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Effects of the soluble fiber complex PolyGlycopleX® (PGX®) on glycemic control, insulin secretion, and GLP-1 levels in Zucker diabetic rats

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ABSTRACT

Aims: The effects of the novel water soluble, viscous fiber complex PolyGlycopleX® [(α-D-glucurono-α-D-manno-β-D-manno-β-D-gluco), (α-L-gulurono-β-D mannurono), β-D-gluco-β-D-mannan (PGX®)] on body weight, food consumption, glucose, insulin, and glucagon-like peptide (GLP-1) levels were determined in Zucker diabetic rats (ZDFs). Such fibers are thought to improve glycemic control through increased GLP-1 induced insulin secretion.
Main methods: ZDFs were treated 12 weeks with normal rodent chow supplemented with cellulose (control, inert fiber), inulin or PGX® at 5% wt/wt and effects on body weight, glycemic control, and GLP-1 determined.
Key findings: In the fed state, PGX® reduced blood glucose compared to the other groups from week 5 until study termination while insulin was significantly elevated when measured at week 9, suggesting an insulin secretagogue effect. Fasting blood glucose was similar among groups until 7–8 weeks when levels began to climb with a modest reduction caused by PGX®. An oral glucose tolerance test in fasted animals (week 11) showed no change in insulin sensitivity scores among diets, suggesting an insulinotropic effect for PGX® rather than increased insulin sensitivity. PGX® increased plasma levels of GLP-1, while HbA_{1c} was markedly reduced by PGX®. Body weights were not changed despite a significant reduction in food consumption induced by PGX® up to week 8 when the PGX®-treated group showed an increase in body weight despite a continued reduction in food consumption.
Significance: PGX® improved glycemic control and reduced protein glycation, most likely due to the insulin secretagogue effects of increased GLP-1.

Introduction

Dietary, non-digestible fiber can alleviate many of the deleterious effects of the metabolic syndrome including improved glycemic control, lipid reduction and in some cases, body weight reduction (Grunberger et al., 2006; Howarth et al., 2001; Wilson et al., 1984; Wiekert and Pfeiffer, 2008). The effect of dietary fiber varies according to fiber type with water soluble, non-digestible fibers that form viscous gels in the gut usually being more efficacious (Blackwood et al., 2000; Jenkins et al., 2000; Panahi et al., 2007). Many dietary fibers increase secretion of intestinal L-cell derived glucagon-like peptide-1 (GLP-1) that will slow gastric-emptying, reduce glucagon secretion, and act as an insulin secretagogue (Baggio and Drucker, 2007; Massimino et al., 1998; Reimer and McBurney, 1996). Therefore some dietary fibers may not only lower lipids (Anderson et al., 1999), but may improve glycemic control either through increases in insulin sensitivity or acting as insulin secretagogues (Kim and Egan, 2008). Recent studies point to the potential importance of GLP-1 in salvaging pancreatic insulin secreting cells (Kim et al., 2007). Furthermore, increased peptide YY (PYY), an anorexigenic peptide also released from intestinal L-cells (Gee and Johnson, 2005), and reduced glucose-dependent insulinotropic polypeptide (GIP) (Morgan et al., 1990) levels have been reported for several dietary fibers. Expression of ghrelin, an orexigenic peptide, can be down-regulated by soluble fibers which would also be expected to contribute to reduced sensations of hunger and delayed gastric emptying (Wang et al., 2007). Dipetidyl peptidase IV (DPPIV) inhibitors such as sitagliptin (Januvia™) inhibit the degradation of GLP-1 in blood and have

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demonstrated increased insulin sensitivity in some animal models (Pospisilik et al., 2002), but generally show increased insulin levels along with improved glycemic control with little effect on body weight (Nonaka et al., 2007; Xerili and Pyon).

In addition to effects on nutrient trapping and satiety, some fibers such as dietary fructans, inulin and other oligosaccharides, may also be subjected to colonic fermentation with subsequent release of short chain fatty acids (SCFA) that can affect glucose and lipid metabolism (Daubioul et al., 2002; Wiekert and Pfeifer, 2008). The major SCFAs butyrate, propionate, and acetate are considered to be responsible for many of the beneficial effects of these dietary fibers (Ramberg et al., 2005; Daubioul et al., 2002).

With the potential effects of viscous, water soluble fiber on body weight loss and improved glycemic control in mind, we examined the effects of the novel water soluble and highly viscous polysaccharide complex (as described in detail by Abdelhameed et al. (2010) and Harding et al. (2010)) PolyGlycopleX® (α-D-glucurono-α-D-manno-β-D-manno-β-D-gluco), (α-L-gulurono-β-D mannurono), β-D-gluco-β-D-mannan marketed under the trade name of (PGX®), InovoBiologic Inc, Calgary, Alberta, Canada). Primary outcomes measures included body weight, food intake, and glycemic and insulinemic control. These were related to the secretion of gut peptides and density of insulin-secreting pancreatic β-cells in a model of type 2 diabetes. The study was performed using Zucker diabetic rats (ZDFs) and PGX® was compared to the inert fiber cellulose or to the water soluble, non-digestible prebiotic fiber inulin which is not as viscous as PGX®, but is probably more readily fermented. PGX® is manufactured from glucomannan, xanthan gum and sodium alginate using a proprietary ratio of starting materials and process (EnviroSimplex®). The resulting polysaccharide exceeds the viscosity of the constituent ingredients as well as other currently known viscous dietary fibers. This fiber is well tolerated in rodents with a maximal tested dose being 5% wgt/wgt in diet (Matulka et al., 2009) and man (Carabin et al., 2007).

Materials and methods

Animals

Male ZDFs (ZDF/Crl-Lepr$^{fa/fa}$) were used as they are considered to be an excellent model of adult-onset obesity with co-morbid type 2 diabetes. ZDFs were also used in this study because of their responsiveness in terms of glucose homeostasis to treatments such as DPPIV inhibitors that increase insulin secretion secondary to increased GLP-1 secretion in man and experimental animals (Thomas et al., 2008; Nonaka et al., 2008). Dietary fibers (Daubioul et al., 2002; Kok et al., 1998) also have been shown to improve glucose homeostasis and reduce adiposity in ZDFs. Thirty male ZDF/Crl-Lepr$^{fa/fa}$ rats were obtained from Charles River (Kingston, NY). The rats were 5 weeks of age upon arrival. The animals were housed singly in suspended wire mesh cages which conformed to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals, DHEW (NIH)*. All studies were approved by the Eurofins institutional animal use and care committee. The animals were conditioned for 1 week, so the studies started at six weeks of age. The animals had access to water and food *ad libitum*. No human subjects or material were used in this study.

Test articles

PGX® test article was shipped to Research Diets (New Brunswick, N.J.) for incorporation into a basic rat chow (D11724); alternate diets incorporated other dietary fiber forms sourced through Research Diets as enumerated below (Table 1). All diets were formulated to be as isoenergetic as possible given the different energy contribution of each fiber source (PGX® and inulin diets provided 3.98 kcal/g and cellulose provided 3.90 kcal/g). After habituation (1 week), rats were randomly assigned to one of three groups (n = 10 for each group) on the basis of initial blood glucose and body weight; each group was given one type of chow (containing either: PGX®, cellulose, or inulin), all at 5% wt/wt of the diet) for a total of 12 weeks. Cellulose was selected as the basic reference fiber that is insoluble and is non-fermentable so it is considered to be an inert reference compound for comparing with other fiber types (Daubioul et al., 2002; Anderson et al., 1994). Inulin is water soluble and non-digestible and some papers have shown efficacy for such prebiotic fibers in lipid reduction and glycemic control, but the results are variable and depend on the degree of prebiotic enrichment and production of short chain fatty acids via fermentation in the colon (Rozan et al., 2008). We used Orafti's Raftiline HP which is a chicory-derived, inulin-type fructan composed of a mixture of oligo- and polysaccharides composed of fructose units linked together by β(2-1) linkages each with terminal glucose units. PGX® is manufactured by a proprietary process from konjac (glucomannan), sodium alginate and xanthan gum to produce a unique complex. The proprietary ratio and manufacturing process produces a product that gram for gram has superior properties compared to other viscous fibers and a viscosity that develops over time (20–30 min). Though complex formation takes place at the secondary and tertiary levels (networking and junction zones), the primary structures of the natural polysaccharides remain unchanged (Harding et al., 2010; Abdelhameed et al., 2010) and PGX® is approved as a Natural Health Product in Canada by Health Canada's NHPD (Natural Health Product Directorate). PGX® is manufactured under Good Manufacturing Procedures (GMP) guidelines making each lot uniform. These polysaccharides form strong interactions leading to a level of viscosity that is higher than any currently known individual polysaccharide. The final product is a novel, soluble, highly viscous polysaccharide complex (functional fiber). Because of the high viscosity of PGX® we used 5% wgt/wgt to compare against the less viscous inulin which is generally efficacious at >10% wgt/wgt (Daubioul et al., 2002; Parnell and Reimer, 2010). A 5% wgt/wgt fiber diet is historically on the low side as most other dietary fibers tested are efficacious at 10–30% (Reimer and McBurney, 1996; Maurer et al., 2009), but because of the high viscosity of PGX®, our objective was to test if the lower dose would be efficacious.

Table 1
Diet composition for the three diets used in the Zucker diabetic rat study.

	PGX® fiber	Insoluble fiber	Soluble, non-viscous fiber
Casein	20%	20%	20%
Methionine	0.3%	0.3%	0.3%
Corn starch	50%	50%	50%
Maltodextrin	15%	15%	15%
Fiber	5% PGX®	5% Cellulose	5% Inulin
Corn oil	5%	5%	5%
Salt/mineral mix	3.5%	3.5%	3.5%
Vitamin mix	1%	1%	1%
Choline bitartrate	0.2%	0.2%	0.2%
Dye	0.1%	0.1%	0.1%

PGX® test article was shipped to Research Diets for incorporation into rat chow (D11724); alternate diets incorporated other fiber forms sourced through Research Diets as enumerated above.

Experimental design

Basic monitoring procedures were conducted throughout the 12 week study (i.e., thrice weekly measurement of food intake; weekly measurement of body weight and collection of blood samples for glucose at study initiation and thereafter starting at 4 weeks after study initiation). Blood samples, other than those for the oral glucose tolerance test (OGTT), were taken from the retro-orbital plexus while being lightly anesthetized with isofluorane. Serum insulin (along

with glucose) was measured at 9 weeks while the animals were non-fasted. The non-fasted state was added due to the observation that greater effects of PGX® on stabilizing glucose levels were seen when the fiber was physically present in the gastrointestinal tract; likely due to the fact that the ZDFs eat continuously both day and night. For all studies, the blood samples used for glucose were taken at approximately the same time of the day at mid-morning which included measurements taken either in the fed state or following 4 h of fasting. Serum insulin was measured in non-fasted animals at week 9 and Homeostasis Model Assessment (HOMA) scores were calculated as mg glucose × insulin (U mg/mL^2). This is a reliable method of showing changes in insulin resistance with lower HOMA scores representing reductions in peripheral insulin resistance. Blood glucose was measured using a Bayer Ascensia Elite Glucometer (Bayer Health Care, Tarrytown, NY) and insulin was measured using an ELISA (Anilytics, Gaithersburg, MD). HOMA scores were calculated using the values of the serum and blood glucose measurements for non-fasted glucose and insulin measurements as mg glucose × insulin (U mg/mL^2) at 9 weeks. At this time a glucose excursion study was done using 1 g/kg oral glucose, although unfortunately many of the glucose values after the glucose load were above the maximal level of our glucometer so these data were not shown. At eleven weeks, a 4 h fasted OGTT was done, serum insulin measured, and HOMA scores calculated using the baseline values of insulin and glucose. We also calculated the composite insulin sensitivity index (CISI) scores for the OGTT studies using the following formula: $CISI = \frac{1000}{\sqrt{(Gluc_{base} \times Ins_{base}) \times (Gluc_{mean} \times Ins_{mean})}}$. This score takes into account glucose excursion and area under the curve with a higher score showing improved insulin sensitivity. The OGTT was induced by oral 1 g/kg glucose. Blood samples were taken at fasting, 30, 60, 90, and 120 min after the glucose load and were analyzed for glucose and insulin levels. Blood samples for glucose and insulin for OGTTs or glucose excursion studies were obtained via tail bleeds.

In addition to effects on glycemic control, we were interested in the effects of PGX® on gut hormones that play an important role in insulin secretion, gut motility, food intake and energy homeostasis. At 12 weeks (allowing recovery from OGTT) an overnight fasted blood sample was drawn and then rats were given glucose orally at 1 g/kg and an hour later gut hormone levels were determined in blood. A multiplex assay (Rat Gut Hormone Milliplex, Millipore, Burlington, MA) was used to simultaneously measure 8 rat gut hormones: GLP-1 (active), GIP (total), PYY (total), ghrelin (active), PP, leptin, insulin and amylin (total). A cardiac blood sample was collected into a syringe containing a DPPIV inhibitor (diprotin A), serine protease inhibitors (AEBSF, Pefabloc SC)), and Sigma Protease inhibitor cocktail (Sigma, St. Louis, MO)]. We removed serum and assayed immediately or stored at −80 °C. For normal samples, no dilution was required. If dilution was necessary, we used Serum Matrix to dilute serum samples prior to assay.

From the terminal cardiac blood sample, HbA_{1c} was also measured in order to show degree of glycation. We measured HbA_{1c} using a Bayer DCA2000+ (Bayer Diagnostics, Tarrytown, NY). This is thought to be a better indicator of true glycemic control as it is a biomarker for protein glycation that correlates with tissue glycation and damage, especially in the kidneys. Diabetic nephropathy is a common problem that individuals with diabetes must face and the damage can be severe and irreversible along with microvascular damage (Nonaka et al., 2007).

At study termination, the pancreas was fixed in 10% neutral buffered formalin. The pancreas was transferred to 70% ethanol after 24 h. Tissues were processed and embedded in paraffin. The pancreas was serial sectioned twice at approximately 5 μm, and were either stained with hematoxylin and eosin (H&E), or immunohistochemically stained with a mouse antibody against rat insulin (1:300, Cell Signaling Technology, Danvers, MA, rabbit anti-rat insulin). Immunohistochemistry was performed according to the following procedure. An isotype control antibody (normal rabbit IgG, R&D Systems, Minneapolis, MN) was used to assess the level of non-specific and background staining. Following deparaffinization, antigen retrieval was performed using Declere® solution (Cell Marque™ Corporation, Rocklin, CA) for 15 min at 120 °C followed by 5 min room temperature in hot Declere® solution. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in deionized water for 10 min. Slides were incubated for 20 min in 5% normal goat serum. The slides were incubated with the primary antibody for 60 min followed by incubation for 30 min in biotinylated goat anti-rabbit antibody. The slides were then incubated in ABC Elite reagent (Vector, Burlingame, CA) for 30 min. Finally, specimens were incubated in diaminobenzidine for 5 min followed by hematoxylin counterstaining.

The pancreatic slides originally stained with H&E were evaluated for morphologic changes related to those commonly observed in ZDFs and graded on a scale from 0 to 5. The percent of the islet area with insulin positive cells was measured on the pancreas slides immunohistochemically stained with anti-insulin antibody. This measurement was performed morphometrically. Ten islets per pancreas were manually outlined by a veterinary pathologist. Areas positive for insulin staining within these islets were similarly outlined and the percent of islet area positive for insulin was calculated using ImagePro® Plus imaging software.

For intestinal L-cell density enumeration, all intestines were removed and fixed in formalin. The colons were transferred to 70% ethanol after 24 h. The colons were trimmed into three regions, one immediately distal to the ileocecocolic junction, and two regions 2 cm distal to the previous one. After embedding in paraffin, all 3 regions were immunohistochemically stained for GLP-1 expression (1:500, Phoenix Pharmaceuticals, Inc., Belmont, CA). Immunohistochemistry was performed according to the following procedure. An isotype control antibody (Negative Control Rabbit Immunoglobulin Fraction, DakoCytomation, Carpinteria, CA) was used to assess the level of non-specific and background staining. Following deparaffinization, antigen retrieval was performed using Declere® solution (Cell Marque™ Corporation, Rocklin, CA) for 15 min at 100 °C followed by 5 min room temperature in hot Declere® solution. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in deionized water for 10 min. Slides were incubated for 20 min in 10% normal goat serum. The slides were incubated with the primary antibody for 60 min followed by incubation for 30 min in 1:500 biotinylated goat anti-rabbit antibody. The slides were then incubated in ABC Elite reagent (Vector, Burlingame, CA) for 30 min. Finally, specimens were incubated in diaminobenzidine for 5 min followed by hematoxylin counterstaining. The slides were counterstained using a diluted 50:50 mixture of distilled water and Hematoxylin 7211 (Richard-Allan Scientific Kalamazoo, MI) for 30 s. The slides were washed in running tap water for one minute, placed in bluing solution (Richard-Allan Scientific Kalamazoo, MI) for one minute, and rinsed again in running tap water for one minute. The slides were then dehydrated through three changes of 100% ethanol at one minute each, and cleared in three changes of xylene at one minute each. GLP-1 positive epithelial cells were counted in the two most proximal sections of the colon. The area of epithelium in these sections was determined by morphometric evaluation using the IPP software system. All slides were examined by a board certified veterinary pathologist with findings ranked as the number of GLP-1 epithelial positive cells per mm^2 of epithelium.

Statistics

Data collected at multiple times were analyzed by two-way repeated measures analysis of variance (ANOVA) and *post hoc* comparisons using Bonferroni's multiple comparison test (MCT). Parameters measured only at one time were analyzed by one-way ANOVA followed by

post hoc comparisons using Dunnett's MCT. Non-interval data (e.g., histology scores) were analyzed by Kruskal Wallis test and Dunn's MCT. Significance was set at $p<0.05$.

Results

The effects of PGX®, cellulose or inulin on body weight and food consumption are shown on Fig. 1A and B. No significant differences in the rate of weight gain were seen for any of the groups until approximately 8 weeks into the protocol when the rate of increase was significantly enhanced by PGX® up to the end of 12 weeks. Interestingly, PGX® reduced food consumption (Fig. 1B) throughout the procedure with a significant reduction seen at several time points compared to the other groups. The drop in food consumption seen at approximately 11 weeks for all groups is due to the stress of two OGTTs and this drop was similar for all experimental groups.

In fasted animals, blood glucose was not elevated for any groups above normal levels until the 8th week (Fig. 2A). While no significant differences were seen at this point, PGX®-treated animals showed a trend towards lower glucose. In non-fasted animals, blood glucose in cellulose- and inulin-treated animals began to rise starting between weeks 4 and 5. The PGX®-treated group showed glucose levels that were significantly lower compared to cellulose- or inulin-treated groups at 5 weeks and later according to 2-way repeated measures ANOVA (Fig. 2B).

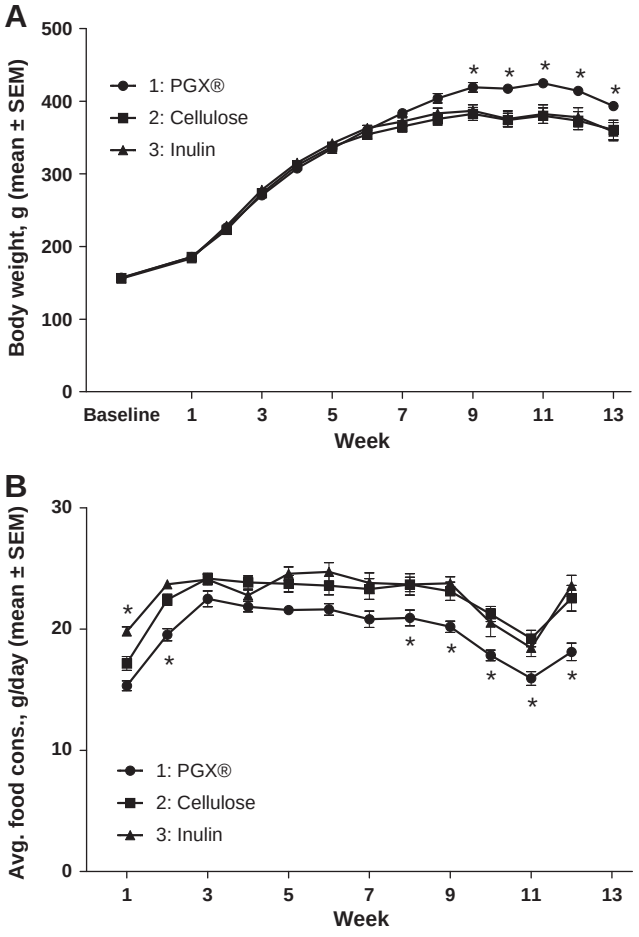

Fig. 1. The effect of PGX®, cellulose or inulin on body weight (A) and food consumption (B) in ZDF rats. Values are mean ± SEM, n = 10. Body weight increased over time with no differences between the groups noted until after 8 weeks. After 8 weeks, PGX®-treated animals showed a significant increase in body weight. * denotes significance ($p<0.05$) from the cellulose-treated animals.

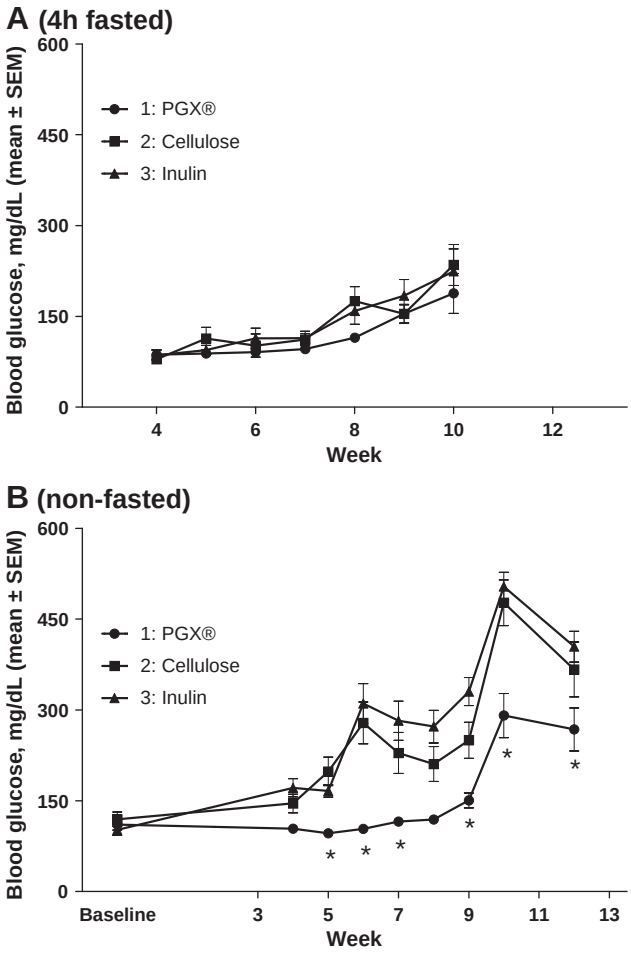

Fig. 2. Effect of PGX® on blood glucose throughout the study. Values are mean ± SEM, n = 10. There were no significant differences among the treatment groups in the fasted state (2A), although PGX® did show a slight tendency to reduce glucose throughout the study. In non-fasted animals (2B), there was a clear reduction in blood glucose by PGX® compared to the other two fibers. * denotes significance ($p<0.05$) from the cellulose-treated animals. Blood was collected from the retro-orbital plexus at mid-morning following withdrawal of food for 4 h (2A) or with no food withdrawn (2B).

Insulin levels were measured in non-fasted animals at 9 weeks and in fasted animals at the baseline points before OGTT performance at 11 weeks. In non-fasted animals, serum insulin was significantly higher and blood glucose levels were significantly lower in PGX®-treated animals compared to cellulose- or inulin-treated animals. Serum insulin values were 13.8 ± 0.7, 9.8 ± 0.8 and 8.7 ± 0.7 (mean ± SEM) for the 9 week non-fasted group for PGX®, cellulose and inulin respectively and 11.2 ± 1.2, 8.4 ± 0.8 and 7.7 ± 0.8 for the 11 week fasted group. The HOMA scores for the non-fasted animals (Table 2) were similar for all groups with a slight trend for the PGX®-treated

Table 2
The effect of cellulose, inulin, or PGX on HOMA scores and CISI[a] scores as well as the percent of pancreatic insulin immunoreactive area.

	PGX	Cellulose	Inulin
HOMA score 9 weeks fasted (U mg/mL^2)	196 ± 24	171 ± 17	149 ± 15
HOMA score 11 weeks non-fasted (U mg/mL^2)	386 ± 41	336 ± 29	348 ± 29
CISI score[a] 9 weeks fasted	0.44 ± 0.05	0.49 ± 0.04	0.56 ± 0.04
Pancreatic percent insulin immunoreactive area (%)	33.2 ± 2.4	28.8 ± 1.2	29.9 ± 1.3

Data are expressed as the mean ± SEM, n = 10/group. No differences between any of the groups were seen for the variables shown below.

[a] $:CISI = \frac{1000}{\sqrt{(Gluc_{base} \times Ins_{base}) \times (Gluc_{mean} \times Ins_{mean})}}$.

group to have higher values which is consistent with the higher insulin levels. In fasted animals, a similar trend was observed before OGTTs determined at 11 weeks, but the differences in HOMA scores were not statistically significant (Table 2). Baseline blood glucose levels were similar for all groups in the fasted animals, although blood glucose showed a strong, but non-significant trend to be lower for the PGX®-treated group compared to cellulose- or inulin-treated animals. For the fasted animals, the OGTT data showed no significant difference in CISI scores (Table 2) between the different fiber-treated groups (see glucose excursion in Fig. 3). Overall, it appeared that PGX®-treated animals had greater glycemic control, particularly in non-fasted animals when the fiber was in the gut and this was correlated with higher insulin levels.

Measurements to determine the effect of PGX®, relative to the other two fibers, was done for several gut peptides at 12 weeks into the protocol. Our main interests were in the incretins GLP-1 and GIP, as well as PYY and ghrelin. The PGX® animals were fasted for 4 h and then gavaged with 1 mg/kg glucose orally and blood was withdrawn for measurement of these peptides. GLP-1 was significantly increased in the PGX®-treated animals compared to the other two fibers, while no differences were seen between cellulose- and inulin-treated animals (Fig. 4A). No increase in intestinal L-cell density was seen for the PGX®-treated group compared to the other groups so increased secretion from existing L-cells is likely (Fig. 4B). No changes in GLP-1 were seen for cellulose- versus inulin-treated groups even though L-cell numbers were significantly increased in the inulin group (Fig. 4B). No differences in PYY were seen for any group (data not shown), but interestingly, ghrelin was markedly and significantly increased by PGX® compared to cellulose- and inulin-treated groups (Fig. 5). No changes in leptin, amylin or PP were seen for any group in this study (data not shown).

For the histological and immunohistological studies of the pancreas, the density of immunoreactive insulin containing cells was slightly, but not significantly increased by PGX® (Table 2). The photomicrographs for the three groups are shown in Fig. 6 and were chosen to be as representative of the numerical scores shown in Table 2 as possible.

While all of the glucose and insulin measurements are of great interest and suggest improved glycemic control, it is important to show whether this reduces protein glycation as measured by HbA_{1c}. At the terminal bleed, blood samples were collected and analyzed for HbA_{1c} levels expressed as a percent of normal hemoglobin. As shown in Fig. 7, PGX® significantly reduced HbA_{1c} compared to both cellulose and inulin-treated groups.

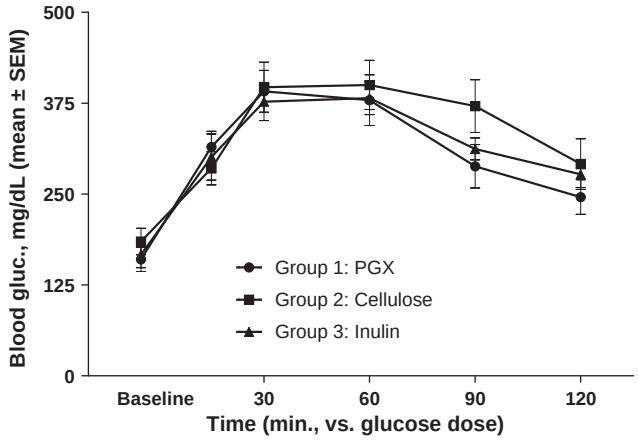

Fig. 3. Glucose and insulin concentrations during an oral glucose tolerance test given in animals fasted for 4 h. Values are mean ± SEM, n = 10. The test was performed at 11 weeks after initiating the 3 different diets. Blood glucose excursion values are shown at specified times after oral 1 g/kg glucose. For insulin, * denotes significance ($p<0.05$) from the cellulose-treated animals.

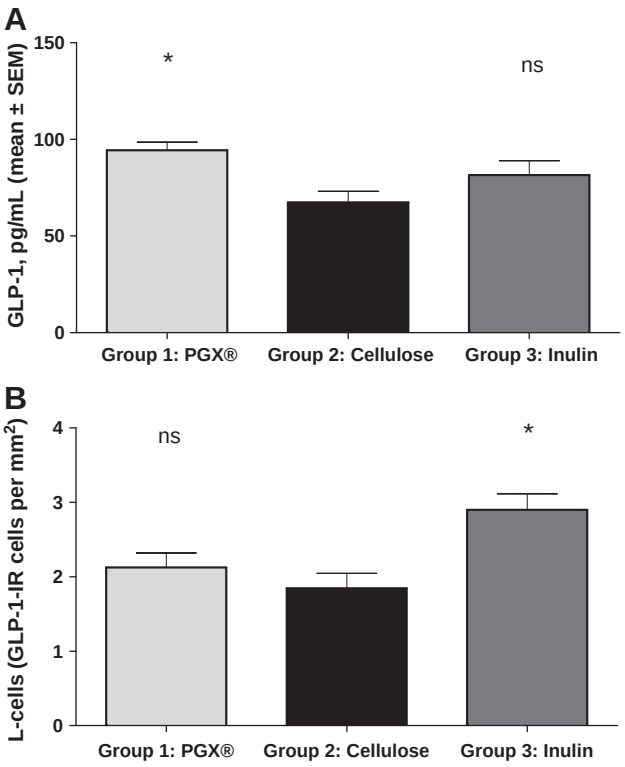

Fig. 4. Effect of dietary fibers on GLP-1 concentration in serum and L-cell density in the gut at week 12. Values are mean ± SEM, n = 10. GLP-1 was measured 1 h following an oral glucose load. PGX® significantly increased GLP-1 (A) compared to cellulose-treated animals (*, $p<0.05$) while inulin only slightly increased levels. L-cell numbers (B) were not different between the cellulose group and PGX® group, while a significant (* $p<0.05$) increase in L-cells were seen for inulin despite its lack of effect on plasma GLP-1.

Discussion

Dietary fiber, particularly water soluble and viscous fibers that are non-digestible have been shown variously to reduce body weight, food intake, nutrient absorption and hepatic and serum lipids including cholesterol and triglycerides (Grunberger et al., 2006; Howarth et al., 2001; Wilson et al., 1984; Wiekert and Pfeiffer, 2008; Panahi et al., 2007; Blackwood et al., 2000; Jenkins et al., 2000; Massimino et al., 1998; Anderson et al., 1999). In addition, improved glycemic control and often increased insulin sensitivity are observed (Panahi et al., 2007; Massimino et al., 1998; Anderson et al., 1999). Results vary depending on fiber type and the model (including man)

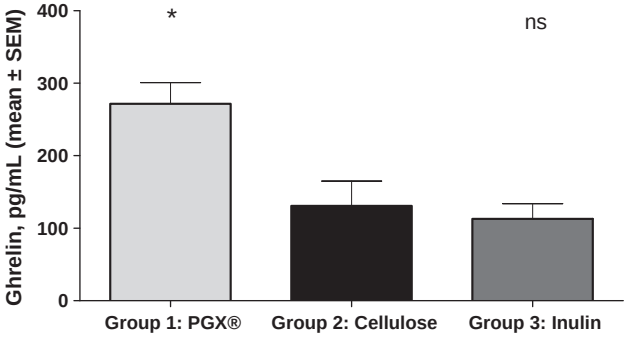

Fig. 5. The effect of dietary fiber on serum ghrelin levels at week 12. Ghrelin was measured 1 h following an oral glucose load. Values are mean ± SEM, n = 10. PGX®-treated animals showed a significantly increased ghrelin level compared to cellulose (*, $p<0.05$) while inulin had a slight, but non-significantly higher ghrelin levels compared to the cellulose groups.

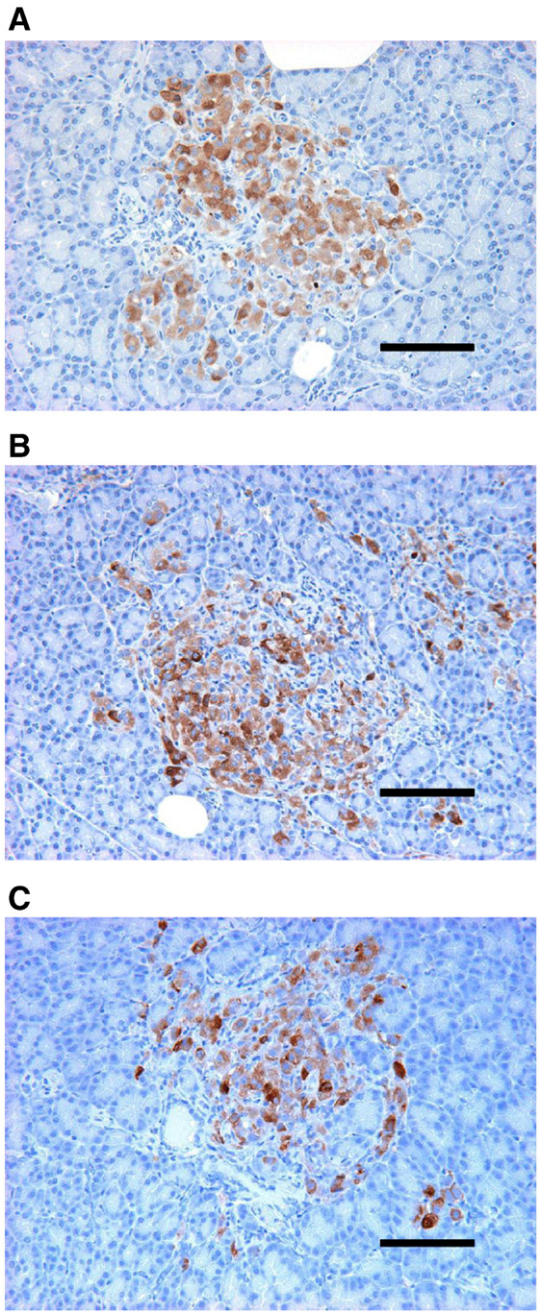

Fig. 6. Photomicrographs of pancreas from PGX (A), cellulose (B) and inulin (C) treated animals. The bars are 100 μm. The brown-stained tissue are positive for insulin-containing cells within the pancreas. While there were no significant differences between the groups, there was a tendency for increased density of insulin-stained cells in the PGX group. Photographs were selected to be as close to the densities reported in Table 2.

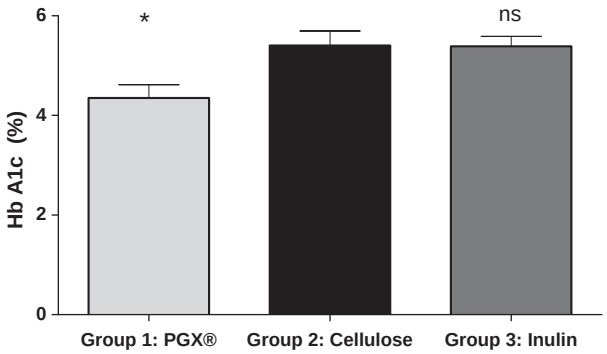

Fig. 7. Effect of dietary fibers on HbA_{1c} levels in blood at study termination. Values are mean ± SEM, n = 10. PGX®-treated animals showed a significant decrease in the percentage of glycated hemoglobin ($p<0.05$) compared to cellulose. Inulin had no effect on HbA_{1c} percentage.

used (Panahi et al., 2007; Jenkins et al., 2000; Morgan et al., 1990; Daubioul et al., 2002; Anderson et al., 1994; Viljanen et al., 2009).

The ability of some fibers to reduce body weight is thought to be due to increased satiety as well as a feeling of fullness (Stevens et al., 1987). In addition, anorexigenic peptides such as PYY are increased in some studies and ghrelin is reduced (Gee and Johnson, 2005; Morgan et al., 2002). Increased GLP-1 production can slow gastric emptying and possibly delay nutrient uptake (Massimino et al., 1998; Baggio and Drucker, 2007). Adsorption of nutrients by the fiber itself may also reduce body weight (Sandberg et al., 1994). It must be pointed out that not all studies with various water soluble, viscous fibers showed body weight reduction so some variability exists either due to model or perhaps to the specific fiber type (Stevens et al., 1987; Schneeman, 1985; Torsdottir et al., 1991; Nishina et al., 1991; Sugatani et al., 2008).

In the present study, despite consistent reductions in food intake in PGX®-treated animals, no significant reductions in body weight were observed up to 8 weeks and even increased body weight thereafter despite a continued reduction in food intake. The mechanism for this is presently unknown. It is possible that the animals reduced metabolic rate to compensate for the reduced food intake, although no overt lethargy was observed. Physical activity and basal metabolic rate changes do not necessarily correlate. A previous study using PGX® showed no change in activity patterns (functional observation battery) and no food aversion in Sprague Dawley rats (Matulka et al., 2009). A similar study published by Reimer and Russell (2008) showed a comparable effect with a prebiotic fiber in JCR:LA-cp (leptin receptor deficient) rats showing reduced caloric intake independent of any weight loss. This lack of effect on body weight with a long-chain prebiotic fiber largely fermented in the distal colon, was in contrast to other reports of weight loss with oligofructose, a shorter-chain prebiotic that is chiefly fermented in the cecum and proximal colon (Cani et al., 2005). The major site of occurrence and degree of fermentation of PGX® is currently unknown, but it is probably fermented in the colon to a lesser extent than inulin, although the effect of this on body weight in PGX® treated animals is unknown. Fermentation may have an effect on lipid metabolism and preliminary evidence from ongoing studies suggests reductions in cholesterol and triglycerides in serum. Furthermore, Reimer and Russell (2008) speculate that the prebiotic fibers may have distinct metabolic effects in monogenic obese models compared to diet-induced obesity and that the inulin-induced increase in GLP-1 seen in the JCR:LA-cp rat may in fact cause weight-loss in other obesity models that, contrary to JCR:LA-cp and ZDF rats, retain a normal hypothalamic–pituitary axis. A study directly measuring metabolic rate might be more revealing (Grover et al., 2003) as in this type of study metabolic rate is increased by thyromimetics without any changes in locomotive activity. While these studies were done in Sprague–Dawley rats, effects in ZDFs might be different.

The effect of increased ghrelin by PGX® may also have contributed to the lack of weight loss as its release, along with the concomitant release of growth hormone, may alter energy metabolism through a variety of mechanisms, including CNS involvement in favor of weight retention (Horvath et al., 2001; Halem et al., 2004). The metabolic effects of the ghrelin-growth hormone axis have been further outlined by Currie et al. (2005) where energy metabolism is significantly altered by ghrelin including changes in metabolic rate and respiratory

quotient. Preliminary studies being completed by us show a clear reduction in body weight (7% weight loss at 7 weeks into the procedure) along with florid diabetes in ZDFs. Preliminary data from these animals shows no change in adiposity with a significant increase in lean body mass as measured by DEXA (Dual energy X-ray absorptiometry).

Food intake was reduced and perhaps the increased ghrelin was a compensatory response. As reviewed by Murphy et al. (2006), ghrelin levels are lower in humans with higher body weights and increase after diet-induced weight loss which may describe our results. It is also important to acknowledge that the measurements of GLP-1 and ghrelin at a single time point (1 h post glucose gavage) are not adequate to fully characterize the response of these hormones to PGX®. Indeed, it would have been ideal to have a full compliment of fasted and postprandial measurements of the satiety hormones to appreciate the role of these hormones in regulating the changes we observed in body weight and food intake. Preliminary data from our lab show that over the course of a 90 min OGTT, the total AUC for ghrelin does not differ between PGX® and cellulose-fed animals.

Some dietary fibers improve glycemic control, usually through improved insulin sensitivity, although increased insulin secretion is also possible due to increased expression of proglucagon, the precursor of GLP-1. In our study, fasted glucose levels did not begin to rise until the end of the study and PGX® showed a strong tendency to reduce glucose at the later times. We saw much clearer hyperglycemia in non-fasted animals and PGX® significantly reduced glucose levels to nearly baseline values. Towards the end of the present study, insulin was significantly increased by PGX®, particularly in the non-fasted state (9 weeks), suggestive of an insulinotropic action. This can be explained by the insulinotropic actions of increased GLP-1 secretion seen in this study. Similar results have been shown for DPPIV inhibitors such as sitagliptin or agonists such as exenatide in animals and in man. Interestingly, sitagliptin does not reduce body weight in man, despite clear effects on blood glucose, glycation and increased insulin and GLP-1 secretion (Pospisilik et al., 2002; Zerilli and Pyon, 2007; Nonaka et al., 2007), although exenatide has been shown to have modest weight lowering activity (Nielson et al., 2004). Our data show no clear effect on insulin resistance by PGX® along with the increased insulin secretion as measured by the fasted CISI score, although an insulin tolerance test might have more clearly shown improved insulin sensitivity. More importantly, PGX® treatment not only reduced blood glucose, but reduced HbA_{1c} levels, showing reduced glycation. GLP-1 reduces blood glucose by increasing insulin production, increasing glucose uptake in peripheral tissue such as skeletal muscle and reducing hepatic gluconeogenesis (Kim and Egan, 2008). The control of GLP-1 is complex (Kim and Egan, 2008) and involves nutrient activation as well as neural control. It increases production of insulin by reduction of β-cell membrane potential, leading to increased calcium uptake and release of insulin. GLP-1 does affect lipid metabolism and we did not measure this in the present study.

The dietary fiber used for comparison was inulin which is a soluble, but relatively non-viscous fiber. Inulin has numerous forms (other oligofructans), but has been shown to reduce body weight, cholesterol, triglycerides and glucose in models of metabolic syndrome similar to the model used in the present study. Inulin may have a somewhat different profile of activity as it works less through forming a viscous gel like PGX®, while fermentation of inulin in the gut will lead to generation of SCFA that induce some of its beneficial effects (Daubioul et al., 2002; Weikert and Pfeifer, 2008). Most studies showing beneficial effects of inulin or related fiber in metabolic syndrome use at least 10% wgt/wgt in the diet (Daubioul et al., 2002; Weikert et al., 2008). Because of the high viscosity of PGX®, 5% wgt/wgt was used and this was also the highest dose tested for safety (Matulka et al., 2009). Preliminary studies in man had also suggested a dose comparable to this dose to be efficacious.

No changes in PYY, amylin, GIP or pancreatic polypeptide were seen in our study. Some dietary fibers alter blood levels of GIP and PYY consistent with weight loss and increased insulin sensitivity (Morgan et al., 1990; Pospisilik et al., 2002). GIP is another important insulinotropic peptide, but its regulation in type 2 diabetes is complex and its lack of change in our study is not presently understood. A recent study in man showed PGX® to increase peptide YY (PYY) in healthy adult humans over the course of a three week study (Reimer et al., 2010). While no changes in GLP-1 were seen, modest increases in insulin sensitivity were seen despite the fact that these subjects were not diabetic. It remains to be seen whether these results will be relevant in obese and/or diabetic patients.

The increased GLP-1 levels were not correlated with an increase in intestinal L-cell density or expression so increased GLP-1 was likely produced by existing cells as it is stored and generally released following a meal and is stimulated in particular by glucose and lipids (Kim and Egan, 2008). It is unknown if GLP-1 half-life in blood was affected, but there are little data to prove this for other dietary fibers that also increase GLP-1. The release of SCFA may account for the increased L-cell density seen in this study for inulin, but this was not associated with an increase in circulating GLP-1. We also determined the density of immunoreactive insulin containing cells and there was no significant increase induced by PGX® although a tendency for increased pancreatic β-cell mass was seen. In unpublished data from our laboratory in a similar model, significant increases in the density of insulin containing cells was seen with PGX® treatment so the trend seen in the current study may be biologically relevant. GLP-1 may reduce the loss of insulin-producing cells (Kim et al., 2007) and perhaps a longer study might have born this out for PGX®. PGX® had little effect on pancreatic histomorphology, although the levels of damage were not great for the cellulose-treated animals.

Conclusions

PGX® is a novel viscous, water soluble fiber complex that reduces food intake, but without reduction in body weight in this study. This fiber improved glycemic control in the ZDFs and appears to act as an insulin secretagogue. This is confirmed by the ability of PGX® to increase circulating GLP-1. As measured by HOMA and CISI scores, insulin sensitivity was not affected, but in future studies insulin tolerance tests should also be done. Confirmation of reduced food intake will be necessary and studies are now in progress to further clarify this issue. The key parameter showing improved glycemic control was the significant reduction of HbA_{1c} by PGX®. The increased GLP-1 was accompanied by an increased ghrelin secretion which may have prevented the weight loss secondary to reduced food intake due to its multiple effects on energy homeostasis.

Conflict of interest statement

Gary Grover Lee Koetzner and Joan Wicks received funding InovoBiologics to perform this study and have no financial interest in PGX®. Roland Gahler is the owner of the Factors Group of Companies, which retains an interest in PGX®. Michael Lyon receives consulting fees from the Factors Group of Companies and Simon Wood receives consulting fees from InovoBiologics Inc.

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