Regulation of GLUT5 gene expression in rat intestinal mucosa: regional distribution, circadian rhythm, perinatal development and effect of diabetes

Anna CASTELLÓ, Anna GUMÁ, Lidia SEVILLA, Marc FURRIOLS, Xavier TESTAR, Manuel PALACÍN and Antonio ZORZANO*
Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda. Diagonal 645, 08028 Barcelona, Spain

1. GLUT5 gene expression was studied in small intestine under a variety of conditions characterized by altered intestinal absorption of monosaccharides. 2. RNA-blotting studies showed that GLUT5 mRNA was abundantly expressed in rat and rabbit intestine and kidney, but it was not detected in heart or brown adipose tissue. GLUT5 mRNA levels were higher in the upper segments of the small intestine (duodenum and proximal jejunum) than in the lower segments (distal jejunum and ileum). 3. The intestinal expression of GLUT5 mRNA in rat proximal jejunum showed circadian rhythm. A 12-fold increase in GLUT5 mRNA levels was detected at the end of the light cycle and at the beginning of the dark cycle when compared with the early light period. In keeping with this, GLUT5 protein content in brush-border membranes was also increased at the beginning of the dark cycle compared with that in the light period. 4. In streptozotocin-induced diabetes an 80% increase in GLUT5 mRNA levels in mucosa from the proximal jejunum was detected under conditions in which enhanced intestinal absorption of monosaccharides has been reported. 5. The intestinal expression of GLUT5 mRNA showed regulation during perinatal development. Levels of GLUT5 mRNA were low during fetal life, increased progressively during the postnatal period and reached levels comparable with the adult state after weaning. Weaning on to a high-fat diet partially prevented the induction of GLUT5 gene expression. 6. Our results indicate that GLUT5 gene expression is tightly regulated in small intestine. Regulation involves maximal expression in the upper part of the small intestine, circadian rhythm, developmental regulation dependent on the fat and carbohydrate content in the diet at weaning and enhanced expression in streptozotocin-induced diabetes. Furthermore, changes observed in intestinal GLUT5 expression correlate with reported alterations in intestinal absorption of fructose. This suggests a regulatory role for GLUT5 in fructose uptake by absorptive enterocytes.

INTRODUCTION

Dietary monosaccharides, e.g. glucose, galactose and fructose, are transported across the enterocyte by the activity of various carrier systems expressed in both the lumenal and basolateral domains of the plasma membrane. In the brush-border-membrane domain of enterocytes the presence of the Na^+/glucose co-transporter and the GLUT5 isoform has been demonstrated [1–4]. In contrast, the basolateral domain of the plasma membrane of the enterocyte expresses GLUT2 but not GLUT5 or the Na^+/glucose co-transporter [4,5].

The Na^+/glucose co-transporter, when functionally expressed in *Xenopus* oocytes, catalyses the Na^+-dependent uptake of glucose and galactose [6–8]. On the basis of expression studies in *Xenopus* oocytes, it has been concluded that GLUT2 catalyses the facilitated transport of glucose, galactose and fructose with low affinity [9–11].

GLUT5 is the only isoform of the facilitative glucose-transporter family that is expressed in the brush border of enterocytes. However, this does not mean that its expression is restricted to enterocytes. In fact, GLUT5 is expressed in various tissues in a species-dependent manner. Thus, in humans, GLUT5 has been detected in intestine, kidney, skeletal muscle, adipose tissue, spermatozoa and brain [12–16]. In contrast, the distribution of GLUT5 in rodents is restricted to intestine, kidney and brain [17,18]. Rat and human GLUT5 amino acid sequences show 81.5% identity and 87.3% similarity [12,17]. Nevertheless, they differ in their biological properties. Thus the expression of human GLUT5 in *Xenopus* oocytes shows selectivity for fructose transport, as determined by inhibition studies [15]. In contrast, the expression of rat GLUT5 in oocytes induces the activity of both fructose and glucose transport [17]. Furthermore, whereas the transport of fructose induced by rat GLUT5 is insensitive to cytochalasin B, the transport of glucose is inhibitable in the presence of cytochalasin B [17].

There is evidence that GLUT5 expression is regulated in intestine. Dietary fructose but not glucose or galactose increases the expression of intestinal GLUT5 mRNA in rodents [18,19]. Furthermore, GLUT5 gene expression is subjected to developmental regulation. Thus expression of human intestinal GLUT5 is low in fetal life [3]. In the rat, there is controversy about the expression of intestinal GLUT5 during perinatal life and whereas some authors have reported high levels of GLUT5 mRNA in early neonatal life [20], others report low levels during fetal and early postnatal life [17]. Here, we have investigated the regulation of intestinal GLUT5 gene expression in response to regional distribution along the small intestine, circadian rhythm, developmental stage and streptozotocin-induced diabetes.

MATERIALS AND METHODS

Materials

[α-^{32}P]dCTP was purchased from ICN. Hybond N and ^{125}I-labelled goat anti-mouse IgG were from Amersham and random-primed DNA-labelling kit was from Boehringer. Immobilon was obtained from Millipore. All electrophoresis reagents and molecular-mass markers were obtained from Bio-Rad and BRL. Most commonly used chemicals were from Sigma.

Animals and tissue sampling

Male Wistar rats (180–220 g) were obtained from our own colony. The rats were fed on Purina Laboratory chow *ad libitum*

Abbreviation used: poly(A)$^+$, polyadenylated.
*To whom correspondence should be addressed.

and housed in animal quarters maintained at 22 °C with a 12 h light, 12 h dark cycle (light cycle started at 08:00 h). Diabetes was induced by an intraperitoneal injection of streptozotocin (70 mg/kg body weight) 1 week before the study. Some animals were starved for 48 h before the day of the experiment. All animals were anaesthetized with sodium pentobarbital (5–7 mg/100 g body weight), and intestine was rapidly excised. In some experiments, heart, brown adipose tissue and kidney were also removed. In some experiments, tissues were also collected from male New Zealand White rabbits. Unless stated, tissues were collected between 09:00 and 11:00 h. After collection, tissues were rapidly frozen and kept in liquid nitrogen until analysis.

Female Wistar rats (150–200 g) obtained from our own colony were mated, and gestation was timed from the appearance of spermatozoa in vaginal smears. At different gestational times (19–21 days), pregnant rats were anaesthetized with sodium pentobarbital (5–7 mg/100 g body weight). Fetuses were removed and samples of intestine were rapidly collected and frozen in liquid nitrogen. When the postnatal period was studied, pups remained with their mothers after delivery and were anaesthetized with sodium pentobarbital at different times, before tissue sampling, which was routinely performed from 09:00 to 11:00 h. Usually the rats were weaned at 21 days on to a regular chow diet (17% proteins, 3% fat, 59% carbohydrates, 4% cellulose, 5% minerals, w/w). In some experiments, the rats were weaned at 21 days on to a high-fat diet (37% proteins, 43% fat, 15% cellulose, 5% minerals, w/w). Since the suckling rats begin to nibble at the food provided for the mother at 15 days of age [21], the mothers of rats weaned on to a high-fat diet were also fed on the high-fat diet from day 15 of lactation until weaning.

Preparation of intestinal brush-border membranes and immunoblotting

Brush-border-membrane vesicles were prepared using the calcium-precipitation method [22]. Brush-border membranes showed a 12-fold enrichment in alkaline phosphatase activity compared with the crude homogenate, in keeping with previous reports [22].

SDS/PAGE was performed on membrane protein by the method of Laemmli [23]. Proteins were transferred to Immobilon as previously reported [24] in buffer consisting of 20% methanol, 200 mM glycine and 25 mM Tris/HCl, pH 8.3. After transfer, the filters were blocked with 5% non-fat dried milk/0.02% NaN_3 in PBS for 1 h at 37 °C and were incubated with a polyclonal antibody against rat GLUT5 (kindly given by Dr. Charles F. Burant, University of Chicago, Chicago, IL, U.S.A.) for the same time at the same temperature. Transfer was confirmed by Coomassie Blue staining of the gel after the electroblot. Detection of antibody–antigen complexes was effected with ^{125}I-Protein A and autoradiography. The autoradiograms were quantified using scanning densitometry (Ultrascan × L enhancer laser densitometer; LKB). Immunoblots were performed under conditions in which autoradiographic detection was in the linear response range.

RNA isolation and Northern-blot analysis

Total RNA from tissues was extracted using the acid/guanidinium thiocyanate/phenol/chloroform method as described by Chomczynski and Sacchi [25]. All samples had an A_{260}/A_{280} ratio above 1.8. Oligo(dT)–cellulose for purification of poly(A)$^+$ RNA was purchased from Boehringer-Mannheim and used by following the manufacturer's protocol.

After quantification, total RNA (25–30 μg) or polyadenylated [poly(A)$^+$] RNA (2.5–10 μg) was denatured at 65 °C in the presence of formamide, formaldehyde and ethidium bromide [26] to allow the visualization of RNA. RNA was separated on a 1.2% agarose/formaldehyde gel and blotted on to Hybond N filters. The RNA in gels and in filters was visualized with ethidium bromide by UV transillumination to ensure the integrity of RNA, to check the loading of equivalent amounts of total RNA and to confirm proper transfer. RNA was transferred in 10 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

Blots were initially prehybridized for 4 h at 45 °C in solution composed of 50% formamide, 5 × Denhardt's (1 × Denhardt's solution is 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.002% BSA), 0.1% SDS, 5 × SSPE (1 × SSPE is 0.15 M NaCl/1 mM EDTA/10 mM NaH_2PO_4, pH 7.4) and 0.2 mg/ml denatured salmon sperm DNA. The blots were then hybridized to the cDNA probes for 12 h at 42 °C in a solution composed of 50% formamide, 5 × Denhardt's, 0.1% SDS, 5 × SSPE, 10% dextran sulphate and 0.2 mg/ml denatured salmon sperm DNA. The human cDNA probe for GLUT-5 was a 1886 bp *Eco*RI fragment and the rat cDNA probe for GLUT-5 was a 2200 bp *Eco*RI fragment (both cDNA probes were obtained from Dr. Graeme Bell, University of Chicago). In some experiments a DNA β-actin probe (*Hin*dIII–*Eco*RI fragment of 4500 bp [27]) and a mouse cDNA probe for keratin 18 (a 1000 bp *Eco*RI fragment [28]) were included. The DNA probes were labelled with [^{32}P]dCTP by random oligonucleotide priming. The probes were included at 2×10^6 c.p.m./ml. Filters were washed for 15 min in 2 × SSC at room temperature, then for 20 min in 0.4 × SSC/0.1% SDS at 55 °C and finally for 25 min in 0.1 × SSC/0.1% SDS at 55 °C. The abundance of specific transcripts was quantified by scanning densitometry of autoradiograms, as described above. Student's t test was used for statistical analysis.

RESULTS

Heterogeneous distribution of GLUT5 mRNA in small intestine

The expression of GLUT5 mRNA was assessed in preparations of total RNA or poly(A)$^+$ RNA obtained from rat tissues by Northern-blot analysis and using either human or rat cDNA probes [12,17]. In keeping with previous observations [17,18], a

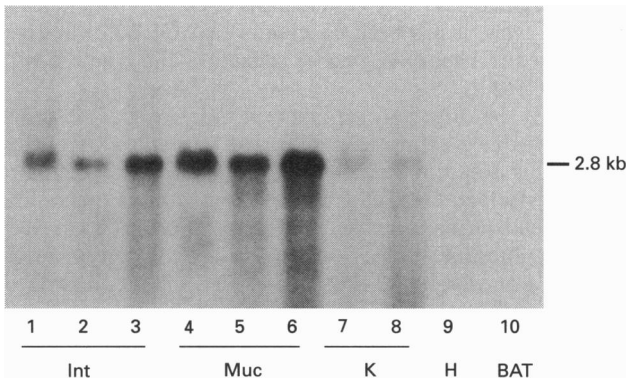

Figure 1 Expression of GLUT5 mRNA in rat tissues

Total RNA and poly(A)$^+$ RNA was purified from total intestinal tissue (Int), intestinal mucosa (Muc), kidney (K), heart (H) and brown adipose tissue (BAT) of normal rats. Then 30 μg of total RNA (lanes 1, 4, 7, 9 and 10), 2.5 μg of poly(A)$^+$ RNA (lanes 2 and 5) or 4.5 μg of poly(A)$^+$ RNA (lanes 3, 6 and 8) was applied to gels. GLUT5 mRNA was detected after hybridization with a rat GLUT5 cDNA probe, as described in the Materials and methods section. A representative autoradiogram from three to five experiments is shown.

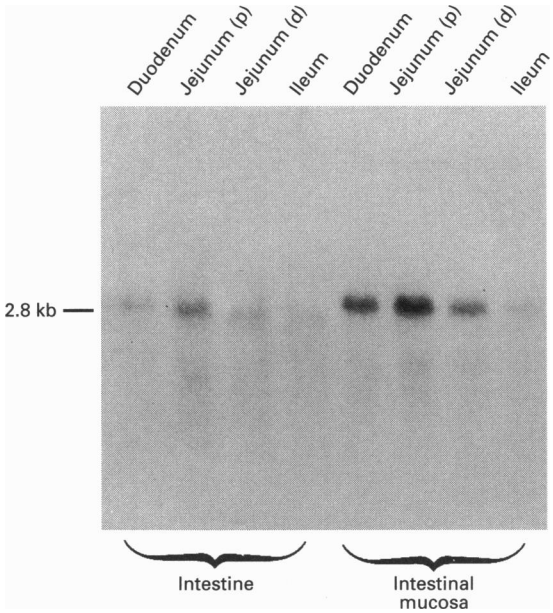

Figure 2 Regional distribution of GLUT5 mRNA in small intestine of the rat

Total RNA was purified from different regions of small intestine or from intestinal mucosa. Then 30 μg of total RNA was applied to gels. GLUT5 mRNA was detected after hybridization with a human GLUT5 cDNA probe, as described in the Materials and methods section. A representative autoradiogram from two separate experiments is shown. Jejunum (p), proximal jejunum; Jejunum (d), distal jejunum.

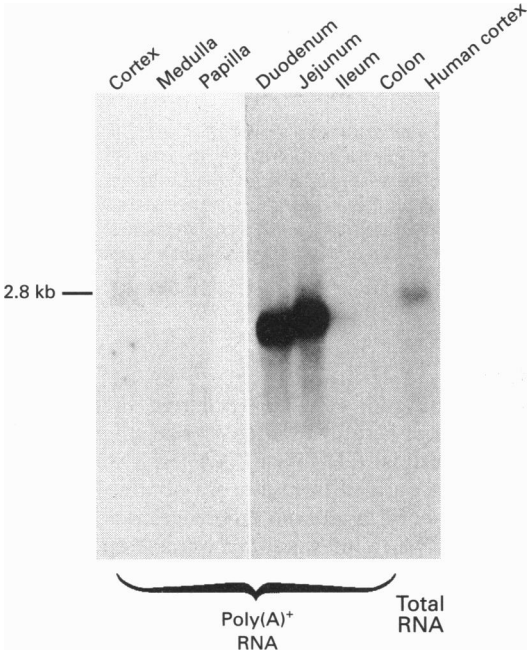

Figure 3 Regional distribution of GLUT5 mRNA in small intestine and kidney of the rabbit

Poly(A)$^+$ RNA was purified from rabbit tissues and total RNA was obtained from human cortex. Total RNA (25 μg) or poly(A)$^+$ RNA (10 μg) was applied to gels. GLUT5 mRNA was detected after hybridization with a human GLUT5 cDNA probe, as described in the Materials and methods section. A representative autoradiogram from two experiments is shown.

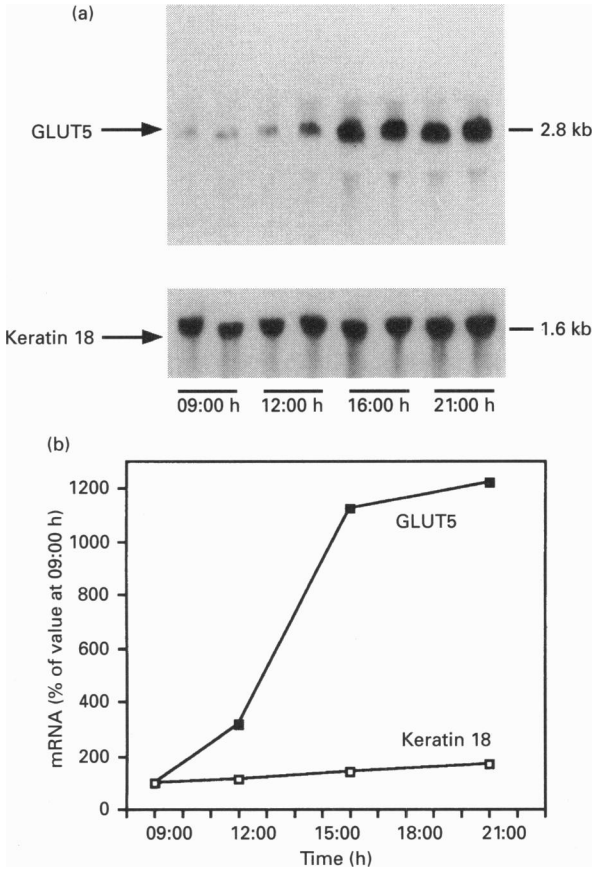

Figure 4 Circadian rhythm of GLUT5 mRNA levels in rat jejunum

Total RNA was purified from rat proximal jejunum obtained at different times during the day (09:00, 12:00, 16:00 and 21:00 h). Total RNA (30 μg) was applied to gels. GLUT5 mRNA was detected after hybridization with a rat GLUT5 cDNA probe (**a**). Cytokeratin 18 mRNA was detected with a mouse cDNA probe, as described in the Materials and methods section (**a**). Autoradiographs were subjected to scanning densitometry. The results of two to five separate experiments are shown, and expressed as a percentage of value at 09:00 h (**b**). Statistical analysis indicated the existence of significant differences at 12:00, 16:00 and 21:00 h compared with the 09:00 h group ($P < 0.05$).

specific band of approx. 2.8 kb was detected in small intestine and kidney and no GLUT5 mRNA was detected in heart or brown adipose tissue (Figure 1). The abundance of GLUT5 mRNA was 2.4-fold greater in small intestine than in kidney (Figure 1). In addition, 2.9-fold greater abundance of GLUT5 mRNA was detected in poly(A)$^+$-RNA preparations of intestinal mucosa compared with preparations of total intestine (Figure 1).

The highest levels of GLUT5 mRNA in rat intestine were found in proximal jejunum (Figure 2). Levels of GLUT5 transcripts in duodenum, distal jejunum and ileum accounted for 51, 32 and 17% of those found in proximal jejunum. Similar results were obtained when total RNA was purified from mucosa from different regions of the intestine, i.e. levels were maximal in proximal jejunum (levels in duodenum, distal jejunum and ileum accounted for 72, 51 and 11% of those found in proximal jejunum) (Figure 2). Poly(A)$^+$ RNA was also obtained from rabbit tissues, and levels of GLUT5 transcripts were determined. High abundance of GLUT5 was found in duodenum and jejunum and much lower levels were detected in ileum (Figure 3). None was detected in colon (Figure 3). Relatively low levels of GLUT5 mRNA were detected in different regions of the rabbit kidney

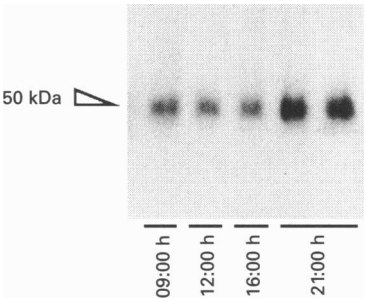

Figure 5 Circadian rhythm of GLUT5 protein levels in rat jejunum

Brush-border membranes were obtained from rat proximal jejunal mucosa at different times during the day (09:00, 12:00, 16:00 and 21:00 h). Identical amounts (30 μg) of membrane proteins from the different experimental groups were applied to 10% acrylamide gels. Proteins from the gels were transferred to immobilon and immunoblotted with a polyclonal antibody raised against the C-terminal peptide of rat GLUT5. Autoradiographs were subjected to scanning densitometry. A representative autoradiogram is shown.

and higher concentrations were detected in human kidney cortex (Figure 3).

Circadian rhythm of GLUT5 gene expression in jejunum

It has been reported that proteins expressed in intestine, such as disaccharidases, show circadian rhythmic changes [29,30]. In order to determine whether GLUT5 gene expression is also subjected to a circadian rhythm, total RNA was obtained from the proximal jejunum taken from rats at different times during the day (09:00, 12:00, 16:00 and 21:00 h). No differences in RNA yield per mg of tissue were detected among groups (results not shown). GLUT5 transcripts increased 3-fold from 09:00 h to 12:00 h (Figure 4b). At 16:00 and 21:00 h GLUT5 mRNA levels increased further and values were 12-fold greater than at 09:00 h (Figure 4b). Under these conditions, the mRNA levels for keratin 18, a marker of epithelial cells [31], increased by 68% (Figure 4b). In order to determine whether GLUT5 protein content is also modified, brush-border membranes were obtained from jejunal mucosa as previously reported [22]. Western-blot analysis with an anti-peptide antibody against rat GLUT5 indicated that GLUT5 protein levels were similar in brush-border membranes obtained from jejunal mucosa at 09:00, 12:00 and 16:00 h (Figure 5). However, a 78% increase was detected in membranes obtained at 21:00 h (Figure 5). No differences in the yield of membrane protein per mg of tissue were found among groups (results not shown).

Increased intestinal GLUT5 gene expression caused by diabetes and starvation

The intestinal absorption of sugars increases in experimental diabetes [32–34]. In order to determine whether the expression of GLUT5 mRNA is enhanced under these conditions, rats were injected with streptozotocin (70 mg/kg body weight) and 7 days later samples of small intestine were taken; GLUT5 mRNA was determined by Northern-blot analysis. Blood glycaemia was 8.6 ± 0.6 and 27.5 ± 0.5 mM in control and streptozotocin-induced diabetic rats respectively. No differences were detected in the yield of total RNA in small intestine in response to diabetes (5.2 ± 0.2 and 4.8 ± 0.4 mg of RNA/g of tissue in the control and diabetic group respectively). Streptozotocin-induced diabetes caused a marked increase (90%) in intestinal GLUT5 mRNA levels (Figure 6a). This occurred in the presence of unaltered levels of keratin 18 mRNA. The stimulatory effect of diabetes on intestinal GLUT5 mRNA levels was also observed when RNA was purified from mucosa obtained from proximal jejunum (Figure 6b). Again, no differences were detected in the yield of total RNA in intestinal mucosa in response to diabetes (7.6 ± 1.6 and 7.4 ± 0.4 mg of RNA/g of mucosa in control and diabetic animals respectively). Under these conditions, GLUT5 mRNA levels were increased 80% in response to diabetes, in the absence of changes in keratin 18 mRNA concentrations (Figure 6b).

In parallel with these studies, the effect of 2 days of starvation was also studied. No change in the yield of total RNA in intestine was observed (results not shown). However, there was a substantial (67%) increase in GLUT5 mRNA levels over the controls, which occurred in the absence of changes in the concentration of keratin 18 mRNA (Figure 6a).

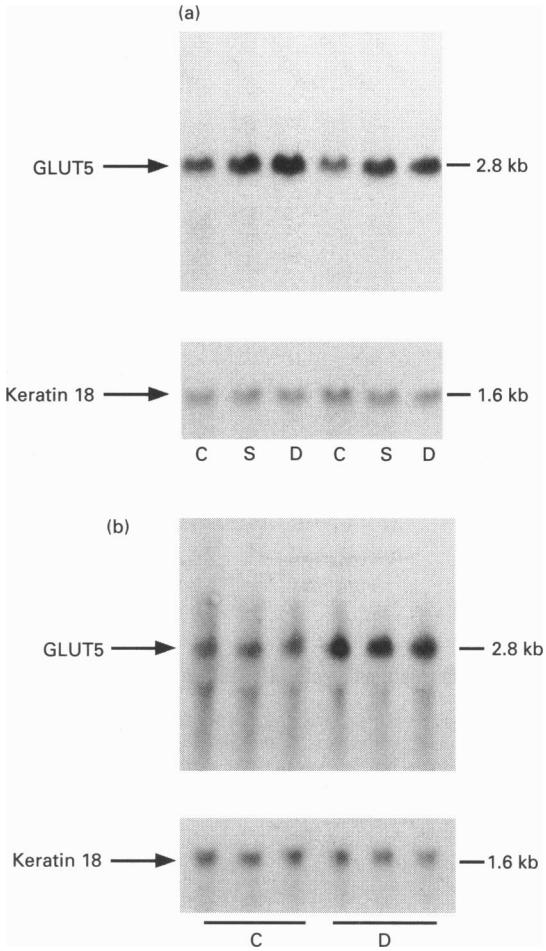

Figure 6 Effect of streptozotocin-induced diabetes and starvation on GLUT5 mRNA levels in rat jejunum

Total RNA was purified from total proximal jejunum (**a**) or mucosa from proximal jejunum (**b**) obtained from control (C), 7-day diabetic (D) and 2-day starved rats (S). Total RNA (30 μg) was applied to gels. GLUT5 and keratin 18 mRNA species were detected after hybridization with specific cDNA probes, as described in the Materials and methods section and legend to Figure 4. Representative autoradiograms from four to five experiments are shown. Differences due to starvation or streptozotocin-induced diabetes were statistically significant ($P < 0.05$).

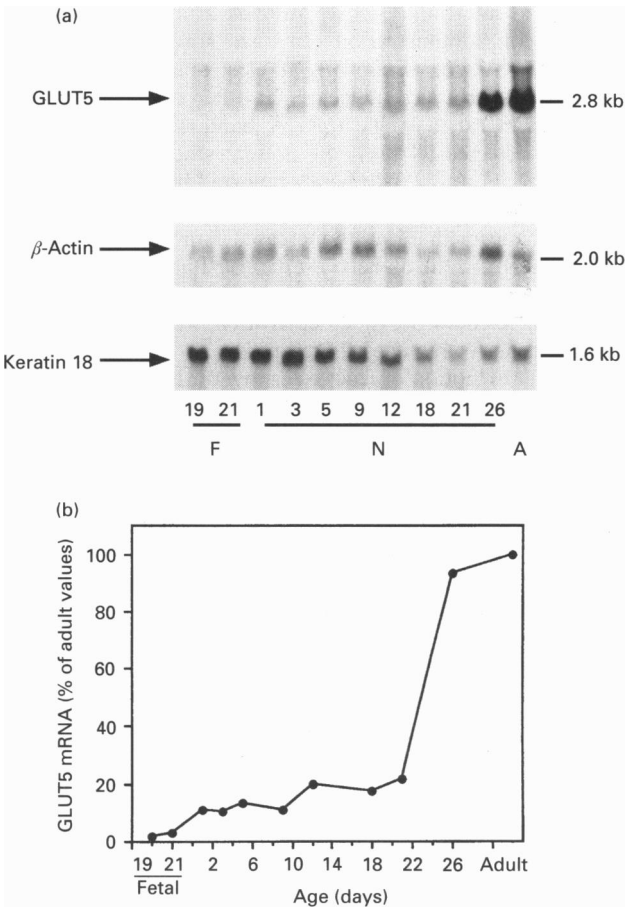

Figure 7 Expression of GLUT5 mRNA in intestine during development

Total RNA was purified from pooled small intestines obtained from rat fetuses (19- and 21-day) and from proximal jejunum obtained from neonatal and adult rats. Total RNA (30 μg) from the different experimental groups was applied to gels. GLUT5, keratin 18 and β-actin mRNA species were detected after hybridization with specific DNA probes, as described in the Materials and methods section (**a**). Autoradiographs were subjected to scanning densitometry. F, fetal group; N, neonatal group; A, adult group.The results of three separate experiments are shown, and expressed as a percentage of adult values (**b**).

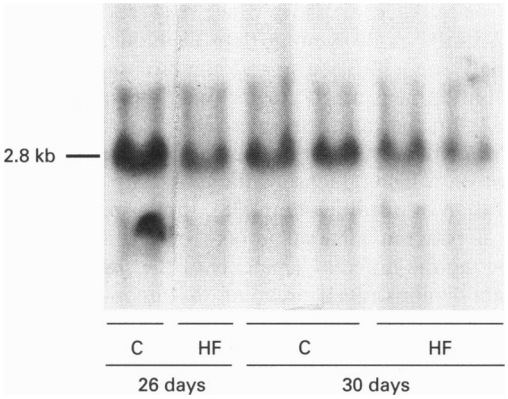

Figure 8 Effect of weaning on to a high-fat diet on intestinal GLUT5 mRNA levels

Total RNA was purified from intestinal jejunum obtained from pups that were weaned at day 21 on to a normal diet (C) or a high-fat diet (HF) and studied at days 26 or 30 after birth. Total RNA (30 μg) from the different experimental groups was applied to gels. GLUT5 mRNA was detected after hybridization with a rat GLUT5 cDNA probe, as described in the Materials and methods section. A representative autoradiogram from two separate experiments is shown. Differences resulting from high-fat feeding were statistically significant ($P < 0.05$).

Developmental regulation of intestinal GLUT5 gene expression

The expression of intestinal GLUT5 mRNA was assessed during development of rats. To this end, total RNA was purified from small intestines obtained from 19- and 21-day fetuses and from proximal jejunum obtained from neonatal and adult rats, and mRNA content was determined. The RNA yield obtained in intestine during development was 5.2 ± 0.6 mg/g of tissue in late fetal life, 3.3 ± 0.1 mg/g of tissue in early neonatal life and 3.3 ± 0.1 mg/g of tissue at the peak of lactation.

GLUT5 mRNA levels were already detectable but low (2% of adult values) at day 19 of fetal life (Figure 7). The abundance of GLUT5 transcripts increased after birth and 1 day postnatal levels accounted for 11% of adult values. Levels of GLUT5 mRNA remained low at the end of the suckling period so by day 21 postnatal levels accounted for 22% of adult values. After weaning (day 26 after birth) levels of GLUT5 mRNA were 93% of adult values. Neither RNA degradation (see β-actin and keratin 18 signals in Figure 7) nor differences in RNA yield could explain this pattern of GLUT5 mRNA expression. The pattern of GLUT5 mRNA during the perinatal phase was similar if data were expressed per μg of RNA or as a ratio of GLUT5/keratin 18, GLUT5/β-actin or GLUT5/rRNA (results not shown).

In order to determine whether the increase in intestinal GLUT5 gene expression detected during weaning was associated with the switch from a high-fat diet to a high-carbohydrate diet, pups were weaned on to a high-fat diet (37% proteins, 43% fat, 15% cellulose, 5% minerals, w/w; i.e. 72% of energy was derived from dietary fat). To this end, either a high-fat diet or a normal (high-carbohydrate diet: 17% proteins, 3% fat, 59% glucids, 4% cellulose, 5% minerals, w/w) was offered to mothers on day 15 after birth. Pups were weaned on day 21 and maintained with either the high-fat diet or the normal diet. At day 26 or 30, animals were anaesthetized and their intestines were removed. Under conditions in which no difference in RNA yield was detected between groups (results not shown), weaning on to a high-fat diet led to a 44 and 51% reduction in GLUT5 mRNA levels at days 26 and 30 respectively (Figure 8).

DISCUSSION

The functional role of GLUT5 in the absorptive enterocyte in the human and rat intestine is not completely understood. Functional studies in *Xenopus* oocytes indicate that human GLUT5 catalyses the facilitated uptake of fructose, whereas rat GLUT5 catalyses fructose and glucose [15,17]. A competitive effect of glucose on the intestinal fructose uptake was detected by Holloway and Parsons [35], who showed a marked decrease in the absorption of fructose caused by the presence of glucose in the lumen of rat intestine. This further supports the view that rat GLUT5 transports both glucose and fructose in the absorptive enterocyte. In keeping with a role for GLUT5 in the intestinal absorption of fructose in the rat, a high-fructose diet enhances fructose but not glucose absorption in intestine [36,37] and, under these conditions, intestinal GLUT5 mRNA and protein levels are markedly increased [18,19].

We have found that in two species, rat and rabbit, GLUT5 mRNA levels are higher in the upper segment of small intestine. Thus GLUT5 mRNA levels reach the maximum in the proximal jejunum of the rat intestine. This is in keeping with previous

studies from Inukai et al. [18] in which they reported higher levels of GLUT5 mRNA in jejunum than in duodenum or in ileum. Interestingly enough, fructose and glucose uptake in proximal–mid small intestine is much greater than in distal small intestine in mice [37]. Furthermore, the intestinal absorption of fructose is 3-fold greater in proximal than in distal jejunum in mice under conditions in which glucose uptake is only 30% greater in proximal than in distal jejunum [38]. In consequence, there is a correlation between lumenal uptake of fructose and GLUT5 mRNA along the different segments of the small intestine.

Our data demonstrate a circadian rhythm in the expression of GLUT5 mRNA and protein in proximal jejunum. Thus GLUT5 mRNA levels are maximal at the late light period of the cycle and at the beginning of the dark period. In parallel, GLUT5 protein levels increase at the beginning of the dark period compared with values during the light period. These data are parallel to intestinal glucose uptake, which is maximum during the night-time feeding period [39,40]. No data are available on changes in the intestinal uptake of fructose during the day.

Circadian rhythmicity has also been observed for the activity of various small-intestine functions, including the activity of disaccharidases such as sucrase-isomaltase, maltase, lactase and trehalase [29,30,40,41], the intestinal absorption of L-histidine [39], the activity of γ-glutamyltransferase and alkaline phosphatase and leucylnaphthylamide-hydrolysing activity [40]. The abundance of sucrase-isomaltase mRNA shows a similar circadian rhythm to that found for GLUT5 mRNA levels and, curiously enough, the two transcripts also share a similar distribution along the crypt–villus axis. Thus the gene expression of sucrase-isomaltase and GLUT5 is maximal in the midvillus whereas in the crypt and the tip of the villus their expression is absent [17,42,43].

Circadian rhythmicity has also been reported for villus height and cell number in small intestine, 1.24 being the ratio of maximal to minimal cell number [40]. The rhythmicity found for enterocyte cell number might explain the pattern found in the levels of keratin 18 mRNA during the day. However, it is unlikely that the circadian rhythm of GLUT5 gene expression, which is much more pronounced, can be explained by alterations in enterocyte cell number. Rather, we favour an explanation in terms of activated intestinal gene expression of GLUT5. Whether the daily rhythm of intestinal GLUT5 mRNA is due to rhythmicity of nutrient presentation or other mechanisms remains unknown.

It has been reported that streptozotocin-induced diabetes causes an enhanced intestinal rate of absorption of glucose, galactose and fructose [34,44–48]. The process of increased intestinal sugar absorption in diabetes appears to be biphasic, with a rapid stimulation of sugar uptake per unit of intestinal mass followed by a slow increase in intestinal mass [49]. Furthermore, the effects of diabetes on intestinal sugar uptake are independent of the hyperphagia associated with this condition [44,46]. In keeping with the accelerated intestinal fructose uptake, we have observed enhanced levels of GLUT5 mRNA in preparations of total intestine from diabetic animals. The greater abundance of GLUT5 mRNA found in the diabetic condition cannot be explained simply by an increase in the relative abundance of mucosa since (a) enhanced GLUT5 mRNA levels were also detected in preparations of intestinal mucosa from diabetic animals and (b) intestinal GLUT5 mRNA concentrations were increased in diabetes under conditions in which keratin 18, an epithelial cell marker, remained unaltered. Therefore our data indicate enhanced expression of GLUT5 gene in intestinal mucosa in streptozotocin-induced diabetes. Our results contradict previous data from Miyamoto et al. [50] who reported no change in intestinal GLUT5 mRNA after 5 or 10 days of streptozotocin administration. In that study three putative GLUT5 mRNA species, showing an electrophoretic mobility of 5.1, 2.8 and 2.0 kb, were detected. In other studies on samples obtained from rat, a single mRNA band with a mobility of 2.8 kb was detected ([17,18]; the present study). Therefore the nature of the 5.1 and 2.0 kb bands reported by Miyamoto et al. [50] is not known.

The mechanisms that lead to increased expression of intestinal GLUT5 mRNA in the diabetic rat deserve further study. On the basis of the fact that increased intestinal GLUT5 mRNA levels were also detected during food deprivation, we suggest insulin as a possible negative regulatory factor.

Intestinal absorption of fructose is low during early postnatal life in species such as rabbit and rat and it increases steeply on weaning [51,52]. We have studied the developmental expression of mRNA encoding GLUT5 in the rat intestine. Our data indicate low levels of transcripts during fetal life and a progressive increase in mRNA during postnatal life. Levels became comparable with those of adults only after weaning. Our results are in keeping with data from Davidson et al. [3], who found low levels of GLUT5 mRNA in fetal human intestine. Our data also agree with those of Rand et al. [17], who reported low concentrations of GLUT5 mRNA in intestine from rat fetuses, and that at days 8 and 14 levels were still below 30% of adult values [17]. However, our data are at variance with those reported by Miyamoto et al. [20], who found high values of GLUT5 mRNA levels during neonatal life. Regarding the mechanisms involved in the acquisition of normal values for fructose absorption, it has been reported that rats prevented from weaning normally by being maintained on dry milk showed normal levels of intestinal fructose uptake [52]. Here, we have presented evidence that weaning on to a high-fat diet attenuates the induction of GLUT5 mRNA levels in intestine, which supports the view that developmental regulation of intestinal GLUT5 gene expression is in some way dependent on the dietary fat content or the quality or quantity of the sugar content. In conclusion, our results indicate a correlation between the pattern of intestinal fructose uptake and GLUT5 gene expression during rat ontogeny [52].

Our data indicate that GLUT5 gene expression is highly regulated in the small intestine. We have provided evidence that it is maximal in intestinal mucosa from proximal jejunum of the rat and shows circadian rhythm. GLUT5 gene expression is subjected to up-regulation by streptozotocin-induced diabetes and starvation, and it shows developmental regulation. The changes in GLUT5 mRNA parallel differences reported in intestinal absorption of fructose along the different segments of the small intestine, during ontogeny and in response to diabetes. These observations support the view that GLUT5 catalyses apical uptake of fructose by the absorptive enterocyte in the rat and, in addition, suggest a regulatory role for GLUT5 in fructose uptake by these cells.

We thank Dr. Graeme I. Bell and Dr. Charles F. Burant (University of Chicago) for providing the rat and human cDNA clones of GLUT5 and a polyclonal antibody against rat GLUT5, respectively. We also thank Robin Rycroft for editorial help, and Dr. Andreas Werner and Dr. Heini Murer (University of Zurich) for the use of rabbit kidney RNA blots. This work was supported by research grant PB92/0805 from the Dirección General de Investigación Científica y Técnica. A.C. was a recipient of a predoctoral fellowship from the Ministerio de Educación y Ciencia.

REFERENCES

1 Semenza, G., Kessler, M., Hosang, M. and Schmidt, U. (1984) Biochim. Biophys. Acta **779**, 343–379

2 Hediger, M. A., Coady, M., Ikeda, T. and Wright, E. (1987) Nature (London) **330**, 379–381

3 Davidson, N. O., Hausman, A. M. L., Ifkovits, C. A., Buse, J. B., Gould, G. W., Burant, C. F. and Bell, G. I. (1992) Am. J. Physiol. **262**, C795–C800
4 Matsumoto, K., Takao, Y., Akazawa, S. et al. (1993) Biochem. Biophys. Res. Commun. **193**, 1275–1282
5 Thorens, B., Cheng, Z. Q., Brown, D. and Lodish, H. F. (1990) Am. J. Physiol. **259**, C279–C285
6 Ikeda, T. S., Hwang, E. S., Coady, M., Hirayama, B., Hediger, M. and Wright, E. M. (1989) J. Membr. Biol. **110**, 87–95
7 Umbach, J. A., Coady, M. and Wright, E. M. (1990) Biophys. J. **57**, 1217–1224
8 Wright, E. M., Turk, E., Zabel, B., Mundlos, S. and Dyer, J. (1991) J. Clin. Invest. **88**, 1435–1440
9 Gould, G. W., Thomas, H. M., Jess, T. J. and Bell, G. I. (1991) Biochemistry **30**, 5139–5145
10 Burant, C. F. and Bell, G. I. (1992) Biochemistry **31**, 10414–10420
11 Colville, C. A., Seatter, M. J., Jess, T. J., Gould, G. W. and Thomas, H. M. (1993) Biochem.J. **290**, 701–706
12 Kayano, T., Burant, C. F., Fukumoto, H. G. et al. (1990) J. Biol. Chem. **265**, 13276–13282
13 Hundal, H. S., Ahmed, A., Gumà, A. et al. (1992) Biochem. J. **286**, 339–343
14 Shepherd, P. R., Gibbs, E. M., Wesslau, C., Gould, G. W. and Kahn, B. B. (1992) Diabetes **41**, 1360–1365
15 Burant, C. F., Takeda, J., Brot-Laroche, L. E., Bell, G. I. and Davidson, N. O. (1992) J. Biol. Chem. **267**, 14523–14526
16 Mantych, G. J., James, D. E. and Devaskar, S. U. (1993) Endocrinology (Baltimore) **132**, 35–40
17 Rand, E. B., Depaoli, A. M., Davidson, N. O., Bell, G. I. and Burant, C. F. (1993) Am. J. Physiol. **264**, G1169–G1176
18 Inukai, K., Asano, T., Katagiri, H. et al. (1993) Endocrinology (Baltimore) **133**, 2009–2014
19 Miyamoto, K., Hase, K., Takagi, T. et al. (1993) Biochem.J. **295**, 211–215
20 Miyamoto, K., Hase, K. Taketani, Y. et al. (1992) Biochem. Biophys. Res. Commun. **183**, 626–631
21 Redman, R. S. and Sweney, L. R. (1976) J. Nutr. **106**, 615–626
22 Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta **506**, 134–154
23 Laemmli, U. K. (1970) Nature (London) **227**, 680–685.
24 Camps, M., Castelló, A., Muñoz, P. et al. (1992) Biochem. J. **282**, 765–772
25 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
26 Rosen, K. M. and Villa-Komaroff, L. (1990) Focus **12**, 23–24
27 Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, Z. and Yaffe, D. (1983) Nucleic Acids Res. **11**, 1759–1771
28 Alonso, A., Weber, T. and Jorcano, J. L. (1987) Roux's Arch. Dev. Biol. **196**, 16–21
29 Saito, M., Sato, H. and Suda, M. (1980) Am. J. Physiol. **238**, G97–G101
30 Inagaki, O., Mori, H., Shimomura, R., Inoue, S. and Fujita, Y. (1982) Nephron **30**, 345–347
31 Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. and Krepler, R. (1982) Cell **31**, 11–24
32 Axelrad, A. D., Lawrence, A. L. and Hazelwood, R. L. (1970) Am. J. Physiol. **219**, 860–864
33 Crane, R. K. (1961) Biochem. Biophys. Res. Commun. **4**, 436–440
34 Csaky, T. Z. and Fischer, E. (1981) Diabetes **30**, 568–574
35 Holloway, P. A. H. and Parsons, D. S. (1984) Biochem. J. **222**, 57–64
36 Bode, C., Eisenhardt, J. M., Haberich, T. J. and Bode, J.Ch. (1981) Res. Exp. Med. **179**, 163–168
37 Solberg, D. H. and Diamond, J. M. (1987) Am. J. Physiol. **252**, G574–G584
38 Ferraris, R. P., Hsiao, J., Hernandez, R. and Hirayama, B. (1993) Am. J. Physiol. **264**, G285–G293
39 Furuya, S. and Yugari, Y. (1974) Biochim. Biophys. Acta **343**, 558–564
40 Stevenson, N. R., Sitren, H. S. and Furuya, S. (1980) Am. J. Physiol. **238**, G203–G207
41 Beam, H. E. and Henning, S. J. (1978) Am. J. Physiol. **235**, E437–E442
42 Chandrasena, G., Sunitha, I., Lau, C., Nanthakumar, N. N. and Henning, S. J. (1992) Cell. Mol. Biol. **38**, 243–254
43 Traber, P. G. (1990) Biochem. Biophys. Res. Commun. **173**, 765–773
44 Olsen, W. A. and Rosenberg, I. H. (1970) J. Clin. Invest. **49**, 96–104
45 Fedorak, R. N. (1990) Can. J. Physiol. Pharmacol. **68**, 630–635
46 Fedorak, R. N., Thomson, A. B. R. and Porter, V. M. (1991) Can. J. Physiol. Pharmacol. **69**, 1143–1148
47 Fedorak, R. N., Cheeseman, C. I., Thomson, A. B. R. and Porter, V. M. (1991) Am. J. Physiol. **261**, G585–G591
48 Ferraris, R. P., Casirola, D. M. and Vinnakota, R. R. (1993) Diabetes **42**, 1579–1587
49 Karasov, W. H. and Diamond, J. M. (1983) Am. J. Physiol. **245**, G443–G462
50 Miyamoto, K., Hase, K., Taketani, Y. et al. (1991) Biochem. Biophys. Res. Commun. **181**, 1110–1117
51 Buddington, R. K. and Diamond, J. (1990) Am. J. Physiol. **259**, G544–G555
52 Toloza, E. M. and Diamond, J. (1992) Am. J. Physiol. **263**, G593–G604

Received 10 January 1995/27 February 1995; accepted 6 March 1995