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Absorption and Oxidation of Glucose Polymers of Different Lengths in Young Infants

ROBERT J. SHULMAN, BENNY KERZNER, HOWARD R. SLOAN, THOMAS W. BOUTTON, WILLIAM W. WONG, BUFORD L. NICHOLS, AND PETER D. KLEIN

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030, and the Department of Pediatrics, Columbus Children's Hospital, Ohio State University, Columbus, Ohio 43205

ABSTRACT. Because mucosal glucoamylase is most active against glucose polymers less than 10 glucose units in length, longer chain polymers may not be completely absorbed by young infants. In order to investigate this possibility, the absorption and oxidation of ^{13}C-rich glucose, short-chain (3 to 8 glucose units in length) and long-chain (average length 43 units) glucose polymers (GP) were compared in 12 healthy, 1-month-old infants. Recovery of the GP and glucose in stool was measured by determining the ^{13}C enrichment of stool. The oxidation of the GP was measured by tracing the increase in breath $^{13}CO_2$ after GP were fed. Carbohydrate malabsorbed in the small bowel was assessed by measurement of breath H_2, a gas formed from the fermentation of carbohydrate in the colon. Analysis of the infants' stools revealed that one infant excreted 9.7% of the dose of glucose, another 6.7% of the dose of short-chain GP, and five infants excreted 2.6 to 18.5% (mean 8.4%) of the dose of long-chain GP. The percent of the administered dose recovered in breath was similar among substrates (mean = 28.7% of the dose fed). A rise in breath H_2 greater than 20 ppm was found in four of the 12 infants after the feeding of glucose, in five of 12 after the short-chain GP, and in six of 12 after the long-chain GP. None of the infants developed diarrhea. The results suggest that healthy young infants do not absorb long-chain GP as completely as they absorb short-chain GP. In the absence of pancreatic amylase, salivary amylase and mucosal glucoamylase are sufficient in some young infants to allow for complete digestion of long-chain GP. (*Pediatr Res* 20: 740–743, 1986)

Abbreviations

GP, glucose polymer
DP, degrees of polymerization

GP commonly are used in infant formulas either as the sole source of carbohydrate or as a caloric supplement. Young infants may not absorb GP completely, because pancreatic amylase activity cannot be detected in the duodenal fluid until 4 to 6 months of age (1, 2). Young infants may digest complex carbohydrates, *e.g.* GP, through alternative processes, such as the actions of salivary amylase, mucosal glucoamylase, and the colonic bacterial flora. The bacterial flora in the colon is capable of fermenting undigested carbohydrate to hydrogen gas and free fatty acids which then can be absorbed through the colonic mucosa (3, 4).

Salivary amylase may aid the digestion of complex carbohydrates by infants (5), but the production of this enzyme in young infants is variable and its survival during passage through the stomach is unknown (6). The amount of glucoamylase in the intestinal mucosa of young infants is comparable to that in children, but the extent to which this enzyme can digest GP *in vivo* remains unclear (7). In addition, glucoamylase has been shown to act preferentially on polymers of <10 glucose units (8). As a consequence, the long-chain component (>11 units), which constitute as much as 35% of the carbohydrate in commercial partial hydrolysates of cornstarch, may not be absorbed completely by young infants. Previous authors who used indirect methods to study glucose polymer utilization, *e.g.* the rise in serum glucose after an oral load of GP (9, 10) or the indirect calculation of the amount of GP in stool (11), have suggested that GP are well absorbed and utilized by young infants. These studies (9–11), however, utilized a mixture of short- and long-chain GP. Our study was undertaken to assess the ability of the young infant to absorb and utilize GP of different chain lengths or DP. Two groups of polymers were studied: a short-chain fraction (DP = 3–8), optimal for digestion by glucoamylase, and a long-chain fraction (DP > 43), more suitable for digestion by pancreatic amylase.

Our study exploited the fact that various foodstuffs contain different amounts of the nonradioactive isotope ^{13}C. When a subject is maintained on a diet low in ^{13}C and a ^{13}C-enriched substrate is then fed, the amount of malabsorbed foodstuff can be calculated directly from the increase in ^{13}C-enrichment in feces (12, 13). Similarly, the appearance of increased $^{13}CO_2$ in breath marks the oxidation of the enriched foodstuff (12). The appearance of hydrogen in breath was used as a marker of small bowel malabsorption of carbohydrate (4).

METHODS

Diet. The basal diet consisted of a soy formula (R.C.F., Ross Laboratories, Columbus, OH) to which beet sucrose had been added as the sole carbohydrate source to achieve a final concentration of 5 g/dl. Soy products and beet sucrose have a naturally low ^{13}C content. The test carbohydrates consisted of glucose, short-chain polymers (DP = 3–8), and long-chain polymers (average DP = 43), which were all derived from corn, a foodstuff naturally rich in ^{13}C.

The short-chain fraction (DP = 3–8) was prepared from the

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Address all correspondence and requests for reprints to Robert J. Shulman, M.D., Section of Nutrition and Gastroenterology, Texas Children's Hospital, 6621 Fannin Street, Houston, TX 77030.
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partial hydrolysate of cornstarch by a combination of yeast fermentation and ethanol fractionation (14). The long-chain fraction was prepared by molecular filtration (14, 15) of a partial hydrolysate of cornstarch (Polycose, Ross Laboratories). Both short- and long-chain fractions constituted approximately one-third of the original cornstarch hydrolysate.

The infants were maintained on the basal diet throughout the study, except for test days when one of the test carbohydrates was selected at random and substituted for the beet sucrose in the basal formula at a dose of 2 g/kg. The formula containing the test carbohydrate was given between 0800 and 0900 after a 3- to 5-h fast. Four hours after this feeding, an additional meal of formula containing 2 g/kg of the same test carbohydrate was given.

Subjects. Twelve infants were enrolled in the study after informed consent was obtained from their parents. Studies were approved by the Baylor College of Medicine and Texas Children's Hospital Human Investigation Committees. All infants were between 3 and 4 wk of age and had normal perinatal histories, growth, and physical examinations.

Design. The ability of the infants to produce hydrogen was tested on day 0 by administering 0.5 g/kg of the nonabsorbable carbohydrate, lactulose. The lactulose was substituted for the beet sucrose in the basal formula. The infants then were sent home with a supply of the basal formuala to which the beet sucrose had been added. They returned on days 3, 6, and 9 at which times they were fed one of the test carbohydrates. Breath samples were collected to determine $^{13}CO_2$ enrichment and H_2 production at −30, −15, and 0 min before and every 30 min, up to 4 h, after the initial test meal was administered. Sample collection and calculation of CO_2 production rates have been described previously (12). All stools were collected in the diapers from day 3 through day 12 and stored in sealed plastic bags in the subjects' home frezers. Based on information derived from preliminary studies, we scheduled test days at 3-day intervals to insure that both breath and stool ^{13}C enrichments had returned to baseline.

Sample analysis. Stool ^{13}C abundance was determined in the CO_2 formed by the combustion of a weighed aliquot of stool after the stool had been lyophilized, homogenized, frozen in liquid nitrogen, and lyophilized once again (12, 13). Breath $^{13}CO_2$ enrichment was determined using gas isotope ratio mass spectrometry (16). Breath H_2 production and percent CO_2 were measured by gas-solid chromatography (17).

Calculations. The calculation of the percent ingested dose of carbohydrate expired in breath or excreted in stool and the calculation of errors have been described previously in detail (12, 13, 18). Statistical analysis utilized a two-way randomized block analysis of variance or Student's *t* test as appropriate for the data. An increase in $^{13}CO_2$ in breath or ^{13}C in stool was considered significant if it were greater than twice the SD of the mean baseline value determined while the infant was on the basal formula (12). Malabsorption was detected when the percent dose found in stool exceeded twice the SD of the measured value (12).

RESULTS

No detectable increase in ^{13}C enrichment of the stools was found in seven of the 12 infants after the administration of the glucose, short- or long-chain GP. Five infants had detectable increases in fecal ^{13}C after the test meals containing the long-chain GP (Table 1); the mean increase in ^{13}C enrichment in stool was 8.4% of the dose fed (Table 1). Malabsorption of the short-chain GP (6.7% of the dose fed) was detected in the stool of one infant and 9.7% of the glucose fed was detected in the stool of a second infant (Table 1).

The time at which a detectable rise in the breath of $^{13}CO_2$ enrichment occurred was 75 ± 34 min (mean ± SD, range 30 to 150 min) after the test meals were administered. There were no consistent differences among the test carbohydrates in the time of appearance of the initial increase in breath $^{13}CO_2$ enrichment. The cumulative percent of the dose fed that was oxidized into breath by the time the initial increase in $^{13}CO_2$ enrichment was first detected was simlar for the three carbohydrates (glucose 2.6 ± 2.5; short-chain GP 2.2 ± 1.7; long-chain 1.8 ± 0.9, mean ± SD). Similarly, the cumulative percent dose that was oxidized into breath over 4 h was similar for glucose and the short- and long-chain GP: 31.4 ± 16.9%, 27.3 ± 6.7%, 27.5 ± 11.5%, respectively (see Table 1, $n = 10$, samples from two infants were lost becuase of faulty collection tubes).

Three infants failed to produce breath H_2 after lactulose was administered, but their results were included because they subsequently produced breath H_2 after ingestion of one or more of the test carbohydrates (Table 1). No differences among the test carbohydrates were demonstrated by the number of infants who produced >20 ppm of H_2 over baseline in the 4 h after the test meals were fed: glucose, four of 12 infants; short-chain GP, five of 12; long chain GP, six of 12. The times (mean ± SD) for

Table 1. *Results of the breath hydrogen tests and cumulative percent dose of the administered carbohydrate recovered in breath and in stool*

	Peak hydrogen (ppm)				% Dose in breath			% Dose in stool		
Patient	L*	G†	SC‡	LC§	G	SC	LC	G	SC	LC
1	28	4	6	21	22	25	23	ND‖	ND	ND
2	7	1	17	4	7	19	13	ND	ND	ND
3	45	135	49	10	33	¶	26	ND	ND	6.1 ± 2.8**
4	12	60	68	9	21	25	21	ND	6.7 ± 2.7	3.7 ± 1.8
5	178	34	36	25	40	20	23	ND	ND	ND
6	6	46	3	23	18	43	21	ND	ND	11.5 ± 3.3
7	194	2	28	3	60	19	¶	ND	ND	ND
8	74	2	12	2	31	26	23	ND	ND	ND
9	24	14	1	36	41	28	36	ND	ND	2.6 ± 1.3
10	77	16	51	29	55	32	53	9.7 ± 2.1	ND	18.5 ± 2.2
11	117	4	8	26	21	29	38	ND	ND	ND
12	245	7	2	11	59	26	23	ND	ND	ND

* L, lactulose.
† G, glucose.
‡ SC, short-chain glucose polymers.
§ LC, long-chain glucose polymers.
‖ ND, not detectable.
¶ Samples lost because of faulty collection tubes (see text).
** Mean ± SD.

appearance of >20 ppm breath H_2 over baseline were: glucose, 60 ± 35 min; short-chain GP, 48 ± 27 min; long-chain GP, 125 ± 84 min. Based on regression analysis, we found no correlation between the percent dose of long-chain GP excreted in stool and the peak breath H_2.

DISCUSSION

The results of our investigation reveal that the absorption of long-chain GP was variable and generally less than that of glucose and of the short-chain GP in the healthy infant population studied. The data from our study and from previous experiments help clarify the process of glucose polymer digestion in young infants.

Kerzner *et al.* (19) have shown in pancreatic amylase-free pocine jejunum that carbohydrate absorption from a partial hydrolysate of cornstarch was less than that from a glucose solution; the bulk of the absorbed carbohydrate had fewer than 12 glucose units. Lebenthal *et al.* (9) measured the amount of glucose produced from an *in vitro* incubation of GP and a homogenate of human small intestinal mucosa with and without duodenal fluid (presumably containing pancreatic secretions). These investigations found that in the absence of duodenal fluid, the hydrolysis of GP was diminished. Both studies (9, 19) utilized a wide range of GP lengths. Auricchio *et al.* (20) have shown that glucose polymers containing more than 30 glucose units accumulate in the intestinal lumen of infants younger than 6 months of age fed amylopectin.

Investigations in animals and adults in which the digestion and absorption of specific lengths of GP have been examined have shown that GP that are 6 to 10 glucose units in length are hydrolyzed and absorbed faster than longer polymers (21, 22). These data agree with the known preference of glucoamylase for GP chain lengths of 5 to 9 glucose units (8). The results from our study support these findings.

Previous investigators have studied the utilization of GP in young infants by examining clinical tolerance (9, 11, 23), weight gain (23), or glycemic response (9, 10). Unlike our study, however, these investigators (9–11, 23) employed cornstarch hydrolysates that were a mixture of both short- and long-chain GP. Although the $^{13}CO_2$ data from our study suggested that similar amounts of short- and long-chain GP were oxidized, the results of the stool analyses revealed that the absorption of the short-chain fraction was greater than that of the long-chain.

Malabsorption of the long-chain GP was detected in the stool analyses of five of the 12 infants. This finding contrasts with the observations of Kien *et al.* (11) who fed premature infants a formula that contained either lactose or lactose and GP in a 50/50 ratio. The infants' stools were analyzed for nitrogen, fat, and total energy excreted. The carbohydrate energy absorption was calculated by difference. The investigators found no differences in the coefficient of carbohydrate energy absorption between the two types of feedings, which suggests that the GP were well absorbed.

Our results are not directly comparable with those of Kien *et al.* (11) because, as in the studies previously cited (9, 10, 23), Kien *et al.* fed a mixture of short- and long-chain GP. In addition, we specifically measured carbon derived from the test carbohydrate.

One infant had detectable malabsorption of both glucose and long-chain GP (Table 1). The reason for the incomplete absorption of glucose is unclear as the infant had no evidence of intestinal dysfunction. A caretaker other than the mother may have fed this infant a foodstuff different from the basal diet, but we were unable to prove this possibility. Because glucose was the last carbohydrate tested in this infant, no further breath collections were made after the infant went home, although stools were collected. Thus, we could not examine the baseline breath $^{13}CO_2$ enrichment which would have reflected a change in the ^{13}C enrichment of the diet.

Breath H_2 measurements have been used to demonstrate the fermentation of unabsorbed carbohydrate by the colonic flora (4). In view of the results of the fecal analyses in our study, more infants would have been expected to have produced >20 ppm breath H_2 after the long-chain GP were fed than after either the glucose or short-chain GP. Our inability to find any differences may have been due to the small number of infants studied.

In summary, our results demonstrate that long-chain GP are not absorbed as completely as short-chain GP by some young infants. Other infants who also are presumed to make no pancreatic amylase, nonetheless, can digest long-chain GP effectively. A number of mechanisms may account for the difference in the infants' ability to digest and absorb the long chain GP, *e.g.* salivary amylase, pancreatic amylase, the colonic flora, and mucosal glucoamylase. The spectrum of activity of salivary amylase is similar to that of pancreatic amylase (6). Recent evidence suggests that some salivary amylase activity may survive passage through the stomach (7). The level of salivary amylase, which is generally low at 1 month of age, is variable among infants. Because of this variability, salivary amylase may contribute to GP digestion in individual infants (6, 7). Although levels of pancreatic amylase at this age also may vary among infants, most studies suggest that the levels are too low at 1 month of age to contribute significantly to the digestion of GP (1, 2). The colonic bacterial flora plays a major role in salvaging carbohydrate malabsorbed in the small bowel (3). The results of breath H_2 studies in these 1-month-old infants after the administration of the nonabsorbable carbohydrate, lactulose, suggest that the ability of the colonic flora to ferment carbohyrate is variable. As a consequence, interindividual variability in the fecal excretion of the long-chain GP would be expected if the carbohydrate were not absorbed completely in the small bowel. Thus, we speculate that the diminished absorption of the long-chain GP primarily reflects the lack of preference of glucoamylase for the long–chain fraction and variability in the ability of the colonic flora of infants this age to ferment carbohydrates. Although the absolute amount of energy lost in the stools of the study infants was not great, infants who have low levels of salivary amylase (*i.e.* premature infants), mucosal glucoamylase (*i.e.* infants with gastroenteritis), or colonic bacterial flora (*i.e.* after antibiotics) may be less able to digest long-chain, and even short-chain, GP than their healthy counterparts.

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