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Role of Rat Intestinal Glucoamylase in Glucose Polymer Hydrolysis and Absorption

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ABSTRACT. Rice starch is a main source of energy in many lesser developed countries. We studied different chain-lengths of rice glucose polymers (GP) to evaluate their possible use in feeding infants in developing countries. The initial GP of rice (G1 = 4.6, G2 = 4.5, G3 = 15.4, G4 = 7.3, G5 = 17.4, G6–G9 = 9.61 and >G9 = 31.3%) was analyzed by HPLC and then separated in a Bio-Gel P-2 column and compared to its short-chain GP of rice (G2 = 22.7, G3 = 28.2, G4 = 14.0, G5 = 16.6, G6 = 11.6, G7–G9 = 6.9%), long-chain GP of rice (>G9 = 100%), and D-glucose. Intraduodenal bolus infusion of 10% solution of short-chain rice GP when compared with long-chain rice GP, the initial rice GP, or D-glucose showed significantly higher values at peak absorption time (0 to 30 min) in the portal venous blood glucose response. The portal venous glycemic response of short-chain rice GP compared with D-glucose was as follows: 2.5 ± 0.1 *versus* 2.0 ± 0.2 cm², area under the portal blood glucose curve at 0–30 min ($p < 0.01$). Glucoamylase, the key enzyme for brush-border hydrolysis of short-chain GP, was assessed with a newly modified glucoamylase assay using GP G5–G8 as substrate. Our finding of faster glucose absorption with short-chain rice GP compared with isocaloric D-glucose might have important physiologic implications for carbohydrate absorption. The osmolality of short-chain rice GP is nearly one-fourth that of glucose. This might have important bearing in the design of infant feeding where increased caloric density with low osmolality is desirable. (*Pediatr Res* 28: 166–170, 1990)

Abbreviations

GP, glucose polymer

Rice starch is one of the main sources of energy in food available in many lesser developed countries. Starch (amylose and amylopectin) is rapidly hydrolyzed by salivary and pancreatic amylases to short GP, maltotriose, maltose, and glucose (1). The brush-border glucoamylase, maltase, and isomaltase act on the products of luminal starch digestion releasing glucose (2).

During the first 4 to 6 mo of life, the key enzyme for starch digestion, pancreatic amylase, is very scarce or nonexistent (3, 4). In addition, brush-border disaccharidases, because of their predominant localization in the proximal small intestine where the mucosal injury from protracted diarrhea of infancy can be the most severe (5), are likely to be diminished during chronic diarrhea in infancy (6–8).

It has been suggested that the availability of glucoamylase in the small intestine can play a role in GP digestion and absorption in early life. Moreover, intestinal glucoamylase is distributed throughout the small intestinal mucosa and tends to be more resistant to intestinal injury than the disaccharidases (9). However, it is not clear whether rice starch hydrolyzed to shorter polymers of glucose will be better absorbed in the small intestinal mucosa in the first 6 mo of life, especially in those with prolonged mucosal injury in persistent diarrhea of infancy.

The aim of our study was to evaluate the effect of different chain-lengths of GP generated from rice on the glucose response in the portal vein of a rat model. Perfusion studies were performed with the initial GP of rice, short-chain GP of rice, long-chain GP of rice, and D-glucose. The GP were separated and prepared from the initial GP of rice using a modified system of gel permeation chromatography and determined by HPLC.

The role of glucoamylase in the specific hydrolysis and absorption of the GP was examined. The study used a new substrate of a purified short-chain GP (chain-length G5–G8) in addition to glucogen for the glucoamylase assay.

MATERIALS AND METHODS

Animals. Adult Sprague-Dawley rats, weighing 250–350 g, were used for all experiments.

GP. GP are a heterogenous group of linear chains of glucose residues, linked predominantly by α-1,4 glucosidic bonds. The nomenclature used in this report is as follows: G1, free glucose; G2, maltose; G3, maltotriose; G4, maltotetraose; G5, maltopentose; G6, maltohexose; G7. maltoseptose; G8, maltoctaose; G9, maltonanose; >G9, GP of longer chain-length than 9 glucose units. The initial GP preparation from rice (donated by Mead Johnson, Evansville, IN) was analyzed by HPLC and composed of G1 = 4.6, G2 = 14.5, G3 = 15.4, G4 = 7.3, G5 = 17.4, G6–G9 = 9.61, and >G9 = 31.3% (Fig. 1*A*). Samples for HPLC analysis were passed through three thicknesses of millipore filters. Twenty μL of the filtrate were then applied to an Aminex HPX-42A HPLC column (Bio-Rad Laboratories, Richmond, CA) for analysis of initial GP of rice, short-chain GP, long-chain GP, corn GP, and glucoamylase substrate at 85°C using specially purified water (HPLC grade, Fisher Scientific, Fairlawn, NJ) as the mobile phase. The profile depicting the individual products was analyzed with a computer-aided data master system from Gilson Medical Electronics (Middleton, WI). Individual components were quantitated as areas under the elution peaks and expressed as percent of total GP measured. GP profiles were integrated using a computer program. Individual components that were integrated include G1, G2, G3, G4, G5, G6, G7, G8, G9, and >G9. The relative percent of each peak was calculated as a fraction of the sum totals.

Short-chain GP and long-chain GP were separated and prepared from the initial rice GP using a modified system of gel

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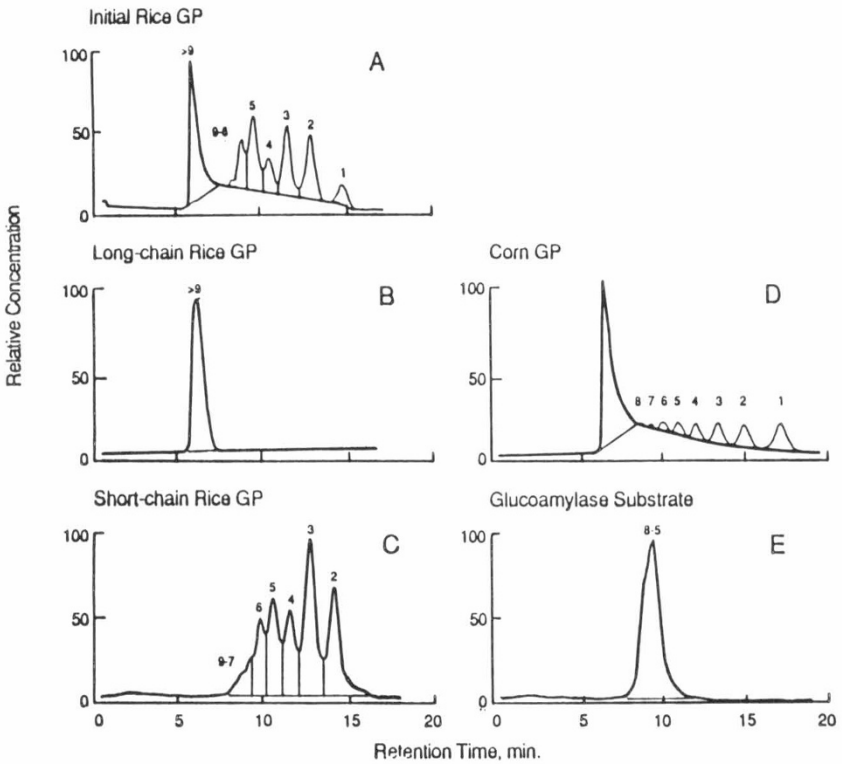

Fig. 1. Representative chromatograms of the initial rice GP (*A*), long-chain rice GP (*B*), short-chain rice GP (*C*), corn GP (*D*), and glucoamylase substrate (G5–G8) (*E*).

permeation chromatography (10). This system separated and classified components on the basis of molecular size. A water-jacketed glass column (2.5 cm × 90 cm) was packed with a preswollen aqueous slurry of Bio-Gel P-2, 200–400 mesh (Bio-Rad Laboratories). When almost fully packed, a rubber stopper (attached to a closed hydrostatic pressure system for elution water delivery) was fitted to take up any dead volume at the top of the column. The jacket temperature was slowly raised to 65°C while the column was eluted with deaerated water. Once the column was fully equilibrated (after ~24 h), the elution with water was continued and 3-mL aliquots of suitably diluted samples were introduced into the elution stream.

The molecular sizes of unknown components of the sample were identified from spectrophotometric reading at 190 nm wavelength and their elution position was compared with a chart for standards.

The eluate fractions that contained G2–G9 GP and >G9 GP were concentrated, lyophilized, and validated by HPLC (Rainin, Woburn, MA). The same procedure was performed to separate and purify the corn GP of G5 to G8 that served as a substrate for determination of glucoamylase activity.

Osmolality measurements of test GP solutions including D-glucose were done by vapor pressure osmometer (Model 5100B, Wescor, Inc. Logan, UT). Osmolality of 10% solutions of D-glucose, the initial rice-GP, short-chain GP, and long-chain GP were 561, 158, 161, and 212 mosmol/kg, respectively.

Infusion technique. After an overnight fast, the animals were anesthetized by intraperitoneal injection of pentobarbital sodium (70 mg/kg body wt) and maintained with a quarter of the initial dosage. The abdomen was opened by a midline incision and the GP was infused through a 21-gauge needle intraduodenally 4 cm proximal to the ligament of Treitz as a 10% solution bolus, at a dose of 0.5 gm/kg body wt within 1 min. Biliary and pancreatic secretions were not excluded from the infused segment. The intestines were kept moist with warm saline-soaked gauze, and the animals were kept warm with an overhead infrared lamp.

Determination of portal venous blood glucose levels. Fifty to 100 μL of portal venous blood were drawn at 30-min intervals from 0 to 120 min in each experiment. Blood glucose was estimated by the glucose oxidase method by Glucometer, Ames, IA. The machine was equilibrated with low and high calibrators before the start of the project and when batteries were changed. Area under the portal venous blood glucose curve was defined as the postinfusional rise above baseline over time.

Preparation of mucosal homogenate. After killing the rats at the end of the study, a 15-cm jejunal segment distal to the ligament of Treitz was taken out, rinsed with cold saline 6 times to get rid of contaminating pancreatic α-amylase as much as possible, and then opened by longitudinal incision. The mucosa was scraped off gently using a glass slide and stored at −70°C. Before enzymatic determinations, the jejunal scrapings were homogenized by hand using a glass tissue homogenizer with 100 volumes of distilled water at 4°C.

Enzymatic determinations. Glucoamylase activity was determined by a modification of the method used by Eggermont *et al.* (11). The substrate solutions contained 0.3 g of GP (G5–G8) or glycogen plus 0.025 gm BSA in 10 mL of 0.15 M sodium citrate buffer at pH 5.4 (to inhibit the pancreatic α-amylase). Activities measured were expressed as μg of glucose liberated per assay mixture after the designated time of incubation. Disaccharidase activities were assayed by the method of Townley *et al.* (12). Glucose was assayed using the diagnostic statzyme reagent (Worthington Biochemical Co., Freehold, NJ). Protein was determined by the technique of Lowry *et al.* (13).

Statistics. All results are expressed as mean ± SEM. Differences in mean values between groups were determined by an unpaired *t* test. *p* values of less than 0.05 were considered significant.

RESULTS

GP. Figure 1 depicts representative chromatograms of the initial GP from rice (Fig. 1*A*), long-chain GP of rice (Fig. 1*B*), short-chain GP of rice (Fig. 1*C*), corn GP (Fig. 1*D*), and the corn GP G5–G8 serving as glucoamylase substrate. The initial GP from rice was composed of G1 = 4.6, G2 = 14.5, G3 = 15.4, G4 = 7.3, G5 = 17.4, G6–G9 = 9.6, and >G9 = 31.3% (Fig.

1*A*). The long-chain GP of rice was composed of >G9 = 100% (Fig. 1*B*). The short-chain GP of rice was composed of G2 = 22.7, G3 = 28.2, G4 = 14.0, G5 = 16.6, G6 = 11.6, G7–G9 = 6.9% (Fig. 1*C*).

Infusion of GP and portal venous glucose response. All GP and glucose produced maximal portal venous glucose peak at 30 min (Fig. 2). After the infusion of the test GP and glucose, portal blood glucose peaked from 63.0 ± 4.8 to 94.4 ± 6.5 mg/dL for initial GP of rice, 60.2 ± 1.5 to 108.2 ± 2.0 mg/dL for short-chain GP, 63.4 ± 2.0 to 88.2 ± 3.5 mg/dL for long-chain GP, and 68.0 ± 2.7 to 105.6 ± 1.1 mg/dL for D-glucose. Portal venous blood glucose then returned to a level that was not significantly different from the baseline at 120 min for each of the carbohydrates studied.

Glycemic response. The glycemic response measured as the mean of the area under the curve revealed a significantly higher value for short-chain GP of rice compared with the initial GP of rice, long-chain rice GP, and D-glucose during the peak absorption time, namely 0 to 30 min (Table 1). At 120 min, the glycemic response values for both short-chain GP and D-glucose did not show any significant difference.

The infusion of the initial GP of rice and the long-chain GP was associated with a wide SD of the portal venous blood glucose levels, whereas those for short-chain GP and glucose revealed a narrow SD.

Glucoamylase activity determination using G5 to G8 as substrate. The adequacy of glucoamylase (E.C. 3.2.1.3) assay used in our study was checked by several methods. The use of an optimal substrate of GP, G5 to G8 units, (Fig. 1*E*) to determine the glucoamylase activity has a great advantage over the glycogen that was used in previous studies (9, 11). Figure 3 compares the time course of glucoamylase activity using G5 to G8 units as substrate with the time course when using glycogen. The sp act of the glucoamylase using G5 to G8 units was found to be linear and constant for the entire 90 min, whereas when using the glycogen as a substrate, there was linearity for 30 min and then an increase. Furthermore, the glucose production using G5 to G8 units compared to glycogen was higher during the 30 and 60 min of the reaction (Fig. 3). This observation is consistent with the finding that the reaction rate of glucoamylase increases with decreasing length of the substrate, glycogen.

Glycerol inhibition studies. Glycerol at 1.2 M concentration caused reductions in glucoamylase activities, using GP G5 to G8 units as substrate, to 45% of its activity under glycerol-free conditions, whereas using glycogen as the substrate resulted in a reduction to 40% of glucoamylase activity under the identical assay system (Fig. 3).

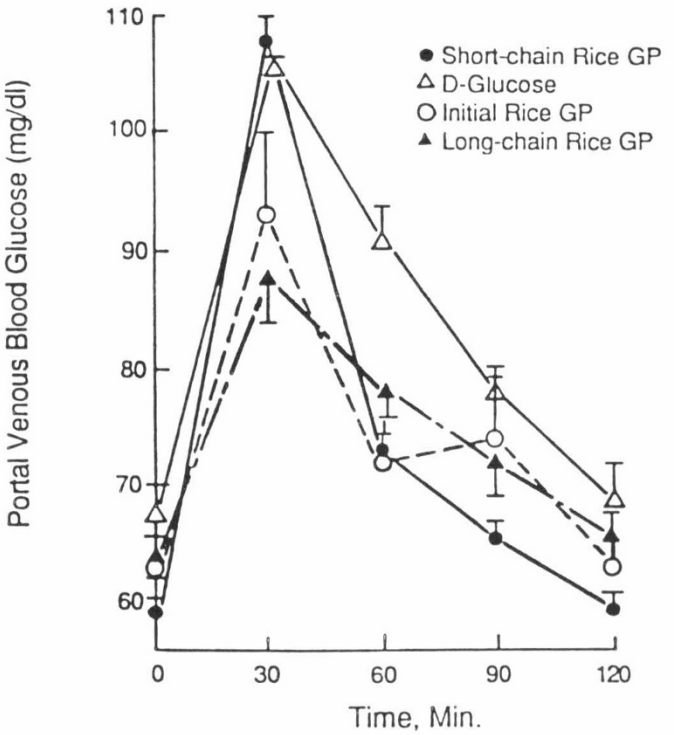

Fig. 2. Portal venous blood glucose response curves for short-chain rice GP (●), D-glucose (△), initial rice GP (○), and long-chain rice GP (▲). All four carbohydrates produced maximal peaks at 30 min. The maximal increase in portal blood glucose is significantly greater for short-chain rice GP infusion in comparison to the initial rice GP, long-chain rice GP, and D-glucose. Maximal increase in portal blood glucose after the initial rice GP infusion is not significantly different from that of D-glucose infusion. Data are expressed as mean values ± SEM.

DISCUSSION

The main finding of our study indicates that short-chain GP of rice composed of G2 to G9 glucose units are hydrolyzed and absorbed in the small intestine faster than isocaloric D-glucose. Our results are similar to those in the study by Jones *et al.* (14) that was performed on humans but with corn GP. In their study, the intestinal absorption and mucosal hydrolysis of a partial and a complete α-amylase hydrolysate of corn starch, simulating the normal intermediary and end products of luminal starch digestion, was undertaken using an *in vivo* steady state jejunal perfusion technique (excluding pancreatic α-amylase) in normal human subjects. Whereas Jones *et al.* (14) used a 25-cm jejunal segment, we used the whole small intestine, including pancreatic α-amylase, simulating a real life situation for digestion and absorption processes of GP. In the study of Jones *et al.*, hydrolysis of the polymer fraction containing more than 10 glucose units was significantly slower than the lower mol wt fraction, and it was postulated that oligosaccharides in the more rapidly hydrolyzed lower mol wt fraction were exerting a kinetic advantage on glucose absorption. Whereas Jones *et al.* (14) used luminal disappearance of the corn GP as evidence of GP hydrolysis and absorption, we measured the effect of GP hydrolysis and absorption as the portal venous blood glucose response. There is evidence that, in rats (15), most of the intraluminally absorbed glucose is absorbed intact into the portal vein and not metabolized to lactate. During peak absorption time, glucose concentration in the portal vein is higher than that in the aorta, suggesting a minimal role of insulin and other regulatory hormones in glucose homeostasis in portal venous blood (15).

The finding of lower total 120-min glycemic response for the initial GP of rice in comparison to D-glucose and no significant difference of glycemic response values at 0 to 30 min is important. The explanation might be related to efficient hydrolysis of GP in the G2–G9 fraction of the initial rice polymer and the slower hydrolysis of the >G9 fraction that might be the limiting factor for efficient glycemic response. That is why we separated the initial rice GP to short-chain GP (G2–G9) and long-chain GP (>G9) of rice. We found that the portal glycemic response was significantly higher for short-chain GP of rice in comparison to long-chain GP of rice both in the 0 to 30 min and in the total 120 min.

The faster short-chain GP absorption in comparison to D-glucose during peak absorption time could be explained by the combined effects of the property of GP, its low osmolality in comparison to D-glucose, and the preferential substrate use by intestinal glucoamylase. GP, due to incorporation of water during hydrolysis, will yield more moles of hexose than the D-glucose solution of the same weight concentration. The osmolalities of our test GP solutions were much less than the D-glucose solution. Daum *et al.* (16) found a larger increment of intraduodenal water content, indicating flux of water, after 10% free D-glucose infusion compared with 10% GP infusion. Even at 30 min, 10% GP solution produced a lower luminal osmolality than that of 10% free glucose. This might be part of the explanation

Table 1. *Area under total portal blood glucose response curve (AUC) above fasting level after intraduodenal infusion of short-chain rice GP, long-chain rice GP, initial rice GP, and D-glucose**

Time (min)	Rise in portal venous blood glucose AUC (cm^2)			
	Short-chain GP ($n = 5$)	Long-chain GP ($n = 5$)	Initial rice GP ($n = 7$)	D-glucose ($n = 6$)
0–30	2.5 ± 0.1†	1.2 ± 0.2	1.6 ± 0.2	2.0 ± 0.1
Total 120	6.9 ± 0.4	4.6 ± 0.6	5.4 ± 0.6‡	7.5 ± 0.5
First ½ h rise (% of total)	35.9 ± 1.3§	26.0 ± 2.8	30.3 ± 1.9	27.5 ± 1.1
First h rise (% of total)	81.2 ± 1.5§	67.2 ± 4.8	69.2 ± 2.9	70.0 ± 1.5

* Values are presented as mean ± SEM. The portal venous glycemic response at 0 to 30 min is significantly greater for short-chain rice GP infusion than for D-glucose. The total 120 min glycemic response is significantly lower for the initial rice GP infusion than for D-glucose infusion. The kinetics of absorption of tested GP, defined as the percentage of the total rise of the area under the portal blood glucose curve over time, reveals significantly higher values both at 1st 30 min and 1st 60 min for short-chain rice GP infusion in comparison to D-glucose infusion.

† $p < 0.01$.

‡ $p < 0.02$.

§ $p < 0.001$.

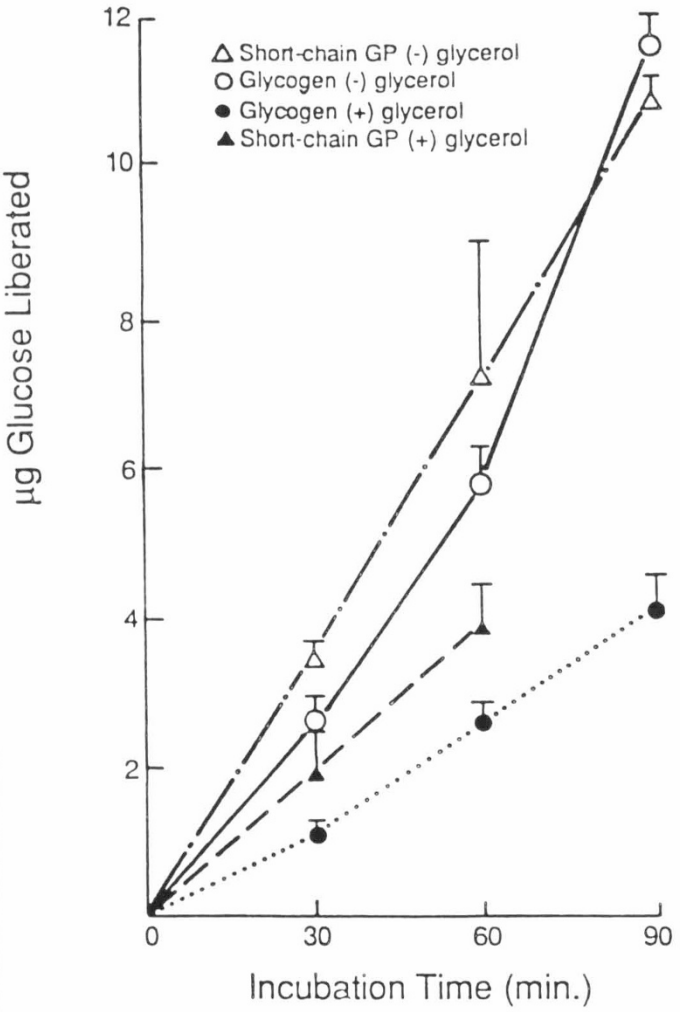

Fig. 3. Comparison of time course of glucoamylase assay using short-chain GP (G5–G8) and glycogen as substrates. The assay was carried out in absence [(△) short-chain GP, (○), glycogen] and presence [(▲) short-chain GP, (●) glycogen] of glycerol. Five µL of mucosal homogenates were used for incubation. Assays were set up as described in Methods. Glycerol was added to a final concentration of 1.2 M in the appropriate assay mixture. For each time point, two assay mixtures were prepared. Assay mixtures were incubated for the designated time. Activities were measured as µg of glucose liberated per assay mixture after the designated time of incubation. Values represent the mean ± 1 SD.

for slower D-glucose absorption in comparison to GP absorption (17). We designed our study to compare the portal venous blood glucose response of the three GP solutions of different chain-lengths and similar osmolalities with D-glucose infusion.

The finding of significantly higher portal venous blood glucose during peak absorption time with short-chain GP in comparison to the initial rice GP or the long-chain rice GP indicates that osmolality alone could not account for the observed difference. This might be an advantage of short-chain GP, when planning infant feeding designs where increased caloric density and lowered osmolality is desirable. One example of the practical relevance of faster short-chain GP absorption over D-glucose would be its use in short bowel syndrome and in patients with rapid intestinal transit time.

In contrast to the human, glucose absorption in the rat small intestine occurs by two independent routes. One is an active electrogenic component that is saturable (luminal glucose concentration ~64 mM) and phlorizin sensitive. The other is a diffusional pathway that is nonelectrogenic, nonsaturable, and phlorizin insensitive. At high luminal glucose concentrations, diffusional component accounts for 50% of glucose absorption (18). The osmolality of our test glucose solution was 561 mosmol/L, which definitely superseded the active electrogenic saturable component. The portal venous blood glucose level might be the combination of both components. The finding of significantly higher glycemic response with short-chain GP at peak absorption time infers that hydrolysis of the short-chain GP might have yielded more glucose at the glucose transport sites than D-glucose. This is in agreement with the finding of Daum *et al.* (16) that GP produces a lower immediate intraduodenal glucose content (conversely higher mucosal glucose content) than a D-glucose solution of the same concentration by weight.

Due to difficulties correlating the *in vivo* perfusion results and *in vitro* enzyme assay in human subjects, our study was designed to correlate the activities of glucoamylase and glucose production *in vitro* by using short-chain GP (G5–G8) and glycogen as substrates. Glucoamylase is the key enzyme for brush-border hydrolysis of short-chain GP (G5–G9); the enzyme sp act decreases with either increasing or decreasing chain-length of the GP (19). We carried out and modified the glucoamylase assay by using the optimal substrate G5–G8, as well as glycogen (prototype of long-chain rice GP), as used in the past (9, 11).

The finding of a linear time-course for glucoamylase activity in the mucosal homogenates over the entire 90-min period with short-chain GP (G5–G8) as substrate in comparison to glycogen indicates preferential use of short-chain GP (G5–G8) by glucoamylase. This is also an advantage of our modification of the glucoamylase assay. This is in agreement with the finding of Kelly and Alpers (19) for human intestinal glucoamylase, where they found linear GP linkages (alpha 1–4) were hydrolyzed more

rapidly than polymers containing (alpha 1–6) cross linkages. They also found a similar Km for all polymers from G5 to G9 and the increase of Km value with either an increase of polymer length (>G9) or a decrease of polymer length (<G5).

The current cost of D-glucose is to some extent prohibitive in lesser developed countries. It is feasible that the use of less expensive, short-chain GP will be more easily afforded by lesser developed countries.

In conclusion, short-chain rice GP with a low osmolality, compared with isocaloric D-glucose, may be an advantageous energy source for children with chronic diarrhea in developing countries.

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