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Dietary Fiber Modulates Intestinal Proglucagon Messenger Ribonucleic Acid and Postprandial Secretion of Glucagon-Like Peptide-1 and Insulin in Rats*

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ABSTRACT

Intestinal hormones stimulate more than 50% of the insulin response after oral glucose administration. Short chain fatty acids stimulate mucosal adaptation and may alter proglucagon messenger RNA and release of the insulin secretagogue, glucagon-like peptide-1 (GLP-1). Sprague-Dawley rats ingested a fiber-free elemental diet or an elemental diet supplemented with 30% fiber providing similar energy and nutrients for 14 days. The cecal and colonic short chain fatty acids contents were significantly higher in the 30% fiber group. Ileal proglucagon messenger RNA levels were significantly higher in the 30% group *vs.* the 0% group (11.47 ± 0.87 *vs.* 6.52 ± 0.87 densitometer units), respectively. Similar trends were seen in the colon (13.36 ± 1.0 *vs.* 10.90 ± 0.77 densitometer units; $P = 0.07$). Plasma GLP-1, insulin, and C peptide levels 30 min postoral glucose were significantly higher in the 30% fiber group *vs.* the 0% group (19.8 ± 1.2 *vs.* 15.4 ± 1.2 pg/ml, 2.67 ± 0.4 *vs.* 1.29 ± 0.5 ng/ml, and 964.4 ± 94.4 *vs.* 530.2 ± 120.4 pM, respectively). Plasma glucose and glucagon did not differ between groups. A diet supplemented with fiber is able to significantly alter proglucagon gene expression and modulate GLP-1 and insulin secretion. These novel findings deepen our understanding of the beneficial role of fiber in improving glucose homeostasis. (*Endocrinology* **137:** 3948–3956, 1996)

IT IS ESTIMATED that more than 50% of postprandial insulin secretion is triggered by intestinal peptide hormones (1, 2). This response is attributed to the incretin effect and is defined as the action of peptide hormones released by the gut in response to oral nutrients to stimulate insulin release in physiologically relevant concentrations. Convincing evidence exists that the truncated form of glucagon-like peptide-1 (GLP-1), GLP-1-(7–37), is a physiologically important incretin (3, 4) and is produced by the human colon (5). In the presence of elevated blood glucose, GLP-1 stimulates the release of insulin by interacting with specific receptors on pancreatic B cells. In addition to potentiating glucose-induced insulin secretion, GLP-1 stimulates proinsulin gene expression and proinsulin biosynthesis (6). By stimulating insulin release and increasing insulin-independent glucose disposal, GLP-1 enhances glucose tolerance (7). The potent actions of this hormone on carbohydrate metabolism make it potentially applicable in the treatment of noninsulin-dependent diabetes mellitus.

Proglucagon, the mammalian glucagon precursor, is a 160-amino acid polypeptide encoded by the glucagon gene (8). The precursor is produced both in the α-cells of the islets of Langerhans and in the L cells of the intestinal mucosa (9, 10). Intestinal L cells produce predominantly glicentin, which corresponds to amino acids 1–69 of the proglucagon precursor [PG-(1–69)], a C-terminal-extended form of glucagon [oxyntomodulin; PG-(33–69)], GLP-1 [PG-(78–108)], GLP-2 [PG-(126–158)], and intervening peptide-2 [PG-(111–123)]. Posttranslational cleavage results in the secretion of amidated and glycine-extended GLP-1, which have similar potencies in terms of insulin secretion. In rats, one third of the GLP-1 immunoreactivity corresponds to glycine-extended GLP-1, and two thirds is in the form of amidated GLP-1 (11). In humans this partitioning is approximately 20% and 80%, respectively (12). Although a gradient of L cells is present throughout the gastrointestinal tract, the principal production of GLP-1 is concentrated in the L cells of the distal small intestine and colon (5, 13). Regulation of the production/secretion of GLP-1 may be subject to changes in intestinal mass and function. These morphological and functional changes, or adaptation, can occur with age, resection, dietary manipulation, and certain disease states, such as diabetes mellitus (14–16). Indeed, D'Alessio *et al.* (17) suggested that differences in basal and stimulated GLP-1 secretion among individuals may account for some of the variation in levels of glucose before as well as after eating. These differences could be amplified by adaptations associated with diet.

Dietary management of diabetes mellitus includes recommendations for increasing levels of complex carbohydrate

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and dietary fiber (18). Therapeutic advantages of increased dietary fiber include lower exogenous insulin requirements, lower fasting and postprandial plasma glucose, and improved glycemic control (19). In studies involving long term ingestion of fiber, improvements in glycemic control are seen with an oral glucose tolerance test after an overnight fast (20). Decreased or unchanged insulin responses to test meals have been reported (21), but with long term ingestion of fiber, the serum C peptide response increased, whereas the serum insulin response remained unchanged. Increased C peptide suggests a stimulation of insulin. The precise mechanisms involved in improved glucose tolerance are not known, but the effect may be mediated via gastrointestinal hormones.

Dietary alterations, such as the addition of certain readily fermentable fibers to an elemental diet, cause a significant proliferative effect in the colon and distal small intestine (22). The effect does not simply reflect the benefits of bulk in the lower gastrointestinal tract, but appears to be related to the production of short chain fatty acids (SCFA) from microbial fermentation of fiber as well (23–26). Crypt cell production rates are significantly increased when fermentable fibers are ingested, suggesting a role for SCFA in the proliferative response. Additionally, the ingestion of fermentable dietary fiber seems to enhance the secretion of the gut trophic hormone, enteroglucagon (27, 28).

The three major SCFA, acetate, propionate, and butyrate, once absorbed are metabolized by the cecal and colonic mucosal epithelial cells. Butyrate is recognized as an important respiratory fuel for the colon in preference to acetate and propionate as well as glutamine, glucose, and ketone bodies, fuels commonly used by intestinal epithelial cells (29, 30). Although incubation of a primary fetal rat intestinal culture with sodium butyrate for up to 2 h did not stimulate the secretion of glucagon-like immunoreactive peptides (31), addition of sodium butyrate to a pancreatic cell line markedly increased glucagon messenger RNA (mRNA) levels after 6 h (32). Glucagon and insulin gene transcription increased by 9.3- and 5.3-fold, respectively. Alterations in secretion and gene expression may be modulated via different mechanisms in the L cell and may explain the differences seen between these two *in vitro* studies. The ability of fermentable carbohydrates, however, to increase plasma enteroglucagon *in vivo* has been documented (26, 28). It remains to be elucidated whether dietary fiber and possibly SCFA similarly regulate the gene expression of gastrointestinal hormones.

The hypothesis that dietary fiber regulates gene expression and secretion of the gastrointestinal peptide hormone, GLP-1, was tested in the present study by comparing the effects of an elemental and 30% fiber diet on proglucagon mRNA. Measurements of plasma GLP-1-(7–37), insulin, C peptide, glucagon, and glucose were determined 30 min after oral glucose administration. Differences in the concentration and total amount of SCFA in cecal and colonic contents were assessed as well.

Materials and Methods

Animals and diets

Female Sprague-Dawley rats (190–220 g) were obtained from the University of Alberta Health Sciences Laboratory Animal Services colony and housed in a temperature- and humidity-controlled room with a 12-h light, 12-h dark cycle. Animals were housed in individual wire mesh-bottomed cages. The protocol was approved by the University of Alberta animal welfare committee.

Animals were maintained on a nonpurified diet (Rodent Laboratory Diet PMI 5001, PMI Feeds, St. Louis, MO) before the experimental period. During the experiment, animals consumed either a fiber-free elemental diet (ICN Chemically Defined Diet 960346, ICN Biomedicals, Mississauga, Canada) or an elemental diet supplemented with 30% fiber [wt/wt, 5% Alphacel plus 25% Fibrad, ICN and Ross Laboratories (Columbus, OH)] for 14 days. Animals had free access to the diet during the experimental period. The compositions of the experimental diets are given in Table 1. When diets are diluted by the addition of fiber, rats

TABLE 1. Compositions of the experimental diets

Ingredient	g/kg diet	
	0% Fiber	30% Fiber
Amino acid mix[a]	178.5	124.95
Corn oil	100.0	70.0
Glucose	443.0	310.1
Sucrose	221.5	155.05
Alphacel		50.0
Fibrad[b]		250.0
Mineral mix[c]	50.0	35.0
Vitamin mix[c]	7.0	4.9
Digestible energy[d] (kJ/g)	17.5	13.8

[a] Supplied (g/178.5 g mix): L-arginine HCl, 13.5; L-histidine HCl · H_2O, 4.5; L-isoleucine, 8.2; L-leucine, 11.1; L-lysine HCl, 18.0; L-methionine, 8.2; L-phenylalanine, 11.6; L-threonine, 8.2; L-tryptophan, 1.7; L-alanine, 3.5; L-aspartic acid, 3.5; L-glutamic acid, 35.0; glycine, 23.3; L-proline, 3.5; L-cystine, 3.5; L-serine, 3; L-tyrosine, 3.5; L-asparagine, 6.0.

[b] Ross Laboratories (Columbus, OH). Contained a mixture of pea fiber, oat fiber, sugar-beet fiber, xanthan gum, and soy lecithin.

[c] Supplied in quantities adequate to meet NRC nutrient requirements.

[d] Digestible energy was calculated from gross energy of diet components and *in vivo* digestible energy measurements for the fiber sources.

TABLE 2. Effect of dietary fiber on length and weight of intestinal segments

Parameter	0% Fiber (n = 10)	30% Fiber (n = 10)	Significance (*P*)
Total small bowel length (cm)	143.30 ± 2.29	138.67 ± 2.41	NS
Total small bowel weight (g)	7.87 ± 0.27	7.79 ± 0.27	NS
Duodenum wt (g)	3.11 ± 0.14	2.78 ± 0.14	NS
Jejunum wt (g)	2.70 ± 0.11	2.52 ± 0.11	NS
Ileum wt (g)	2.04 ± 0.12	2.50 ± 0.12	<0.05
Cecum wt (g)	0.63 ± 0.02	0.89 ± 0.06	<0.05
Total colon length (cm)	14.60 ± 0.88	19.00 ± 0.99	<0.05
Total colon wt (g)	0.93 ± 0.09	1.47 ± 0.09	<0.05

Values are the mean ± SEM. Total small bowel was partitioned under tension with a 15-g weight into three equal segments for duodenum, jejunum, and ileum, respectively.

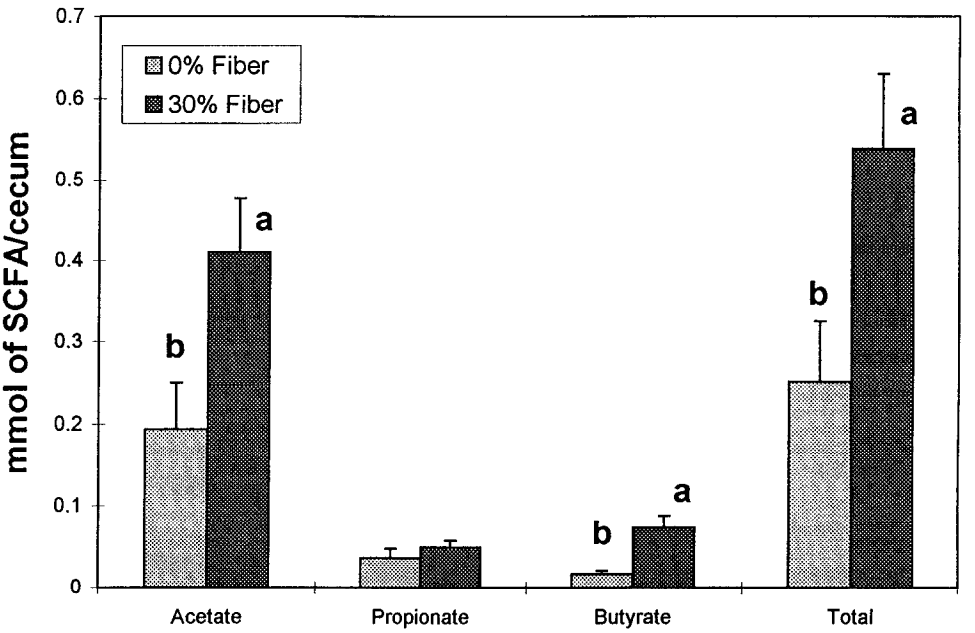

FIG. 1. Effect of fiber supplementation on SCFA content in the cecum of rats. Values are the mean ± SEM (n = 6 rats/diet treatment). Total SCFA are the sum of all individual SCFA analyzed (acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproic). The amount (millimoles) of SCFA was determined by multiplying the mass of cecal contents with concentrations (millimoles per mg wet content). For each individual SCFA, values with different letters are significantly different ($P < 0.05$).

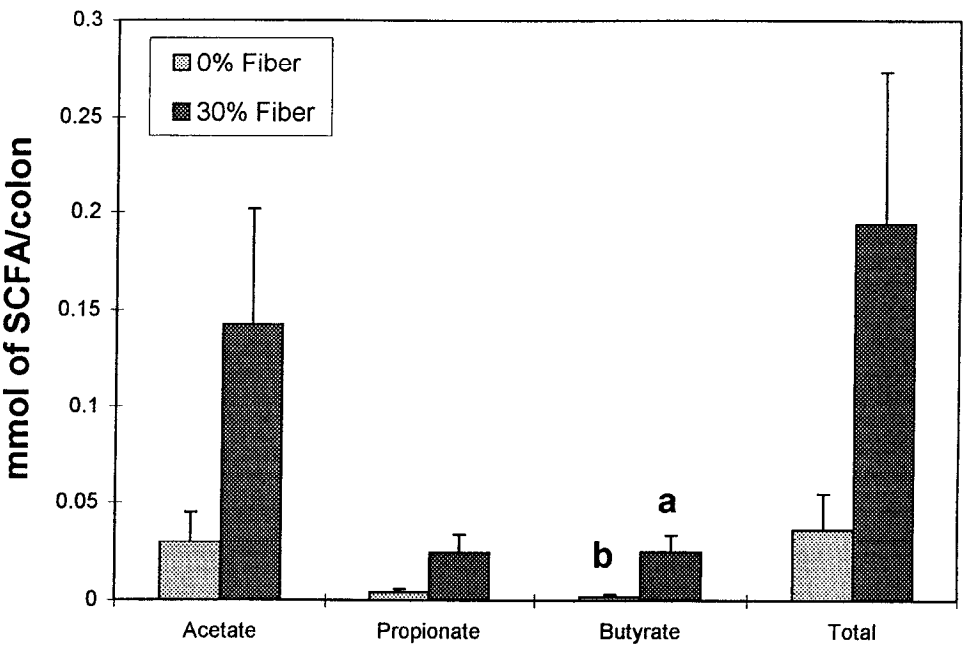

FIG. 2. Effect of fiber supplementation on SCFA content in the colon of rats. Values are the mean ± SEM (n = 6 rats/diet treatment). Total SCFA are the sum of all individual SCFA analyzed (acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproic). The amount (millimoles) of SCFA was obtained by multiplying mass of colonic contents with concentrations (millimoles per mg wet content). For each individual SCFA, values with different letters are significantly different ($P < 0.05$).

increase their food intake to achieve similar energy intakes (33, 34). Thus, the 30% fiber diet was formulated so that animals consuming a constant digestible energy intake would have identical nutrient and nutrient: digestible energy intakes on both diets.

Oral glucose gavage and sample collection

After an overnight fast, all animals were given 50% dextrose by gavage at a dose of 2 g glucose/kg. Thirty minutes postgavage, animals were anesthetized, and blood was taken by cardiac puncture. A 3-cm segment of distal ileum and proximal colon was immediately excised, flushed with ice-cold saline, immersed in liquid nitrogen, and stored at −72 C for later mRNA analysis. Cecal and colonic contents were extruded into vials for later SCFA analysis. One hundred microliters of 0.17% phosphoric acid and 1 ml water were added to the contents. Empty ceca and colons were rinsed and weighed.

Isolation of total RNA

Total RNA was isolated from each segment using a procedure described by Chirgwin *et al.* (35) with modifications. Briefly, approximately 0.3 g intestine was added to guanidium isothiocyanate (GIT) containing 0.5% Antifoam A and homogenized at top speed for 20 sec. To each sample 0.2 ml NaOAc (2 M), 2 ml buffered phenol, and 0.4 ml chloroform/iso-amyl alcohol were added. Tubes were placed on ice for 15 min and then centrifuged at 9000 × *g* for 20 min at 4 C. DNA will be at the interface with proteins and lipids in the organic (lower) phase. About 2.2 ml of the RNA containing upper phase were recovered to a new microcentrifuge tube. An equal volume of ice-cold isopropanol was added to precipitate the RNA overnight at −20 C. Microfuge tubes were centrifuged at 9000 × *g* for 20 min at 4 C, the liquid was drawn off, and the pellet was resuspended in 0.6 ml GIT. Tubes were placed in a 60 C water bath to dissolve the pellet. Ice-cold isopropanol (0.6 ml) was again added, and RNA was allowed to

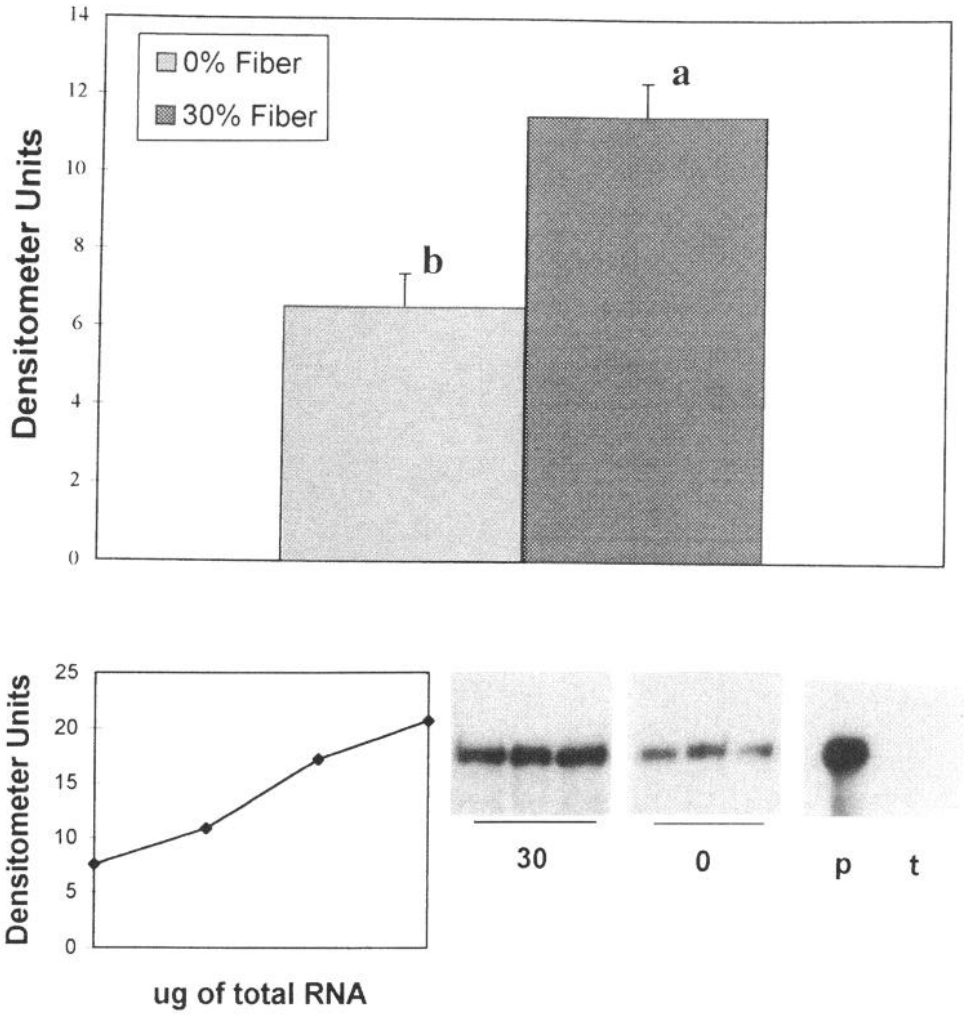

FIG. 3. Effects of increasing levels of fiber supplementation on ileal proglucagon mRNA expression in rats. Values are the mean ± SEM (n = 6 rats/diet treatment). Values with different letters are significantly different ($P < 0.05$). *Inset* of autoradiograph. Each lane (or well) of the gel was loaded with 15 μg of total RNA. See *Materials and Methods* for explanation of controls. 0 = 0% fiber; 30 = 30% fiber; t = tRNA; p = probe alone.

precipitate for 1 h at −20 C. Microfuge tubes were centrifuged for 20 min at 9000 × *g*, and the liquid drawn off. A standard phenol-chloroform-iso-amyl alcohol (25:24:1) extraction was performed by redissolving pellet in 400 μl TE (10 mM Tris-Cl and 1 mM EDTA). RNA was ethanol precipitated overnight, and pellet was dissolved in 400 μl/g tissue STE (10 mM Tris-HCl, 5 mM EDTA, and 0.1% SDS). The quantity and purity of RNA were determined by UV spectrophotometry at 260, 280, and 230 nm.

Ribonuclease (RNase) protection assay

Glucagon mRNA in all samples was measured in a RNase protection assay procedure as described by Gilman (36) with modifications. The glucagon complementary RNA probe (15) was a gift from Peter J. Fuller of Prince Henry's Institute of Medical Research (Melbourne, Australia). Aliquots of 15 μg sample total RNA were hybridized overnight with 3 × 10^6 dpm [^{32}P]CTP (DuPont Canada, Markham, Canada)-labeled rat glucagon riboprobe and then incubated for 1 h at 30 C in 350 μl digestion buffer containing 10 mmol/liter Tris-HCl, 300 mmol/liter NaCl, 5 mmol/liter EDTA, 0.04 g/liter RNase A (Pharmacia LKB Biotechnology, Uppsala, Sweden), and 0.002 g/liter RNase T1 (Sigma Chemical Co., St. Louis, MO). The protected fragments were then size-fractionated by electrophoresis on a denaturing 6% polyacrylamide-7 M urea sequencing gel using 40 watts constant power and approximately 1000 V. After electrophoresis, the gel plates were separated, and the gel was rinsed in 10% glacial acetic acid (vol/vol) and 10% methanol (vol/vol) for 15 min. After draining for 15 min, the gel was transferred to 3MM Whatman filter paper (Whatman International, Maidstone, UK), heat sealed into a plastic bag, and exposed to Kodak XAR 5 film at −70 C using an intensifying screen (Dupont Canada).

Controls

To confirm the authenticity of protected fragments in the protection assay, samples containing only yeast transfer RNA (tRNA) in amounts equal to those used in all other RNA samples were included in each assay and treated exactly as all other RNA samples. There is no mRNA present in yeast tRNA; consequently, any protected bands detected reflect nonspecific binding of the probe. The riboprobe alone (6000 dpm) was run in a separate lane and served as a positive control. A dose-response curve of 10, 15, 25, and 35 μg total RNA from one animal was treated as all other samples and run on each gel as well. This ensures that the probe was present in molar excess over the target fragment in the hybridization reaction, and the intensity of the protected fragment was directly proportional to the amount of complementary RNA in the sample mixture.

Consistent with other published work using RNase protection assays (37, 38) and the nature of the assay, separate probes such as actin were not used as loading controls. Like Winesett *et al.* (38), we confirmed the integrity of total RNA and loading accuracy by running separate RNA formaldehyde-denaturing agarose gels to visualize 28S ribosomal RNA (rRNA) and 18S rRNA bands. The use of 28S rRNA as a control for total RNA loading has been reported to be equivalent or superior to the use of constitutively expressed RNA such as ubiquitin or actin (39) and a more reliable and reproducible control, because signals of interest can be obscured by the high background often produced with constitutively expressed mRNA such as actin mRNA (38).

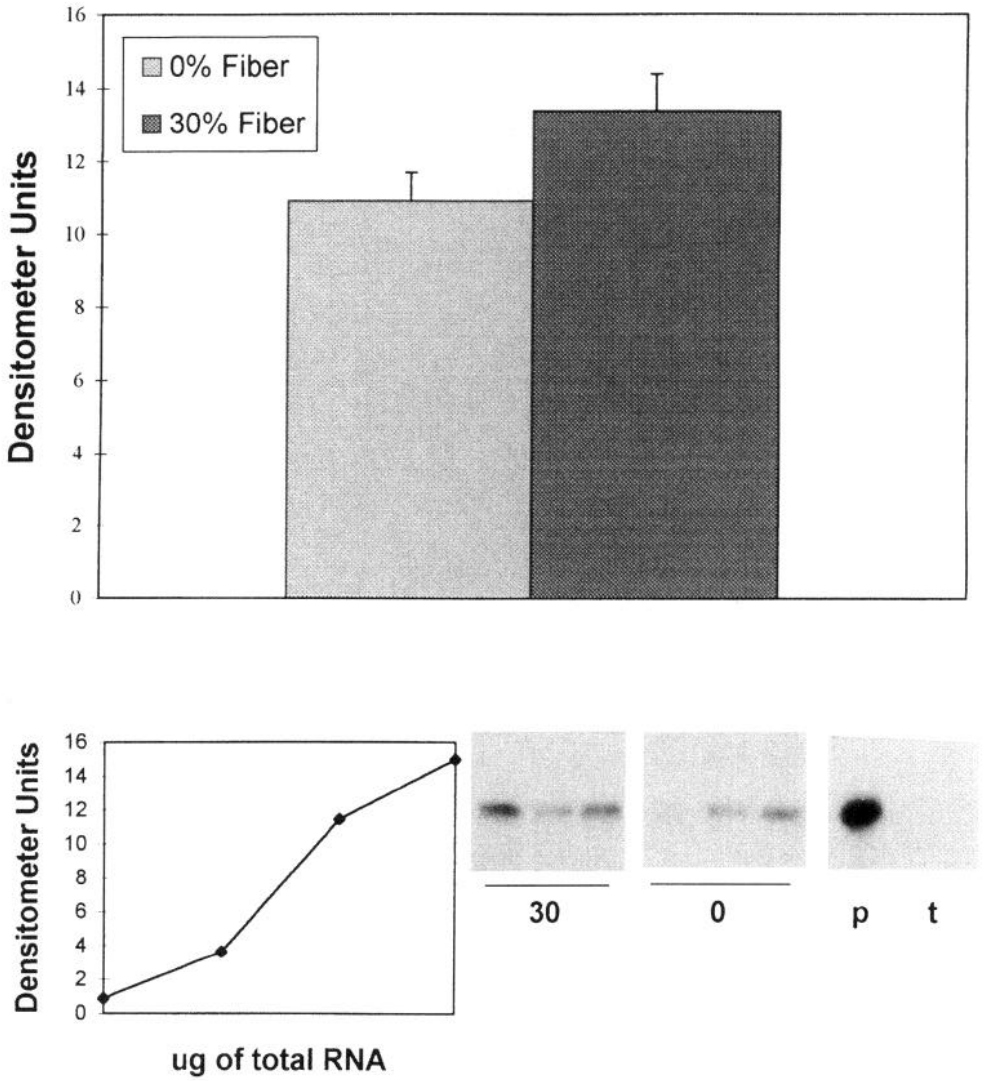

FIG. 4. Effect of increasing levels of fiber supplementation on colonic proglucagon mRNA expression in rats. Values are the mean ± SEM (n = 12 rats/0% diet treatment; n = 8 rats/30% diet treatment). Values with different letters are significantly different ($P < 0.05$). *Inset* of autoradiograph. Each lane (or well) of the gel was loaded with 15 μg total RNA. See *Materials and Methods* for explanation of controls. 0 = 0% fiber; 30 = 30% fiber; t = tRNA; p = probe alone.

SCFA analysis

At the time of analysis, 0.5 ml (3 mg/ml) isocaproic acid was added as an internal standard to each sample. Samples were frozen overnight and then centrifuged in Eppendorf tubes until a clear supernatant was obtained. One milliliter of supernatant was run on gas chromatography (column: Stabilwax-DA; id, 30 m × 0.25 mm; temperature, 120–170 C at 10 C/min; injector: 170 C; detector: 190 C; split ratio, 20:1).

RIAs

Approximately 8 ml blood were collected from each rat into a chilled syringe. Approximately 6 ml blood were collected with the addition of EDTA (1 mg/ml blood) and aprotinin (500 kallikrein inhibitor units/ml blood; Sigma Chemical Co.). The remaining 2 ml were mixed with 80 μl Heparin Leo (1000 IU/ml; Leo Laboratories Canada, Ajax, Canada) and 120 μl NaF (0.025 g/ml; Fisher Scientific, Fairlawn, NJ). Blood was centrifuged at 1600 × *g* for 15 min at 0 C, and aliquots were taken for GLP-1, glucagon, and C peptide determinations from the EDTA samples. The NaF samples were divided to provide aliquots for insulin and glucose determinations. Samples were stored at −70 C.

GLP-1-(7–37)

GLP-immunoreactive peptides were extracted from 2.5 ml plasma using a Sep-Column containing 200 mg C_{18} (catalog no. RIK-SEPCOL 1, Peninsula Laboratories, Belmont, CA) with buffer A (0.1% trifluoroacetic acid; catalog no. RIK-BA-1, Peninsula Laboratories) and buffer B (60% acetonitrile; catalog no. RIK-BB-1, Peninsula Laboratories) as elution solvents. The extraction was performed according to the protocol provided with the GLP-1-(7–37) RIA kit. The recovery rate of the extracted peptide was 50% using this method. Concentrations of GLP-1-(7–37) were measured using a double antibody RIA kit (Peninsula Laboratories; catalog no. RIK-7123). This kit measures GLP-1-(x-37) with less than 0.01% cross-reactivity with GLP-1-(7–36). The ED_{50} for this assay is 45 pg/tube at a binding of 98.2% (defined as the mean total bound minus 2 SD from the mean total bound). The intraassay coefficient of variance was 6.76%.

C Peptide

Plasma levels of C peptide were quantified in a single RIA using a commercial rat C peptide RIA kit (Linco Research, St. Louis, MO). The ED_{50} for this assay is 397 pM/ml at a binding of 98.8% (defined as the mean total bound minus 2 SD from the mean total bound). The intraassay coefficient of variance was 2.10%.

Insulin and glucagon

Plasma insulin and glucagon concentrations were measured at the Muttart Diabetes Research Center, University of Alberta (Edmonton,

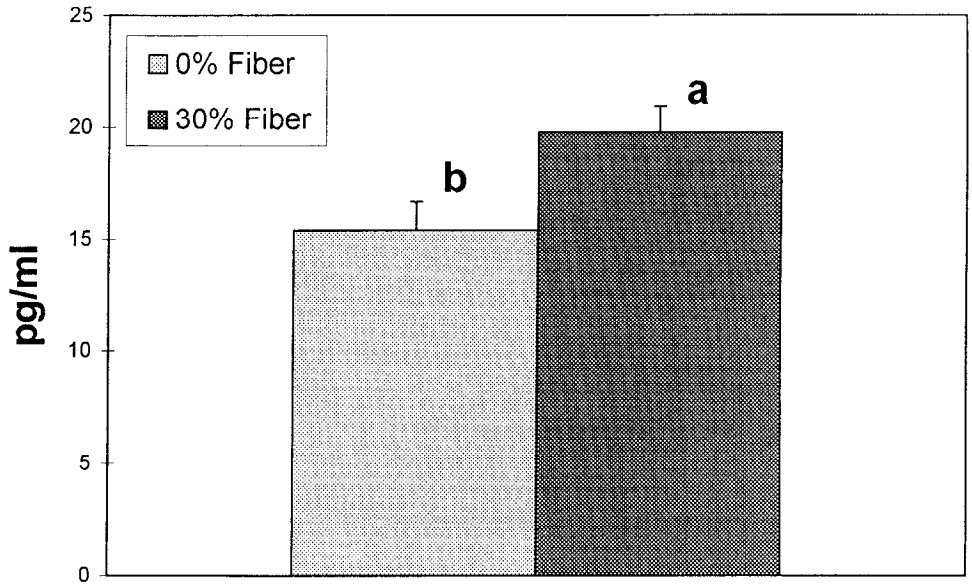

FIG. 5. Plasma levels of GLP-1-(7–37) 30 min postoral glucose load in rats fed either a 0% or 30% fiber diet. Values are the mean ± SEM (n = 8 rats/0% diet treatment; n = 9 rats/30% diet treatment). Values with different letters are significantly different ($P < 0.05$).

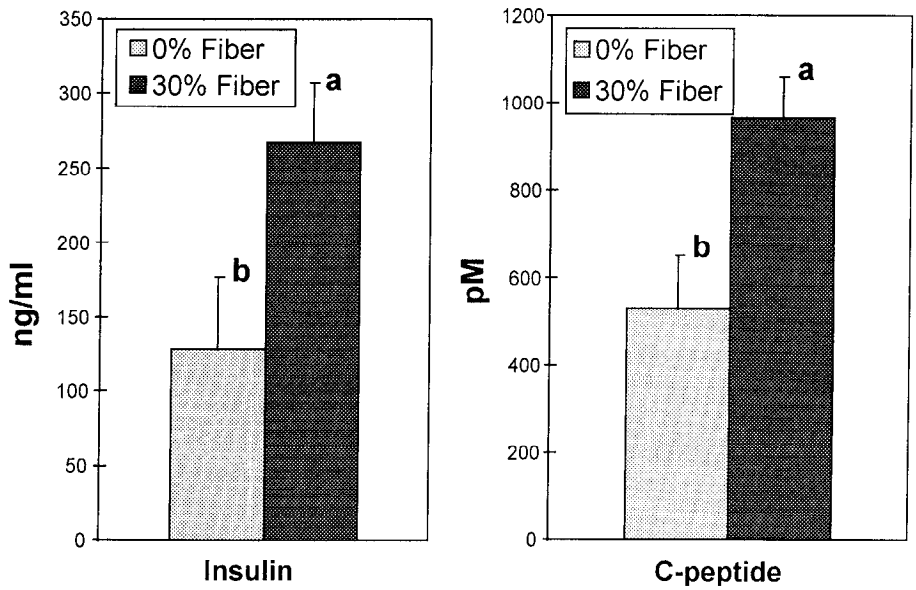

FIG. 6. Plasma levels of insulin and C peptide 30 min postoral glucose load in rats fed either a 0% or 30% fiber diet. Values are the mean ± SEM (for insulin: n = 9 rats/0% diet treatment and n = 13 rats/30% diet treatment; for C peptide: n = 8 rats/0% diet treatment and n = 13 rats/30% diet treatment). For each individual peptide, values with different letters are significantly different ($P < 0.05$).

Alberta, Canada). Insulin was determined using a commercial double antibody RIA kit (Linco Research, St. Louis, MO) for rat insulin with a detection limit of less than 2 μU/ml. Plasma levels of glucagon were determined using a commercial double antibody RIA kit (Diagnostic Products Corp., Los Angeles, CA).

Plasma glucose determination

Plasma glucose was determined using Sigma Diagnostics glucose (Trinder) reagent for the enzymatic determination of glucose at 505 nm.

Statistical analysis

All data are given as the mean ± SEM. Differences between treatments were determined using the one-way ANOVA model in the general linear model procedure in SAS (version 6.04, SAS Institute, Cary, NC). Statistical significance is defined as $P \leq 0.05$.

Results

Diet intake and growth

Animals consumed 15.2 ± 0.9 and 19.5 ± 0.9 g/day of the 0% and 30% fiber diets, respectively. These means were significantly different ($P < 0.05$). Under *in vitro* fermentation conditions, Fibrad, a moderately fermentable fiber source, produced 4.50 mmol SCFA/g organic matter (40). The digestible energy of the fiber mixture was determined experimentally *in vivo* to be 1.17 Cal/g (Marsman, K. E., and M. I. McBurney, unpublished data). Nutrient intakes, with the exception of dietary fiber, did not differ between diets because of the greater intake on the 30% fiber diet. The animals eating the 0% and 30% fiber diets ingested similar amounts of energy (64.9 ± 3.4 *vs.* 65.0 ± 3.3 Cal/day), protein (2.7 ± 0.1 *vs.* 2.4 ± 0.1 g/day), lipid (1.5 ± 0.1 *vs.* 1.4 ± 0.1 g/day), sugars (10.1 ± 0.5 *vs.* 9.0 ± 0.5 g/day), and vitamin and

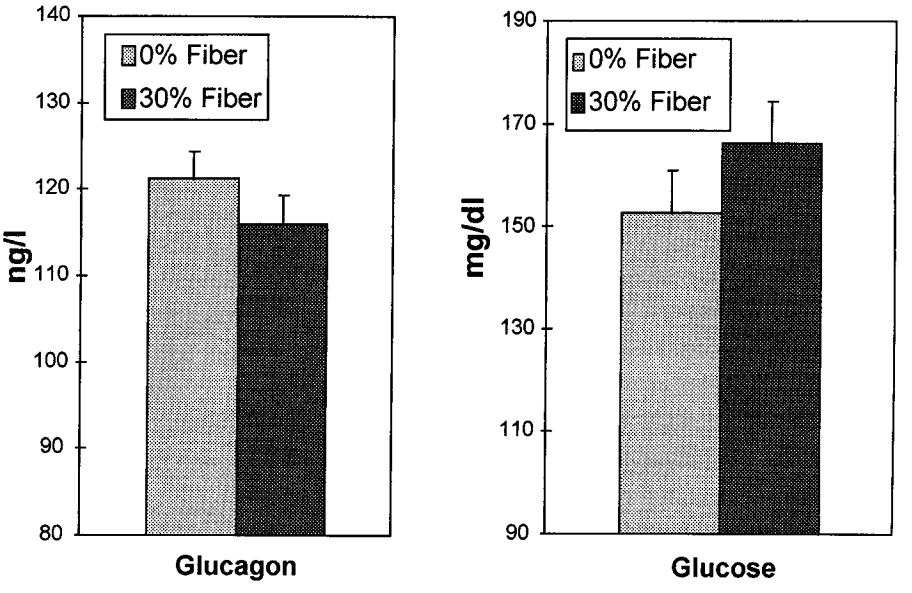

FIG. 7. Plasma levels of glucagon and glucose 30 min postoral glucose load in rats fed either a 0% or 30% fiber diet. Values are the mean ± SEM (n = 10 rats/0% diet treatment; n = 9 rats/30% diet treatment for glucagon; n = 9 rats/ diet treatment for glucose). For each individual peptide, values with different letters are significantly different ($P < 0.05$).

mineral mix (0.9 ± 0.04 *vs.* 0.8 ± 0.04 g/day), respectively. Animals gained 2.9 ± 0.3 and 3.5 ± 0.3 g/day on the 0% and 30% fiber diets, respectively. Weight gain was not significantly different.

Intestinal segment characteristics

Total small intestine weight did not differ between the two groups due to preferential weight gain in the duodenum and jejunum with the 0% fiber diet and in the ileum with the 30% fiber diet (Table 2). In the distal gut, the 30% fiber diet resulted in a significant increase in the mass of the ileum, cecum, and colon. The total RNA content of the colon was greater with the high fiber diet than with the elemental diet (1.9 ± 0.1 *vs.* 1.6 ± 0.1 mg/g wet tissue; $P < 0.05$).

SCFA analysis

The concentration of butyric acid was significantly higher (0.03 ± 0.004 *vs.* 0.02 ± 0.003 mmol/mg wet wt) in the cecum of the 30% fiber animals ($P < 0.05$). As well, animals fed the 30% fiber diet had a greater mass of cecal contents and a higher amount (millimoles) of the predominant SCFA and summed total SCFA. The effect of fiber on the amount (millimoles) of SCFA found in the cecum is shown in Fig. 1. Differences due to diet were found for acetate, butyrate, and total SCFA ($P \leq 0.05$).

Concentrations (millimoles per g wet wt) of SCFA in colonic contents were unaffected by diet. Figure 2 shows the amount (millimoles) of predominant and total SCFA in the colon. Only butyrate was increased by the 30% fiber diet ($P \leq 0.05$). Although not significant, all other SCFA assayed were consistently higher with the fiber diet.

Proglucagon mRNA

The protected proglucagon mRNA fragment of 340 bases was readily detected in the total RNA from colon and ileal samples. As shown in Figs. 3 and 4, statistical analysis of the mean densitometric readings confirmed that feeding a 30% fiber diet significantly increases the amount of proglucagon mRNA in the ileum ($P \leq 0.05$), but in the colon, this only reached a P value of 0.07. Ileal levels of proglucagon mRNA in the 30% fiber animals were nearly double those in the 0% fiber animals (11.5 ± 0.9 *vs.* 6.5 ± 0.9 densitometer units). Colonic levels of proglucagon mRNA in the 30% fiber animals were approximately 20% higher than those in the 0% fiber animals (13.4 ± 1.0 *vs.* 10.9 ± 0.8 densitometer units).

RIAs

Figure 5 shows the increased levels of GLP-1 present in the blood of rats fed the 30% fiber diet *vs.* the 0% fiber group ($P = 0.02$). Insulin levels were significantly higher in the high fiber fed group (Fig. 6). As observed with the insulin responses, C peptide levels in rats consuming the 30% fiber diet were significantly higher than those in the 0% fiber-fed group (Fig. 6).

There was no significant difference in the levels of glucagon between the two groups (Fig. 7). At the 30 min point, no difference was found in plasma glucose levels between the two groups (Fig. 7).

Discussion

Ileal proglucagon gene expression is known to adapt (41), with increases occurring after massive small bowel resection (15). As well, levels of proglucagon mRNA in the developing ileum increase during the postnatal period, peaking at weaning and then decreasing somewhat before reaching adult levels (15). The present study demonstrates that long term adaptation to dietary fiber modulates proglucagon mRNA abundance and subsequent GLP-1-(7–37) secretion after an oral glucose load.

In this study, animals ate equivalent amounts of nutrients, and only dietary fiber intake differed. SCFA contents in the cecum and colon were increased with fiber as expected (23–25). Indeed, luminal concentrations of SCFA underestimate total production because approximately 95% of those produced are absorbed (42). The predominant SCFA, acetate, propionate, and butyrate, account for approximately 90–95% of SCFA production. The localization of L cells to the distal gut places them in a strategic position to respond to short

chain fatty acids. Our results confirm a higher content of SCFA in the cecum and colon of the animals fed the 30% fiber diet, which may be responsible for changes in proglucagon gene expression and postprandial GLP-1 secretion. Luminal pectin, but not SCFA, stimulates the release of GLP-1-(7–36) amide in the isolated vascularly perfused colon (43). Therefore, we cannot determine whether the release of GLP-1 is modulated by SCFA production or the entry of fiber into the large intestine. However, iv infusions of SCFA with total parenteral nutrition (TPN) in rats after massive small bowel resection significantly increased proglucagon mRNA abundance 3 and 7 days postoperatively (Tappenden, K. A., and M. I. McBurney, unpublished data).

Increases in gene expression are physiologically meaningful if concomitant alterations are also seen in the resulting peptide. The commercial antibody used in this RIA is directed toward the C-terminal end and thus does not differentiate between pancreatic GLP-1-(1–37) and intestinal GLP-1-(7–37). However, after a meal, nearly all of the GLP-1-immunoreactive material is caused by changes in the secretion of GLP-1 moieties from the small intestine rather than the pancreas (44). Thus, our RIA predominantly reflects the responses of the intestinal insulinogenic forms of GLP-1 immunoreactivity rather than those of the noninsulinogenic pancreatic forms. The acceptance that approximately 70% of GLP-1 secretion from the small intestine of the rat (11) and 80% in humans (12) are in the amidated form clearly indicates that the newly available RIA for GLP-1-(7–36) amide is the most accurate means of determining GLP-1 in a single RIA. It is probable that the RIA used in this experiment underestimates total small intestinal GLP-1 secretion, because GLP-1-(7–36) amide is not quantified. Pancreatic GLP-1-(1–37) is not insulinogenic (3), so we conclude that the changes observed in plasma insulin and C peptide concentrations reflect increased intestinal proglucagon expression and GLP-1 secretion.

Plasma concentrations of GLP-1 vary with meal pattern (12). In healthy human volunteers, fasting GLP levels are 30–40 pmol/L and rise about 2-fold after an oral glucose load, with a peak occurring at 30 min and declining to basal values after 180 min (44). In the rat, we observed plasma values of GLP-1-(7–37) in the range of 15–20 pM 30 min after oral glucose administration. As the GLP-1 RIA requires 6 ml whole blood for extraction of the peptide and another 2 ml were required for glucose, insulin, glucagon, and C peptide, we could only obtain one plasma sample per rat. We chose the 30 min point, when GLP-1 concentrations are elevated (44), to determine diet-induced differences in GLP-1 secretion. Unfortunately, due to the constraints on blood sampling, it was physically impossible to obtain multiple samples and determine incremental areas under the curve for metabolites of interest. In this study, all animals were gavaged with 50% glucose solutions. D'Alessio *et al.* (45) have shown that plasma GLP-1 concentrations are increased with luminal nutrients, but not water or saline. Our comparisons are only after an oral glucose load. The increase in GLP-1 observed support the findings of Gee *et al.* (28) that fermentable, but not viscous, carbohydrates result in increased plasma enteroglucagon concentrations.

Studies in the rat insulinoma cell line RIN 1046–38 showed that GLP-1 increased levels of proinsulin mRNA (6). In addition, it has been shown that GLP-1 stimulates proinsulin biosynthesis in insulinoma cells (13). Consistent with the known biological action of GLP-1, insulin and C peptide secretion was elevated in animals fed the 30% fiber diet. Many studies have shown that long term ingestion of fiber improves glucose tolerance. Groop *et al.* (20), in contrast with some reports (46) and in agreement with others (47, 48), did not find changes in serum insulin concentrations; however, in response to oral glucose, C peptide increased after guar gum treatment. C Peptide measurements provide a better estimate of insulin secretory rate than peripheral insulin measurements and represent a true enhancement of insulin secretion (22, 49).

In summary, we suggest that reported improvements in glycemic control after adaptation to dietary fiber can be explained by changes in SCFA production, which modulates intestinal proglucagon abundance and postprandial GLP-1 secretion. McBurney *et al.* (50) used the frequently sampled iv glucose tolerance test to demonstrate that iv infusions of SCFA do not affect insulin sensitivity, glucose disposal, or insulin secretion. However, iv SCFA do increase proglucagon mRNA abundance after massive small bowel resection (Tappenden, K. A., and M. I. McBurney, unpublished data). Thus, in demonstrating that SCFA do not directly affect insulin secretion or glucose metabolism, McBurney *et al.* (50) confirmed previous observations (2) that the enteroinsular axis can be uncoupled by circumventing the intestine with iv infusions. In this study, we show that long term ingestion of dietary fiber by rats ingesting similar amounts of energy, protein, lipid, glucose, vitamins, and minerals stimulates SCFA production and proglucagon mRNA abundance and increases postprandial GLP-1, insulin, and C peptide concentrations. We propose a mechanism to explain improvements in glycemic control observed with chronic consumption of dietary fiber.

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