A Physiological Level of Rhubarb Fiber Increases Proglucagon Gene Expression and Modulates Intestinal Glucose Uptake in Rats¹,²

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ABSTRACT Previous work demonstrated that a high fiber diet upregulates proglucagon mRNA and secretion of glucagon-like peptide-1 [GLP-1(7–37)] and insulin compared with an elemental fiber-free diet. This study examined whether similar intakes of fibers differing in physiochemical and fermentative properties alter the expression of intestinal hormones and intestinal absorptive properties. Sprague-Dawley rats were fed either a 50 g/kg cellulose or rhubarb fiber diet for 14 d. Ileal proglucagon mRNA levels were significantly higher in rats fed rhubarb fiber than in those fed cellulose fiber (9.3 ± 0.9 vs. 6.2 ± 1.0 densitometer units). Proglucagon mRNA in the colon did not differ between diet treatments. Plasma c-peptide concentrations were significantly higher 30 min after an oral glucose tolerance test in the rhubarb vs. cellulose group (1627 ± 67 vs. 1290 ± 71 pmol/L). Passive permeability, measured by the uptake of L-glucose, was significantly higher in the jejunum of rats fed cellulose compared with those fed rhubarb fiber. Adjusting total glucose uptake for passive permeability and unstirred water layer resistance resulted in a higher K_m being calculated for the jejunum and ileum of the cellulose fiber group. Jejunal and ileal carrier-mediated uptakes (V_max) were not altered by diet and reflected the lack of difference between groups in sodium-dependent glucose cotransporter (SGLT-¹) and sodium-independent glucose transporter (GLUT2) mRNA levels. Replacing cellulose fiber with rhubarb fiber in a diet upregulated ileal proglucagon mRNA and resulted in a reduced passive permeability but did not affect glucose transport of the small intestine. This work establishes the importance of dietary fiber fermentability in modulating intestinal proglucagon expression and possibly glucose homeostasis. J. Nutr. 127: 1923–1928, 1997

KEY WORDS: • proglucagon • rhubarb fiber • rats • glucose uptake

Current recommendations for the dietary management of diabetes mellitus include increasing the consumption of complex carbohydrate and fiber (American Diabetes Association 1987). Increasing dietary fiber confers such benefits as lower exogenous insulin requirements, lower fasting and postprandial plasma glucose concentrations and improved glycemic control (Vinik and Jenkins 1988). The more highly soluble fibers, including pectin, psyllium and guar gum, appear to have a greater effect on glucose tolerance because of their ability to slow glucose absorption in the small intestine (Jenkins et al. 1978, Pastors et al. 1991). After long-term ingestion of fiber, however, improvements in glycemia can be recognized even when fiber is not physically present in the intestine, i.e., after an overnight fast (Groop et al. 1993, Pastors et al. 1991). Only recently has it been suggested that the effects of dietary fiber on glucose transport, insulin secretion and glycemia may be mediated by changes in gastrointestinal hormones as well.

The addition of fermentable fiber to an elemental diet causes a significant proliferative effect in the colon and distal small intestine (Jacobs and Lupton, 1984). The trophic effect appears to be related to the production of short-chain fatty acids (SCFA), which result from the microbial fermentation of dietary fiber in the gut (Rombeau and Kripke 1990, Sakata, 1987). Indeed, both ingestion of a high fiber diet and supplementation of total parenteral nutrition (TPN) with SCFA upregulate proglucagon mRNA (Reimer and McBurney 1996, Tappenden et al. 1996). It remains to be elucidated if the ingestion of different fiber types also alters proglucagon mRNA abundance.

Proglucagon, synthesized by L cells found in the distal ileum and colon, is post-translationally processed into glucagon-like peptides (GLP-1, GLP-2) and is released into the circulation in response to increases in the blood glucose concentration.

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peptide-1 [GLP-1(7–37)], a potent insulin secretagogue and other peptides (Holst 1994). We have recently demonstrated in rats that ingestion of a high fiber diet increases plasma levels of GLP-1(7–37), insulin and c-peptide after oral glucose compared with a fiber-free diet (Reimer and McBurney 1996). Recent evidence suggests that another proglucagon-derived peptide, glucagon-like peptide-2 (GLP-2), may mediate small intestinal glucose transport (Cheeseman and Tsang 1996). The potent actions of this hormone on carbohydrate absorption and metabolism make it a potential candidate in the regulation of glucose homeostasis.

We hypothesized that changes in proglucagon gene expression and postprandial secretion of insulin and c-peptide would differ with the ingestion of physiologic intakes of fibers with different fermentative properties. To test this hypothesis, we compared the effects of a highly fermentable rhubarb stalk fiber with a less fermentable cellulose fiber (50 g/kg diet) on proglucagon mRNA; plasma levels of insulin and c-peptide were measured. Under in vitro fermentation conditions, rhubarb stalk and cellulose produce 6.5 and 2.5 mmol SCFA/g, respectively (unpublished data). To determine if fiber type might modulate glucose homeostasis via changes in small intestinal glucose transport, we measured sodium-dependent glucose cotransporter (SGLT-1) and sodium-independent glucose transporter (GLUT2) mRNA and in vitro glucose uptake.

### MATERIALS AND METHODS

**Animals.** Male Sprague-Dawley rats (220–250 g) were obtained from the University of Alberta Health Sciences colony (University of Alberta, Edmonton, AB, Canada). Animals were individually housed in wire mesh bottom cages in a temperature and humidity controlled room with a 12-h light:dark cycle. The protocol was approved by the University of Alberta Animal Welfare Committee.

Rats were given free access to a nonpurified diet (Rodent Laboratory diet PMI #5001, PMI Feeds, St. Louis, MO) and water before the experimental period. During the experiment rats were given a 50 g/kg fiber diet containing either cellulose or rhubarb stalk fiber and water for ad libitum consumption (n = 8 per diet). Rhubarb fiber was prepared according to procedures described by Basu et al. (1993). Compositions of the experimental diets are given in Table 1.

The rats in this study served as controls in a larger study in which diabetes was induced by intravenous penile injection of streptozotocin (65 mg/kg body weight; Sigma Chemical, St. Louis, MO) in an acetate buffer (pH 4.5). The rats in this study therefore received a sham injection of the acetate buffer alone on d 3 of the experiment. The rats were fed their respective diets for an additional 14 d.

Food was withheld 16 h before the experiment at which time a tail nick blood sample was taken for fasting plasma glucose; on d 14, 500 g/L dextrose by gavage, at a dose of 2 g/kg, was administered to all rats. At 30 min postgavage, rats were anaesthetized and blood taken by cardiac puncture. A 5-cm segment of distal duodenum, jejunum and ileum and proximal colon was excised, flushed with ice cold saline, immersed in liquid nitrogen and stored at −72°C for later mRNA analysis.

**Chemicals.** Trizol was purchased from Gibco BRL (Burlington, ON, Canada). All other chemicals used in northern blot analysis and transport kinetic assays were purchased from Sigma Chemical, BDH Chemical (Toronto, ON, Canada) or Gibco BRL. Radiosotopees were purchased from Amersham Canada (Oakville, ON).

**Isolation of total RNA.** Total RNA was isolated using Trizol (Gibco BRL). Isolation was according to the protocol provided with the reagent. RNA was dissolved in diethyl pyrocarbonate–treated water and quantity and purity determined by ultraviolet spectrophotometry at 260 and 280 nm.

**Northern blot analysis.** Messenger RNA in all samples was measured with the use of a Northern blot analysis procedure described by Fuller et al. (1989) with modifications. Aliquots of 15–μg total RNA were dissolved in 10 μL gel loading buffer (500 mL deionized formamide), 2 mol/L formamide, 13 mL/L glycerol, 0.02 mol/L morpholinopropanesulfonic acid (MOPS), 5 mmol/L sodium acetate, 1 mmol/L EDTA and 1 g/L bromophenol blue), boiled for 2 min to denature the RNA and then placed on ice for 5 min. Samples were centrifuged at 10,000 g for 10 min to remove the reagent. RNA was then fractionated according to size by electrophoresis in the presence of a recirculating running buffer containing 0.02 mol/L MOPS, 5 mmol/L sodium acetate and 1 mmol/L EDTA (5 h at 100 V). After electrophoresis, the gels were soaked in two changes of 10X standard saline citrate (1.5 mol/L NaCl, 0.15 mol/L trisodium citrate, pH 7.0) and then blotted onto an MSI Nitropure nitrocellulose membrane (MSI Laboratories, Westboro, MA), with the use of the capillary method described by Southern (1975). The RNA was then fixed onto membranes by baking in vacuo at 80°C for 2 h.

Membranes were prehybridized for 2 h at 65°C in prehybridization buffer [6X SSPE (0.18 mol/L NaCl, 0.01 mol/L sodium phosphate, 1 mmol/L EDTA, pH 7.4), 1 g/L SDS, 5X Denhart’s solution (0.5 g Ficoll 400, 0.5 g poly vinyl pyrrolidone, 0.5 g bovine serum albumin (fraction V)]. After prehybridization, membranes were incubated for 16 h at 65°C in an identical volume of fresh hybridization buffer with the [32P]ATP-labeled cDNA probe. The 440-bp cDNA proglucagon probe (Taylor et al. 1990), 3.8-kb GLUT2 cDNA probe (donated by G. I. Bell, Howard Hughes Medical Institute, University of Chicago, IL) and 4.8-kb SGLT-1 cDNA probe (donated by N. Davidson, University of Chicago, IL) were labeled by nick translation (Random Primers DNA Labelling System, Life Technologies, Burlington, ON, Canada) with [32P]dATP (111 TBq/mmol, Amersham Canada).

After hybridization, membranes were washed three times for 20 min each at room temperature with 2X SSPE, 1 g/L SDS. They were then washed once at 65°C for 20 min with 0.1 X SSC, 1 g/L SDS. Membranes were heat-sealed in plastic bags and then exposed at −70°C to KODAK XAR5 film (Eastman Kodak, Rochester, NY) using an intensifying screen (Dupont Canada, Mississauga, ON). For statistical analysis, the signals were quantified using laser densitometry [Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada), Mississauga, ON]. The 28S and 18S ribosomal bands were quantified from negatives of photographs of the membranes. These bands confirm the integrity of the RNA and were used as a denominator to determine mRNA levels of GLP-1(7–37), insulin and c-peptide after oral glucose were briefly centrifuged at 10,000 g for 10 min to remove the reagent. RNA was dissolved in diethyl pyrocarbonate–treated water and quantity and purity determined by ultraviolet spectrophotometry at 260 and 280 nm.

**Measurement of transport kinetics.** Transport kinetics were determined as previously described (Thomson and Rajotte 1983). Briefly, the segment (15 cm) of jejunal plus ileum was opened along its mesenteric border and carefully rinsed with ice cold saline. Pieces of intestine were cut from the segments and mounted as flat sheets in preincubation chambers containing oxygenated Krebs-bicarbonate buffer (pH 7.4) at 37°C. After 10 min, the chambers were transferred to other chambers containing [1H]-insulin and various [14C]-probe molecules in oxygenated Krebs-bicarbonate buffer (pH 7.4) at 37°C. The concentrations of D-glucose and fructose were 4, 8, 16, 32 and 64

### Table 1: Composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Cellulose g/kg diet</th>
<th>Rhubarb g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>648</td>
<td>648</td>
</tr>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Corn oil</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fiber2</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

1 Supplied in quantities adequate to meet NRC requirements and described in Marsman and McBurney (1996).

2 Cellulose was supplied as Alphacel nonnutritive bulk (ICN Biomedicals, Aurora, OH). Rhubarb stalk fiber was prepared in B. Ooarikul’s laboratory at the University of Alberta (Edmonton, Canada).
mmol/L. The maximal transport rates ($V_{\text{max}}$) and the apparent Michaelis constants ($K_m$) were estimated using nonlinear regression and D-glucose values. Linear regression was used to obtain the slope of the linear relationship describing d-fructose uptakes. L-Glucose uptakes at 4°C and aliquots taken for glucagon and c-peptide determinations were measured at the Muttart Diabetes Research Center, University of Alberta. Insulin was determined with the use of a commercial double antibody RIA kit (Linco Research) for rat insulin with a detection limit of 0.5 pmol/L (Sigma Chemical). Statistical significance is defined as $P < 0.05$. Nutrient uptakes were measured with the use of a commercially available statistics program (SYSTAT, Evanston, IL) applying a nonlinear regression method. This program weights individual data points to generate a single curve per treatment; outliers (>1 SD) were removed, resulting in $n = 6$ rats per diet.

**RESULTS**

**Diet intake and growth.** Diet intake and weight gain during the experimental period did not differ between groups. Rats consumed 26.1 ± 0.5 and 26.1 ± 0.9 g/d of cellulose and rhubarb diet, respectively. Weight gain at d 7 was 3.8 ± 0.4 and 4.2 ± 0.2 g/d, and at d 14 was 6.6 ± 0.3 and 6.5 ± 0.3 g/d in rats fed cellulose and rhubarb diets, respectively. Dietary fiber source did not affect total weight of jejunum, ileum and colon or percentage of mucosa in the jejunum and ileum (Table 2).

**Blood variables.** Postprandial c-peptide levels in rats consuming the rhubarb fiber diet were significantly higher than in those consuming the cellulose fiber diet (Fig. 1). Plasma insulin concentrations did not differ between cellulose and rhubarb diet–fed rats (120 ± 9 vs. 160 ± 20 pmol/L, respectively). Consumption of cellulose vs. rhubarb fiber did not alter levels of plasma glucagon (93.6 ± 5.1 vs. 98.4 ± 3.3 ng/L), fasting glucose (5.4 ± 0.3 vs. 5.5 ± 0.3 mmol/L) and plasma glucose concentrations 30 min after an oral glucose gavage (9.0 ± 0.3 vs. 8.7 ± 0.4 mmol/L).

**mRNA abundance.** The 440-bp proglucagon mRNA fragment was readily detected in total RNA from ileum and colon. Densitometric readings for proglucagon mRNA were significantly higher than controls in the ileum of rats fed rhubarb fiber (Fig. 2), but values did not differ in the colon (Fig. 3). Fiber type did not affect the expression of the brush border glucose transporter, SGLT-1 (6.0 ± 0.8 vs. 6.4 ± 0.6 densitometer units; cellulose vs. rhubarb, respectively) or the basolateral glucose transporter, GLUT2 (7.8 ± 0.9 vs. 8.3 ± 0.8 densitometer units; cellulose vs. rhubarb, respectively).

**In vitro nutrient uptake.** The apparent passive permeability coefficient, estimated with 1-glucose, was significantly higher in rats fed cellulose compared with those fed rhubarb fiber diets.

<table>
<thead>
<tr>
<th>Table 2: Intestinal characteristics of rats fed diets containing rhubarb or cellulose</th>
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<tbody>
<tr>
<td>Diet</td>
</tr>
<tr>
<td>Cellulose</td>
</tr>
<tr>
<td>Weight, mg/cm</td>
</tr>
<tr>
<td>Mucosa, g/100 g</td>
</tr>
<tr>
<td>Total weight, g</td>
</tr>
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</table>

$^1$ Values are means ± SEM, $n = 6$. Sigma Diagnostics Glucose (Trinder) Reagent for the enzymatic determination of glucose at 505 nm (Sigma Chemical).

**Figure 1:** Plasma c-peptide concentrations 30 min after an oral glucose gavage in rats fed rhubarb or cellulose fiber diets. Values are means ± SEM, $n = 8$. *Significantly different from rats fed rhubarb ($P < 0.05$).
TABLE 3

<table>
<thead>
<tr>
<th>Diet</th>
<th>Cellulose</th>
<th>Rhubarb</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(\text{nmol/(100 mg tissue \cdot min)})</td>
<td></td>
</tr>
<tr>
<td><strong>L-Glucose</strong></td>
<td></td>
<td></td>
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<tr>
<td>Jejunum 1 mmol/L</td>
<td>12.7 ± 1.1*</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>Ileum 1 mmol/L</td>
<td>10.5 ± 1.2</td>
<td>8.6 ± 1.0</td>
</tr>
<tr>
<td>16 mmol/L</td>
<td>202.8 ± 17.0*</td>
<td>122.8 ± 15.4</td>
</tr>
<tr>
<td>D-Fructose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum 1 mmol/L</td>
<td>10.4 ± 0.2</td>
<td>10.9 ± 0.6</td>
</tr>
<tr>
<td>Ileum 1 mmol/L</td>
<td>12.3 ± 0.9</td>
<td>13.4 ± 0.8</td>
</tr>
</tbody>
</table>

*Significantly different than rats fed rhubarb (\(P < 0.05\)).

Values are means ± SEM, \(n = 6\).

DISCUSSION

We have previously shown that feeding a high fiber diet upregulates proglucagon mRNA compared with a fiber-free elemental diet (Reimer and McBurney 1996). In this study, the level of fiber was similar in both diets. We now report that ingestion of a more fermentable dietary fiber, rhubarb fiber, at physiologic levels (50 g/kg diet) is associated with increased ileal proglucagon mRNA and postprandial c-peptide concentrations compared with the cellulose control.

These results suggest that fiber fermentability, in addition to the level of fiber intake, regulates intestinal proglucagon gene expression. Indeed, supplementing TPN with SCFA significantly increases proglucagon mRNA abundance in rats after massive small bowel resection (Tappenden et al. 1996). Moreover, plasma concentrations of enteroglucagon have been shown to be increased in rats ingesting fermentable dietary fiber (Gee et al. 1996, Southon et al. 1987). We suggest that SCFA resulting from the fermentation of dietary fiber in the large intestine may modulate proglucagon gene expression. The apparent lack of response in colonic proglucagon mRNA levels may relate to diurnal variation in colonic production of SCFA. Indeed, Tappenden (1996) found increased abundance

TABLE 4

<table>
<thead>
<tr>
<th><strong>In vitro D-glucose uptake kinetics in intestine of rats fed diets containing rhubarb or cellulose fiber(^1,2)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(V_{\text{max}})</strong></td>
</tr>
<tr>
<td>(\text{nmol/(mg tissue \cdot min)})</td>
</tr>
<tr>
<td>Jejunum</td>
</tr>
<tr>
<td>Ileum</td>
</tr>
</tbody>
</table>

*Significantly different than rats fed rhubarb (\(P < 0.05\)).

\(^1\) Values are means ± SEM, \(n = 6\).

\(^2\) \(K_m\) and \(V_{\text{max}}\) were corrected for apparent passive permeability and unstirred water layer resistance as described by Thomson and Rajotte (1983).
of proglucagon mRNA within 6 h of intravenous SCFA administration. The variability in SCFA production from the fermentation of dietary fibers may explain the often larger error associated with proglucagon mRNA measurements in the colon versus the ileum.

Nutrient movement across the intestinal epithelium occurs via passive permeation or carrier-mediated uptake. Tight junctions (paracellular pathway) and the enterocyte (transcellular pathway) mediate passive permeation (Ballard et al. 1995, Karasov and Diamond 1983, Pappenheimer and Reiss 1987). Although the physiological importance of the paracellular pathway has been questioned (Fedorak et al. 1990), the greater t-glucose uptake of jejunal segments isolated from rats fed cellulose in this study suggests that fiber type may alter nutrient delivery per se or intestinal permeability. Unstirred water layer resistances, measured by in vitro uptake of fatty acid (12:0), were significantly lower in the ileum of rats fed cellulose but not in the jejunum. Fructose uptakes were unaffected by diet, indirectly providing support for the concept that changes in passive permeability may reflect the unstirred water layer resistance. The Km, which is corrected for passive permeability and unstirred water layer resistance, was significantly lower in both the jejunum and ileum of rats fed rhubarb. This suggests that at low luminal concentrations of glucose, intestinal glucose carriers from rats fed rhubarb have a higher affinity for glucose than those of rats fed cellulose. However, protein-mediated transport is predominantly altered by changes in the maximum transport rates (Vmax) (Karasov and Diamond 1983), and glucose uptakes after a meal are largely determined by Vmax. The maximum transport rates and expression of the glucose transporters, SGLT-1 and GLUT2, were unaffected by diet, suggesting that the quantities or activities of transporters were not altered with ingestion of the two fiber types examined in this study.

More fermentable dietary fibers appear to enhance glucose homeostasis via several mechanisms. First, the increase in proglucagon mRNA and resultant secretion of GLP-1 (7–37) should delay gastric emptying and stimulate insulin secretion to enhance the disposition of absorbed glucose into peripheral tissues. Earlier work demonstrated a significant enhancement of GLP-1 (7–37) secretion in rats fed high fiber diets (Reimringer and McBurney 1996). C-peptide measurements provide a better estimate of insulin secretory rate than peripheral insulin measurements (Polonsky and Rubenstein 1984), and higher concentrations of c-peptide were observed with the more fermentable rhubarb fiber.

Many studies have shown that the long-term ingestion of fiber improves glucose tolerance (Groot et al. 1993, Pastors et al. 1991). Until recently, it was thought that the physical properties of fibers within the lumen of the intestine were solely responsible for improvements in glucose homeostasis. Viscous fibers delay diffusion of glucose from dialysis bags, whereas particulate fibers have little effect (Jenkins et al. 1980 and 1984). However, alterations in postprandial secretion of gastrointestinal hormones and diet-related changes in intestinal transport capacity may also be important. Total intestinal glucose uptakes were not altered with the experimental diets in this study, but the lower Km for D-glucose in both jejunum and ileum of rats fed rhubarb fiber may reflect an increase in unstirred water layer resistance, theoretically slowing the rate of glucose absorption.

Willms et al. (1996) recently demonstrated that the potent actions of GLP-1 (7–37) on glucose homeostasis may also be due in large part to significant reductions in gastric emptying. We measured pancreatic secretion (c-peptide) and not gastric emptying rates. Nevertheless, we propose that increased intestinal proglucagon gene expression may explain overall improvements in glucose homeostasis reported with the long-term ingestion of dietary fiber. Clearly, the nutrient absorptive capacity of the small intestine changes in response to many physiologic and pathologic states, including dietary changes, pregnancy and lactation, intestinal resection and in diabetes and starvation (reviewed by Philpott et al. 1992). The precise signals for the regulation of nutrient absorption and utilization are not yet completely understood. In this study, we have demonstrated that the ingestion of a more fermentable fiber resulted in an upregulation of ileal proglucagon mRNA and enhanced c-peptide secretion 30 min after an oral glucose load but did not affect in vitro measurements of total glucose uptake.

**LITERATURE CITED**


