Differential responses of intestinal glucose transporter mRNA transcripts to levels of dietary sugars

Ken-ichi MIYAMOTO,*‡ Kyoko HASE,* Toshimitsu TAKAGI,† Takeru FUJII,† Yutaka TAKETANI,* Hisanori MINAMI,* Tatsuzo OKA* and Yukihiro NAKABOU*

*Department of Nutrition, School of Medicine, University of Tokushima, Kuramoto-Cho 3, Tokushima 770, and †Department of Cell Biology, Teikoku Seiyaku Co, Sambonmatsu, Kagawa 567, Japan

Dietary sugars are known to stimulate intestinal glucose transport activity, but the specific signals involved are unknown. The Na^+-dependent glucose co-transporter (SGLT1), the liver-type facilitative glucose transporter (GLUT2) and the intestinal-type facilitative glucose transporter (GLUT5) are all expressed in rat jejunum [Miyamoto, Hase, Taketani, Minami, Oka, Nakabou and Hagihira (1991) Biochem. Biophys. Res. Commun. **181**, 1110–1117]. In the present study we have investigated the effects of dietary sugars on these glucose transporter genes. A high-glucose diet stimulated glucose transport activity and increased the levels of SGLT1 and GLUT2 mRNAs in rat jejunum. 3-*O*-Methylglucose, D-galactose, D-fructose, D-mannose and D-xylose can mimic the regulatory effect of glucose on the SGLT1 mRNA level in rat jejunum. However, only D-galactose and D-fructose increased the levels of GLUT2 mRNA. The GLUT5 mRNA level was increased significantly only by D-fructose. Our results suggest that the increase in intestinal transport activity in rats caused by dietary glucose is due to an increase in the levels of SGLT1 and GLUT2 mRNAs, and that these increases in mRNA may be caused by an enhancement of the transcriptional rate. Furthermore, for expression of the SGLT1 gene, the signal need not be a metabolizable or transportable substrate whereas, for expression of the GLUT2 gene, metabolism of the substrate in the liver may be necessary for signalling. Only D-fructose is an effective signal for expression of the GLUT5 gene.

INTRODUCTION

There is much evidence that intestinal nutrient transporters are adaptively regulated by the dietary levels of their substrates [1–5]. For example, a high-carbohydrate diet stimulates glucose transport in the small intestine [4–12]. The effect of glucose is specific to the maximal velocity of the glucose transporter and does not influence the mucosal mass or the rate of passive glucose movement [4–6,10–12]. This dietary signal is perceived by the crypt cells, which irreversibly determine the glucose transporter activity of enterocytes [12]. However, the specific signals involved are still unknown.

The DNAs for the Na^+-dependent glucose co-transporter (SGLT1) and at least five facilitative glucose transporters (GLUT1–GLUT5) have been isolated [13–16]. We have reported that three glucose transporter genes (SGLT1, GLUT2 and GLUT5) are expressed in the rat jejunum [17,18]. SGLT1 is located in the brush-border membrane of intestinal epithelial cells [16]. GLUT2 is present in the liver, small intestine, kidney and islets of Langerhans [19]. It is also located in the basolateral membrane of intestinal epithelial cells, and is responsible for the release of glucose across the basolateral membrane [19]. GLUT5 is present at highest concentrations in human small intestine and kidney [20]. In rat jejunal mucosal cells, the GLUT5 mRNA level is highest on day 10 after birth and then decreases, reaching the adult level by day 20 [18], suggesting that GLUT5 is important for intestinal glucose transport in the neonatal period [18]. Davidson et al. [21] found that GLUT5 is located in the brush-border membranes of human small intestine. Furthermore, Burant et al. [22,23] reported that the kinetic properties and tissue distribution of GLUT5 indicate that it is a fructose transporter, but its role in intestinal hexose transport is unknown.

There are many reports on the regulation of intestinal glucose transporters by dietary sugars [4–12], but nothing is yet known about changes in the mRNA levels of SGLT1, GLUT2 and GLUT5 caused by dietary sugars. In the present study we have examined whether the increase in glucose transport activity in rats given a high-glucose diet is due to increases in the levels of glucose transporter mRNAs and if so, whether these increases are due to an increase in the transcriptional rate. We also investigated whether other hexoses can mimic the regulatory effect of glucose on the glucose transporter genes.

MATERIALS AND METHODS

Animals and diets

Male Sprague–Dawley rats (150–160 g; Japan SLC, Shizuoka, Japan) were kept in wire-bottomed cages in an animal room maintained at 22 °C with a 12 h light period (08:00 to 20:00 h). They were given a carbohydrate-free diet (24% protein, 55% fat, 9% cellulose, 4% mineral mixture and 1% vitamins) for 2 weeks and then test diets for 5 days [9]. The test diets consisted of 24% protein, 4% mineral mixture, 1% vitamin, 9% cellulose and 55% sugars (D-glucose, D-galactose, 3-*O*-methylglucose, D-fructose, D-mannose or D-xylose). On day 5, the animals (final body weight 250–260 g) were killed at 18:00 h, and the entire small intestine was quickly removed and washed with saline. The upper jejunum was used for RNA preparation and transport experiments.

Transport experiments

The entire small intestine was quickly removed and washed with saline. The first 5 cm length from the pylorus was cut off and

Abbreviation used: SGLT, Na^+-dependent glucose co-transporter.

‡ To whom correspondence should be addressed.

discarded. The next 10 cm length was used for RNA purification as described below, and the lower part of the jejunum was used for transport studies. The rate of intestinal absorption was determined as described previously [24]. Briefly, a sac of everted intestine was filled with a measured volume of Krebs–Ringer phosphate buffer, pH 7.4, containing 5 mM D-galactose, and placed in a 50 ml Erlenmeyer flask containing 5 ml of the same solution with D-[U-^{14}C]galactose (1 μCi; 50 mCi/mmol; Amersham Japan). The flask was gassed with 100% O_2 and incubated with shaking for 50 min at 37 °C. The rate of transport was expressed as the net increase in the substance on the serosal side, with units of μmol/h per 100 mg wet weight [24].

Northern blot analyses

Total RNA was isolated by the guanidium isothiocyanate/CsCl method [25]. Poly(A)$^+$ RNA was purified on an oligo(dT)–cellulose column. Poly(A)$^+$ RNA (4.5 μg) was denatured in a solution of 2.2 mM formaldehyde and 50% formamide by heating at 95 °C for 2 min, size-fractionated in a 1.2% formaldehyde agarose gel, and transferred to a nylon membrane (Gene Screen, NEM). The cDNA probes were labelled with ^{32}P with a Megaprime labelling system kit (Amersham) following the recommendations of the supplier. Hybridization to the labelled probes were performed overnight in a solution of 50% deionized formamide, 10% dextran, 10 × Denhardt's solution, 40 mM Tris/HCl (pH 7.5), 10 mg/ml salmon sperm DNA and 0.1% SDS at 42 °C. The membranes were washed twice for 10 min each time with 2 × SSC/0.1% SDS, and twice for 10 min each time with 0.1 × SSC/0.1% SDS at 55 °C (1 × SSC is 0.15 M NaCl/0.015 M sodium nitrate) [18]. Quantification was performed by scanning densitometry [17,18].

In vitro transcription in isolated nuclei

Isolation of nuclei from rat small intestine and subsequent *in vitro* transcription assays were performed by modifications of previously described methods [26,27]. Briefly, intestinal nuclei (30 absorbance units at 260 nm) were incubated for 30 min at 26 °C in 100 μl of reaction buffer containing 400 μCi of [^{32}P]UTP (3000 Ci/mmol; Amersham Japan), 1 mM each of ATP, CTP and GTP (Pharmacia), 30% (v/v) glycerol, 30 units of RNasin, 10 mM phosphocreatine, 1.2 mM dithiothreitol, 0.4 mM EDTA, 0.2 M NaCl, 4 mM $MnCl_2$, 4 mM $MgCl_2$, 0.3 mM $(NH_4)_2SO_4$ and 50 mM Hepes, pH 7.9. Yeast tRNA (1 mg/ml) and 50 units of DNAase I were added for 15 min at 37 °C. The reaction mixtures were deproteinized by treatment at 42 °C for 10 min with 100 μl of TES (20 mM Tris/HCl, pH 7.9, 20 mM EDTA, 1% SDS) and proteinase K (0.5 mg/ml). RNA was extracted with phenol/chloroform (1:1, v/v) and precipitated with ethanol. [^{32}P]RNA was hybridized to 3 μg of cDNA and pUC19 bound to a filter (prehybridized for 2 days at 42 °C) in 500 μl of TES-containing hybridization buffer (50% formamide, 5 × SSC, 0.5% SDS, 10 × Denhardt's solution, 1 mg/ml salmon testis DNA). Then the filters were washed with three changes of 2 × SSC/0.5% SDS at 35 °C for 10 min, 0.2 × SSC/0.5% SDS at 42 °C for 30 min, and 0.1 × SSC/0.5% SDS at 42 °C for 30 min. They were then treated with 10 μg/ml ribonuclease A in 2 × SSC at 37 °C for 20 min, and washed with 2 × SSC at 50 °C for 30 min. The filters were then air-dried and autoradiographed.

cDNA probes

The probes used were the 1396 bp *Eco*RI insert from phGT2-1 for GLUT1 cDNA [28]; the 1850 bp *Eco*RI insert from pLGT-1 for GLUT2 cDNA [29]; the 2190 bp *Eco*RI insert of phMGT-31 for GLUT3 cDNA [30]; the 1730 bp *Eco*RI insert from phJHT-3 for GLUT4 cDNA [31]; the 1890 bp *Eco*RI insert from hjHT-5 for GLUT5 cDNA [20]; and the 2142 bp (nt 201–2343) *Eco*RI insert of hSGLT1 for SGLT1 cDNA [32].

Statistical analysis

Analysis of variance was used to determine the significance of differences ($P < 0.05$) between a group given the high-carbohydrate diet and a group given the carbohydrate-free diet. The significance of differences between mean values of measurements was examined by one-way ANOVA followed by the Student–Newman–Keuls test.

RESULTS AND DISCUSSION

Time courses of changes in D-galactose transport activity and levels of glucose transporter mRNAs in rats given a high-glucose diet

Rats were given the carbohydrate-free diet for 2 weeks and then switched to the high-glucose diet. The D-galactose transport activity in the jejunum was significantly increased 3 days after the change to the high-glucose diet (Figure 1). This was due to an increase in the maximal velocity ($V_{max.}$) of D-galactose transport (3 days of high-glucose diet, 21.3 ± 4.1 nmol/min per mg; control, 7.8 ± 1.6 nmol/min per mg; $n = 6$). We measured transport *in vitro* at 37 °C as the transport of D-[^{14}C]galactose by everted sacs of small intestine [24]. In these preparations transporter activities are maintained at high levels, but transport represents that across two cell membranes: uptake from the intestinal lumen across the brush-border membrane into enterocytes, and exit from the enterocytes across the basolateral membrane into the bloodstream. The glucose transporters in the brush-border and basolateral membrane have quite different properties [33].

Next we investigated the levels of SGLT1 and GLUT2 mRNAs in these animals. As shown in Figures 1 and 2, at 3 days after the change to the high-glucose diet, the levels of SGLT1 and GLUT2 mRNAs had increased about 2.4- and 2.1-fold respectively. The time course of these changes in the mRNA levels for the two glucose transporters paralleled those of the changes in D-galactose transport activity (Fig 1). When the diet was changed back to a carbohydrate-free diet, the levels of SGLT1 and GLUT2 mRNAs decreased to the original levels within a similar time frame: 7 days, 220 ± 32% (SGLT1) and 168 ± 21% (GLUT2) of control values; 9 days, 110 ± 19% (SGLT1) and 104 ± 16% (GLUT2).

Figure 3 shows the rates of transcription of the SGLT1 and GLUT2 mRNAs in rats given the high-glucose diet. The transcriptional rate of SGLT1 began to increase 3 days after switching to the high-glucose diet, and reached a maximum 3.0-fold increase compared with the control level at 5 days after the change to the high-glucose diet. The transcriptional rate of GLUT2 was significantly increased 5 days after changing to the high-glucose diet. Thus the increases in the levels of SGLT1 and GLUT2 mRNAs in rats given a high-glucose diet seemed to be due mainly to increased rates of transcription of their mRNAs, although the stabilities of these mRNAs may also have changed. The transcriptional rate and mRNA level of GLUT5 did not change after switching to the high-glucose diet.

Effects of various dietary sugars on the level of SGLT1 mRNA

Rats were maintained for 2 weeks on the carbohydrate-free diet and then given diets containing 55% (w/w) concentrations of various dietary sugars for 5 days. Figure 4 and Table 1 show the

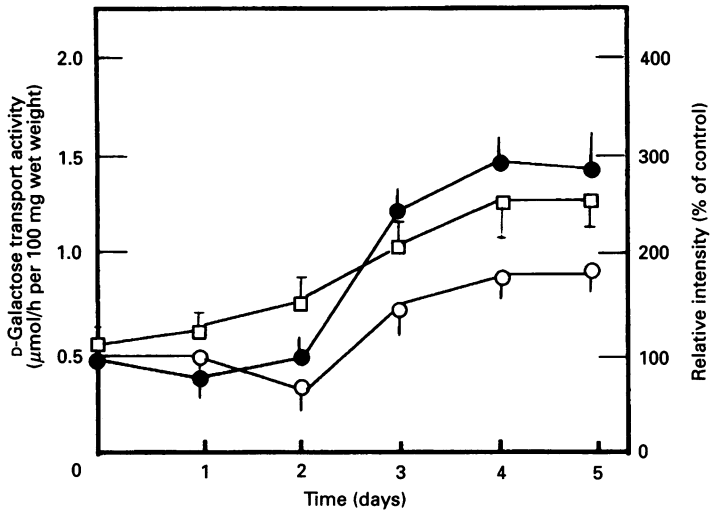

Figure 1 Time courses of the changes in D-galactose transport activity and glucose transporter mRNA levels in the jejunum of rats given a high-glucose diet

Intestinal D-galactose transport activity was determined as described in the Materials and methods section. The concentrations of SGLT1, GLUT2 and β-actin mRNAs were determined by densitometric scanning of autoradiograms, and are expressed as percentages of control values (obtained from rats on a carbohydrate-free diet). □, D-Galactose transport activity; ●, SGLT1 mRNA; ○, GLUT2 mRNA. Values are means ± S.E.M.; $n = 6$.

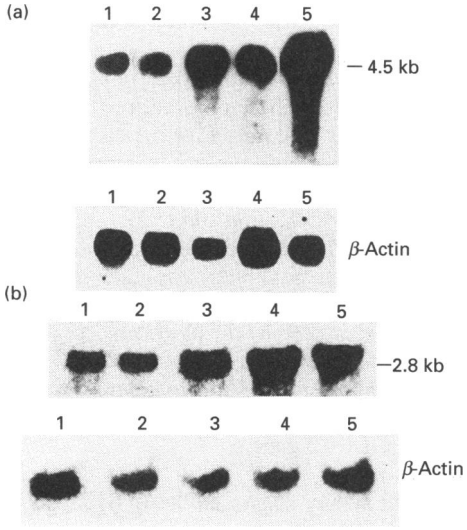

Figure 2 Northern blot analyses of SGLT1 and GLUT2 mRNAs in the jejunum of rats given a high-glucose diet

Animals were given a carbohydrate-free diet for 2 weeks and then were switched to a high-glucose diet. Each lane contains 4.5 μg of poly(A)$^+$ RNA from the jejunal mucosa. (**a**) SGLT1 and β-actin mRNAs; (**b**) GLUT2 and β-actin mRNAs. The periods of administration of the high-glucose diet were: lane 1, control (carbohydrate-free diet); lane 2, 2 days; lane 3, 3 days; lane 4, 4 days; lane 5, 5 days.

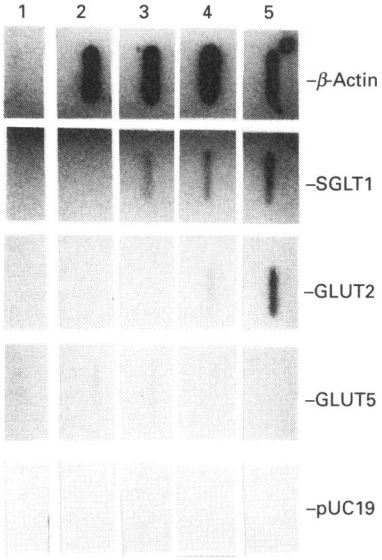

Figure 3 *In vitro* transcription in isolated nuclei of jejunal cells from rats fed on a high-glucose diet

Nuclei were isolated from the jejunal mucosa of rats at 0, 3, 4 and 5 days after the change to the high-glucose diet, and were assayed for transcription *in vivo* in the presence and absence of 1 μg/ml α-amanitin. ^{32}P-labelled transcripts were hybridized to control plasmid pUC19; to pGEM4Z with inserted human SGLT1 cCNA; to pGEM7Z with inserted rat GLUT2 cDNA; and to pUC19 with inserted β-actin cDNA. Lanes show levels in the presence of α-amanitin (lane 1), and in its absence, of β-actin, SGLT1, GLUT2, GLUT5 and pUC19 on day 0 (lane 2), day 3 (lane 3), day 4 (lane 4) and day 5 (lane 5) after the change to the high-glucose diet.

D-galactose transport activities and levels of SGLT1 and GLUT2 mRNAs in the jejunum of these rats 5 days after the change to these diets. The test groups showed no appreciable differences from the controls in body weight, blood glucose levels, intestinal weight or intestinal length. Food intakes were slightly, but not significantly, lower in the groups given D-galactose and D-xylose than in the other groups (control group, 20.7 ± 8.9 g/day per 100 g body weight; D-galactose group, 18.4 ± 8.1 g/day per 100 g body weight; D-xylose group, 17.5 ± 8.8 g/day per 100 g body weight).

D-Galactose transport activity in the jejunum was significantly increased in all groups of rats given test diets. These results were consistent with those of other workers [9,34]. Human SGLT1 cDNA hybridized to a 4.5 kb transcript in rat jejunal mucosa as described previously [17,32]. Administration of D-glucose or D-galactose increased the SGLT1 mRNA level to about 3 times

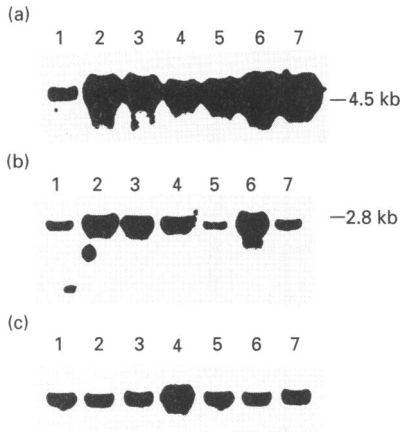

Figure 4 Effects of different dietary sugars on the levels of SGLT1 and GLUT2 mRNAs

Animals were given a carbohydrate-free diet for 2 weeks and then switched to a high-glucose diet. Each lane contains 4.5 μg of poly(A)$^+$ RNA from the jejunal mucosa of rats after 5 days on diets containing various sugars. (**a**) SGLT1 mRNA, (**b**) GLUT2 mRNA, (**c**) β-actin mRNA. Carbohydrates: lane 1, none (carbohydrate-free diet); lane 2, D-glucose; lane 3, D-galactose; lane 4, 3-*O*-methylglucose; lane 5, D-mannose; lane 6, D-fructose; lane 7, D-xylose.

Table 1 Effects of dietary sugars on the levels of intestinal D-galactose transport activity and transporter mRNAs

D-Galactose transport activities were determined in rats fed on diets containing different sugars for 5 days. The concentrations of SGLT1, GLUT2, GLUT5 and β-actin mRNAs were determined by densitometric scanning of autradiograms. Results are expressed as percentages of control values (obtained from rats on a carbohydrate-free diet). Values are means ± S.E.M. ($n = 6$); * indicates a significant difference ($P < 0.05$) compared with the control. Abbreviation: 3-*O*-MG, 3-*O*-methylglucose.

Sugar in diet	Transport activity (μmol/h per 100 mg wet weight)	Relative intensity (% of control)		
		SGLT1 mRNA	GLUT2 mRNA	GLUT5 mRNA
Control (none)	0.54 ± 0.12	100	100	100
D-Glucose	1.27 ± 0.41*	320 ± 53*	224 ± 59*	72 ± 46
D-Galactose	1.14 ± 0.33*	287 ± 57*	217 ± 62*	79 ± 37
3-*O*-MG	1.49 ± 0.28*	195 ± 47*	89 ± 22	106 ± 28
D-Fructose	2.06 ± 0.66*	362 ± 121*	219 ± 58*	246 ± 39*
D-Mannose	1.56 ± 0.52*	270 ± 36*	81 ± 29	114 ± 48
D-Xylose	1.37 ± 0.23*	265 ± 40*	105 ± 24	92 ± 16

that of control rats given the carbohydrate-free diet. 3-*O*-Methylglucose also increased the SGLT1 mRNA level (Figure 4). Surprisingly, D-fructose was the strongest inducer, with D-mannose and D-xylose also increasing the level of SGLT1 mRNA. D-Fructose uptake has a specific carrier system that is distinct from the Na^+-dependent glucose co-transport system(s) [33]. Therefore, in rat small intestine, non-metabolizable hexoses, such as 3-*O*-methylglucose, D-fructose, D-mannose and D-xylose, can mimic the regulatory effect of glucose on the SGLT1 mRNA level. Solberg and Diamond [9] suggested that in the transport of glucose across the brush-border membranes of mice fed on different dietary sugars, regulatory signals need not be transported substrates and need not be metabolizable. Therefore hexoses may stimulate expression of the SGLT1 gene directly.

Effects of different dietary sugars on the level of GLUT2 mRNA

The GLUT2 mRNA level was increased by D-glucose, D-galactose and D-fructose, but was not affected by 3-*O*-methylglucose, D-mannose or D-xylose (Figure 4 and Table 1). This glucose transporter is located in the basolateral membrane of intestinal epithelial cells, and is responsible for the release of glucose across the basolateral membrane [19,35]. Cheesman and Harley have recently reported that the number of D-glucose-inhibitable [^{3}H]cytochalasin B binding sites in the basolateral membrane of the rat jejunum was increased by D-glucose and D-fructose, but not by D-galactose or 3-*O*-methylglucose, which are not metabolized in small intestinal cells [36]. Therefore these workers suggested that intracellular metabolism is necessary for signalling changes in the transport of glucose across the basolateral membrane [36]. On the other hand, our results indicated that D-galactose significantly increased the GLUT2 mRNA level, whereas 3-*O*-methylglucose did not. D-Galactose is known to be metabolized in rat liver [37], but 3-*O*-methylglucose is not metabolized at all [34]. Furthermore, in humans and rats, D-fructose is metabolized primarily in the liver, although both the small intestinal mucosa and kidney also contain the enzymes necessary for its catabolism [38]. Therefore the metabolism of D-galactose and D-fructose in the liver might be necessary for induction of intestinal GLUT2 mRNA. This possibility is supported by the finding that the level of GLUT2 mRNA in rat liver was significantly increased in rats given the high-D-fructose or -D-galactose diets (K.-i. Miyamoto and co-workers, unpublished work). Recently, Asano et al. [39] showed that the GLUT2 mRNA level in primary cultured hepatocytes was increased by D-glucose, D-fructose and D-mannose, but not by non-metabolized hexose. These observations suggest that sugar metabolism in the liver is required for regulation of the GLUT2 mRNA level in both liver and intestine. However, D-galactose is metabolized slowly in the small intestine over long periods of dietary manipulation [37], and this slow metabolism could provide a signal which is localized to the small intestine. Experiments to test this possibility are under way.

Leturque et al. [40] reported that the level of GLUT2 mRNA in the liver may be regulated by the blood glucose level. However, in the present experiments, the levels of blood glucose in rats given different sugars were similar. We are now studying the role of metabolism of these hexoses in the rat liver in the expression of the intestinal GLUT2 gene.

Effects of different dietary sugars on the GLUT5 mRNA level

As shown in Figure 5 and Table 1, the GLUT5 mRNA level was not changed by any of the dietary sugars tested, except for D-fructose. Burant et al. [22,23] found that injection of synthetic human GLUT5 mRNA into *Xenopus* oocytes stimulated the uptake of D-fructose, but not of D-galactose, α-methyl-glucopyranoside or 3-*O*-methylglucose, suggesting that GLUT5 is a high-affinity fructose transporter.

We found that the mRNA level of this transporter in jejunal mucosal cells was highest in neonatal rats [18]. However, we could not detect any facilitative glucose transport activity in neonatal rat jejunum cells. Kayano et al. [20] suggested that the GLUT5 transporter may be the second transporter system, SII, described by Brot-Laroche et al. [41]. However, this system was detected in guinea pig jejunal brush-border membranes but not in rat or mouse jejunum. In the present study, the GLUT5 gene was shown to respond only to D-fructose. The brush border has a fructose transporter which is distinct from the aldohexose transporters whose activities also increase in mice and rats given a high-fructose diet [2,9]. Therefore GLUT5 may be a D-fructose

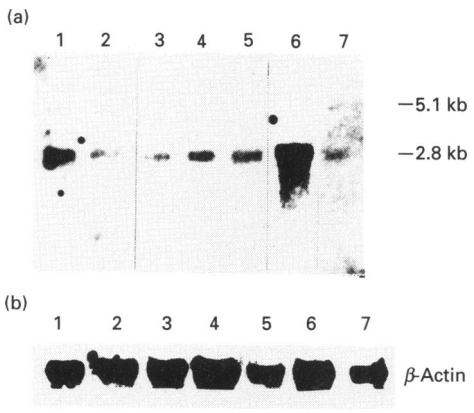

Figure 5 Effects of different dietary sugars on the level of GLUT5 mRNA

Each lane contains 9 μg of poly(A)$^+$ RNA from the jejunal mucosa of rats after 5 days of administration of various dietary sugars. (**a**) GLUT5 mRNA, (**b**) β-actin mRNA. Sugars: lane 1, none (carbohydrate-free diet); lane 2, D-glucose; lane 3, D-galactose; lane 4, 3-*O*-methylglucose; lane 5, D-mannose; lane 6, D-fructose; lane 7, D-xylose.

transporter. This possibility is supported by the fact that the GLUT5 mRNA level is higher in the jejunum of rabbits, which are herbivores with natural diets containing much D-fructose, than in the jejunum of rats and mice, which are omnivores. Furthermore we found that injection of poly(A)$^+$ RNA isolated from rabbit intestinal mucosa into *Xenopus* oocytes induces Na^+-independent D-fructose transport activity, and that hybrid depletion with a rabbit GLUT5 antisense oligonucleotide greatly inhibits the mRNA-dependent induction of uptake of D-fructose (K.-i. Miyamoto and co-workers, unpublished work).

No transcripts of GLUT1, GLUT3 and GLUT4 could be detected by our assay in the jejunum of rats fed on the various diets, as described previously [17].

In conclusion, the increase in glucose transport activity in rat given a high-carbohydrate diet was shown to be due to an increase in the level of SGLT1 and GLUT2 mRNAs. These increases might be due to increases in transcriptional rates in isolated nuclei. Our results also show that the signals for expression of SGLT1 gene did not need to be metabolizable or transportable substrates, whereas metabolism of substrates in the liver appeared to be required for expression of the intestinal GLUT2 gene. Only D-fructose served as an effective signal for expression of the GLUT5 gene.

We thank Dr. S. Seino and Dr. G. I. Bell (University of Chicago) for providing cDNA clones.

REFERENCES

1 Diamond, J. M. and Karasov, W. H. (1984) J Physiol. (London) **349**, 419–440
2 Bode, C., Eisenhardt, J. M., Haberich, T.-J. and Bode, J. C. (1981) Res. Exp. Med. **179**, 163–168
3 Stein, E. D., Chang, D. D. and Diamond, J. M. (1987) Am. J. Physiol. **252**, G626–G635
4 Karasov, W. H., Pond, R. S., Solberg, D. H. and Diamond, J. M. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 7674–7677
5 Ferraris, R. P. and Diamond, J. M. (1986) J. Membr. Biol. **94**, 77–82
6 Csaky, T. Z. and Fischer, E. (1984) Biochim. Biophys. Acta **722**, 259–263
7 Debnam, E. S. (1982) Pflügers Arch. **393**, 104–108
8 Debnam, E. S. (1985) Digestion **31**, 25–30
9 Solberg, D. H. and Diamond, J. M. (1987) Am. J. Physiol. **252**, G574–G584
10 Cheeseman, C. I. and Maenz, D. D. (1989) Am. J. Physiol. **256**, G878–G883
11 Maenz, D. D. and Cheesman, C. I. (1986) Biochim. Biophys. Acta **860**, 277–285
12 Ferraris, R. P. and Diamond, J. M. (1992) Am. J. Physiol. **262**, G1069–G1073
13 Hediger, M. A., Coady, M. J., Ikeda, J. S. and Wright, E. M. (1987) Nature (London), **330**, 379–381
14 Bell, G. I., Kayano, T., Buse, J. B., Burant, C. F., Takeda, J., Lin, D., Fukumoto, H. and Seino, S. (1990) Diabetes Care **13**, 198–208
15 Thorens, B., Charron, M. J. and Lodish, H. F. (1990) Diabetes Care **13**, 209–218
16 Wright, E. M., Turk, E., Zabe, B., Moundlos, S. and Dyer, J. (1991) J. Clin. Invest. **88**, 1435–1440
17 Miyamoto, K.-i, Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagihira, H. (1991) Biochem. Biophys. Res. Commun. **181**, 1110–1117
18 Miyamoto, K.-i, Hase, K., Taketani, Y., Minami, H., Oka, T., Nakabou, Y. and Hagihira, H. (1992) Biochem. Biophys. Res. Commun. **183**, 626–631
19 Thorens, B., Chen, Z. Q., Brown, D. and Lodish, H. F. (1990) Am. J. Physiol. **259**, C279–C285
20 Kayano, T., Burant, C. F., Fukumoto, H., Gould, G. W., Fan. Y.-S., Eddy, R. L., Byers, G. Shows, T. B., Seino, S. and Bell, G. I. (1990) J. Biol. Chem. **265**, 13276–13282
21 Davidson, N. O., Hasuman, A. M., Ifkovits, C. A., Buse, J. B., Gould, G. W., Brunt, C. F. and Bell, G. I. (1992) Am. J. Physiol. **262**, C795–C800
22 Burant, C. F., Brot-Laroche, E., Ifkovits, C. and Davidson, N. O. (1992) Diabetes, **41** (Suppl.), 11A
23 Burant, C. F., Takeda, J. Brot-Laroche, E., Bell, G. I. and Davidson, N. O. (1992) J. Biol. Chem. **267**, 14523–14525
24 Nakabou, Y., Ishikawa, Y., Misake, A. and Hagihira, H. (1980) Metab. Clin. Exp. **29**, 181–185
25 Chirgwin, J. M., Pryzybyla, A. E., MacDonald, R. J. and Rutter, W. J. (1979) Biochemistry **18**, 5249–5299
26 Lamers, W. H., Hanson, R. W. and Meisner, H. M. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 5137–5141
27 Schibler, U., Hagenbuchle, O., Wellaver, P. K. and Pittet, A. C. (1983) Cell **33**, 501–508
28 Shows, T. B., Eddy, R. L., Byer, M. G., Fukushima, Y., DaHaven, C. R., Murray, J. C. and Bell, G. I. (1987) Diabetes **36**, 546–549
29 Thorens, B., Sarkav, H. K., Koback, H. R. and Lodish, H. F. (1988) Cell **55**, 281–290
30 Kayano, T., Fukumoto, H., Eddy, R. L., Fan, Y.-S., Byers, M. G., Shows, T. B. and Bell, G. I. (1988) J. Biol. Chem. **263**, 15245–15248
31 Fukumoto, H., Kayano, T., Buse, J. B., Edwards, Y., Pilch, P. F., Bell, G. I. and Seino, S. (1989) J. Biol. Chem. **264**, 7776–7779
32 Coady, M. J., Pajor, A. M. and Wright, E. M. (1990) Am. J. Physiol. **259**, C605–C610
33 Hopfer, U. (1987) in Physiology of the Gastrointestinal Tract, 2nd edn. (Johnson, L. R., ed.), pp. 1499–1526, Raven Press, New York
34 Roy, C. C. and Dubois, R. S. (1972) Proc. Soc. Exp. Biol. Med. **139**, 888–896
35 Miyamoto, K., Takagi, T., Fujii, T., Matsubara, T., Hase, K., Taketani, Y., Oka, T., Minami, H. and Nakabou, Y. (1992) FEBS Lett. **314**, 466–470
36 Cheeseman, C. I. and Harley, B. (1991) J. Physiol. (London) **437**, 563–571
37 Rancour, N. J., Hawkins, E. D. and Well, W. W. (1979) Arch. Biochem. Biophys. **193**, 232–241
38 Hallfrisher, J. (1990) FASEB. J. **4**, 2652–2660
39 Asano, T., Katagiri, H., Tsukada, K., Lin, J.-L., Ishihara, H., Yazaki, Y. and Oka, Y. (1992) Diabetes **41**, 22–25
40 Leturque, A., Postic, C., Burcelin, R., Pegorier, J.-P., Loizeau, M. and Girard, J. (1992) Diabetes **41** (Suppl.), 44A
41 Brot-Laroche, E., Serrano, M.-A., Delhomme, B. and Alvarado, F. (1986) J. Biol. Chem. **261**, 6168–6176

Received 19 April 1993; accepted 30 April 1993