# Effect of Dietary Fiber Concentrated from Celery, Parsnip, and Rutabaga on Intestinal Function, Serum Cholesterol, and Blood Glucose Response in Rats

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Wheat bran, oat bran, or 80% ethanol-insoluble residues from celery, parsnip, or rutabaga were incorporated in purified diets to reach a 6% fiber level. Rats were fed one of the fiber diets or a fiber-free diet. Intestinal function was improved with all fiber diets. Fecal moisture content was the highest with the parsnip fiber diet; fecal density was the lowest with the wheat bran diet. Fiber fermentability was inversely correlated with percent wet volume or dry matter. Total fat excretion was increased with all fiber-containing diets compared with controls. Serum and HDL cholesterol levels were unchanged. Blood glucose levels tended to be reduced. The 6% level of the vegetable fibers was well tolerated, but the weights of the heart, spleen, and liver were significantly decreased by parsnip, parsnip and rutabaga, and rutabaga, respectively, suggesting that the safety of these vegetable fiber concentrates merits further investigation.

It has been recommended that the U.S. and Canadian populations increase their intake of dietary fiber from a variety of sources, including vegetables (HPB, 1985; FDA, 1987). Investigation of the physiological effects of dietary fiber has been rapidly progressing over the last 15 years (Leeds, 1985). Although a dozen or so Western diseases were previously attributed to lack of fiber in the diet (Trowell and Burkitt, 1975), major physiological effects of dietary fiber are those relating to intestinal function, blood glucose response, and blood lipid levels (HPB, 1985).

In spite of the fact that the per capita consumption of vegetables has risen over the past decade (Bingham, 1986), the potential physiological effects of vegetable fiber have remained largely uninvestigated. Part of the reason is that most vegetables have a relatively low fiber content compared with that of cereal grains. In order to assess the potential health benefits of vegetable fibers, therefore, it is necessary to concentrate their dietary fiber content for incorporation into the test diets.

The present work was undertaken to evaluate the potential effects of fiber concentrates from rutabaga, parsnip, and celery, compared with those of wheat bran and oat bran, on the intestinal function, blood glucose response, and blood lipid levels of rats, and, for safety assessment, on the weight of vital organs.

## EXPERIMENTAL SECTION

Animals. Fourty-eight young male Sprague-Dawley rats (Cr1:CD (SD) BR; Charles River Canada Inc., St. Constant, Québec), weighing  $100 \pm 10$  g, were housed individually in Nalgene metabolic cages to minimize food spillage and optimize separation of feces from urine. Illumination was provided automatically from 7 a.m. to 7 p.m. Room temperature was controlled at  $22 \pm 1$  °C and humidity at 45-55%. Animals were randomly divided into six groups and fed control, vegetable fiber, or cereal bran containing purified diets for up to 21 days. Food and tap water were provided ad libitum. Body weight was measured twice a week.

**Preparation of Ethanol-Insoluble Residues from Vegetables.** Fibers from celery (Aspium graveolem), parsnip (Curcubita maxima), and rutabaga (Brassica mapibrassica) were prepared by the Food Research Centre, Agriculture Canada, as follows: Fresh celery, parsnips, and rutabaga were obtained from a local distributor. Whole parsnips and rutabaga were peeled and diced prior to extraction; only the stalks of the celery were used. Samples weighing 800 g (wet weight) were extracted in a Waring blender for 20 min with 1.5 L of boiling 80% ethanol. The hot solutions were filtered on a Buchner funnel of medium porosity. Five more similar extractions yielded ethanol-insoluble solids, which were then washed with acetone to produce fluffy white materials. These materials were air-dried on trays, blended thoroughly, and stored in ground-glass stoppered bottles. They are called fiber concentrates in this paper. A total of 500 g of fiber concentrate from celery, 1200 g from parsnip, and 600 g from rutabaga was prepared.

**Diets.** AIN-76 diets were modified as shown in Table I. The basal diet contained 20% casein, 10% corn oil, 3.5% salt mixture (AIN mineral mixture 76; ICN Nutrition Biochemicals Ltd., Cleveland, OH), 1% vitamin mixture (AIN vitamin mixture 76, ICN), 0.3% DL-methionine, and 0.2% choline bitartrate. Fiber sources were exchanged for corn starch in amounts calculated to obtain a fiber polysaccharide content of approximately 6%. The level of corn starch was also decreased by the amount of starch contained in each fiber source.

Hard red spring certified wheat bran was purchased from the AACC (American Association of Cereal Chemists, St. Paul, MN 55121). Oat bran (Mother's oat bran) was provided by the Quaker Oats Co., Chicago, IL 60654. Total food intake was measured twice each week.

Fiber and Nutrient Analyses and Fermentability Estimates. Moisture contents of vegetable fiber concentrates were determined by drying in vacuo to constant weight at 56 °C.

Ash and nitrogen contents were determined by the method of Steyermark (1961).

Starch was hydrolyzed to glucose with use of  $\alpha$ -amylase and amyloglucosidase as described by Batey (1982) and the amount determined by an automated glucose oxidase procedure, based on the method of Lloyd and Whelan (1969) with the Technicon Autoanalyser II system (Method No. 42B-76A).

The insoluble fiber content of the diets and test fiber sources was measured in duplicate by the detergent system of Goering and Van Soest (1970) as outlined by Mongeau and Brassard (1982). The modifications include rapid treatment (65 min) with  $\alpha$ -amylase from hog pancreas (Sigma Chemical Co., St. Louis, MO 63178; Catalog No. A6880 or A3176).

Fermentability of the insoluble fiber was estimated from the neutral detergent fiber (NDF) in the diet and in the feces. (The fermentability of soluble fiber is not usually estimated since

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Table I. Composition of Diets  $(g/100 g)^{\alpha}$ 

	CTRL	С	Р	R	W	0
basal diet	35.0	35.0	35.0	35.0	35.0	35.0
cornstarch	65.0	57.3	48.3	56.7	55.0	35.6
source of fiber	0.0	7.7	16.7	8.3	10.0	29.4

<sup>a</sup> See text. Key: CTRL, fiber-free control; C, P, and R, fiber concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran.

soluble fiber is extensively fermented in the colon.)

The total fiber content of the diets was determined by the above described NDF procedure supplemented with a rapid procedure for soluble fiber (Mongeau and Brassard, 1986, 1989). In addition, the dietary nonstarch polysaccharide contents of the vegetable fiber concentrates were measured using the Englyst method (1982) as follows: The nonfiber material was removed by enzymatic treatment, and the fiber residue (water-soluble and insoluble) was hydrolyzed with sulfuric acid. The constituent sugars were determined by GC as alditol acetates. The total monomer content (expressed as polysaccharides) was taken to represent total dietary fiber. This measurement would include water-soluble and insoluble fiber, but not lignin.

Uronic acid was analyzed by Englyst's colorimetric procedure and also by the decarboxylation technique of Castagne and Siddiqui (1975), with modifications as described by Siddiqui and Morris (1979). The decarboxylation technique has been successfully applied for the analysis of a number of acidic polysaccharides of plant, bacterial, fungal, and animal origin.

Fecal Characteristics. In order to evaluate the effects of the fiber sources on intestinal function, several fecal parameters were measured. The consistency of up to 17 fecal pellets for each group (average 2/rat) was estimated on days 8 and 9. The fresh samples were immediately sealed in a small, tared plastic bag, and their deformability was measured with a "consistometer". This apparatus, developed by the Food Directorate, Health Protection Branch, Ottawa, consists of two horizontal plates that are finely machined so that the upper plate can be slowly and steadily lowered by a gear system and a stepping motor. The motor is controlled by a microcomputer connected with a strain gauge (Sartorius 1020) located in the lower plate. A camera (Microneye) is placed above a plexiglass window in the upper plate; the lower plate has a translucent window equipped with a light. The camera system measures the area of the surface shaded by the sample when placed on the translucent window. The pellet weight, smallest diameter, and consistency (deformability at 600 g pressure between the plates) were also automatically measured and recorded by the software. The pressure between the plates is equivalent to placing a weight of 600 g on the sample. Consistency was defined as the extent of displacement from initial to final pressure since measurement of diameter was more precise than that of surface area. The measurement of displacement with use of rubber samples was shown to be precise and reproducible. The extent of displacement increased with the softness of the sample. The rate of increase in pressure to 600 g was shorter with a hard sample (e.g., a few seconds) than with a sample with a soft consistency (e.g., 20 s). At 600 g pressure, the deformation resulted in flattening, but not rupture, of the pellets.

On days 12-15, the feces of individual rats were collected once each morning and their weight and volume were determined immediately. Wet volume was measured as previously described (Mongeau and Brassard, 1984). Density was calculated by dividing wet weight by wet volume. Water content was estimated by freeze-drying. These data were determined for each rat over the 4-day period and expressed per 100 g of food ingested (total fecal output divided by total food intake  $\times$ 100 for the same rat). This reduces variations in fecal output due to variations in food intake. The results are expressed as the means (±SD) for each group of *n* rats for the 4-day collection period.

The feces from the animals of each group were pooled and kept frozen for analyses of pH and composition.

**Blood Glucose and Cholesterol Determination.** After 17 days of feeding, an equal number of animals from each group

Table II. Dietary Fiber Content of Diets (g/100 g)<sup>a</sup>

	CTRL	С	Р	R	W	0
total dietary fiber <sup>b</sup>	0.78	5.72	5.86	6.46	7.07	6.75
water-soluble	0.32	2.39	3.43	3.36	1.23	3.18
	$(41)^{c}$	(42)	(59)	(52)	(17)	(47)
water-insoluble	0.46	3.33	2.43	3.10	5.84	3.57
	(59)	(58)	(41)	(48)	(83)	(53)

<sup>a</sup> Key: CTRL, fiber-free control; C, P, and R; concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran. <sup>b</sup> Using the method of Mongeau and Brassard (1986, 1989). <sup>c</sup> Percent of total dietary fiber as water-soluble and water-insoluble given in parentheses.

was used for each series of tests. Each series of tests was started at 9:00 a.m. on consecutive days. Animals were fasted for 18 h, weighed, and given, by stomach tube, a solution containing glucose (0.25 g/mL) and fiber concentrate from celery, parsnip, or rutabaga or oat bran (0.01 g/mL, 1 mL/100 g of body weight). The control group received the glucose solution alone. The animals from the wheat bran fed group were fasted but were not intubated, since wheat bran is inappropriate for tube feeding.

A few drops of blood were collected by tail puncture at times 0 and 60 min following gastric intubation. Whole blood glucose was measured with use of a portable dextrometer (Ames). At 120 min, animals were anesthetized with 2% fluothane (Ayerst, Montréal, Québec) in oxygen. Blood was collected from the abdominal aorta, and a few drops were used immediately for measuring glucose concentration. The remaining blood was centrifuged in heparinized tubes; the resulting plasma was frozen and kept at -70 °C for subsequent glucose and cholesterol analyses. Plasma glucose was measured on an ABA (Abbott bichromatic analyzer) using the A-Gent glucose-UV test kit (Abbott Laboratories Ltd., Mississauga, Ontario). These latter results are not reported since they confirm those made on whole blood at 120 min using the dextrometer. Total plasma cholesterol and HDL cholesterol (high-density lipoprotein) were measured on an ABA using the A-Gent cholesterol and HLD test kits (Abbott), respectively.

**Organ Weights.** The hearts, spleens, livers, and kidneys were excised, cleaned of obvious fat, and weighed. The cecum was weighed with its contents.

Statistical Analyses. The Mann and Whitney nonparametric test was used to determine the statistical significance of differences among values for test and control dietary groups (Stat Plus, 1982).

#### RESULTS

Table II shows the proportions of water-soluble and insoluble fibers in the vegetable concentrate and cerealbran diets. These are roughly equal for the vegetable fiber and oat bran diets; the wheat bran diet had a relatively high proportion of insoluble fiber. Table III illustrates the nutritional composition of the vegetable concentrates, including dietary fiber constituents, as analyzed by two methods. The celery and rutabaga concentrates were predominantly fiber; however, the parsnip concentrate consisted of almost 50% starch.

The mean daily food consumption for each group of rats ranged from  $15.3 \pm 2.3$  g for the rutabaga diet to  $16.8 \pm 1.2$  g for the control diet (results not shown). After 16 days on the diets, the mean body weight gain of rats ranged from  $111 \pm 19$  g on the rutabaga diet to  $125 \pm 15$  g on the oat bran diet (results not shown). None of these differences were statistically significant ( $P \ge 0.14$ ).

The effects of the test diets on consistency and other fecal characteristics are shown in Tables IV and V, respectively. The consistency of individual fecal pellets was estimated from measurement of the extent of displacement from initial to final pressure. The extent of displacement increased with the softness of the sample. The displacement (P < 0.01), the weight (P < 0.02), and the

Table III. Composition of Concentrates from Celery, Parsnip, and Rutabaga (g/100 g)

	celery	parsnip	rutabaga
yield of residue	1.59	12.73	3.22
moisture	11.57	9.02	11.48
ash	8.83	2.81	4.55
protein $(N \times 5.8)$	9.75	9.94	7.13
starch	0.34	48.22	7.27
dietary fiber			
method 1, insoluble (NDF <sup>a</sup> )	37.33	13.54	39.70
hemicellulose	6.52	3.18	7.85
cellulose	28.29	9.16	29.10
lignin	2.27	0.84	1.24
cutin	0.25	0.36	1.51
method 2, $NSP^{b}$	64.72	29.68	60.72
rhamnose	2.24	0.77	2.26
ribose	0.24	0.00	0.09
arabinose	4.38	5.09	5.60
xylose	4.16	1.02	2.54
mannose	1.90	0.61	1.48
galactose	5.20	3.90	5.15
glucose	29.98	9.10	23.25
uronic acid	16.64	9.20	20.36
uronic anhydride <sup>c</sup>	25.51	18.17	25.00

<sup>a</sup> Rapid neutral detergent fiber method (Mongeau and Brassard, 1982). <sup>b</sup> Nonstarch polysaccharides (Englyst et al., 1982). <sup>c</sup> Method of Siddiqui and Morris (1979).

Table IV.Effect of the Test Diets on Consistency of FecalPellets As Measured by Deformation at 600 g Pressure<sup>a</sup>

	CTRL	С	Р	R	W	0
n	16	13	17	14	16	16
pellet wt,	74 <sup>b</sup>	83 <sup>ъ</sup>	99ь	92 <sup>ь</sup>	88 <sup>6</sup>	134°
mg	$(\pm 25)$	(±28)	(±42)	(±36)	(±26)	(±37)
pellet diam,						
mm						
init	3.84 <sup>b</sup>	4.46°	4.43°	4.38°	4.87 <sup>d</sup>	4.77 <sup>d</sup>
	$(\pm 0.45)$	$(\pm 0.34)$	$(\pm 0.62)$	(±0.43)	(±0.25)	$(\pm 0.31)$
init – final <sup>ø</sup>	1.97 <sup>b</sup>	2.47 <sup>b</sup>	2.92°	2.97°	2.72 <sup>bc</sup>	3.69 <sup>d</sup>
	$(\pm 1.23)$	(±0.39)	(±0.66)	(±0.58)	(±0.51)	(±0.43)
area shaded surf, mm <sup>2</sup>						
init	26.9 <sup>b</sup>	32.7°	32.1 <sup>bc</sup>	29.5 <sup>ьс</sup>	34.0°	41.4 <sup>d</sup>
	( <b>±6</b> .2)	(±6.8)	$(\pm 12.2)$	$(\pm 12.9)$	(±6.8)	(±8.8)
final – init <sup>c</sup>	35.8 <sup>bc</sup>	32.8 <sup>b</sup>	35.4 <sup>bc</sup>	45.2°	37.2 <sup>bc</sup>	82.4 <sup>d</sup>
	$(\pm 21.4)$	(±4.0)	$(\pm 24.4)$	(±15.4)	(±11.6)	(±37.0)

<sup>a</sup> Key: CTRL, fiber-free; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran; n, number of pellets. (b-d) Values in the same line with different superscripts are significantly different (P < 0.05). See text. Mean (±SD). <sup>b</sup> Degree of displacement from initial to final pressure. <sup>c</sup> Change from initial to final pressure.

shaded surface (P < 0.02) with the fecal pellets from Ofed rats were clearly different from that of rats fed all other diets; a difference (P < 0.05) was also seen in the initial diameter except for group W (Table IV). Oat bran feeding resulted in longer fecal pellets (results not shown). The displacement with the fecal pellets from P- and Rfed rats was significantly larger (P < 0.03) than that of rats fed the control and celery diets; other differences were not significant. The changes in the area of shaded surface paralleled the above results. The softness of the fecal pellets from O-fed rats was reflected by a 3-fold increase in the area of shaded surface (Table IV).

As indicated in Table V, all fiber diets at least doubled daily fecal weight (P = 0.001) and volume  $(P \le 0.002)$  compared with controls; wheat bran induced a moderate fecal weight (2.1-fold increase) but the highest fecal volume (3.8-fold increase). The relatively high fecal volume of P-fed rats was associated with a high water content (Table V). All fiber diets increased dry matter (P)

 Table V.
 Effect of the Test Fiber Diets on Fecal

 Characteristics<sup>a</sup>

	CTRL	С	Р	R	W	0
n	8	7	8	8	8	8
wet wt, g	4.19 <sup>b</sup>	$9.72^{cd}$	13.51 <sup>d</sup>	8.68°	10.82 <sup>d</sup>	11.83 <sup>d</sup>
	(±0.87)	$(\pm 2.66)$	$(\pm 5.06)$	(±4.42)	(±1.77)	$(\pm 2.31)$
wet vol, mL	3.79 <sup>b</sup>	10.63 <sup>cd</sup>	13.41 <sup>de</sup>	8.95°	14.36°	11.44 <sup>cd</sup>
	(±1.04)	$(\pm 2.93)$	$(\pm 5.26)$	(±4.71)	(±1.80)	$(\pm 2.60)$
wet density,	1.17°	0.92 <sup>cd</sup>	1.02 <sup>e</sup>	0.99 <sup>de</sup>	0.81 <sup>ь</sup>	1.04°
g/mL	(±0.32)	(±0.07)	(±0.06)	(±0.10)	(±0.07)	(±0.05)
water, g	2.01 <sup>b</sup>	4.54 <sup>ce</sup>	7.74 <sup>ef</sup>	4.55 <sup>cd</sup>	5.33 <sup>df</sup>	6.63 <sup>f</sup>
	(±0.50)	(±1.52)	(±3.51)	$(\pm 2.85)$	(±0.35)	(±1.63)
dry matter,	2.18 <sup>b</sup>	5.18 <sup>cd</sup>	5.77 <sup>de</sup>	4.13°	6.18 <sup>e</sup>	5.20 <sup>d</sup>
g	(±0.39)	(±1.17)	(±1.64)	(±1.58)	$(\pm 0.42)$	(±0.72)

<sup>a</sup> Fecal data determined for each rat and expressed per 100 g of food ingested during the 4-day collection period. Key: CTRL, fiber-free control; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran. Mean for n animals ( $\pm$ SD). (b-d) Values in the same line with a different super-script are significantly different (P < 0.05). See text.

Table VI. Fecal pH and Composition<sup>a</sup>

	CTRL	С	Р	R	W	0
pH % insol fiber <sup>b</sup> total fat excretion, mg/ret per dev	6.64 3.05 62	6.42 21.34 93	6.04 22.40 91	6.15 14.57 98	6.47 44.81 106	6.14 21.57 117
total fat concn in wet feces						
g/100 g g/100 mL	17.1 9.68	$\begin{array}{c} 10.8\\ 5.25 \end{array}$	10.0 4.24	15.2 6.97	10.3 4.42	14.3 6.46

<sup>a</sup> Pooled data from animals in each group. Key: CTRL, fiberfree control; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran. <sup>b</sup> Grams of NDF/ 100 g of dry fecal weight.

Table VII. Apparent Fermentability of Insoluble Fiber Contained in Vegetable Fiber Concentrates and Cereal Brans<sup>a</sup>

	CTRL	С	Р	R	W	0
NDF	85.0	66.5	46.1	80.2	52.2	67.8
cellulose	86.8	69.6	44.2	82.3	27.4	57.1
hemicellulose	94.9	70.1	64.8	82.2	62.2	74.8

<sup>a</sup> Duplicate determinations in a pool from each group, percent of disappearance (grams ingested minus grams recovered). Key: CTRL, fiber-free control; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran.

= 0.001) and water excretion ( $P \le 0.002$ ). The fecal characteristics of vegetable fiber fed rats were more variable than those of the W fed rats.

With respect to the composition of the feces (Table VI), insoluble fiber represented 44.8% of the weight of the fecal dry matter from W-fed rats—this is twice the proportion in any other group. All diets increased total fat excretion (mg/rat per day); however, due to the concomitant increase in fecal volume, fecal concentration of fat decreased, e.g. the O diet increased fat excretion by 89% and decreased its concentration (g/mL) by 33%. The W and P diets induced the largest decreases in fat concentration.

The apparent fermentability of the insoluble fiber content of the diets is given in Table VII. The percent of disappearance of insoluble fiber was inversely correlated with the fecal dry matter (y = 8.58 - 0.05x; r = -0.92; P< 0.05) and wet volume (y = 21.14 - 0.15x; r = -0.94; P< 0.02; n = 5 fiber groups).

The mean fasting blood glucose level for the R-fed rats was lower (P < 0.02) than that of control-fed rats (Table

Table VIII. Blood Glucose Response to Gastric Feeding of Glucose and Fiber Sources  $(mg/dL (\pm SD))^a$ 

	CTRL	С	Р	R	0
n	8	7	8	8	8
fasting	91°	84 <sup>bc</sup>	79 <sup>bc</sup>	75 <sup>b</sup>	81 <sup>bc</sup>
	$(\pm 24)$	(±13)	(±13)	(±11)	$(\pm 15)$
60 min	143°	150°	149°	147°	154 <sup>b</sup>
100	$(\pm 16)$	(±23)	(±12)	(±29)	$(\pm 13)$
120 min	163° (+32)	157~	$(\pm 17)$	$(\pm 22)$	$108^{\circ}$ (+37)
	(402)	$(\pm 20)$	(41)	$(\pm 22)$	(±01)

<sup>a</sup> Key: CTRL, fiber-free; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran; n, number of animals. (b, c) Values in the same line with a different superscript are significantly (P = 0.015) different.

Table IX. Effect of the Test Diets on Blood Cholesterol Levels (mg/dL (±SD))<sup>a</sup>

	CTRL	C	р	R	W	0
<u>.                                    </u>						
n	8	7	8	8	8	8
cholesterol	103 <sup>b</sup>	98 <sup>b</sup>	96 <sup>b</sup>	102 <sup>b</sup>	114 <sup>b</sup>	104 <sup>b</sup>
	$(\pm 19)$	$(\pm 22)$	$(\pm 16)$	(±16)	$(\pm 29)$	$(\pm 31)$
HDL	59.4 <sup>b</sup>	58.6 <sup>b</sup>	59.0 <sup>b</sup>	56.0 <sup>b</sup>	65.0 <sup>b</sup>	58.0 <sup>b</sup>
	$(\pm 8.0)$	$(\pm 15.0)$	$(\pm 13.3)$	$(\pm 7.1)$	$(\pm 11.9)$	$(\pm 11.4)$
cholesterol/	1.75 <sup>bc</sup>	1.68 <sup>bc</sup>	1.65 <sup>b</sup>	1.83°	$1.74^{bc}$	$1.77^{bc}$
HDL	$(\pm 0.24)$	$(\pm 0.22)$	$(\pm 0.23)$	$(\pm 0.19)$	$(\pm 0.14)$	$(\pm 0.21)$

<sup>a</sup> Key: CTRL, fiber-free control; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran, n, number of animals. (b, c) Values in the same line with a different superscript are significantly (P = 0.04) different.

Table X. Effect of the Test Diets on Organ Weight (Mean  $(\pm SD))^a$ 

	CTRL	С	Р	R	w	0
n	8	7	8	8	8	8
heart, mg	999°	949 <sup>bc</sup>	898 <sup>b</sup>	904 <sup>bc</sup>	968 <sup>bc</sup>	945 <sup>bc</sup>
, 0	$(\pm 63)$	$(\pm 64)$	$(\pm 95)$	$(\pm 100)$	$(\pm 186)$	(±81)
spleen. mg	770°	691 <sup>bc</sup>	599 <sup>b</sup>	575 <sup>b</sup>	649 <sup>bc</sup>	735°
, ,	$(\pm 55)$	$(\pm 101)$	$(\pm 106)$	$(\pm 118)$	$(\pm 135)$	$(\pm 84)$
liver, g	9.16°	8.53 <sup>bc</sup>	7.91 <sup>bc</sup>	7.75 <sup>b</sup>	8.17 <sup>bc</sup>	8.84 <sup>bc</sup>
	$(\pm 0.81)$	$(\pm 1.13)$	$(\pm 1.06)$	$(\pm 1.24)$	$(\pm 1.32)$	$(\pm 0.57)$
kidney, g	2.07 <sup>b</sup>	2.16 <sup>b</sup>	1.99 <sup>b</sup>	2.01 <sup>b</sup>	$2.01^{b}$	2.11 <sup>b</sup>
578	$(\pm 0.19)$	$(\pm 0.18)$	$(\pm 0.18)$	$(\pm 0.21)$	$(\pm 0.25)$	$(\pm 0.11)$
cecum, g	1.61 <sup>b</sup>	1.91 <sup>bc</sup>	2.13°	2.00 <sup>bc</sup>	1.91 <sup>bc</sup>	2.19°
/ 8	$(\pm 0.24)$	$(\pm 0.45)$	$(\pm 0.72)$	$(\pm 0.75)$	$(\pm 0.24)$	$(\pm 0.41)$
body, g	240 <sup>b</sup>	238 <sup>b</sup>	230 <sup>b</sup>	227 <sup>b</sup>	235 <sup>b</sup>	247 <sup>b</sup>
	$(\pm 11)$	$(\pm 22)$	$(\pm 20)$	$(\pm 26)$	$(\pm 26)$	$(\pm 18)$

<sup>a</sup> Key: CTRL, fiber-free; C, P, and R, concentrates from celery, parsnip, and rutabaga, respectively; W, wheat bran; O, oat bran; n, number of animals. (b, c) Values in the same line with a different superscript are significantly (P < 0.05) different. See text.

VIII). Blood glucose levels in all groups were not different from that of control fed rats at 60 or 120 min after gastric intubation (Table VIII).

Total and HDL cholesterol levels were comparable in all groups (Table IX).

Although the body weight was not significantly  $(P \ge 0.1)$  different among the various groups, the weights of the heart, spleen, and liver were significantly decreased by parsnip (P < 0.03), parsnip and rutabaga (P < 0.01), and rutabaga (P < 0.02), respectively, compared to the control group (Table X). The highest decrease (25%) was in the spleen weight of R-fed rats. Cecum weight was increased in all groups compared with the control group, but the difference was significant only with P-and O-fed rats (P < 0.03, 0.01, respectively) (Table X). Cecal weight was not directly related to the extent of fiber fermentability.

#### DISCUSSION

Dietary fiber has been associated with particular beneficial gastrointestinal effects such as increasing softness and volume of feces (Wozasek and Steigmann, 1942), as well as flattening of blood glucose responses (Kiehm et al., 1976; Jenkins, 1980; Anderson, 1980) and lowering of blood lipid levels (Kay and Strasberg, 1978; Anderson, 1980). Wheat bran provides mostly insoluble fiber, which is about 50% fermentable. It is known to exert effects on the lower gut and on fecal characteristics, while the soluble portion of oat bran has been shown to exert metabolic effects such as the lowering of blood lipid levels (Anderson et al., 1984). The wheat and oat bran diets used in the present study, therefore, provided a good basis for comparison when effects of the vegetable fiber preparations are evaluated.

Although improved intestinal function can be described as increased softness and volume of the feces, effective and adequate techniques to determine these characteristics in humans have not yet been established. The rat is a useful animal model for evaluating the effects of fibers on intestinal function, since rats and humans have similar segmented colonic musculature (Bing, 1976) and are subject to bowel disorders such as diverticulosis resulting from the lack of suitable kinds and amounts of dietary fiber (Carlson and Hoelzel, 1949; Painter, 1982).

Several parameters were used to evaluate intestinal function in the present study. In addition to those conventionally used in studies of the same type (Table V), a new technique involving a consistometer was employed to estimate the relative softness of feces resulting from feeding the different fiber sources. This is a practical instrument that can provide objective data in seconds and with great ease. Using this technique, and comparing the weights and the diameters and surface area of individual fecal pellets before and after deformation, it was found that the oat bran diet induced softer and longer fecal pellets. The fact that these differences were not evident from the results shown in Table V illustrates the desirability of a more complete characterization of feces, including measurement of consistency.

The measurement of pellet diameters has implications for the study of diseases involving changes in colonic pressure in humans, e.g., diverticulosis. According to the La Place law (P = t/r), where t is the tension on the bowel wall, increasing the radius (r) of the bowel lumen decreases intraluminal pressure (P) in humans (*Fed. Regist.*, 1975). The larger fecal diameters observed with the C, P, R, W, and O diets suggest that intraluminal pressure was decreased with fiber feeding.

Fecal volume represents a key factor in evaluating laxation effects, since the volume of material in the rectal lumen is responsible for triggering bowel movements in humans (Phillips and Devroede, 1979). In the present study, wet volume was the most sensitive indicator of change in intestinal function due to fiber feeding, and wheat bran resulted in the highest volume with the lowest variability. The high sensitivity of the fecal volume measurement agrees with previous reports (Mongeau and Brassard, 1985) and is consistent with the observation, initially made by Fantus and Frankl in 1941 and confirmed in later human studies (Phillips and Devroede, 1979; Wyman et al., 1976) that the difference in stool volume is more obvious than the difference in stool weight.

The results of the present study suggest that the parameters shown in Tables IV and V provide unique information relating to intestinal function and are not necessarily correlated. The large weights of individual fecal pellets from oat bran fed rats, for example, were not associated with larger diameters. The data from these measurements of fecal characteristics, however, may be used to

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partially explain the relative fecal bulking and softening effects of the various fiber sources as indicated by fecal volume and deformability. For example, the fecal density and water data indicate that the larger volume of feces from the parsnip and wheat bran fed rats was attributable to water retention and inclusion of dispersed gases, respectively. The low variability of the volume and water content in the feces of wheat bran fed rats observed in the present study was remarkable (Table V). Similarly, the relatively soft feces of the parsnip and rutabaga fed rats could be explained by their higher fecal water and fat contents, respectively. The very soft feces of the oat bran fed rats could have been due to a combination of these two factors. The inclusion of dispersed gases may have influenced the consistency of the feces of wheat bran fed rats as well as their volume.

The fecal composition and fermentability data (Tables VI and VII) give additional information regarding possible mechanisms involved in fecal bulking. Wheat bran, for example, contains a relatively high proportion of both fermentable and nonfermentable fiber. Its moderate fermentability (Table VII) leads to the presence of products of fiber fermentation and unfermented fiber in the lower gut. The relatively high recovery of NDF in feces (45%, Table VI) suggests that the presence of a large number of undegraded particles is probably responsible for the normalizing effects of wheat bran on colonic function observed in humans (Devroede, 1978). The inverse correlation between the fermentability of fiber and fecal volume observed in the present study suggests that changes in volume are not necessarily explained by the extent of fermentability for the different fiber sources (46-85%, Table VII); nevertheless, fermentability remains an important fiber characteristic (Hellendoorn, 1978; Cummings, 1981; Van Soest, 1984).

Compared with the control diet, none of the fiber diets tested induced a flattening effect on blood glucose response following glucose and fiber feeding by stomach tube (Table VIII). Since the known flattening effect of oat bran was not observed, no firm conclusion can be drawn from these data. The significantly lower fasting blood glucose levels of the parsnip fed rats suggests that parsnip fiber may modify the carbohydrate metabolism of normal rats, but in a different way from wheat bran, which induced a relatively high level (121  $\pm$  29 (mean  $\pm$  SD); results not shown). This latter result is in agreement with Delorme et al. (1987) who observed an increased fasting serum glucose level in beagle dogs with chronic ingestion of wheat bran.

The cholesterol lowering effect of oat fiber was not observed here, possibly because of the limited number of days (17-21 days) on the O diet. Since the metabolism of cholesterol in rats is more closely controlled than in humans, it is more difficult to induce changes in blood cholesterol levels in this animal.

The observed decrease in organ weights may have been due to a decrease in both dry and wet weight or to a dehydrating effect. More data are needed on the nature of the change in the weight of tissues of fiber-fed animals. In addition, the possible role of compounds (fiberassociated or not), e.g., those in parsnip and rutabaga, in reducing organ weights should be examined. The organ weight data of the present study suggest that the safety of these vegetable fiber concentrates merits further investigation.

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#### LITERATURE CITED

- Anderson, J. W. Dietary fiber and diabetes. In Medical Aspects of Dietary Fiber; Spiller, G. A., Kay, R. M., Eds.; Plenum Medical: New York, London, 1980.
- Anderson, J. W.; Story, L.; Sieling, B.; Chen, W.-J. L.; Petro, M. S.; Story, J. Hypocholesterolemic effects of oat-bran or bean intake for hypocholesterolemic men. Am. J. Clin. Nutr. 1984, 40, 1146-1155.
- Batey, I. L. Starch analysis using thermostable alpha-amylase. Staerke 1982, 34, 125-127.
- Bing, F. C. Dietary fiber in historical perspective. J. Am. Diet. Assoc. 1976, 69, 498-505.
- Bingham, S. Patterns of Dietary Fiber Consumption in Humans. In Handbook of Dietary Fiber in Human Nutrition; Spiller, G. A., Ed.; CRC Press: Boca Raton, FL, 1986.
- Carlson, A. J.; Hoelzel, F. Relation of diet to diverticulosis of the colon in rats. Gastroenterology 1949, 12, 108-115.
- Castagne, A. E.; Siddiqui, I. R. Uronic acid determination. Carbohydr. Res. 1975, 42, 382–386.
- Cummings, J. H. Short chain fatty acids in the human colon. Gut 1981, 22, 763-779.
- Delorme, C. B.; Barbeau, M.; Barette, D.; Larivière, N. The effect of chronically high levels of dietary fibre from wheat bran on glycemia, triglyceridemia and cholesterolemia in Beagle dogs. Presented at the 30th Annual Meeting of the Canadian Federation of Biological Societies, Canadian Society for Nutritional Sciences, Winnipeg, Manitoba, 1987; Paper TU-PO-34.
- Devroede, G. Dietary fiber, bowel habits, and colonic function. Am. J. Clin. Nutr. 1978, 31, S157-S160.
- Englyst, H.; Wiggins, H. S.; Cummings, J. H. Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatographyofconstituentsugarsasalditolacetates. Analyst 1982, 107, 307-318.
- Fantus, B.; Frankl, W. The mode of action of bran. I. Effect of bran upon composition of stools. J. Lab. Clin. Med. 1941, 26, 1774-1777.
- FDA. Physiological effects and health consequences of dietary fiber; Pilch, S. M., Ed.; Food and Drug Administration, Health and Human Services: Washington, DC, June 1987.
- Fed. Regist. 1975, 40, 56, 12907.
- Goering, H. K.; Van Soest, P. J. Forage fiber analyses. In United States Department of Agriculture Handbook 379; USDA: Washington, DC, 1970.
- Hellendoorn, E. W. Fermentation as the principal cause of the physiological activity of indigestible food residue. In *Topics* in Dietary Fiber Research; Spiller, G. A., Amen, R. J., Eds.; Plenum Press: New York, London, 1978.
- HPB. Report of the Expert Advisory Committee on Dietary Fibre; Health Protection Branch, Health and Welfare Canada: Ottawa, Ontario, 1985.
- Jenkins, D. J. A. Dietary fiber and carbohydrate metabolism. In *Medical Aspects of Dietary Fiber*; Spiller, G. A., Kay, G. A., Eds.; Plenum Medical: New York, London, 1980.
- Kay, R. M.; Strasberg, S. M. Origin, chemistry, physiological effects and clinical importance of dietary fibre. *Clin. Invest. Med.* 1978, 1, 9-24.
- Kiehm, T. G.; Anderson, J. W.; Ward, K. Beneficial effects of a high carbohydrate, high fiber diet on hyperglycemic diabetic men. Am. J. Clin. Nutr. 1976, 29, 895-899.
- Leeds, A. R. In Dietary Fibre Perspectives. Reviews & Bibliography 1; Avenell, A., Ed.; John Libbey: London, Paris, 1985.
- Lloyd, J. B.; Whelan, W. J. An improved method for enzymatic determination of glucose in the presence of maltose. Anal. Biochem. 1969, 30, 467-470.

- Mongeau, R.; Brassard, R. Determination of neutral detergent fiber in breakfast cereals: pentose, hemicellulose, cellulose and lignin content. J. Food Sci. 1982, 47, 550-555.
- Mongeau, R.; Brassard, R. Effect of dietary fiber from Shredded and Puffed Wheat breakfast cereals on intestinal function in rats. J. Food Sci. 1984, 49, 507-509.
- Mongeau, R.; Brassard, R. Dietary fiber and fecal characteristics in rats: effect of level and particle size of bran. J. Food Sci. 1985, 50, 654–656.
- Mongeau, R.; Brassard, R. A rapid method for the determination of soluble and insoluble dietary fiber: comparison with AOAC total dietary fiber procedure and Englyst's method. J. Food Sci. 1986, 51, 1333-1336.
- Mongeau, R.; Brassard, R. A comparison of three methods for analyzing dietary fiber in 38 foods. J. Food Compos. Anal. 1989, accepted for publication.
- Painter, N. S. Diverticular disease of the colon. S. Afr. Med. J. 1982, 61, 1016-1020.
- Phillips, S. F.; Devroede, G. J. Functions of the large intestine. In International Review of Physiology, Gastrointestinal Physiology III, 19; Crane, R. K., Ed.; University Park Press: Baltimore, MD, 1979.

- Siddiqui, I. R.; Morris, G. Optimal analysis of alginates by decarboxylation. Carbohydr. Res. 1979, 69, 330-332.
- Stat Plus. In A General Statistics Program for the Apple II; Human Systems Dynamics: Northridge, CA, 1982.
- Steyermark, A. I. In Quantitative Organic Microanalysis, 2nd ed.; Academic Press: New York, London, 1961.
- Trowell, H. C.; Burkitt, D. P. Concluding considerations. In Refined Carbohydrate Foods and Desease: Some Implications of Dietary Fibre; Trowell, H. C., Burkitt, D. P., Eds.; Academic Press: London, 1975.
- Van Soest, P. J. Some physical characteristics of dietary fibers and their influence on the microbial ecology of the human colon. *Proc. Nutr. Soc.* 1984, 43, 25-33.
- Wozasek, O.; Steigmann, F. Studies on colon irritation. III. -Bulk of feces. Am. J. Digest. Dis. 1942, 9, 423-425.
- Wyman, J. B.; Heaton, K. W.; Manning, A. P.; Wicks, A. C. B. The effect on intestinal transit and the feces of raw and cooked bran in different doses. Am. J. Clin. Nutr. 1976, 29, 1474– 1479.

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## Importance of Lactose in Yogurt for Mineral Utilization

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Mineral utilization was assessed in mature female rats fed yogurt-based diets or casein-based diets that contained quantities of sucrose, lactose, or lactose's component sugars (glucose, galactose) equivalent to the amount of lactose found in yogurt and one of two levels of calcium (4 or 8 mg of Ca/g of diet). Ingestion of the high level of calcium depressed the efficiency of absorption of calcium, magnesium, and zinc. Although ingestion of purified lactose, but not yogurt, improved apparent absorption of magnesium and zinc, the effect of lactose on calcium absorption is questionable. An effect of lactose on calcium absorption is questionable. An effect of lactose on calcium absorption could only be observed among rats fed the high level of calcium if less rigorous statistical tests (LSDs) were applied. Diet digestibility was strongly correlated with apparent absorption of calcium, magnesium, and zinc. However, changes in the colon induced by the dietary treatments (i.e., pH and  $\beta$ -glucurondiase activity of colonic contents) were not correlated to apparent absorption of minerals.

Lengemann et al. (1957) suggested that the greater bioavailability of calcium from milk than from plant sources was probably due to the lactose content of milk. A number of investigators have reported that lactose improved calcium absorption in intestinal preparations (Vaughan and Filer, 1960; Favus and Angeid-Backman, 1984; Armbrecht and Wasserman, 1976). However, recent studies have shown few statistically significant differences in the utilization of calcium from dairy products containing lactose and from calcium supplements not containing lactose (Greger et al., 1987; Sheikh et al., 1987; Recker et al., 1988; Behling and Greger, 1988). Scrimshaw and Murray (1985) summarized the apparently inconsistent data on lactose-calcium interactions in this manner: "The bulk of the evidence indicates a favorable or neutral effect of lactose on Ca absorption.

Some of the controversy over the importance of lactose may reflect differences in the physiological state of experimental animals or human subjects. Lactose is not believed to affect the saturable transcellular process of calcium absorption that is regulated by vitamin D but does appear to affect the nonsaturable paracellular pathway of calcium absorption in the gut (Bronner, 1987). As calcium intakes increase, the relative importance of the nonsaturable paracellular transport system increases (Bronner, 1987); presumably sensitivity to lactose also increases.

The maturity of animals may also affect responses to lactose. Armbrecht et al. (1979) noted that active transport of calcium and the level of vitamin D dependent calcium-binding protein decreased in the intestine of rats as they matured. This would presumably increase the relative importance of the nonsaturable process in mature animals. However, as rats mature, intestinal lactase levels tend to decline (Leichter, 1973). Several investigators have observed that dietary lactose did not improve calcium absorption among lactase-deficient humans (Kocian et al., 1973; Cochet et al., 1983). However, other researchers have observed that carbohydrates that escape absorption in the proximal gut enhance calcium absorption distally (Kelly et al., 1984; Amman et al., 1988).

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