Carbohydrate Malabsorption

Its Measurement and Its Contribution to Diarrhea

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Abstract

The major purpose of this research was to gain insight into the effect of carbohydrate malabsorption on fecal water output. To do this we measured daily fecal output of total carbohydrate, reducing sugars, and organic acids (a product of bacterial fermentation). Normal subjects were studied in their native state and when diarrhea was induced by mechanisms that did and did not involve carbohydrate malabsorption. Patients with malabsorption syndrome were also studied. We concluded that: (*a*) Excretion of carbohydrate and its breakdown products can be expressed as a single number by converting organic acids to their monosaccharide equivalents. (*b*) Diarrhea per se causes only a trivial increase in fecal carbohydrate excretion. (*c*) The molar output of osmotic moieties in feces due to unabsorbed carbohydrate can be determined by adding fecal reducing sugars to organic acids and their obligated cations. This expression parallels almost exactly the effect of increasing doses of lactulose (a nonabsorbable sugar) on fecal water output; one excreted millimole obligates 3.5 g of stool water. This relationship can be used to predict the effect of carbohydrate malabsorption on stool water output in patients with diarrhea. (*d*) 12 of 19 patients with malabsorption syndrome due to various diseases had excessive fecal excretion of carbohydrate and its breakdown products; of the diseases that cause malabsorption syndrome, combined small and large bowel resection is most likely to result in excessive fecal excretion of carbohydrate and monosaccharide equivalents. In 6 of these 19 patients carbohydrate malabsorption appeared to be the major cause of diarrhea. (*J. Clin. Invest.* 1990. 86:1936–1944.) Key words: organic acids • reducing sugars • starch • lactulose • steatorrhea

Introduction

Carbohydrate (CHO)[1] that is not absorbed in the small intestine can be metabolized by colonic bacteria to organic acids (OA), some of which are absorbed across the colonic mucosa (1, 2). CHO that is not metabolized by colonic bacteria to OA, and OA that are not absorbed by the colon, would remain in the colonic lumen and could lead to osmotic diarrhea. The actual magnitude of fecal excretion of CHO and OA in malabsorption syndrome has not been well quantitated in the past. Therefore, it is uncertain to what extent CHO malabsorption is responsible for the diarrhea that can be a major feature of malabsorption syndrome.

The purpose of this research was to gain a more comprehensive picture of the magnitude and significance of carbohydrate malabsorption than has been possible in the past. To do this, we measured fecal output of total CHO, reducing sugars, and OA. Total CHO was assayed using anthrone, a method that measures all hexose CHO, whether excreted as monosaccharides, disaccharides, or oligosaccharides (3, 4). The unit of expression is grams of fecal CHO excreted per day; it thus provides a measure of total fecal CHO excretion, regardless of molecular size. In contrast, the reducing sugar assay detects the reducing ends of CHO molecules; 1 mol of starch (> 50,000 g of CHO) and 1 mol of glucose (180 g of CHO) would give the same result. Therefore, the reducing sugar assay provides information on the number of moles of excreted CHO and is useful in determining the osmotic effect of fecal CHO. Fecal output of OA was measured to detect the fraction of CHO that is excreted in stool as a bacterial product of unabsorbed CHO. These analytical methods were applied to stools that were quantitatively collected by normal subjects studied in their native state and when diarrhea was induced by mechanisms that did and did not involve CHO malabsorption. Patients with malabsorption syndrome secondary to various diseases were also studied and the magnitude and osmotic activity of unabsorbed CHO and OA were compared with stool water losses.

Methods

Stool collections. Stools were collected quantitatively using preweighed containers. The stools were kept cooled in an ice chest or in a portable refrigerator. Previous studies have shown that bacterial metabolism of CHO under these storage conditions is minimal (5); these data are shown in the inset in Fig. 1. Preservative and antibiotic solutions (6) were not used because they were found to interfere with an evaluation of stool consistency and with some of the analytical procedures necessary for this research.

Experimental diarrhea in normal subjects. Normal subjects volunteered for these experiments. All had had previous experience in clinical research and in the techniques of quantitative stool collection. They were paid a fee for their participation. These studies were approved by the Institutional Review Board for Human Protection of Baylor University Medical Center.

While subjects continued to eat their normal diets, osmotic diarrhea was induced by ingestion of a solution containing either 105 g/liter polyethylene glycol (PEG) 3350 (J. T. Baker Chemical Co., Phillipsburg, NJ), 50 mmol/liter sodium sulfate (Mallinckrodt Inc., Paris, KY) or 50 or 70 g/liter lactulose (Fluka Chemie AG, Buchs, Switzerland). 48 mmol/liter NaCl, 5 mmol/liter KCl, and 17 mmol/liter $NaHCO_3$ were added to each solution to ensure against the development of electrolyte deficiency due to fecal losses. On four consecu-

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Received for publication 30 May 1990 and in revised form 27 July 1990.

1. *Abbreviations used in this paper:* CHO, carbohydrate; OA, organic acids.

J. Clin. Invest.

0021-9738/90/12/1936/09 $2.00
Volume 86, December 1990, 1936–1944

tive days the subjects drank specified volumes of one of the solutions with each of three meals and a bedtime snack for a total daily dose of 53, 95, 189, or 252 g/d of PEG, 45, 95, or 125 g/d of lactulose, or 90 mmol/d of sulfate. Secretory diarrhea was induced on four consecutive days by ingestion of 240 mg of phenolphthalein (Mission Pharmaceutical Co., San Antonio, TX) four times daily (960 mg/d). Subjects were weighed and serum electrolytes, creatinine, and urea nitrogen were determined at the start and end of the experiment; there were no significant changes. Stools collected during the second to fourth days of the study were pooled and analyzed.

Diarrhea was also induced by cecal infusion of two isotonic CHO solutions. The first solution contained 217 g/liter of Polycose, an oligosaccharide with a mean of 6 glucose units per molecule (Ross Laboratories, Columbus, OH). The second solution contained 70 g/liter of starch (Starch Soluble; Mallinckrodt Inc.) supplemented with electrolytes (100 mmol/liter NaCl, 35 mmol/liter $NaHCO_3$, and 5 mmol/liter KCl) to achieve isotonicity. Subjects swallowed a polyvinyl tube that had a mercury weight and an inflatable balloon on its tip. The location of the tip of the tube was checked fluoroscopically. The balloon was inflated as soon as the tip of the tube reached the descending part of the duodenum. After the tip of the tube reached the cecum the balloon was deflated and the subject ingested a meal. Beginning 1 h after the meal, 144 ml (31.2 g) of the polycose solution was infused at a constant rate into the cecum over a 2-h period. Subjects ingested subsequent meals every 6 h, followed by polycose infusion starting 1 h after each meal. The total amount of polycose infused in the 30-h study period was 156 g (125 g per 24-h period). 15 h (from the start of the first meal until the end of the third infusion) were allowed for defecation of bowel contents that were already in the colon before infusion was begun. After finishing the third infusion a 24-h stool collection was obtained. The same protocol was followed for the infusion of the starch solution, except that each infusion period lasted 4 h, during which 450 ml of starch solution (31.5 g of starch) were infused after each meal (125 g per 24-h period).

Patients. We studied 19 patients with chronic diarrhea, with disorders expected to cause malabsorption. Diagnosis was established after a complete protocol evaluation that has been previously described (7, 8). The patients had pancreatic disease, villous atrophy, or intestinal resection. Those with pancreatic insufficiency, normally maintained on pancreatic enzymes, were off enzymes for 2 d before and during the 3-d stool collections. The villous atrophy patients were studied before the institution of therapy. Six patients with microscopic/collagenous colitis were studied as a comparison group with diarrhea and no expected malabsorption. While the patients ate a normal diet, containing a mean of 2,490 kcal, 287 g of CHO, and 110 g of fat, stools were collected quantitatively for 72 h. Table I contains clinical information and dietary intakes of these patients.

Analytical methods. Stool was homogenized using a commercial blender (Waring Products, New Hartford, CT), and samples were analyzed immediately or after freezing or lyophilization (Freezemobile 12; VirTis Co., Inc., Gardiner, NY). To analyze lyophilized samples, specimens were reconstituted to their original weight by adding deionized water. To obtain supernate for analysis, samples were spun at 30,000 rpm for 45 min.

The method of van de Kamer et al. was used to measure fecal fat (9). OA were measured by a titration method that was described previously (10) and validated in our laboratory (5). Total CHO was analyzed using anthrone by a modification of a spectrophotometric method (3). Reducing substances were analyzed by the method of Nelson-Somogyi (11). Since PEG interfered with the reducing substance assay, PEG was extracted from the supernatants of PEG-induced diarrhea with an equal volume of chloroform; after extraction, excess chloroform was evaporated in a boiling water bath before analysis for reducing substances. OA did not react as a reducing substance in the procedure. Glucose was determined using an enzymatic kit (Boehringer Mannheim Biochemicals, Indianapolis, IN), and osmolality was measured by freezing point depression (Micro Osmometer; Advanced Instruments, Inc., Needham Heights, MA).

To assess the extent to which OA were lost from fecal samples due to evaporation, Na acetate, Na propionate, and Na butyrate solutions (100 mmol/liter) were stored at room temperature at their original pH ($\sim$ 7.5) and after adjusting the pH of the solutions to 4.0 using hydrochloric acid. According to the Henderson-Hasselbach equation, at pH 7.5 almost 100% of acetate, propionate, and butyrate are dissociated (salt form), whereas at pH 4.0 > 80% are undissociated (acid form). After storage for 48 h in a closed container, there was complete recovery of OA from solutions at pH 7.5 and 4.0. In contrast, when these OA solutions were lyophilized, only 20% could be recovered from solutions with an initial pH of 4.0; from solutions at pH 7.5 the recovery of OA from lyophilized samples was 100%. From these results we concluded that during storage of whole stool samples no significant loss of OA due to evaporation would occur. In contrast, depending on fecal pH, considerable amounts of OA could be lost during lyophilization, so that lyophilized stool samples could not be used to measure OA except when stool pH was high.

The amount of cations obligated by fecal OA (to maintain electroneutrality) was determined by a previously published relationship established for lactulose- and PEG-induced diarrhea, where 1 mmol of fecal OA obligated an average of 0.6 mmol of fecal cations consisting of 0.30 mmol of Na^+, 0.21 mmol of Ca^{2+}, 0.07 mmol of K^+, and 0.02 mmol of Mg^{2+} (5).

Fecal solute output was calculated by multiplying fecal concentration of solute (measured in supernate) by stool water output. Stool water output was calculated by multiplying stool weight by percent water content (as measured by lyophilization).

Results

Carbohydrate and monosaccharide equivalents. A fermentation equation (1) was used to calculate the amount of CHO excreted as OA in stool. This equation predicts that bacterial metabolism of 1 mol monosaccharide yields 1.855 mol OA. By dividing measured OA (in millimoles) by 1.855, the amount of monosaccharide (in millimoles) that was metabolized to generate these OA could be calculated. To convert monosaccharide from millimoles to grams (which was necessary to allow adding them to total CHO measured with anthrone), the amount in millimoles was multiplied by 0.18 (because the molecular weight of dietary monosaccharides is 0.18 g/mmol). Therefore, the formula used to calculate the monosaccharide equivalent (in grams per liter) of fecal OA was:

$$\text{monosaccharide equivalent (grams per liter)} = \frac{\text{OA (millimoles per liter)}}{1.855} \times 0.18 = \text{OA} \times 0.097.$$

To evaluate the validity of this equation, seven freshly passed stool specimens from two normal volunteers were homogenized with an equal weight of solution containing 30 g/liter of glucose (n = 5) or 30 g/liter of polycose (n = 2). The stool homogenate was stored in a closed container at room temperature for 48 h to allow bacteria to metabolize monosaccharides to OA. Total CHO (anthrone) and OA were analyzed and monosaccharide equivalents were calculated. Fig. 1 shows that the significant decrease ($P < 0.001$) in CHO concentration during storage of samples at room temperature was accompanied by a significant increase ($P < 0.001$) in OA concentration; the sum of the measured concentration of total CHO plus calculated monosaccharide equivalents did not change significantly. Therefore, the magnitude of carbohydrate malabsorption (which is the sum of fecal CHO per se plus monosaccharide equivalents) can be calculated and expressed as a single

Table I. Clinical Information and Dietary Intake in Patients with Malabsorption Syndrome or Microscopic/Collagenous Colitis

Patient No., ID	Age	Sex	Dietary intake					Etiology and comment
			Kilocalories	Protein	Fat	CHO	Fiber	
	yr							
Chronic pancreatitis								
1, MJ	60	F	1,903	65	70	255	8	Idiopathic
2, VD	41	M	1,526	73	82	131	4	Alcoholism
3, JS	63	M	3,647	132	202	335	29	Alcoholism
Pancreatic cancer								
4, HM	76	F	1,721	80	96	141	11	
5, CS	73	M	2,262	89	88	282	8	
Villous atrophy								
6, BF	18	F	2,410	119	135	187	9	Collagenous sprue; refractory to gluten-free diet
7, BH	35	M	2,553	111	81	331	18	Villous atrophy due to acquired hypogammaglobulinemia; refractory to gluten-free diet
8, JI	64	F	2,316	55	84	341	8	Celiac sprue
9, BH	52	F	2,586	92	91	349	7	Celiac sprue
10, TS	52	M	2,772	118	102	354	11	Celiac sprue
11, WR	61	M	2,297	89	92	278	8	Celiac sprue
Massive small bowel resection; intact colon								
12, BR	43	F	1,368	53	59	161	4	Infarction due to adhesions and obstruction; all but 3 ft of proximal small bowel resected
Ileal and right colon resection								
13, LM	52	M	4,023	124	171	503	19	Crohn's disease
14, MS	68	F	3,542	119	164	404	12	Crohn's disease
15, AP	69	M	4,356	131	209	535	14	Crohn's disease
16, KM	45	M	2,910	124	156	251	6	Crohn's disease; antrectomy and B-II anastomosis for peptic ulcer disease; afferent limb of B-II obstructed possibly causing functional pancreatic insufficiency; rapid small intestinal transit
Small bowel resection, total colectomy and ileostomy								
17, BM	60	F	1,553	63	68	178	7	Crohn's disease
18, SD	28	F	2,460	103	106	274	5	Crohn's disease
19, JS	36	F	2,754	74	151	289	12	Crohn's disease
Microscopic/collagenous colitis								
20, JF	67	F	1,596	47	52	202	9	
21, LH	38	F	3,800	113	191	396	17	
22, PG	70	F	721	35	27	132	8	
23, TM	64	F	1,491	52	69	168	7	
24, CF	59	F	2,161	70	81	209	11	
25, JK	51	F	2,771	97	111	351	9	

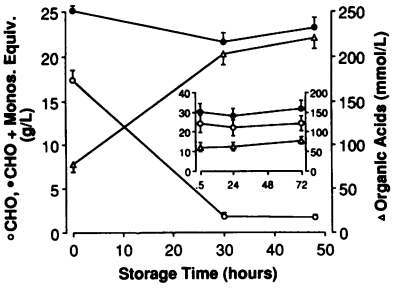

Figure 1. Concentration of total CHO (*open circles*), OA (*triangles*), and CHO plus monosaccharide equivalents (*closed circles*) in seven stool samples during storage at room temperature for 48 h. Samples were obtained from normal people and were homogenized at time 0 with an equal volume of a solution containing 30 g/liter glucose (n = 5) or 30 g/liter polycose (n = 2). The inset shows results when stool is refrigerated for 72 h; data are from five diarrheal specimens induced with 125 g/d of lactulose (5).

number. The inset to Fig. 1 shows that storage under refrigerated conditions markedly diminishes bacterial metabolism of fecal CHO.

Average normal values and upper limits of normal (defined as the mean + 2 SD) for outputs of CHO, OA, monosaccharide equivalents, and other relevant parameters were established in normal subjects in their native state and when diarrhea was induced by ingestion of PEG, phenolphthalein, or sodium sulfate. These values were compared with those from normal subjects with experimental CHO malabsorption and patients with malabsorption syndrome or microscopic/collagenous colitis. Results in these five groups are shown in Tables II and III.

Fig. 2 summarizes fecal output of CHO plus monosaccharide equivalents in individual subjects and patients. In normal subjects in their native state CHO plus monosaccharide equivalent output was < 5 g/d, and with non–CHO-induced diarrhea it was as high as 9 g/d. With CHO-induced diarrhea, 10 of 15 normal subjects excreted > 9 g/d of CHO plus monosaccharide equivalents. Five normal subjects with CHO-induced diarrhea excreted < 9 g/d; these subjects had mild diarrhea, with stool weights < 400 g/d (6, 7, 9, 13, and 15 in Table II).

12 of the 19 patients with malabsorption syndrome had a

Table II. Fecal Output of Carbohydrates and Organic Acids in Normal Subjects

	Stool weight/water/frequency/ consistency	Total CHO	OA	CHO + monosaccharide equivalent	Reducing sugars	Reducing sugars + OA + cations
	g/d, g/d, times/d	*g/d*	*mmol/d*	*g/d*	*mmol/d*	*mmol/d*
Control (n = 6)						
Mean±SEM	157±24/122±20/0.9±0.1/F + SF	0.5±0.1	27±5	3±0.5	1.4±0.3	44±8
Upper limit of normal*	232/190/2/F + SF	0.8	50	5	3	81
PEG (n = 13)-, phenolphthalein (n = 5)-, and SO_4 (n = 3)-induced diarrhea‡						
Mean±SEM	788±91/661±72/4±0.3/SF, SL, L	1.0±0.2	40±3	5±0.4	2.9±0.6	53±8
Upper limit of normal for non-CHO-induced diarrhea*	Not Applicable	2.4	70	9	8	127
Carbohydrate-induced diarrhea						
Lactulose, 125 g/d						
1 AS	1,042/927/3/L	37.0	77	44	111	234
2 DD	1,596/1,468/4/L	48.0	192	68	144	451
3 CC	1,285/1,157/6/L	38.0	140	52	147	371
Lactulose, 95 g/d						
4 AS	747/672/4/SL	24.0	80	32	69	197
5 CP	621/540/3/SL	8.0	118	19	17	206
6 RR	282/234/3/SL	2.0	56	7	6	96
Lactulose, 45 g/d						
7 PF	222/175/2/SL	0.2	41	4	2	68
8 CP	356/285/2/SL	1.9	82	10	3	134
9 JL	261/214/3/SL	0.7	39	4	2	64
Polycose, 125 g/d						
10 HH	474/403/4/SL	7.0	112	18	21	200
11 PS	672/578/3/L + SL	18.0	130	31	62	270
12 CS	663/577/4/L + SL	17.0	107	27	30	201
Starch, 125 g/d						
13 CS	248/206/2/SL + SF	0.7	43	5	1	70
14 PS	897/816/8/L	40.0	38	43	142	203
15 CP	354/290/2/SL	0.9	77	8	6	129

Consistency of stools was described as formed (F), semi-formed (SF), semi-liquid (SL), and liquid (L). * Upper limit of normal for controls and non–CHO-induced diarrhea was derived from the mean + 2 SD. ‡ Results are an average of all 21 studies with induced diarrhea.

Table III. Fecal Output of Carbohydrates, Organic Acids, Reducing Sugars, and Fat in Patients with Malabsorption Syndrome or Microscopic/Collagenous Colitis

	Stool weight/stool water	Total CHO	OA	CHO + monosaccharide equivalent	Reducing sugars	Reducing sugars + OA + cations	Fat
	g/d	*g/d*	*mmol/d*	*g/d*	*mmol/d*	*mmol/d*	*g/d*
Upper limit of normal for controls*	232/190	0.8	50	5	3	81	—
Upper limit of normal for non-CHO-induced diarrhea*	—	2.4	70	9	8	127	—
Patient							
Chronic pancreatitis							
1, MJ	484/324	0.4	25	3	2	42	69
2, VD	978/662	1.3	131	14	10	220	113
3, JS	550/387	1.5	91	10	7	153	70
Pancreatic cancer							
4, HM	664/596	0.8	41	5	3	69	30
5, CS	1,270/1,143	12.0	95	21	59	211	37
Villous atrophy							
6, BF	364/261	0.9	34	4	1	55	24
7, BH	1,212/1,063	5.6	87	14	7	146	41
8, JI	3,302/2,950	73.0	310	103	287	783	60
9, BH	409/339	2.2	35	5	2	58	14
10, TS	367/273	0.2	49	5	3	81	36
11, WR	482/416	0.8	53	6	2	87	15
Massive small bowel resection; intact colon							
12, BR	656/544	2.2	34	6	1	56	34
Ileal and right colonic resection							
13, LM	2,378/2,002	47.0	312	78	48	547	44
14, MS	950/782	3.1	93	13	2	151	62
15, AP	646/553	2.9	79	11	2	128	41
16, KM	1,729/1,449	19.0	185	37	51	347	99
Partial small bowel resection, total colectomy, and ileostomy							
17, BM	1,467/1,366	13.0	56	18	25	115	29
18, SD	1,009/935	18.0	35	22	40	96	6
19, JS	2,572/2,418	12.0	111	22	15	193	24
Microscopic/collagenous colitis							
20, JF	405/365	0.7	62	7	2	101	7
21, LH	391/344	0.6	41	4	1	67	2
22, PG	675/610	1.8	76	9	1	123	8
23, TM	838/804	0.6	35	4	1	57	5
24, CF	504/451	0.5	54	6	1	87	5
25, JK	603/545	0.1	71	7	2	116	8

* Upper limits of normal from Table II.

fecal CHO plus monosaccharide equivalent output higher than the upper limit of normal for non-CHO-induced diarrhea. Included in these 12 were 3 of 5 patients with pancreatic disease (2, 3, and 5), 2 of 6 patients with villous atrophy (7 and 8), and 7 of 8 patients with small bowel resection. There was no significant correlation between fecal fat output and CHO plus monosaccharide equivalent output. The control patient group with microscopic/collagenous colitis had values in the non-CHO-induced diarrhea range.

Fecal calorie loss due to CHO malabsorption can be calculated by multiplying CHO plus monosaccharide equivalent output by 4 kcal/g. In the 19 patients with malabsorption syndrome CHO malabsorption caused caloric losses between 12 and 412 kcal/d. In two patients (8 and 13) fecal calorie loss due to CHO malabsorption approached fecal calorie loss due to fat malabsorption.

Reducing sugars and organic acids. Experiments were performed to establish whether the Nelson-Somogyi procedure for reducing substances can be used to determine the osmotic activity of CHO in stool. The first study was done to establish the correlation between osmolality and reducing substance concentration in solutions containing 50 g/liter of starch, dextran, maltose, lactose, lactulose, sucrose, glucose, fructose, or galactose and 50 or 100 g/liter of polycose. Except for sucrose, which is not a reducing substance, the correlation was excellent (slope = 0.96, r = 0.99). Therefore, the concentration of reducing sugars in fecal fluid should reflect the osmotic activity of fecal CHO except when the fecal sugar is sucrose. A second

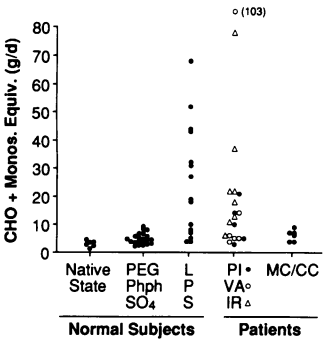

Figure 2. Output of CHO plus monosaccharide equivalents in normal subjects in their native state; in normal subjects with PEG-, phenolphthalein (*Phph*)-, sodium sulfate (SO_4)-, lactulose (*L*)-, polycose (*P*)-, and starch (*S*)-induced diarrhea; and in patients with pancreatic insufficiency (*PI*), villous atrophy (*VA*), intestinal resection (*IR*), and microscopic/collagenous colitis (*MC/CC*).

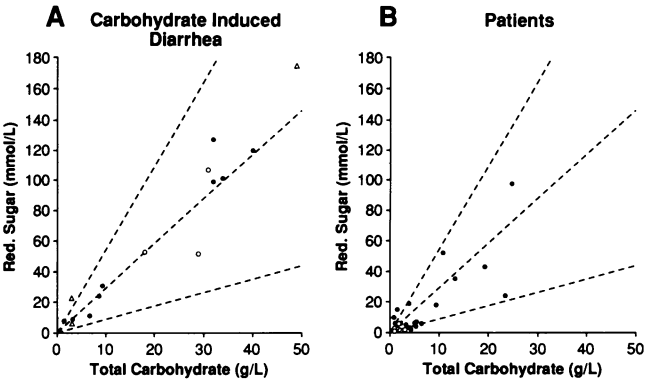

Figure 3. Relation between concentration of total CHO and reducing sugar in (*A*) CHO-induced diarrhea (*closed circles*, lactulose; *open circles*, polycose; *triangles*, starch), and (*B*) patients with malabsorption syndrome (*closed circles*) or microscopic/collagenous colitis (*open circles*). The three reference lines reflect results if all CHO were monosaccharides (steepest line), disaccharides (middle line), or heptasaccharides (lowest line).

experiment was done to establish whether the Nelson-Somogyi procedure could be used to measure reducing sugars in stool. Urine is known to contain non-CHO reducing substances (12) and we expected their presence also in stool. We postulated that some of these non-CHO reducing substances might be volatile (like sulfides or low molecular weight aldehydes), and might be removed from stool by lyophilization. We therefore measured reducing substances in supernatant obtained both from fresh or fresh frozen stool and from stool that was lyophilized and then reconstituted to its original weight by adding water. Table IV shows concentrations of reducing substances, glucose, and total CHO (measured with anthrone) in phenolphthalein-, sodium sulfate–, and lactulose-induced diarrhea. Concentration of reducing substances was reduced 21±5 mmol/liter by lyophilization ($P < 0.002$); since glucose and total CHO concentration did not change significantly, this reduction of reducing substances must be due to volatilization of non-CHO reducing substances. In most samples of diarrhea fluid induced by phenolphthalein or sodium sulfate, the concentration of reducing substances in lyophilized stool was similar to the concentration of glucose, which makes it likely that no considerable amounts of non-CHO reducing substances were left in stool after lyophilization. In lactulose-induced diarrhea the concentration of reducing substances was much higher than the glucose concentration, reflecting fecal excretion of lactulose and/or its monosaccharide components, fructose and galactose. These results show that the Nelson-Somogyi procedure can be used to measure reducing sugar concentration in lyophilized but not fresh or frozen stool samples.

Table IV. Concentration of Reducing Substances, Glucose, and Total Carbohydrate in Lyophilized and Fresh/Frozen Stool Samples

		Reducing substances			Glucose			Total CHO		
	Stool weight	Fresh/frozen	Lyophilized	Change	Fresh/frozen	Lyophilized	Change	Fresh/frozen	Lyophilized	Change
	g/d		*mmol/liter*			*mmol/liter*			*g/liter*	
Phenolphthalein										
JO	496	16	6	−10	7	7	0	2.1	3.1	+1.0
DD	386	31	14	−17	27	23	−4	3.9	4.4	+0.5
MF	562	36	14	−22	18	16	−2	2.7	5.3	+2.6
PS	581	35	8	−27	9	8	−1	5.2	5.2	0
Sulfate										
CP	773	17	12	−5	21	15	−6	2.3	3.2	+0.9
DD	924	8	4	−4	4	4	0	0.5	1.2	+0.7
PS	360	27	9	−16	15	11	−4	4.5	4.8	+0.3
Lactulose, 125 g										
AS	1,042	192	120	−72	2	3	+1	37.0	33.9	−3.1
DD	1,596	117	99	−18	2	2	0	27.6	26.8	−0.8
CC	1,285	149	127	−22	2	5	+3	32.4	33.1	+0.7
Lactulose, 95 g										
AS	1,021	134	106	−28	3	14	+11	32.0	29.9	−2.1
CP	682	73	38	−35	17	19	+2	11.5	12.1	+0.6
Lactulose, 45 g										
PF	254	5	7	+2	—	—		1.7	3.0	+1.3
				−21±5			0±1			+0.2±0.4

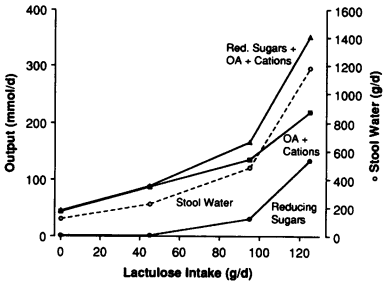

Figure 4. Relation between lactulose intake (grams per day) and fecal outputs of reducing sugars (*closed circles, solid line*), OA plus obligated cations (*squares, solid line*), reducing sugars plus OA plus obligated cations (*triangles, solid line*), and water (*open circles, dotted line*). Water output is in grams per day, solute outputs are in millimoles per day.

The relation between the concentration of total CHO and of reducing sugars in lactulose-, polycose-, and starch-induced diarrhea is shown in Fig. 3 *A*. This figure shows three reference lines calculated from molecular weights; the steepest line reflects results if all CHO were monosaccharides, the middle line if all CHO were disaccharides, and the lowest line if all CHO were heptasaccharides. Most samples of lactulose-induced diarrhea (*closed circles*) were near the middle line, suggesting that most of the excreted CHO was lactulose, rather than its monosaccharide components. Five of the six subjects with polycose- or starch-induced diarrhea were close to or above the middle line, suggesting that most of the excreted CHO were mono- or disaccharides, rather than polycose or starch. Fig. 3 *B* shows this correlation for patients with malabsorption syndrome. The points varied rather widely between the lines for monosaccharides and heptasaccharides.

Fig. 4 shows the effect of increasing doses of lactulose on fecal output of (*a*) reducing sugars, (*b*) OA plus their obligated cations, and (*c*) the sum of reducing sugars plus OA plus their obligated cations. Fecal water output is shown for comparison, by the dotted line. The effect of increasing doses of lactulose on stool water output was paralleled almost exactly by fecal output of the sum of reducing sugars plus OA plus their obligated cations. This lent support to the supposition that fecal water output increased in CHO malabsorption because of the combined osmotic effect of reducing sugars, OA, and obligated cations, and suggested that the fraction occupied by the three components of this combined effect had been accurately apportioned. Fig. 4 also shows that it was OA and obligated cations, and not CHO, that accounted for nearly all CHO-derived osmotic moieties when lactulose intake was 0 and 45 g, suggesting that all of the CHO that reached the colon had been metabolized to OA. At higher lactulose intakes, reducing sugars accounted for an increasing fraction of CHO-derived osmotic moieties that appeared in fecal fluid, indicating that metabolism of CHO to OA was incomplete.

In Fig. 5 *A*, fecal output of reducing sugars plus OA plus obligated cations is plotted against stool water output in subjects with induced diarrhea. There was no significant correlation between these variables in subjects with diarrhea due to PEG, phenolphthalein, or sodium sulfate (*open circles*). However, in lactulose-, polycose-, and starch-induced diarrhea (*solid circles*) the correlation was excellent ($r = 0.94$, $P < 0.001$); 1 mmol of reducing sugar plus OA plus cations obligated an average of 3.5 g of stool water. Fig. 5 *B* shows similar data in the patients. In six patients (2, 3, 5, 8, 13, and 16 in Table III) results fell within or close to the extrapolated range for CHO-induced diarrhea; this suggests that in these patients CHO malabsorption was a major cause of diarrhea. Results in the other patients fell within or close to the range of non–CHO-induced diarrhea; in these patients there was therefore no evidence that CHO malabsorption contributed in a major way to the development of diarrhea (as measured by excess stool water output).

Discussion

Unabsorbed dietary CHO can appear in stool as either CHO per se or as OA (see Introduction). To obtain an overall measure of carbohydrate malabsorption, it is useful to derive a single value that takes into account both fecal CHO and OA. Our in vitro studies (Fig. 1) show that fecal excretion of OA can be converted to their monosaccharide equivalents with the use of a previously published fermentation equation (1); when this value is added to total fecal CHO measured by anthrone, a single value (CHO plus monosaccharide equivalents in grams per day) is obtained that reflects both fecal CHO per se and CHO excreted as OA.

Fecal excretion of CHO plus monosaccharide equivalents in normal subjects under control conditions averaged 3 g/d. Since normal people eat ~ 275 g of CHO per day (5), and 25 g may reach the colon physiologically (13), the human intestine and its bacterial flora are very efficient in digesting, metabolizing, and absorbing CHO and OA. We also studied normal subjects when they had diarrhea due to ingestion of either PEG, phenolphthalein, or sodium sulfate; fecal excretion of CHO plus monosaccharide equivalents rose only slightly, to an average of 5 g per day, despite a marked increase in fecal water output. Digestion, metabolism, and absorption of CHO and

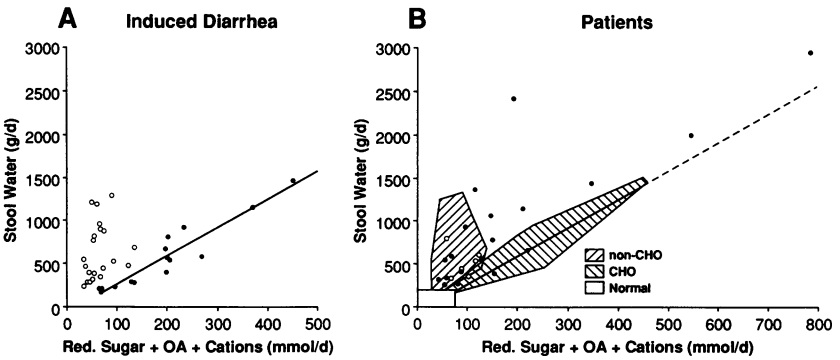

Figure 5. Relation between the sum of fecal osmotic solutes secondary to carbohydrate malabsorption (reducing sugars, organic acids, obligated cations) and stool water output. (*A*) Normal subjects with CHO- (*closed circles*) and non–CHO-induced (*open circles*) diarrhea. (*B*) Patients with malabsorption syndrome (*closed circles*) or microscopic/collagenous colitis (*open circles*). The regression line shown in *A* and *B* is calculated from experimental CHO-induced diarrhea. The hatched areas in *B* represent the range of data presented in *A*. The shaded rectangle represents the range of normals from Table II.

OA therefore remains efficient in normal people, even in the face of severe diarrhea.

Diarrhea was also induced in normal subjects by feeding them lactulose, a disaccharide that is not absorbed by the small intestine, or by infusing polycose or starch into the cecum. When 125 g/d of lactulose was ingested, an average of 55 g of CHO plus monosaccharide equivalents was excreted in stool; the other 70 g were presumably metabolized by colonic bacteria to OA which were absorbed by the colon. When 125 g/d of polycose or starch were infused into the cecum, fecal excretion of CHO plus monosaccharide equivalents averaged 25 and 19 g/d, respectively, about half the amount excreted when 125 g of lactulose were ingested. These results might reflect better bacterial metabolism of starch and polycose than of lactulose; however, we cannot rule out some reflux of infused starch or polycose from the cecum into the ileum, where digestion (by amylase) and absorption of sugar per se (by the small intestine) might have occurred. Variable degrees of reflux could also be responsible for the rather large differences in results between different subjects who received the same intracecal dose of polycose and starch; variable colonic motility might also contribute to these differences.

When < 125 g of lactulose was ingested, fecal excretion of CHO plus monosaccharide equivalents fell progressively, to 19 g/d with 95 g/d of lactulose, and to 6 g/d with 45 g/d of lactulose. The latter value is about the same as for normal subjects with non-CHO-induced diarrhea. Therefore, it seems reasonable to conclude that bacterial metabolism in the colon and colonic absorption of OA can almost totally prevent excess fecal excretion of CHO plus monosaccharide equivalents when 45 g of unabsorbable CHO are ingested per day (in four divided doses). When larger amounts of unabsorbable CHO are ingested, there is excess fecal excretion of CHO plus monosaccharide equivalents.

We also studied patients with malabsorption syndrome due to various diseases. Patients with combined small bowel and colon resection consistently had abnormally high fecal excretion of CHO plus monosaccharide equivalents. Two factors probably contribute to the tendency to high fecal excretion of CHO plus monosaccharide equivalents after small bowel plus colon resection. First, the shortened small intestine of these subjects might absorb an abnormally small fraction of ingested dietary CHO, and second, their colonic conversion of CHO to OA and their colonic absorption of these OA might be reduced. In support of this second possibility, one patient (12 in Table III) with a massive small bowel resection but an intact colon excreted normal amounts of CHO plus monosaccharide equivalents.

Three of five patients with pancreatic disease also had excessive fecal excretion of CHO plus monosaccharide equivalents, but in these cases excess fecal CHO excretion was relatively mild compared with fecal fat excretion. Two of six patients with villous atrophy had excessive fecal excretion of CHO plus monosaccharide equivalents, and one of these (8 in Table III) had the highest fecal CHO excretion of the entire group.

In two patients (8 and 13 in Table III) fecal calories due to CHO plus monosaccharide equivalents approached fecal calories due to fat. It should be noted that calorie loss due to CHO malabsorption has to be calculated from CHO plus monosaccharide equivalent loss. Calculation from measured fecal CHO and OA would underestimate calorie loss, because some of the energy contained in CHO is lost from the body as colonic bacteria convert CHO to OA.

As noted in the Introduction, fecal CHO measured by the anthrone assay does not reflect the osmotic activity of fecal CHO. Therefore, the anthrone assay for CHO does not give reliable insight into the extent to which fecal CHO contributes to the development of osmotic diarrhea. However, the moles of fecal CHO (as opposed to the grams of fecal CHO) can theoretically be determined by the amount of reducing substances in feces. Using in vitro studies, we showed that feces contain rather large concentrations of non-CHO reducing substances, but that these can be removed by lyophilization. Since lyophilization did not reduce fecal CHO or fecal glucose, the amount of reducing substances in lyophilized stool must reflect fecal reducing sugars. Therefore, with the exception of sucrose (which is not a reducing sugar), the concentration of reducing substances in lyophilized feces should accurately reflect the molar concentration of fecal CHO.

By comparing fecal concentration of total CHO measured by the anthrone method with fecal concentration of reducing substances we were able to estimate the average molecular size of fecal CHO (Fig. 3). In lactulose-induced diarrhea, fecal CHO existed as either disaccharides or monosaccharides. Surprisingly, this was also usually the case when polycose or starch was infused into the cecum. This latter observation suggests that bacterial metabolism of starch to small CHO moieties is a rapid process; the conversion of monosaccharides to OA (rather than the conversion of poly- and oligosaccharides to monosaccharides) appears to be the rate-limiting step in the overall conversion of poly- and oligosaccharides to OA. This was also true in many patients with malabsorption who excreted excessive amounts of CHO.

When molar output of CHO, as measured by reducing substances, is added to molar output of OA and obligated cations, an expression for total molar output of osmotic moieties in feces due to unabsorbed CHO is derived. Provided that the analytical methods are valid, and that the three components of this expression have been accurately apportioned, in subjects with diarrhea due solely to CHO malabsorption there should be a direct and close correlation between the output of fecal reducing sugars plus OA plus obligated cations, and the output of fecal water. This proved to be the case when diarrhea was induced in normal subjects by feeding lactulose in varying amounts or by infusing polycose or starch intracecally (Figs. 4 and 5 *A*). On average, 1 mmol of fecal reducing sugar plus OA plus obligated cations obligated 3.5 g of fecal water. This means that in diarrhea due exclusively to CHO malabsorption, the concentration of reducing sugar plus OA plus obligated cations in fecal water is 286 mmol/liter.

In passing, it is interesting to note that only if both organic acids (and their obligated cations) and reducing sugars are considered, is there an excellent agreement between the fecal output of osmotic moieties and fecal water (Fig. 4). This strongly supports the original view that OA play a role in the pathogenesis of diarrhea due to CHO malabsorption (14–16), rather than the more recent view that they probably do not (17–19).

Three of the five patients with pancreatic diseases had values for fecal reducing sugars plus OA plus obligated cations which fell within or close to the range of values for CHO-in-

duced diarrhea in normal subjects (Fig. 5 *B*). (In two of the three it was mainly OA rather than reducing sugar that was excreted in excessive amounts.) Thus, in these three patients with exocrine pancreatic insufficiency, increased fecal water output might be due mainly or even exclusively to CHO malabsorption. If this is the case, their high fecal fat output did not contribute in a major way to high fecal water output, presumably because it was not present in a molecular form that inhibits fluid absorption or stimulates fluid secretion by colonic mucosa (20). The results in two of the patients with intestinal resection and in one of the patients with villous atrophy fell close to the extrapolated line for CHO-induced diarrhea; in these patients also, CHO malabsorption might be the dominant mediator of increased fecal water output. In the other 13 patients with malabsorption syndrome, the data do not suggest a major role for CHO malabsorption as a mediator of increased fecal water output even though several of them had increased fecal output of CHO plus monosaccharide equivalent.

These experiments show that excessive fecal excretion of CHO and OA is common in patients with malabsorption syndrome. CHO malabsorption can contribute significantly to fecal calorie loss in these patients, and can be the major cause of their diarrhea.

Acknowledgments

The authors wish to thank Diana Santa Ana for technical assistance, Beverly Peters for preparing the graphics, and Sharon Michael for preparing the manuscript.

This work was supported by U. S. Public Health Service grant 5-RO1-DK37172-05 from the National Institute of Diabetes and Digestive and Kidney Diseases and by the Southwest Digestive Disease Foundation. Dr. Hammer was supported by an Erwin Schroedinger stipendium (J-0251 M and J 0358 M) from the Austrian Fonds zur Förderung Wissenschaftlicher Forschung.

References

1. Miller, T. L., and M. J. Wolin. 1979. Fermentations by saccharolytic intestinal bacteria. *Am. J. Clin. Nutr.* 32:164–172.

2. Ruppin, H., S. Bar Meir, K. H. Soergel, C. M. Wood, and M. G. Schmitt. 1980. Absorption of short chain fatty acids by the colon. *Gastroenterology.* 78:1500–1507.

3. Ameen, V. Z., and G. K. Powell. 1985. A simple spectrophotometric method for quantitative fecal carbohydrate measurement. *Clin. Chim. Acta.* 152:3–9.

4. Ameen, V. Z., G. K. Powell, and L. A. Jones. 1987. Quantitation of fecal carbohydrate excretion in patients with short bowel syndrome. *Gastroenterology.* 92:493–500.

5. Hammer, H. F., C. A. Santa Ana, L. R. Schiller, and J. S. Fordtran. 1989. Studies of osmotic diarrhea induced in normal subjects by ingestion of polyethylene glycol and lactulose. *J. Clin. Invest.* 84:1056–1062.

6. Bo-Linn, G. W., C. A. Santa Ana, S. G. Morawski, and J. S. Fordtran. 1983. Purging and calorie absorption in bulimic patients and normal women. *Ann. Intern. Med.* 99:14–17.

7. Read, N. W., G. J. Krejs, M. G. Read, C. A. Santa Ana, S. G. Morawski, and J. S. Fordtran. 1980. Chronic diarrhea of unknown origin. *Gastroenterology.* 78:264–271.

8. Arrambide, K. A., C. A. Santa Ana, L. R. Schiller, K. H. Little, W. C. Santangelo, and J. S. Fordtran. 1989. Loss of absorptive capacity for sodium chloride as a cause of diarrhea following partial ileal and right colon resection. *Dig. Dis. Sci.* 34:193–201.

9. Van de Kamer, J. H., H. B. Huinink, and H. A. Weyers. 1949. Rapid method for the determination of fat in feces. *J. Biol. Chem.* 177:347–355.

10. Collin, D. P., and P. G. McCormick. 1974. Determination of short chain fatty acids in stool ultrafiltrate and urine. *Clin. Chem.* 20:1173–1180.

11. Nelson, N. 1944. A photometric adaption of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153:375–380.

12. Folin, O., and H. Berglund. 1922. A colorimetric method for the determination of sugars in normal human urine. *J. Biol. Chem.* 51:209–211.

13. Caspary, W. F. 1986. Diarrhea associated with carbohydrate malabsorption. *Clin. Gastroenterol.* 15:631–655.

14. Weijers, H. A., J. H. Van de Kamer, W. K. Dicke, and J. Ijsseling. 1961. Diarrhea caused by deficiency of sugar splitting enzymes. *Acta Paediatr.* 50:55–71.

15. Bustos-Fernandez, L. B., E. Gonzalez, A. Marzi, and M. I. L. De Paolo. 1971. Faecal acidorrhoea. *N. Engl. J. Med.* 284:295–298.

16. Fordtran, J. S. 1971. Organic anions in fecal contents. *N. Engl. J. Med.* 284:329–330.

17. Cummings, J. H. 1981. Short chain fatty acids in the human colon. *Gut.* 22:763–779.

18. Fleming, S. E., and D. S. Arce. 1986. Volatile fatty acids: their production, absorption, utilization, and roles in human health. *Clin. Gastroenterol.* 15:787–814.

19. Vernia, P., G. Latella, F. M. Magliocca, and R. Caprilli. 1987. Fecal organic anions in diarrhoeal diseases. *Scand. J. Gastroenterol. Suppl.* 129:105–109.

20. Ammon, H. V., and S. F. Phillips. 1973. Inhibition of colonic water and electrolyte absorption by fatty acids in man. *Gastroenterology.* 65:744–749.