot indicated 3 clusters: insulin positively affected by PC 1 and negatively by PC 2, glucose and incretin positively affected by both PC 1 and PC 2, and SCFA positively affected by PC 2 and negatively by PC 1. The latter was negatively correlated to the other 2 clusters (angles between arrows was >90°). The relations of portal glucose with portal insulin \((R^2 = 0.70; P < 0.001)\), C-peptide \((R^2 = 0.78; P < 0.001)\), GIP \((R^2 = 0.81; P < 0.001)\), and GLP-1 \((R^2 = 0.88; P < 0.001)\) indicated positive and significant relations (Supplemental Fig. 3A–D, respectively). However, portal SCFA was strongly and negatively related to portal glucose \((R^2 = 0.52; P < 0.01)\) and portal GLP-1 \((R^2 = 0.54; P < 0.001)\) (Supplemental Fig. 3E,F, respectively).

**Discussion**

Dietary oat β-glucan lowers glucose and insulin responses and thus affects the entero-insular axis and also stimulates fermentation. The association of incretins to these responses is poorly understood. To our knowledge, the present study provides novel evidence that the incretins are associated with the reduced net glucose flux and insulin secretion caused by oat β-glucan in a dose-dependent manner.

**Porto-arterial catheterization model.** Oat is considered a functional food, because it is linked to lower blood cholesterol (23) and glucose (24). However, effects on lowering glucose and insulin were not consistent among short-term studies (3,25,26). Studies in humans with collected peripheral blood (4,25) did not allow the quantification of the association between kinetics of nutrient absorption and insulin and incretin secretion. Hence,
the porcine porto-arterial catheterization model was used following dietary supplementation of oat β-glucan. Portal blood allows quantitative measurements of absorbed nutrients, albeit following epithelial utilization, whereas arterial blood represents the basal concentration of nutrients reaching the intestine; their difference is the qualitative nutrient flux (27). Simultaneous blood flow measurements allowed quantification of the net nutrient flux in long-term studies (13). Interestingly, the same biological mechanism applies for insulin and incretins, because both are drained into the portal vein (28).

**Kinetics of glucose absorption.** In the present study, oat β-glucan altered the kinetics of glucose absorption. Six-percent dietary oat β-glucan reduced the peak and total net glucose flux during first hour after feeding, similar to soluble NSP in pigs (18) and in normal (4,29), obese (3), and diabetic humans (2). The high viscosity of β-glucan may explain the lower glucose flux (30), because increased digesta viscosity reduces gastric emptying rate (31) and also slows digestion and absorption (32). Specifically, high digesta viscosity decreases enzyme diffusion (33) and stimulates the formation of the unstirred water layer (34) and thereby decreases the transport of glucose to the enterocyte. Thus, 6% dietary oat β-glucan lowers the glucose response in pigs fed a wheat-based diet, confirming that oat β-glucan is a functional food for diabetes management.

**Insulin responses.** Insulin responses are generally assessed with plasma insulin concentrations. However, C-peptide that is released in equimolar amounts from proinsulin in the pancreas is considered as a more reliable indicator of insulin release into systemic blood, because C-peptide has a lower hepatic extraction than insulin (35). The lower apparent production of C-peptide in pigs fed BG6 indicated clearly that insulin release was reduced even though portal insulin did not differ between pigs fed BG6 and BG0. This contrast emphasizes the importance of measuring C-peptide simultaneously with insulin (35). The lower pancreatic insulin response in pigs fed BG6 was probably due to reduced glucose absorption that directly reduced pancreatic insulin release (18), similar to observations in humans after eating β-glucan–enriched breads (3) or beverages (29). The glucose and insulin responses in pigs fed BG6 but not BG3 highlighted the importance of dose-dependent responses similar to those observed in humans (2,30).

**Incretins.** The incretins GIP and GLP-1 are released from enteroendocrine K and L cells, respectively, stimulate glucose-induced insulin secretion (36) and are thus important in glucose homeostasis (28). Carbohydrate intake and intraluminal glucose stimulate incretin release (37). The lower apparent production of GIP and GLP-1 in pigs fed BG6 is thus in agreement with other viscous NSP decreasing the early postprandial GIP flux (29,38,39) and subsequent lower insulin release (40). In contrast, fermentable NSP increased secretion of GLP-1 in intestinal mucosa and blood (12,41) that is required for stimulation for GLP-1 incretin secretion (8). The inverse association between portal SCFA and GLP-1 thus challenge the paradigm that increased SCFA stimulates GLP-1 secretion.

**SCFA.** The most interesting change in net SCFA flux was observed in the late postprandial phase that was achieved via the 12-h collection. The feeding of a diet containing soluble fiber increased production of SCFA in the gastrointestinal tract and thereby increased portal propionate and butyrate absorption and net SCFA flux (22). The disappearance of β-glucan by the end of the large intestine (data not shown) indicated that β-glucan is fermented completely in the intestine. The increased butyrate flux is important, because butyrate is a major source of energy to colonic mucosa (43) and an important link to gut health via stimulation of cell proliferation (44), promotion of apoptosis, and prevention of colon cancer (11,45). Propionate acts as a substrate for hepatic gluconeogenesis and inhibits cholesterol synthesis in the liver (45). Dietary oat β-glucan may thus improve intestinal and metabolic health.

**Glucose link to hormones.** The PC analysis demonstrated strong correlations among portal glucose and insulin, incretins, and SCFA, with SCFA inversely related to all others. Further specific regression analyses between portal glucose and portal insulin, C-peptide, GIP, and GLP-1 indicated that glucose in portal blood acts as a stimulant for the secretion of incretins and insulin. Glucose sensors in the arterial blood supplying the β-cells of Islets of Langerhans and in the portal vein may stimulate the insulin secretion (46).

This study demonstrated that 6% oat β-glucan lowered peak net glucose flux in pigs concurrently with an attenuated incretin and insulin response, thereby explaining some of the underlying physiology and metabolism of dietary NSP. The PC analysis of portal variables indicated strong associations among glucose and incretins, providing further evidence that portal glucose acts as a strong stimulus for incretin release. Incretins in turn affect insulin release and thereby enforce the existence of the enteroinsular axis or cross-talk between intestine and pancreas to control blood glucose. Finally, increased fermentation and net flux of propionate and butyrate provided solid evidence that oat β-glucan are fermented and are beneficial to manage human metabolic diseases and gut health.

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**Literature Cited**

Supplemental Material can be found at: