Dietary carbohydrates enhance lactase/phlorizin hydrolase gene expression at a transcription level in rat jejunum

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We have previously shown that dietary sucrose stimulates the lactase/phlorizin hydrolase (LPH) mRNA accumulation along with a rise in lactase activity in rat jejunum [Goda, Yasutake, Suzuki, Takase and Koldovsky (1995) *Am. J. Physiol.* **268**, G1066–G1073]. To elucidate the mechanisms whereby dietary carbohydrates enhance the LPH mRNA expression, 7-week-old rats that had been fed a low-carbohydrate diet (5.5 % of energy as starch) were given diets containing various monosaccharides or sucrose for 12 h. Among carbohydrates examined, fructose, sucrose, galactose and glycerol elicited an increase in LPH mRNA accumulation along with a rise in lactase activity in the jejunum. By contrast, glucose and α -methylglucoside were unable to elicit a significant increase in LPH mRNA levels. To explore

a transcriptional mechanism for the carbohydrate-induced increases in LPH mRNA levels, we employed two techniques currently available to estimate transcriptional rate, i.e. RNA protection assays of pre-mRNA using an intron probe, and nuclear run-on assays. Both assays revealed that fructose elicited an increase in transcription of the LPH gene, and that the transcription of LPH was influenced only slightly, if at all, by glucose intake. These results suggest that certain monosaccharides such as fructose or their metabolite(s) are capable of enhancing LPH mRNA levels in the small intestine, and that transcriptional control might play a major role in the carbohydrate-induced increase of LPH mRNA expression.

INTRODUCTION

Lactase/phlorizin hydrolase (LPH) is expressed almost exclusively in the small intestine of mammals. Lactase activity is high during the suckling period, and it declines to a low level after weaning [1]. LPH expression during postnatal development seems to be regulated at the level of transcription [2,3]. In adult rats, lactase activity in the small intestine has been shown to be influenced by carbohydrate intake [4–7]. Recently, it has been demonstrated in our laboratory that the diet-induced increase in lactase activity in rat jejunum was accompanied by a concomitant increase in LPH protein synthesis and LPH mRNA levels [8]. A short-term force-feeding experiment revealed that sucrose elicited an increase in LPH mRNA level in the jejunum within 12 h, but α -methylglucoside, a non-metabolizable monosaccharide, was unable to enhance the LPH mRNA levels [8]. This result suggested that particular carbohydrates might give rise to an increase in LPH gene expression. However, it was unclear whether the carbohydrate-induced increase in LPH mRNA involved a transcriptional regulation, or if it was associated with an alteration of a post-transcriptional event. In the present study, we have sought possible alterations of the transcription of the LPH gene in the jejunum of rats fed various carbohydrates, by ribonuclease protection assays of preprocessed LPH mRNA (LPH pre-mRNA) using an intron probe, and by nuclear run-on assays of LPH gene transcription. The results of this study show that LPH expression is enhanced by particular metabolizable carbohydrates, including fructose, through the stimulation of the transcription rate of the LPH gene.

MATERIALS AND METHODS

Animals and diets

Six-week-old male rats (Sprague–Dawley strain; Japan SLC, Hamamatsu, Japan) were housed in wire-bottomed cages in a room maintained with a 12 h light period (06:00 to 18:00 h). At

7 weeks of age the animals were fed a low-carbohydrate (5.5 % of energy as cornstarch), high-fat (73 % of energy as corn oil) diet [8] for 7 days. They were subsequently force-fed the low-carbohydrate, high-fat diet or the diets containing glucose, fructose, sucrose, galactose or α -methylglucoside (40 % of energy or equivalent weight as carbohydrate, 37 % of energy as corn oil, 22 % of energy as casein) three times, over a 12 h period, as described previously [9,10]. The rats were killed by decapitation between 10:00 and 11:00 h. To examine whether a decrease in the fat content in the carbohydrate diets affected lactase activity and LPH mRNA levels, another group of animals were force-fed a diet identical with the low-carbohydrate diet except for the amount of fat (reduced to the level in the high-carbohydrate diets; low-fat diet). The experimental procedures used in this study met the guidelines of the animal use committee of the University of Shizuoka.

Preparation of intestinal samples

The entire small intestine was removed, and the duodenum extending from the pylorus to the ligament of Treitz was discarded. The jejunoileum was divided into three segments of equal length. The proximal two-thirds (jejunum) of the jejunoileum was flushed with ice-cold 0.9 % NaCl solution. A 1.0 cm segment (100 mg) was excised from the middle region of the jejunal segment and immediately used for RNA extraction. An adjacent 1.0 cm segment was excised, and quickly frozen in liquid nitrogen and stored at -20°C for the preparation of brush-border membranes. This segment was homogenized in 0.5 ml of ice-cold 2 mM Tris/HCl buffer (pH 7.1) containing 50 mM mannitol, 50 $\mu\text{g}/\text{ml}$ leupeptin and 3 $\mu\text{g}/\text{ml}$ aprotinin, and the brush-border membranes were prepared according to the method of Kessler et al. [11]. Aliquots of the homogenate and the brush-border membranes were stored at -20°C for the assay of lactase activity. The mucosa was scraped from the remaining part of the jejunal segment with a glass microscope slide. The scraped mucosa was immediately subjected to nuclei preparation.

Abbreviations used: LPH, lactase/phlorizin hydrolase; GLUT5, glucose transporter type 5; SGLT, sodium-dependent glucose transporter.

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Enzyme assays

Lactase activity was assayed as described by Dahlqvist [12] with 28 mM lactose as substrate. The lactase assay mixture contained *p*-hydroxymercuribenzoate to inhibit any residual lysosomal β -galactosidase [13]. Protein was measured by the method of Lowry et al. [14].

RNA extraction and Northern blot hybridization

Total RNA was extracted as described by Chomczynski and Sacchi [15]. For Northern blot analysis, aliquots (20 μ g) of total RNA were denatured with 2.2 M formaldehyde, fractionated by electrophoresis on 1% agarose gels and transferred to a nylon membrane (Hybond-N⁺, Amersham) as described previously [8]. Hybridization was performed using ³²P-labelled rat LPH cDNA as described previously [8].

The cDNA probe was labelled with [α -³²P]dCTP (3000 Ci/mmol; ICN Biomedicals, Costa Mesa, CA, U.S.A.) using a random primer DNA labelling system (Takara Shuzo Co., Kyoto, Japan). The radioactivity on the membrane was analysed with an image analyser (BAS 2000, Fuji Film, Tokyo, Japan). Control hybridizations were carried out using a rat β -actin cDNA.

PCR amplification and cloning of LPH intron cDNA

LPH intron cDNA was amplified from 600 ng of rat genome DNA prepared from the liver by PCR using *Taq/Pwo* DNA polymerase (Expand Hi-Fi, Boehringer Mannheim), and the gene-specific primers. The gene-specific primers for the PCR were designed from the rat LPH intron 1 cDNA sequence as follows [16]: PCR 5' oligo, 5'-GAATTCATATTCCTCTCCACCTA (*Eco*RI site underlined); PCR 3' oligo, 5'-GGATTCGATGTGCTGGCATAATC-3' (*Eco*RI site underlined). The reaction mixture was denatured at 96 °C for 2 min, annealed at 52 °C for 2 min and polymerized at 73 °C for 3 min. Thirty-five cycles were performed, followed by a 7 min extension at 73 °C. The PCR product was digested with *Eco*RI, subcloned into pBluescriptII SK⁺ (Stratagene) and sequenced by the dideoxynucleotide termination method [17].

RNase protection assays

RNase protection assays were carried out by modifications of the method described by Krasinski et al. [2]. The plasmid-containing LPH intron cDNA insert was linearized with *Xba*I. Antisense RNA probe was synthesized by the incubation of the linearized plasmid template (1 μ g) with transcription buffer containing 2.5 mM each of ATP, GTP and CTP, 40 μ mol of [α -³²P]UTP (600 Ci/mmol; ICN Biomedicals) and 10 units of DNA-dependent RNA polymerase in a final volume of 20 μ l for 1 h at 37 °C. To synthesize the full length transcript, 0.5 units of RNA polymerase was added to the mixture, and further incubation was carried out for 30 min. After treatment with 10 units of DNase I to digest the DNA template, the full length RNA probe was purified on a 5% polyacrylamide gel. The probe was extracted from the gel in a gel extraction buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1% SDS and 1 mM EDTA), and precipitated in ethanol. The purified probe was counted by a liquid-scintillation counter (LSC-3100; Aloka, Tokyo, Japan). Antisense RNA probe (1.0 $\times$ 10⁵ c.p.m.) was added to sample total RNA (30 μ g), denatured for 5 min at 85 °C and hybridized for 20 h at 68 °C. Unhybridized RNA was digested with RNase A (40 μ g/ml) and RNase T1 (2 μ g/ml), and the protected fragment was separated on 6% denaturing poly-

acrylamide gel. The gel was dried for 1 h at 80 °C under vacuum and exposed to an image plate (Fuji Film). The radioactivity of the protected fragment was analysed with an image analyser (BAS 2000, Fuji Film). Control RNase protection assays were performed using a 18 S rRNA antisense probe.

Isolation of intestinal nuclei

Nuclei were isolated from jejunal mucosa by modifications of the method described by Traber et al. [18] and Krasinski et al. [2]. The mucosa was homogenized in 5 volumes of homogenization buffer (50 mM Tris/HCl, pH 7.5, 25 mM potassium chloride, 5 mM magnesium chloride, 0.32 M sucrose, 5 mM dithiothreitol, 0.1 mM PMSF) using a Teflon homogenizer with five strokes of a tight pestle. After centrifugation at 9000 *g* for 5 min at 4 °C, the fat layer was removed and the pellets were resuspended in 10 volumes of sucrose buffer (50 mM Tris/HCl, pH 7.5, 25 mM potassium chloride, 1 mM calcium chloride, 2.4 M sucrose) containing 0.2% (v/v) Nonidet P-40, and homogenized with ten strokes of a loose pestle. An aliquot of the homogenate was inspected for complete cell lysis under a light microscope. The lysed cells were filtered through a layer of nylon mesh, layered on the sucrose buffer and ultracentrifuged at 75000 *g* for 30 min at 4 °C in a SW28SA rotor (Hitachi, Tokyo, Japan). The nuclei pellets were resuspended in 500 μ l of nuclei storage buffer (40% glycerol, 50 mM Tris/HCl, pH 8.0, 0.1 mM EDTA, 5 mM magnesium chloride, 0.1 mM PMSF) and filtered through nylon mesh to remove clusters of nuclei before freezing in liquid nitrogen.

Nuclear run-on assays

Nuclear run-on assays were carried out by modifications of the method described by Traber et al. [18]. The numbers of nuclei were adjusted to 5 $\times$ 10⁷ for each assay. Transcription reactions were initiated by addition of 50 μ l of 6 $\times$ transcription buffer (30 mM Tris/HCl, pH 8.0, 15 mM magnesium chloride, 900 mM potassium chloride and 1.5 mM each of ATP, GTP and CTP, and 200 units of ribonuclease inhibitor), and 10 μ l of [α -³²P]UTP (3000 Ci/mmol, 100 Ci) to 230 μ l of nuclei suspension. Reaction mixtures were incubated at 30 °C for 30 min, and the labelled RNA transcripts were isolated. Purified cDNA inserts of interest were applied on a nitrocellulose membrane using a dot-blot manifold (Immunodot, Atto, Tokyo, Japan) and denatured by soaking in 0.4 M NaOH for 20 min. The membranes were prehybridized for 2 h at 42 °C. The membranes were then immersed in the hybridization buffer containing the labelled RNA transcripts (1 $\times$ 10⁷ c.p.m./ml). Hybridization was continued for 48 h at 42 °C. The membranes were washed twice with 2 $\times$ SSPE (1 $\times$ SSPE is 0.18 M NaCl, 0.01 M sodium phosphate and 1 mM EDTA, pH 7.7)/0.1% SDS at 42 °C for 15 min, once with 2 $\times$ SSPE containing 10 μ g/ml RNase A at 37 °C for 30 min and twice with 1 $\times$ SSPE/0.1% SDS at 42 °C for 15 min. The radioactivity retained on the membranes was analysed with the image analyser (BAS 2000; Fuji Film). The LPH cDNA and β -actin cDNA probes used for run-on assays were the same as used for Northern blot hybridization. The rat glucose transporter type 5 (GLUT5) cDNA clone was a generous gift of Dr. K. Miyamoto (Tokushima University).

Statistical analysis

All results were subjected to one-way analysis of variance. Differences in mean values among groups were tested using Tukey's multiple range test [19], and were considered statistically different at *P* < 0.05.

RESULTS

Lactase activity

To examine whether certain types of carbohydrates in the diet are able to increase lactase activity in the small intestine, we force-fed the diets containing sucrose or various monosaccharides to the rats which had been fed a low-carbohydrate, high-fat diet. Since a previous study [8] suggested that the distribution of lactase activity in the brush-border membranes and the intracellular membranes was changeable by dietary manipulations, we determined lactase activity in both the mucosal homogenate and the brush-border membrane preparation. Lactase activity in the jejunal homogenate was significantly elevated ($P < 0.01$) in rats fed glucose (100%), fructose (167%), sucrose (133%), galactose (106%) and glycerol (83%) compared with controls (Table 1). The animals fed the fructose diet exhibited a greater lactase activity in the jejunal homogenate ($P < 0.05$) than those fed the glucose diet. In contrast, lactase activity was unaffected by feeding a low-carbohydrate, low-fat diet, nor by feeding the diet containing α -methylglucoside, a non-metabolizable sugar (Table 1). In the brush-border membranes of the jejunal segment, lactase activity was also significantly elevated in rats fed the diets containing glucose (111%), fructose (156%), sucrose (144%), galactose (89%) and glycerol (83%) compared with that of controls (Table 1). Lactase activity in the brush-border membranes of rats fed the low-carbohydrate, low-fat diet and those fed the α -methylglucoside diet did not differ significantly from that of control groups (Table 1). Thus, the diet-induced increases in lactase activity in the brush-border membranes closely reflected those in the mucosal homogenate, indicating that there were not marked alterations in the post-translational modifications of LPH among the dietary groups.

Northern blot hybridization

To determine whether the increase of lactase activity observed in animals given certain types of sugars was accompanied by a rise in LPH mRNA accumulation, we assessed LPH mRNA abundance in the jejunal segment. The levels of LPH mRNA were significantly elevated ($P < 0.01$) in rats given the diet containing fructose (140%), sucrose (99%), galactose (100%) or glycerol (131%) compared with controls (Figure 1). LPH mRNA accumulation tended to be increased in the rats fed glucose diet. By contrast, the LPH mRNA levels of rats fed the diet containing α -methylglucoside were similar to those of controls. The LPH

Figure 1 Effects of force-feeding various carbohydrates on LPH mRNA levels in the jejunum

Total RNA was extracted from the jejunal segment of individual rats fed a low-carbohydrate, high-fat control diet (Cont), low-carbohydrate, low-fat diet (LF) and high-carbohydrate diets containing glucose (Glu), fructose (Fru), sucrose (Suc), galactose (Gal), α -methylglucoside (α -MG) or glycerol (Gly). Representative Northern blots for LPH mRNA and β -actin mRNA are shown (upper panel). The results for each sample normalized for the β -actin mRNA abundance are expressed as arbitrary units, representing the mean value of the control group as 100% (lower panel). Values represent means $\pm$ S.E.M. for eight animals. Values not sharing a common letter, a–c, are significantly different at $P < 0.05$ by Tukey's multiple range test.

mRNA levels were unaffected by feeding the low-carbohydrate, low-fat diet (Figure 1). Thus, the increases in lactase activity induced by various carbohydrates occurred in parallel with the rises in LPH mRNA levels.

LPH pre-mRNA levels and transcriptional activity

To explore whether the sugar-induced increases in LPH mRNA levels involved transcriptional control of LPH gene, we first attempted to determine the relative abundance of LPH pre-mRNA by RNase protection assays. The antisense probe was obtained by amplification of the LPH intron 1 region (254 bp) followed by subcloning into plasmids and *in vitro* transcription. Treatment of the amplified LPH cDNA with *EcoRI* and *HindIII* produced a reduced size (242 bp) of DNA, which was subsequently cloned in the plasmid vector. Sequencing of the cloned cDNA revealed that it contained the 5' region of the expected LPH intron sequence. RNase protection assays were performed to detect the LPH pre-mRNA. The antisense probe gave a major protected fragment and minor fragments of a smaller size (Figure 2). The protected fragment of smaller size might have been generated by a transcription product which was not full length, or it might be due to degradation of the antisense probe. To determine which one of the bands corresponded to the LPH intron sequence of full length, the antisense probe was hybridized with the sense probe transcribed with T3 promoter, and the RNA–RNA hybrid was subjected to RNase treatment and PAGE. This analysis indicated that the bands located on the top in the RNase protection assays corresponded to the original antisense probe. Therefore, the bands located on the top were subjected to the quantification of LPH pre-mRNA, following the normalization for 18 S ribosomal RNA. LPH pre-mRNA abundance in rats fed fructose was 148% greater than that of controls

Table 1 Effects of feeding diets containing various monosaccharides or sucrose on lactase activity in homogenate and brush-border membranes in jejunum of rats

Results are means $\pm$ S.E.M. for four animals in each group. Values not sharing a common superscript letter, a–d, are significantly different at $P < 0.05$ by Tukey's multiple range test.

Sugar in diet	Lactase activity in jejunal homogenate (μ mol/h per mg of protein)	Lactase activity in brush-border membranes (μ mol/h per mg of protein)
Control (none)	0.18 $\pm$ 0.01 ^a	2.58 $\pm$ 0.17 ^a
Low-fat (none)	0.22 $\pm$ 0.02 ^a	3.58 $\pm$ 0.32 ^{a,b}
D-Glucose	0.36 $\pm$ 0.03 ^b	5.78 $\pm$ 0.43 ^c
D-Fructose	0.48 $\pm$ 0.02 ^c	9.48 $\pm$ 0.98 ^d
Sucrose	0.42 $\pm$ 0.03 ^{b,c}	7.56 $\pm$ 0.60 ^{c,d}
D-Galactose	0.37 $\pm$ 0.03 ^b	5.93 $\pm$ 1.06 ^{b,c}
α -Methylglucoside	0.17 $\pm$ 0.02 ^a	3.53 $\pm$ 0.60 ^{a,b}
Glycerol	0.33 $\pm$ 0.03 ^b	6.72 $\pm$ 0.75 ^c

Figure 2 Effects of force-feeding glucose (Glu) diet and fructose (Fru) diet on LPH pre-mRNA levels in the jejunum

(A) RNase protection assays of RNA isolated from the jejunal segment of individual rats were carried out using LPH intron 1 RNA probe labelled with [α - 32 P]UTP. The size of the protected fragment for LPH pre-mRNA is shown on the left. (B) Quantitative determination of LPH pre-mRNA in rat jejunum. The results for each sample normalized for the 18 S rRNA abundance are expressed as arbitrary units, with the mean value of the control group as 100%. Values represent means $\pm$ S.E.M. for three animals. Values not sharing a common letter, a or b, are significantly different at $P < 0.05$ by Tukey's multiple range test.

($P < 0.05$) (Figure 2). Feeding the glucose diet resulted in only a marginal and insignificant increase in LPH pre-mRNA abundance (Figure 2).

LPH gene transcription was measured directly in the jejunum of rats fed the low-carbohydrate diet (control), glucose diet and fructose diet, by nuclear run-on assays (Figure 3). In these assays, the RNAs undergoing transcription were labelled with [32 P]UTP by the action of endogenous RNA polymerase and hybridized to the cDNAs of interest that were fixed onto the membrane. Thus, the intensities of the signals on the dot blots are considered as the estimates of transcription rate of the genes. Whereas only small variations were observed in β -actin and 28 S rRNA transcription rate, the transcription rate of LPH gene was apparently elevated in rats given the fructose diet (Figure 3). When normalized with β -actin signals, the transcription rate of the LPH gene in rats fed the fructose diet was 168% greater than that of control. By contrast, feeding the glucose diet did not cause an appreciable increase in the transcription rate of LPH gene (Figure 3). When the transcription rate of LPH gene was normalized for 28S rRNA the results were practically the same (results not shown). The transcription rate of GLUT5, a fructose transporter in the

Figure 3 Effects of force-feeding glucose (Glu) diet and fructose (Fru) diet on transcription rates of LPH and GLUT5

(A) Nuclear run-on assays were performed on the nuclei isolated from the jejunum of individual rats. β -Actin gene transcription was also monitored for the normalization of LPH and GLUT5 transcription rates. (B) Quantitative determination of transcription rate of LPH gene in rat jejunum. (C) Quantitative determination of transcription rate of GLUT5 gene in rat jejunum. The results for each sample normalized for the β -actin are expressed as arbitrary units, with the mean value of the control group as 100%. Values represent means $\pm$ S.E.M. for three animals. Values not sharing a common letter, a or b, are significantly different at $P < 0.05$ by Tukey's multiple range test.

brush-border membranes of enterocytes [20], was remarkably elevated in rats fed the fructose diet (231%), but the influence of glucose diet on the transcription rate of GLUT5 was less evident (Figure 3).

DISCUSSION

The present study has shown that lactase activity and LPH mRNA level in the jejunum are co-ordinately increased by consumption of various carbohydrates, including sucrose, fructose, galactose and glycerol. Feeding the glucose diet caused a slight increase in lactase activity, accompanied by an elevation of LPH mRNA levels. By contrast, α -methylglucoside, a non-metabolizable sugar, was also unable to induce an elevation in

LPH mRNA levels. Thus the results of this study confirm and extend previous studies from this laboratory [8], which demonstrated that LPH mRNA levels are elevated in rats given sucrose as a dietary carbohydrate source, but not in those given α -methylglucoside. We found in this study that, of the two constituent monosaccharides of sucrose, fructose is more active in enhancing LPH mRNA levels than glucose. Therefore it is most likely that the sucrose-induced increases in the LPH mRNA levels are attributable to fructose that is produced by the hydrolysis of sucrose. These results prompted us to hypothesize that certain carbohydrates or their common metabolite(s) might play a pivotal role in the regulation of LPH gene expression.

To explore a transcriptional mechanism for the carbohydrate-induced increases in LPH mRNA levels, we employed two techniques currently available to estimate transcriptional rates, i.e. RNase protection assays of pre-mRNA using an intron probe, and nuclear run-on assays. These two techniques have been successfully utilized to offer independent measures of the transcription rate of LPH [2]. Both RNase protection assays of pre-mRNA using an intron probe and nuclear run-on assays showed that fructose was able to elicit an increase in transcription of the LPH gene, and that the transcription of LPH was influenced only slightly, if at all, by glucose intake. Taken together, these data strongly suggest that gene transcription plays a central role in the carbohydrate-induced increase in LPH mRNA abundance.

As far as we know, this is the first report that shows evidence for a diet-mediated transcriptional regulation of LPH gene. A conspicuous finding of this study was that LPH gene expression is stimulated by the carbohydrates (e.g. fructose, sucrose and glycerol) which are irrelevant to its own substrate, i.e. lactose. Thus it is apparent that the carbohydrate-induced alteration of LPH gene expression is not a 'substrate-mediated induction'. The carbohydrate-induced change in LPH gene expression might be a phenomenon common to intestinal disaccharidases and hexose transporters. Previous studies demonstrated that the mRNA levels of sucrase/isomaltase [21,22] and sodium-dependent glucose transporter type 1 (SGLT1) [22] were elevated in rats given a high-sucrose diet. Miyamoto et al. [23] showed that feeding a diet rich in fructose to rats caused a co-ordinate rise in the mRNA levels of intestinal hexose transporters, including SGLT1, GLUT2 and GLUT5. In this study, we found that fructose was able to enhance the transcription rate of both LPH and GLUT5. This result may suggest that fructose not only enhances the transcription of various genes related to intestinal carbohydrate digestion and absorption, but also induces its own transporter. This may result in a positive feedback of fructose transport and a pronounced effect of fructose. We speculate that consumption of certain carbohydrates causes an alteration of transcription of LPH, sucrase/isomaltase and hexose transporters in small intestine, presumably through a common regulatory mechanism.

Dietary carbohydrates, including fructose and glycerol, are known to stimulate the gene expression of L-pyruvate kinase, a key enzyme for the glycolytic pathway, at the transcriptional level in the liver [24]. In the 5' flanking region of the L-pyruvate kinase gene, three positive regulatory *cis*-elements were found to respond to administration of carbohydrates, especially fructose and their metabolites, leading to designation of the *cis*-elements as 'carbohydrate-response elements' [25]. Experiments using transgenic mice revealed that fructose stimulated L-pyruvate kinase expression in the small intestine, but glucose had little effect [26]. Because the hexokinase in the small intestine shows a much lower Michaelis constant than that of the liver [27], the rate of conversion of glucose into glucose 6-phosphate may be readily

saturated in the small intestine. Therefore, it is likely that most of the excessive amount of glucose in the small-intestinal cells is transferred to the blood vessels without efficient utilization in the small intestine. On the other hand, fructose and glycerol may be metabolized faster than glucose, because they are able to skip two rate-determining steps in the glycolytic pathway [28]. Alternatively, it is also possible that glucose represses the expression of LPH through some putative *cis*-element, in analogy with the regulatory elements responsible for the glucose-dependent repression reported in the 5' flanking region of the sucrase/isomaltase gene [29,30]. Hence, we speculate that an intermediate metabolite of the glycolytic pathway may be involved in the regulation of LPH gene transcription.

In conclusion, this study demonstrated that certain metabolizable carbohydrates were capable of enhancing LPH mRNA levels in the small intestine, and that transcriptional control plays a major role in the carbohydrate-induced alterations of LPH mRNA expression. Further studies are required to determine whether a 'carbohydrate response element' is present in the LPH gene, and what kinds of *trans*-acting factors are involved.

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