Luminal fructose modulates fructose transport and GLUT-5 expression in small intestine of weaning rats

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Luminal fructose modulates fructose transport and GLUT-5 expression in small intestine of weaning rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G232–G239, 1998.—In neonatal rats, precocious introduction of dietary fructose significantly enhances brush-border fructose transport rates and GLUT-5 mRNA levels during early weaning. In this study, these rates and levels were more than two times higher in the anastomosed intestine compared with those in the bypassed loop of weaning pups that underwent Thiry-Vella surgery and consumed high-fructose (HF) diets. In Thiry-Vella pups fed fructose-free (NF) diets, uptake rates and mRNA levels in the anastomosed intestine were very low and similar to those in the bypassed loop. In sham-operated littermates, transport rates and mRNA levels were similar between intestinal regions that corresponded to anastomosed and bypassed loops in Thiry-Vella pups and were two to three times greater in pups fed HF than in those fed NF diet. In contrast, rates of brush-border glucose transport and levels of SGLT-1 and of GLUT-2 mRNA were independent of diet and were similar between bypassed and anastomosed regions. Changes in GLUT-5 expression did not follow a distinct diurnal rhythm. When pups were fed HF diet after 12 h of starvation to empty the intestinal lumen, fructose transport rates increased with feeding duration and reached a plateau 12–24 h after feeding; in contrast, GLUT-5 mRNA levels were highest within 4 h after arrival of chyme in the jejunum and then decreased gradually and returned to baseline levels 24 h later. In littermates fed NF diet, mRNA levels and uptake rates were each independent of feeding duration. Luminal, and not endocrine, signals regulate GLUT-5 expression in weaning pups.

Moreover, regulation of intestinal nutrient transporters differs between neonates and adults (27). The mechanisms and signals underlying regulation of nutrient transporters during early development, however, are unknown.

Intestinal brush-border transporters may be classified into two main categories. Prenatal onset transporters are those that are present and functional before birth, whereas late onset transporters are those that become functional only after birth or after certain stages of the life cycle have been completed, e.g., weaning. The brush-border fructose transporter GLUT-5 (3) is a classical example of an intestinal transporter expressed at significant levels only after completion of weaning (4, 23, 25, 28). Expression of fructose transporter activity after completion of weaning occurs independent of dietary signals and is apparently “hard-wired”; a hardwired ontogenetic timing mechanism also exists for expression of rat intestinal sucrase activity after the suckling stage (29). We used the fructose transporter as a model not only to investigate dietary effects on transporter development but also to demonstrate the potential for precocious enhancement of late onset transporters.

We have previously shown that if a high-fructose (HF) diet is introduced early during weaning, brush-border fructose transport rate and GLUT-5 mRNA levels are each significantly increased before weaning is completed (9, 25); levels of SGLT-1 (the brush-border glucose transporter, Ref. 14) and GLUT-2 (the basolateral glucose and fructose transporter, Ref. 6) mRNA, as well as rates of brush-border glucose uptake, are each independent of diet. We then showed that a dietary concentration of 10–30% fructose is required for enhancement. Thus intestinal fructose transport is turned on independent of dietary signals by the end of weaning but can be turned on well before completion of weaning by dietary fructose.

In this report we studied the signal(s) underlying this dramatic increase by using the Thiry-Vella surgical method to create a bypassed loop of small intestine. This method would allow us to determine the nature of the signal for upregulation of intestinal fructose transport by distinguishing between luminal and hormonal factors. We also investigated the time course of dietary enhancement of fructose transport rate and GLUT-5 mRNA level by briefly fasting the pups and then killing them at various times after refeeding. This would enable us to determine the time it would take for mRNA levels and uptake rates to increase and whether the increase in mRNA levels and the increase in uptake rates are simultaneous or sequential. Finally, recent reports of a significant circadian rhythm in GLUT-5

DURING POSTNATAL DEVELOPMENT, the rat gradually shifts from a diet containing low-carbohydrate, high-fat levels to one containing high-carbohydrate, low-fat levels (1). To digest and absorb nutrients properly, the small intestine adapts by regulating membrane properties, enzyme activities, and transporter activities at levels capable of processing diets typically consumed at each developmental stage (1, 15, 20). The increase in sucrase activity and decrease in lactase activity during weaning are now known to be modulated by glucocorticoids and to a lesser extent by thyroxine (22, 30). Modulation by diet plays a more modest role in the development of sucrase and lactase in the small intestine (15).

In contrast to what is known about regulation of brush-border enzymes, there is scant information about regulation of nutrient transporters during development. In cats, frogs, rabbits, and rats, changes in activity of nutrient transporters and in absorptive capacity for various nutrients allow these animals to adapt to developmentally related shifts in diet (1, 11).
mRNA and transporter levels in adult rats (4, 8) led us to determine whether neonatal GLUT-5 mRNA levels and fructose uptake also varied with time of day.

**MATERIALS AND METHODS**

**Animals**

Adult male and female Sprague-Dawley rats weighing 200–220 g were purchased from Charles River Laboratories (Wilmington, MA). They were kept in a 12:12-h light-dark cycle and fed a standard chow diet ad libitum. The diet of female rats was also supplemented with cheese (2 g/day per rat) before and during pregnancy; cheese supplementation improved female rat fecundity, pup growth, and pup survival. The male and female rats were bred in the Research Animal Facility. Once the female rats were pregnant they were separated from the males and caged individually. The progress of pregnancy was carefully monitored, and the date of birth was noted and considered as day 0.

Thiry-Vella study. Pups were kept with their mother in the same cage until they were 20 days old. They were then separated from the dam and starved overnight (12 h) before surgery. The six litters used in this experiment were killed at different dates but all treatment groups (see below) were represented in each litter. After an overnight recovery, pups that were already 22 days old were fed either HF or NF (fructose-free) diets.

Circadian rhythm study. Pups were kept with the dam until they were 22 days old and then killed at various times of the day: 0900, 1200, 1500, 1800, 2100, and 0300.

Intestinal motility study. Measuring the time course of enhancement depended highly on whether pups were consuming the HF and the control NF diets. Pups just removed from the dam displayed little inclination in eating the pelletized diets, and therefore we had to briefly starve them. We determined not only the time it took to empty the stomach and small intestine (8–12 h for both diets in a preliminary experiment) but also the time it took for chyme to reach the distal ileum after refeeding. Pups were kept with the dams until they were 22 days old. Pups were removed from the dams, subjected to overnight (12 h) starvation (which emptied their small intestine), refed with HF pelletized diets, and then killed at 1-, 2-, 3-, 4-, 5-, and 6-h intervals after refeeding. The distances that the chyme (particulate matter) reached in the intestinal lumen were recorded. We did not repeat the study using NF diets not only because gut emptying times are similar between NF and HF diets but also because food intake between pups consuming NF and HF diets did not differ (see below).

Time course study. Pups were kept with the dam until they were 22 days old. There were three litters, and each litter was randomly separated into six groups. All groups except one were initially starved for 12 h. The first group was killed immediately without refeeding, and the last group stayed with the dams. Groups 2, 3, 4, or 5 were killed 4, 8, 12, or 24 h, respectively, after refeeding. One-half of the pups in groups 2–5 were fed HF diet, and the other one-half were fed NF diet. In this experiment, we observed no diet-related difference in chyme movement along the intestinal lumen. Littermates that stayed with the dam were killed at random between 4 and 24 h after refeeding of other groups.

**Diets**

The NF and HF diets (Dyets, Bethlehem, PA) were supplied in pelletized form. HF diets consist of 65% fructose and 20% casein (other components are 5% corn oil, 5% cellulose, 3.5% salt mix, 1% vitamin mix, 0.3% DL-methionine, and 0.2% choline bitartrate). NF diets contain 10% glucose, 74.2% casein, and 1.1% DL-methionine. The remainder of the ingredients are the same. Standard chow is composed of 46.1% calculated complex carbohydrate, 24% crude protein, 4% crude fat, and 5% crude fiber (Purina Mills, Richmond, IN).

**Thiry-Vella Loop Surgery**

Rat pups were anesthetized by injection of ketamine cocktail (20% ketamine and 12.5% xylazine in 0.9% NaCl, 0.2 ml/100 g ip), and a midline abdominal incision was made under sterile conditions. A 12-cm segment of jejunum about 8 cm distal to the ligament of Treitz was excised and brought to the surface as a double jejunostomy, with its vascular stalk left intact (2). The remaining bowel was anastomosed end-to-end. The pups received 2-ml subcutaneous injections of lactated saline immediately after surgery and were allowed to recover overnight without food. They were then fed either HF or NF diet for 5 days. Sham-operated animals were treated similar to those with Thiry-Vella loop constructs, except that instead of constructing a bypassed loop, the excised segment was rejoined to the remaining bowel.

**Uptake Measurements**

Glucose and fructose uptake rates into the small intestinal mucosa were determined by using the everted sleeve technique described in detail elsewhere (18, 25). Rats were anesthetized with pentobarbital sodium (0.1 ml/30 g body wt ip). The small intestine was isolated and flushed with ice-cold Ringer solution (composition in mM, 128 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, and 20 NaHCO3; pH 7.3–7.4, osmolarity 290 mosmol/kg) gently. The total weight and total length (from pyloric sphincter to ileocecal junction) of the small intestine were recorded.

The small intestine of pups in the time course study was then divided into three regions: proximal (20% of intestinal length distal to the pylorus), distal (20% of intestinal length proximal to the cecum), and middle (the remaining 60% of small intestine). The middle small intestine was everted on a glass rod, and two 1-cm sleeves were obtained from the proximal end. One gram of the proximal middle intestine was saved (at 80°C) for Northern blot analysis. In Thiry-Vella pups, both bypassed and anastomosed regions were everted. The bypassed loop was used in its entirety: 1 cm each for glucose and fructose uptakes and the remaining 10 cm were stored (at 80°C) for Northern blot analysis. From the remaining bowel, two 1-cm sleeves were taken proximal to the anastomosis for uptake experiments. For later Northern blot analysis, an additional 5 cm were taken proximal and another 5 cm distal to the anastomosis. In sham-operated pups, the reanastomosed region (equivalent to the bypassed loop) was also taken out in its entirety and processed in exactly the same manner as the bypassed loop. The remaining intestine (equivalent to the remnant intestine of Thiry-Vella pups) was also processed as above, so that the sleeves used for uptake experiments and the tissues saved for Northern blots were taken from similar regions as the in Thiry-Vella pups.

Each sleeve was mounted on a grooved steel rod (3-mm diam) and preincubated at 37°C for 5 min in mammalian Ringer solution bubbled with 95% O2-5% CO2. The sleeve was then incubated at 37°C in an oxygenated, stirred (1,200 rpm) solution containing either D-[14C]glucose for 1 min or D-[3H]fructose for 2 min. To reduce the radioactive label in the adherent fluid, there was a 20-s rinse in 30 ml ice-cold Ringer solution immediately after the incubation. L-[3H]glucose was...
used to correct for adherent fluid and passive diffusion of glucose or fructose. All radioisotopes were purchased from Du Pont-NEN (Boston, MA). The uptake rates of both D-glucose and D-fructose were determined at 50 mM because this is the concentration that yields the maximal velocity that is not affected by errors caused by unstirred layers (18).

Northern Blot Analysis

Briefly, total RNA was isolated from rat small intestine by the phenol-chloroform-guanidium method (7). Poly(A)+ RNA was then extracted from total RNA by using oligo(dT) cellulose column. About 10–15 µg mRNA from each sample were subjected to 1% agarose-6% formaldehyde electrophoresis and then transferred to a nitrocellulose membrane by capillary action. cDNA probes of rat GLUT-2, GLUT-5, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; control for loading and transfer) were labeled with [32P]dCTP by using the random primer labeling kit (GIBCO-BRL). The hybridized membrane was washed, air dried, exposed to X-ray film for 24–48 h, and checked for X-ray film saturation. Quantification was performed by densitometry system (IS-1000 Digital Imaging System, Alpha Innotech). Results were first normalized to corresponding levels of loading control GAPDH, and then the normalized values were expressed as a percentage of the value of the mRNA level in the control group.

Statistical Analysis

Results are presented as means ± SE. A two- or three-way analysis of variance (ANOVA) was used to determine the significance (P < 0.05) of differences. If we found a significant difference with the higher level ANOVA, we reanalyzed the results with a one-way ANOVA followed by a least-significant difference test to identify heterogeneous means. Statistical analysis was conducted using the STATGRAPHICS Program (Statistical Graphics, Princeton, NJ).

RESULTS

Effects of Luminal Fructose

Body weight and food intake. Body weight (Fig. 1A) was independent of diet (P > 0.06 by three-way ANOVA) and type of surgery (Thiry-Vella or sham, P = 0.63) but was highly dependent on time after surgery (P < 0.0001). Pups in all groups slightly lost body weight the day after surgery. Pups not only returned to presurgical body weights within 2 to 3 days after surgery but also had significant increases in body weight thereafter, explaining the highly significant P value.

Food intake (Fig. 1B) was affected by time after surgery (P < 0.0001 by three-way ANOVA) and diet (P < 0.001) but not by type of surgery (P = 0.11). As would be expected, food intake in all groups was very low the day after surgery. It then increased rapidly and significantly with time (P < 0.0002 by one-way ANOVA for each diet) as pups recovered from surgery and grew. Food intake was modestly affected by diet on days 3 and 5 (P < 0.05 by one-way ANOVA) of the experiment.

GLUT-5 gene expression and fructose uptake. Increased GLUT-5 expression can be observed in the anastomosed segment of Thiry-Vella pups fed HF diet as well as in both “anastomosed” and “bypassed” segments of sham-operated pups fed HF diet (Fig. 2). In contrast, lower levels of GLUT-5 mRNA were expressed in the bypassed loop of Thiry-Vella pups fed HF diet, in both bypassed loop and anastomosed intestines of Thiry-Vella pups fed NF diet, and in equivalent regions of sham-operated pups fed NF diet.

Fig. 1. A: body weight of pups that were sham operated (SH) or underwent Thiry-Vella surgery (TV) and then fed high fructose (HF) or fructose-free (NF) diet. Bars, means ± SE of body weight (n = 5–8). Day 0, day of surgery (rats were weighed before surgery); days 1–5, 1 to 5 days after surgery. B: feeding rate of pups.

Fig. 2. Expression of GLUT-5 mRNA in bypassed (by) or anastomosed (an) small intestine of pups subjected to Thiry-Vella (TV) surgery in and same intestinal regions of littermates subjected to sham (SH) surgery. Pups were fed either HF or NF diet for 5 days after surgery and before being killed. Top: poly(A)+ RNA isolated from either anastomosed and bypassed jejunum were hybridized with [32P]-labeled rat GLUT-5 cDNA probe and exposed to X-ray film overnight. Arrow, 2.8 kb. Bottom: hybridization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as loading control confirmed equivalence in loading and transfer.
To demonstrate that luminal fructose is needed to enhance GLUT-5 mRNA levels or fructose uptake rates, we compared bypassed and anastomosed intestinal regions of pups fed HF diet (Fig. 3, A and B). A one-way ANOVA showed higher levels of GLUT-5 mRNA (P = 0.03) and rates of fructose uptake (P = 0.0005) in the anastomosed region compared with those in the bypassed region of Thiry-Vella pups fed HF diet.

To show that in the absence of luminal fructose in the anastomosed segment GLUT-5 mRNA levels and fructose uptake rates would be similar between anastomosed and bypassed segments, we compared levels and rates in bypassed and anastomosed intestinal regions of pups fed NF diet (Fig. 3, A and B). There were no differences in GLUT-5 mRNA levels (P = 0.30) and fructose uptake rates (P = 0.52) between these regions.

To demonstrate that the absence of an enhancement by luminal fructose in the bypassed loop was due neither to surgery nor to an endogenous characteristic of the region that was removed and to demonstrate high uptake rates and mRNA levels, we compared the reanastomosed area, which would have been the bypassed intestinal region, with the rest of the small intestine of sham-operated pups fed HF diet (Fig. 3, A and B). GLUT-5 mRNA levels (P = 0.54) and fructose uptake rates (P = 0.43) were similarly high in both intestinal regions.

Interestingly, a one-way ANOVA of all treatments was highly significant (P < 0.001 for both uptake rates and mRNA levels). When followed by a post hoc test, fructose uptake rates and GLUT-5 mRNA levels in the bypassed loop of Thiry-Vella pups fed HF diet were equal to those exhibited by all intestinal regions of pups fed NF diet, whereas those in the anastomosed loop of Thiry-Vella pups fed HF diet were equal to those exhibited by both regions of sham-operated pups fed HF diet. In all cases, changes in fructose uptake rates per centimeter were similar to those of uptake rates expressed per milligram and discussed in detail previously.

Glucose uptake. Glucose uptake per milligram in the bypassed loop was similar to that in the anastomosed loop in Thiry-Vella pups fed HF diet (P = 0.73 by one-way ANOVA) and in those fed NF diet (P = 0.86). Glucose uptake per milligram in the reanastomosed intestinal region was also similar to that in the remainder of the small intestine in sham-operated pups fed HF diet (P = 0.84) and in those fed NF diet (P = 0.34). In fact, a one-way ANOVA of all treatments show that means are statistically similar (P = 0.28). In all cases, changes in glucose uptake rates per centimeter were similar to those of uptake rates expressed per milligram (Fig. 3C).

SGLT-1 and GLUT-2 gene expression. Like glucose uptake rates, two Northern blots showing mRNA levels of GLUT-2 and SGLT-1 were each similar between anastomosed and bypassed regions of Thiry-Vella pups fed HF diet (results not shown).

Fig. 3. Effect of luminal fructose on GLUT-5 mRNA expression (A), fructose uptake (B), and glucose uptake (C) by jejunum of Thiry-Vella (TV) or sham-operated (SH) rat pups fed HF or NF diet. A: means ± SE of abundance of GLUT-5 mRNA relative to that of sham-operated, NF-fed rats (n = 5–8). Within same treatment, bars with different letters are significantly different. Densities of mRNA blots were first normalized to corresponding levels of GAPDH mRNA and normalized values were then expressed as percentage of those in sham-operated, NF-fed rats (arbitrarily designated as 100%). B: means ± SE of effect of luminal fructose on fructose uptake rate in jejunum of Thiry-Vella or sham-operated rat pups (n = 5–8). C: means ± SE of effect of luminal fructose on glucose uptake rate in jejunum of Thiry-Vella or sham-operated rat pups (n = 5–8).
Circadian Rhythm

Levels of GLUT-5 mRNA, measured at 0900, 1200, 1500, 2100, and 0300, were independent of time of day (P > 0.29, results not shown). Fructose uptake rates, measured at similar intervals, were also independent of time of day (results not shown).

Intestinal Motility

From 1 to 3 h after refeeding, chyme remained mainly in the stomach lumen (results not shown). Four hours after refeeding, chyme containing particulates was clearly found in the proximal 20% of the small intestine. Thereafter, chyme moved rapidly down the intestinal lumen, and by 6 h almost the entire length of the small intestine had luminal particulate contents.

Time Course of Induction

Clinical parameters. Body weight, intestinal weight, and intestinal length were each independent of diet (P > 0.40, results not shown) and duration of refeeding (P > 0.10). As would be expected, food intake was initially very rapid and increased with duration of refeeding. There was a modest diet difference in initial food consumption (P < 0.05) at 4 and 8 h; there were no diet-related differences by 12 and 24 h (this study) and after several days (25).

GLUT-5 gene expression as function of time after refeeding. By two-way ANOVA, there was a significant effect of duration of refeeding (P < 0.05) and diet (P < 0.0001) on levels of GLUT-5 mRNA (Fig. 4A). The significance of the effect of duration could have been much greater except that GLUT-5 mRNA levels in NF-fed pups were independent of time after refeeding (P = 0.10 by one-way ANOVA). These NF levels were also similar (P = 0.15) to those in pups killed before refeeding and in pups remaining with the dams and having access to mother’s milk and chow. In contrast, GLUT-5 mRNA levels in HF-fed pups were highly dependent on time after refeeding (P = 0.0037 by one-way ANOVA). Least-significant difference tests showed that the most significant increase in mRNA levels occurred just 4 h after refeeding on a HF diet. Levels of mRNA then declined subsequently but were still significantly greater (P ranging from <0.007 to <0.0001) than those in pups fed NF diet at the same time interval. After 24 h of HF feeding, however, levels of GLUT-5 mRNA declined to levels consistently exhibited by control and NF-fed pups and by pups with access to mother’s milk and chow (P = 0.74).

Fructose uptake. By two-way ANOVA, fructose uptake per milligram was highly dependent on diet (P < 0.0001) but was independent of time after refeeding (P = 0.17). As in GLUT-5 mRNA levels, subsequent one-way ANOVA revealed that fructose uptake per milligram was only affected by time after refeeding if pups were fed with HF (P = 0.003) and not with NF (P = 0.71) diet. The pattern of the diet-induced increase (Fig. 4B) in rates of fructose uptake, however, differed from that in levels of GLUT-5 mRNA. Four hours after feeding on a HF diet, rates of fructose uptake increased modestly but kept on increasing gradually with time until these reached an asymptote 12 h after refeeding. On the other hand, in pups fed NF diets, rates of fructose uptake per milligram remained not only independent of time after refeeding (overall mean pooled from 4- to 24-h time points: 0.20 ± 0.06 nmol·min⁻¹·mg⁻¹) but also similar (P > 0.40) to those of...
littermates killed immediately before refeeding (0.37 ± 0.22) and only slightly less (P = 0.03) than those of littermates that stayed with the dams (0.50 ± 0.11). The overall mean pooled from 4- to 24-h time points for HF-fed pups was 1.60 ± 0.12 nmol·min⁻¹·mg⁻¹, which is much greater (P < 0.001) than each of the above pooled means.

Changes in rates of fructose uptake per centimeter (results not shown) parallel those of fructose uptake per milligram, suggesting that diet-induced increases in fructose uptake are not due to changes in mucosal mass per centimeter.

Glucose uptake. Uptake per milligram was independent of diet (P = 0.11 by two-way ANOVA) and time after refeeding (P = 0.21). Subsequent one-way ANOVA revealed that glucose uptake per milligram was affected by time after refeeding if pups were fed with either HF (P = 0.0014) or with NF (P = 0.05) diet. The pattern of the diet-induced increase (Fig. 4C) in rates of glucose uptake, however, differed from those of fructose uptake. Rates of glucose uptake increased 4-8 h after feeding on either diet; uptake rates remained generally the same 12 and 24 h later for the HF diet but decreased for the NF diet. Pups fed HF diet (4.35 ± 0.16 nmol·min⁻¹·mg⁻¹, overall mean pooled from 4-24 h time points) had uptake rates similar (P > 0.10) to those of littermates that stayed with the dams (3.90 ± 0.21) but had uptake rates greater (P < 0.001) than those of littermates killed immediately before refeeding (2.61 ± 0.28).

Changes in rates of glucose uptake per centimeter (results not shown) parallel those of glucose uptake per milligram, suggesting that diet-induced changes in glucose uptake are not due to changes in mucosal mass per centimeter.

**DISCUSSION**

Luminal Fructose is Required for Enhancement

We have clearly demonstrated that luminal fructose is necessary for enhancement of transport activity because fructose transport rate and GLUT-5 gene expression are significantly increased only in continuous segments of the small intestine of Thiry-Vella pups fed HF diet, because fructose transport rates and GLUT-5 mRNA levels are low in both bypassed and continuous segments of Thiry-Vella pups fed NF diet, and because these low rates and levels are equivalent to those in the bypassed loop of their HF-fed littermates. Luminal fructose is also required for GLUT-5 expression in adult rats (2, 17, 21). Luminal glucose is required for SGLT-1 protein, but not mRNA, expression in sheep and for maintenance of SGLT-1 protein in postweaning lambs that otherwise would no longer have intestinal luminal glucose because of ruminant digestion (24). The results also suggest that the effect of dietary fructose on its transporter does not employ a secondary signal released to the circulatory system because no increase in fructose uptake rate nor GLUT-5 mRNA level is observed in the bypassed segment. Our results, however, cannot distinguish between direct enhancement of fructose transport by luminal fructose or indirect enhancement by paracrine factors released by luminal fructose.

The negative response in the bypassed loop of Thiry-Vella pups fed HF diet is certainly not due to the mucosal loss observed in that segment, because glucose uptake and SGLT-1 gene expression are similar between bypassed and anastomosed segments. The similarity of SGLT-1 and GLUT-2 gene expression between bypassed and anastomosed segments also suggests that the effect of dietary fructose is specific for GLUT-5. We can also discount potential inhibitory effects of surgery on GLUT-5 expression in the bypassed loop, because its equivalent segment in sham-operated pups fed an HF diet had high rates of fructose uptake and high levels of GLUT-5 mRNA. Finally, the tight correlation between the mRNA response and the functional response suggest regulation by changes in transcription rates or mRNA stability.

Is the enhancement due to dietary fructose alone, to high levels of dietary carbohydrate, or to low levels of dietary protein? We have previously shown that rates of intestinal fructose uptake and levels of GLUT-5 mRNA each increased markedly in response to a 65% fructose diet, modestly to a 65% sucrose diet, and not to a 65% glucose diet (9, 25). Moreover, a stepwise substitution of dietary glucose by fructose (all other dietary components remaining the same) would result in parallel, stepwise increases in fructose uptake rates and GLUT-5 mRNA levels. Finally, fructose uptake in 0% carbohydrate, 70% protein diets was similar to that in 65% glucose, 20% protein diets. These results suggest that fructose uptake and GLUT-5 mRNA specifically respond to altered dietary fructose levels and not to carbohydrate or protein levels.

Response to Luminal Signal is Immediate

Because ~4 h is required for a fructose diet to reach the jejunal lumen and because levels of GLUT-5 mRNA increase 4 h after refeeding, luminal fructose must stimulate a dramatic increase in jejunal GLUT-5 mRNA immediately after reaching the lumen. The increase in rates of fructose uptake and levels of GLUT-5 mRNA is specific for dietary fructose and not due to starvation followed by refeeding on any diet, because there is no increase if starvation is followed by refeeding on a NF diet. It is also not due to stress of separation from the dams or to precocious weaning to a pelleted diet, because rates and levels are much greater in HF-fed pups than those in littermates staying with the dams and having access to chow and milk. The prompt, direct response of the small intestine to dietary fructose supports our suggestion of a local mechanism mediating the effect of luminal fructose on GLUT-5. Luminal glucose and fructose are also known to modulate the expression of GLUT-2 located in the basolateral membrane (5).

Starvation-refeeding enhancement of GLUT-5 mRNA level and of fructose uptake rates is sequential and not simultaneous. This study depicts a rapid, almost five times enhancement of GLUT-5 mRNA 4 h after refeeding, followed by a gradual decrease and a return to
before the onset of peak feeding (6 PM to 6 AM) in adult and GLUT-5 mRNA levels to increase two to eight times. Moreover, Corpe and Burant (8) found SGLT-1, GLUT-2, GLUT-5 mRNA are highest at 9 PM and lowest at 9 AM. The timing of fructose transporter protein and GLUT-5 mRNA are highest at 9 PM and lowest at 9 AM. This sequential pattern suggests that the increased level of GLUT-5 mRNA is probably followed by an increase in transporter number, although only actual measurements of GLUT-5 protein levels can establish the time course of protein expression. This sequential pattern contrasts sharply with present and previous results depicting tight linkage between GLUT-5 mRNA levels and fructose uptake rates (or proteins) during diet-induced changes in transport. This tight linkage has been demonstrated many times in experiments in which dietary regulation of fructose transport has been investigated (2, 4, 25), and we know that 72–96 h after switching from a mother’s milk and chow diet to HF, both fructose uptake rates and GLUT-5 mRNA levels increase two to four times, although we do not know what happens between 0 and 72 h after the switch. It is only in this study, however, that an early time course of changes in uptake rates and mRNA levels was studied and that rat pups have been starved for 12 h before refeeding a HF diet. Thus the sequential pattern we observed here may represent an early response to the HF diet so that GLUT-5 mRNA synthesis would increase again after 24 h. The alternative explanation for rapid increases and subsequent decreases in levels of GLUT-5 mRNA is a substrate-induced or starvation-induced change in its degradation rate (mRNA stability). The importance of this latter mechanism has been demonstrated for regulation of GLUT-1 and GLUT-4 expression (19).

Brush-border glucose uptake increased with time in pups fed either HF or NF diets. In fact, glucose uptake rates at each time interval were similar between HF and NF diets except at 12 h when uptake in NF pups decreased suddenly. Diet does not seem to regulate glucose uptake during early development because uptake remains the same when pups are switched from a mother’s milk and chow diet to a variety of glucose-containing diets (9, 25). It is possible that the diet-independent increase in glucose uptake in these pups is a response to refeeding after a brief period of starvation, especially since starvation can alter enterocyte metabolism.

Circadian Rhythm is Not Yet Established in Weaning Pups

Circadian rhythm has been reported and is seemingly well established for various small intestinal functions in the adult rat. Castello et al. (4) found that the levels of fructose transporter protein and GLUT-5 mRNA are highest at 9 PM and lowest at 9 AM. Moreover, Corpe and Burant (8) found SGLT-1, GLUT-2, and GLUT-5 mRNA levels to increase two to eight times before the onset of peak feeding (6 PM to 6 AM) in adult rats, but found GLUT-5 protein levels to remain low throughout the 24-h study. Activities of several intestinal brush-border enzymes, mainly disaccharidases (26) and the intestinal L-histidine transporter (13) are also subject to circadian rhythm in adult rats. This discrepancy between adult and weaning rats may be explained by the undeveloped physiological circadian rhythm at the age of weaning. Unlike adult rats that consume 90% of their food during the night (12), rat pups feed virtually all the time. Other studies also found that food intake in neonatal rats is not subject to circadian rhythm and that the typical rhythm of food intake of adults begins only when postweaning pups reach 29 days old (16).

Future Studies and Unsolved Problems

We have previously found no effect of diet on intestinal glucose uptake by neonatal rats (9, 25); our results in the time course study may have been confounded by the brief starvation period before refeeding. We have also found a very strong response to dietary fructose in the middle 60% of the small intestine just 4 h after refeeding. Our motility study showed that particulate matter from the food was clearly visible only in the anterior 20% and not in the middle 60% and distal 20% of small intestine 4 h after refeeding. This suggests the possibility that fructose from the diet has been solubilized and that dissolved fructose in the luminal fluid preceded the appearance of particulate matter in the lumen.

Although we have clearly shown that dietary fructose precociously enhances both fructose uptake and steady-state GLUT-5 mRNA levels in weaning rats, there are other steps in the metabolic pathway that represent targets for regulatory processes [e.g., transcription rate, mRNA or protein degradation rates, translation rates (19)]. Some of these steps are presently being investigated in our laboratory. The developmental aspects of the regulatory process are still unclear and should be investigated further. For example, unlike that in weaning pups, luminal fructose cannot enhance intestinal fructose transport and GLUT-5 mRNA levels in suckling pups (10). Finding the mechanism underlying this fundamental difference will advance our understanding of the highly interesting events that occur during development of intestinal nutrient transporters.

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