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Intestinal sodium-dependent D-glucose co-transporter: dietary regulation

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Co-transporteur du D-glucose sodium-dépendant dans l'intestin: régulation alimentaire

RÉSUMÉ

La fonction du co-transporteur de glucose et de Na^+ (SGLT1) est d'absorber le D-glucose et le D-galactose de l'alimentation à travers la membrane luminale en bordure en brosse des entérocytes. Le SGLT1 est une protéine intégrale glycosylée de la bordure en brosse. Elle a été clônée à partir d'intestins de différentes espèces. Ces protéines SGLT1 partagent entre elles un extraordinaire degré de similitude.

Il est bien établi que les glucides de l'alimentation peuvent moduler les niveaux d'expression de SGLT1 dans la plupart des espèces de mammifères. En utilisant l'intestin de mouton, qui est un modèle unique, nous avons déterminé les mécanismes moléculaires impliqués dans les réponses de l'entérocyte aux changements des glucides alimentaires. En effet, la formation du rumen est une manière naturelle et efficace de priver l'intestin de monosaccharides.

Nous avons montré que ceci mène à la perte de la fonction de transport et de la protéine SGLT1 dans la bordure en brosse des entérocytes; il s'y associe une diminution significative des niveaux d'ARNm de la SGLT1.

Nous avons également montré que l'introduction du D-glucose ou d'un éventail d'analogues du D-glucose dans le contenu luminal d'intestins d'ovins stimule la synthèse de la SGLT1 fonctionnelle. Le signal émis par le monosaccharide luminal est perçu par un détecteur de monosaccharide situé sur la face externe des cellules, dans la région des cryptes de l'intestin. Le signal transmis stimule la transcription du gène de la SGLT1, le transfert de l'ARNm de la SGLT1 et l'insertion de la protéine SGLT1 dans la bordure en brosse des entérocytes situés au-dessous de la jonction entre les cryptes et les villosités.

L'expression de l'ARNm de la SGLT1 et de la protéine fonctionnelle SGLT1 dans l'intestin grêle du fetus d'agneau est détectée dès le 80ème jour après la conception. L'expression de la SGLT1 régulée par transcription au cours du développement prénatal varie dans le même sens que les concentrations de monosaccharides dans le liquide amniotique avalé par le fétus.

En conclusion, le clonage et le séquençage du co-transporteur du glucose et du Na^+

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intestinal ont apporté des outils pour comprendre les méchanismes cellulaires du contrôle de l'absorption intestinale du glucose. C'est le modèle unique du mouton au moment du sevrage qui nous a permis de comprendre comment les monosaccharides présents dans la lumière intestinale régulent à la fois l'expression et l'activité du transporteur intestinal du glucose.

Dietary sugars, D-glucose and D-galactose, are transported from the lumen of the intestine into the enterocytes by the brush-border membrane Na^+–glucose co-transporter protein (SGLT1). The energy for this step is obtained by coupling sugar transport to the Na^+ and electrochemical gradients (membrane potential, $\Delta\mu$ Na^+ approximately -120 mV). The Na^+ gradient is maintained by the active transport of Na^+ out of the cell, across the basolateral membrane of enterocytes, by Na^+/K^+-ATPase (*EC* 3.6.1.37; Crane *et al.* 1961; Wright *et al.* 1994). D-Glucose and D-galactose exit across the basolateral membrane, down their concentration gradients into the systemic system via the facilitated sugar transporter, GLUT2 (Thorens, 1993).

It is well established that, in most species, dietary carbohydrates can modulate the levels and activities of the intestinal brush-border membrane SGLT1 (Ferraris & Diamond, 1989; Shirazi-Beechey *et al.* 1995). Recent advances in the molecular and cellular biology of intestinal sugar transport, and their application to an appropriate animal model, have increased our understanding of the molecular mechanisms involved in the regulation of intestinal glucose transport by lumen sugars. In the present paper I report findings on the molecular mechanisms involved in the control of development and the synthesis of the intestinal SGLT1 in response to signals initiated by lumen sugars.

STRUCTURAL IDENTIFICATION OF INTESTINAL SODIUM–D-GLUCOSE CO-TRANSPORTER PROTEIN

SGLT1 is found in the small intestine of most mammalian species. The rabbit intestinal brush-border membrane SGLT1 was the first to be cloned using the expression-cloning technique (Hediger *et al.* 1987). Subsequently, the human clone was isolated from an intestinal Lambda gt10 library, using the rabbit cDNA as a probe. The rabbit and human intestinal SGLT1 consist of 662 and 664 amino acids respectively (see Table 1). There are 84% identity and 94% similarity between the sequences of these proteins.

Table 1. *Comparison of amino acid sequences of sodium–D-glucose co-transporter proteins*

Tissue source	Identity* (%)	Similarity* (%)	No. of amino acids	Reference
Ovine intestine	100	100	664	Shirazi-Beechey *et al.* (1995), Tarpey *et al.* (1995)
Ovine parotid	100	100	664	Tarpey *et al.* (1994, 1995)
Rabbit intestine	85	93	662	Hediger *et al.* (1987)
Rabbit kidney	85	93	662	Coady *et al.* (1990), Morrison *et al.* (1991)
Rat kidney	88	94	665	Lee *et al.* (1994)
Human intestine	86	94	664	Hediger *et al.* (1989)
Pig kidney	89	95	662	Ohta *et al.* (1990), Wright *et al.* (1994)

* The sequence comparisons are relative to the ovine intestinal sequence.

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Table 2. *Monosaccharide transporters of the small intestine and their substrates* (Modified from Hopfer, 1987)

Sugar transporter	Site	Substrate	Excluded substrates
SGLT1	Brush border	D-Glucose D-Galactose Methyl-α,D-glucose 3-*O*-Methyl-glucose	L-Glucose D-Mannose 2-Deoxy-D-glucose
GLUT2	Basolateral	D-Glucose D-Galactose 2-Deoxy-D-glucose D-Fructose	L-Glucose Methyl-α,D-glucose
D-Fructose (GLUT5)	Brush border	D-Fructose	

SGLT1, sodium–glucose co-transporter protein.

The rabbit intestinal SGLT1 was used also as a probe to screen a lamb intestinal cDNA library. The library did not yield a full length cDNA, and the 5′ end of the cDNA was isolated by a polymerase-chain-reaction-based strategy (Wood *et al.* 1994; Shirazi-Beechey *et al.* 1995).

The composite cDNA sequence of lamb intestinal SGLT1, derived from overlapping clones, encodes a protein of 664 amino acids which exhibits homologies of 85% identity with and 93% similarity to the rabbit intestinal SGLT1 sequence (Table 1). The SGLT1 proteins isolated thus far share an extraordinary degree of similarity, and are stucturally distinct from the facilitative glucose transporters, the GLUT family.

The proposed secondary structure model for SGLT1 indicates that the protein spans the plasma membrane twelve times. Each hydrophobic transmembrane region (M1–M12) is composed of twenty-one amino acid residues arranged in an α-helix. Both the amino and carboxyl termini are on the cytoplasmic face of the membrane, and the single N-linked glycosylation site, at asparagine 248, is on the hydrophilic domain between M5 and M6. Glycosylation increases the mass by about 15 kDa, and the mature protein runs on SDS–PAGE with an apparent mass of approximately 75 kDa (Shirazi-Beechey *et al.* 1991*a*; Wright *et al.* 1994).

The antibody to the synthetic peptide, the sequence of amino acids 402–420, which is identical in rabbit and ovine intestinal SGLT1, has been used to study the distribution of this epitope in different species (Hirayama *et al.* 1991; Shirazi-Beechey *et al.* 1991*a*; Pajor *et al.* 1992). Similarly, the cDNA encoding the rabbit SGLT1 transporter has been used in Northern analysis to examine the distribution of related mRNA in various species (Lescale-Matys *et al.* 1993; Vazquez *et al.* 1993). The results have indicated that the SGLT1 DNA and protein sequences are highly conserved throughout evolution.

TRANSPORTED SUBSTRATES OF SODIUM–D-GLUCOSE CO-TRANSPORTER PROTEIN

The hexose sugars with an equatorial hydroxyl at position C-2 are the preferred substrates of SGLT1. These include the natural dietary sugars, D-glucose and D-galactose and non-metabolized sugars, 3-*O*-methyl-α,D-glucopyranoside and methyl-α,D-glucopyranoside (Semenza *et al.* 1984).

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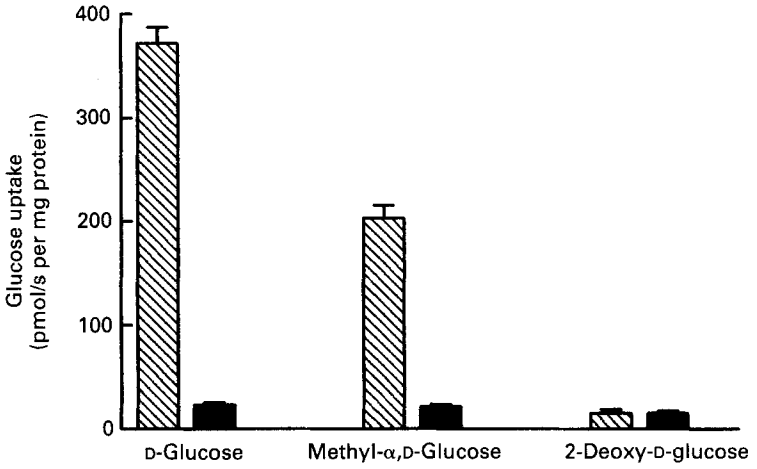

Fig. 1. The initial rate of uptake of D-glucose and various analogues by brush-border-membrane vesicles isolated from the intestinal tissue of ruminant sheep, maintained on a roughage diet, but with their intestine infused with D-glucose through duodenal cannulas for 4 d. This treatment would lead to the induction of functional sodium–D-glucose co-transporter protein in the brush border of enterocytes (see pp. 172 & 173). The uptakes of hexoses into membrane vesicles were measured using incubation media containing either NaSCN (□) or KSCN (■). Values are means with their standard errors represented by vertical bars.

Table 2, taken from a review by Hopfer (1987), depicts the transported and the excluded substrates of the intestinal sugar transporters, SGLT1, GLUT5 and GLUT2. Since then these have been confirmed in various laboratories using a variety of techniques. It is well established that 2-deoxy-D-glucose is not transported by SGLT1 and that methyl-α-D-glucopyranoside, the exclusive substrate of SGLT1, is not handled by GLUT transporters.

To illustrate the substrate specificity of ovine SGLT1 we have demonstrated that membrane vesicles isolated from ovine intestine can transport D-glucose, D-galactose, 3-*O*-methyl-α,D-glucopyranoside, but not 2-deoxy-D-glucose in a Na^+-dependent manner (see Fig. 1).

SHEEP INTESTINE: A UNIQUE MODEL SYSTEM FOR STUDIES ON THE DIETARY REGULATION OF INTESTINAL SUGAR ABSORPTION

In non-ruminant species, the level of sugars reaching the intestinal lumen is maintained during adult life from the digestion of dietary carbohydrates, such as starch and various mixtures of maltodextrins, sucrose, lactose and fructose. Complex carbohydrates are digested in the gut through the action of pancreatic α-amylase (*EC* 3.2.1.1), and brush-border hydrolases. The resultant monosaccharides, D-glucose, D-galactose and D-fructose, are absorbed across the brush-border membrane by SGLT1 and GLUT5 respectively (Shirazi-Beechey *et al.* 1990, 1991*a*; Davidson *et al.* 1992).

Similarly, in preruminant animals such as lambs, the milk sugar, lactose, is hydrolysed by intestinal lactase (*EC* 3.2.1.108) into D-glucose and D-galactose. These sugars are transported by SGLT1 and the rate of absorption is very high (Scharrer, 1975; Shirazi-Beechey *et al.* 1989, 1991*a*).

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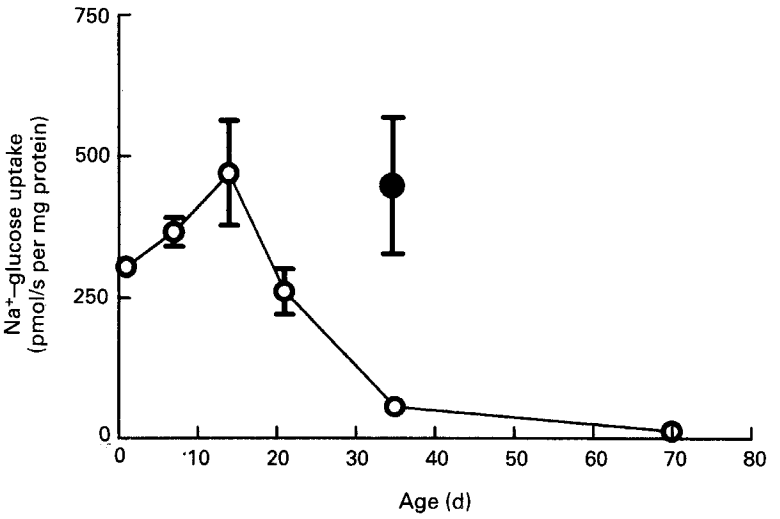

Fig. 2. Initial rates of sodium–D-glucose co-transporter (SGLT1) transport activity measured in brush-border membrane vesicles isolated from the intestinal tissue of lambs reared normally (○) or maintained on a milk replacer diet beyond the weaning period (●). The abundance of the SGLT1 protein correlates with the SGLT1 transport activity. Values are means with their standard errors represented by vertical bars. (From Shirazi-Beechey *et al.* 1991*a*.)

Lambs normally wean at 3–8 weeks and, as their diet changes from milk to grass, the rumen develops. In ruminant sheep, the ingested carbohydrates are fermented to volatile fatty acids in the rumen and as a consequence hardly any monosaccharides reach the small intestine (Leat, 1970; Bassett, 1975). The development of the rumen is associated with a 100–500-fold decline in both SGLT1 function and protein in the brush-border membrane of enterocytes (Scharrer *et al.* 1979; Shirazi-Beechey *et al.* 1989, 1991*a*) and a significant decline in SGLT1 mRNA levels (Freeman *et al.* 1993; Lescale-Matys *et al.* 1993). The reduction in the capacity of the enterocytes to transport D-glucose and D-galactose during postnatal development was correlated with the diminution of monosaccharides reaching the intestine. In contrast, the transport of L-lysine (Scharrer *et al.* 1979) and L-proline (Shirazi-Beechey *et al.* 1989) remained constant. These changes cannot be attributed to modifications in the structure of the absorptive surface (Scharrer *et al.* 1979; Shirazi-Beechey *et al.* 1991*b*); neither can they be explained in terms of an age-related process. Maintaining lambs on a milk replacer diet beyond the normal weaning period prevented the regression of both SGLT1 protein abundance and SGLT1 transport function (Shirazi-Beechey *et al.* 1991*a*; see Fig. 2).

Interestingly, maintaining lambs on a milk replacer diet beyond the normal weaning period had no effect on the pattern of postnatal change in digestive enzyme activities (Shirazi-Beechey *et al.* 1991*b*). In particular, it was notable that the activity of lactase, the brush-border hydrolase which hydrolyses the milk sugar, lactose, to constituent monosaccharides D-glucose and D-galactose, decreases during postnatal development (see Fig. 3). Whilst maintaining lambs on a milk replacer diet beyond the normal weaning period retards the decrease in glucose transport (see Fig. 2), it has no effect on the decline in lactase activity (Fig. 3). Equally, the selective increase in the activity and

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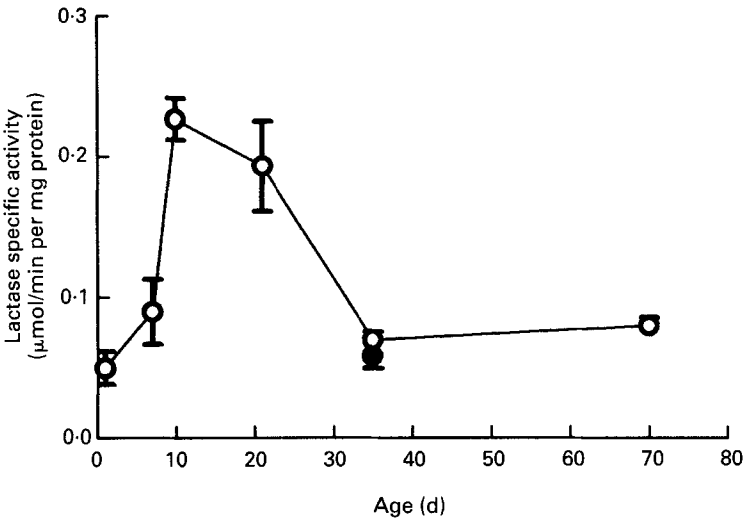

Fig. 3. The specific acitivity of lactase (*EC* 3.2.1.108) in brush-border membrane vesicles isolated from the intestinal tissue of lambs during normal postnatal development (○). The activity of lactase declines to the same level in the intestinal brush border of lambs maintained on a milk replacement diet beyond the normal weaning period (●) compared with the age-matched controls maintained on a roughage diet (○). Values are means with their standard errors represented by vertical bars.

abundance of intestinal brush-border dipeptidyl peptidase IV (*EC* 3.4.14.5) during normal development from preruminant to ruminant state, is unaffected in lambs maintained on a milk replacer diet (Shirazi-Beechey *et al.* 1991*b*). These results indicate the differences between the 'programmed' and 'diet-induced' effects on the enterocyte expression of lactase and the SGLT1.

LUMEN SUGARS AS DIETARY SIGNALS

In order to determine the potential role and the specificity of lumen sugars on the induction of functional SGLT1, the intestinal lumen of ruminant sheep maintained on a roughage diet were infused with glucose and various analogues. Solutions (30 mM, on dilution with intestinal digesta) of D-glucose, D-galactose, methyl-α,D-glucopyranoside, 3-*O*-methyl-α,D-glucopyranoside, D-fructose, 2-deoxy-D-glucose, D-sorbitol and D-mannitol were infused though duodenal cannulas into the intestine for 4 d, whilst the animals were maintained on a roughage diet. The brush-border membrane vesicles isolated from the intestine of these animals were screened for Na^+–glucose co-transport activity and the abundance of SGLT1 protein (Dyer *et al.* 1994). Fig. 4 shows a typical result.

Infusing the intestine with either D-glucose or D-galactose resulted in induction of functional SGLT1, whilst infusion with D-mannitol and D-sorbitol (non-transported, non-metabolizable alditols) did not. The induction of functional SGLT1 in the brush-border membrane by infusion of methyl-α,D-glucopyranoside or 3-*O*-methyl-α,D-glucopyranoside, non-metabolizable substrates of SGLT1, indicates that there is no prerequisite for the substrate to be metabolized by the enterocytes. Methyl-α,D-glucopyranoside cannot be transported out of the cell via GLUT2 into the systemic

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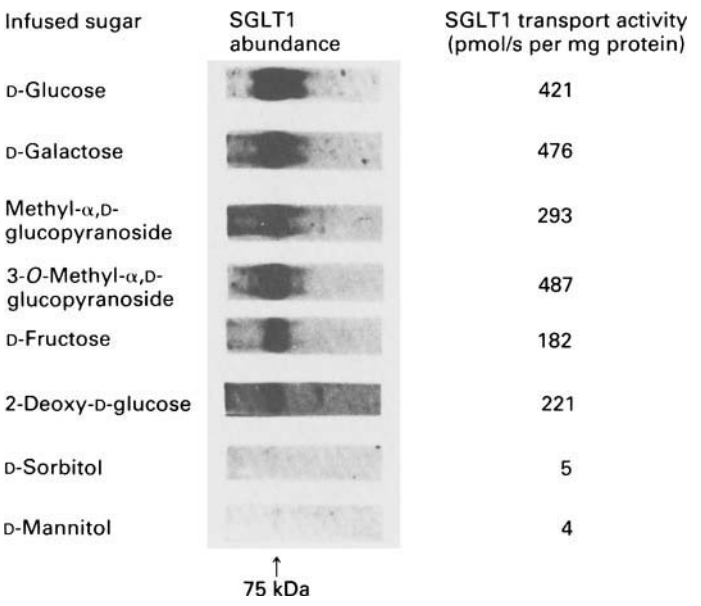

Fig. 4. Induction of functional sodium–D-glucose co-transporter (SGLT1) protein by various sugars. The intestines of ruminant sheep were infused with 30 mM solutions of specified sugars, for 4 d through duodenal cannulas. The values shown are the initial rates of SGLT1 transport activity and the abundance of SGLT1 protein in brush-border membrane vesicles isolated from the intestinal tissues.

system, to impose any systemic effects. Induction of functional SGLT1 by D-fructose and 2-deoxy-D-glucose, implies that the inducing sugar need not necessarily be a substrate of SGLT1. From these results one can speculate the presence of a sugar-sensing system, which has a different sugar specificity from that of SGLT1. Since 2-deoxy-D-glucose is not transported by any known intestinal brush-border membrane protein (see Table 2; Hopfer, 1987; Fig. 1; Wright *et al.* 1994), the induction by 2-deoxy-D-glucose implies that the sugar sensor is located on the external face of the luminal membrane. It should be noted that infusing the systemic system of the ruminant sheep with either D-glucose, methyl-α,D-glucopyranoside or 2-deoxy-D-glucose did not lead to the induction of luminally-located SGLT1.

Immunocytochemical and *in situ* hybridization techniques have been used to determine the profile of the expression of SGLT1 protein and mRNA along the crypt–villus axis of preruminant lamb intestine and ruminant sheep intestine after lumenal infusion with D-glucose (Freeman *et al.* 1993; Shirazi-Beechey, 1995; Shirazi-Beechey *et al.* 1995). Cells isolated sequentially from along the crypt–villus axis were employed for the isolation of brush-border membrane vesicles, and the determination of Na^+-dependent D-glucose transport activity (Dyer *et al.* 1994; Shirazi-Beechey *et al.* 1995). It was shown that the distribution of SGLT1 mRNA along the crypt–villus axis is concurrent with the expression of functional SGLT1 protein. This implies that the SGLT1 gene is transcribed, the mRNA is translated and the functional protein is inserted into the brush-border membrane of enterocytes below the crypt–villus junction.

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The molecular basis of the diet-induced changes in Na^+-dependent D-glucose transport activity were examined by measuring the abundance of brush-border SGLT1 protein, by Western blotting, and mucosal SGLT1 mRNA by Northern hybridization analysis. The diet-induced changes in Na^+glucose co-transporter activity were proportional to the brush-border SGLT1 protein abundance (Shirazi-Beechey *et al.* 1991*a*), but were not matched by corresponding changes in intestinal mRNA levels (Freeman *et al.* 1993; Lescale-Matys *et al.* 1993). It was concluded, therefore, that the principal level of SGLT1 regulation by lumen sugars is post-transcriptional.

EPITHELIAL LOCATION OF THE SUGAR SENSOR

Experiments were designed to determine where, along the crypt–villus axis, the dietary sugar signal is perceived. In one set of experiments, daily biopsies were taken through duodenal cannulas, from the intestine of the sheep which were infused continuously for 4 d with D-glucose, whilst they were maintained on a roughage diet. The functional SGLT1 protein was detected in the brush-border membranes isolated from mature enterocytes (upper villus enterocytes) after 3 d; it was maximal after 4 d.

In a wide-ranging series of experiments it was noted that an infusion of D-glucose into the intestinal lumen of ruminant sheep for 2 h had no effect on the ability of existing mature enterocytes to transport D-glucose. However, the presence of the functional SGLT1 was detected 4 d later in the newly-formed mature enterocytes (Shirazi-Beechey *et al.* 1994, 1995).

The simplest interpretation of these results is that the signal-receiving site is localized within the crypt. The programming of the crypt enterocytes to synthesize SGLT1 is rapid and the observed lag for the appearance of functional SGLT1 in the upper villus enterocytes is correlated with cell migration time along the crypt–villus axis; the migration time being 3–4 d in ovine intestine (Attaix & Meslin, 1991). One cannot exclude, however, a possible villus location of the receptor. In this case the receptor could be linked to crypt events via a neural or paracrine mechanism.

LUMEN SUGARS AND THE EXPRESSION OF INTESTINAL SODIUM-D-GLUCOSE CO-TRANSPORTER PROTEIN IN THE PRENATAL STATE

The initial appearance of the intestinal SGLT1 differs among various species, with those species with long gestation periods expressing the transporter earlier. Using everted sacs of the jejunum and the ileum of human fetuses, it has been shown that the human fetal intestine is capable of actively transporting D-glucose as early as the second quarter of gestation (Koldovsky *et al.* 1965).

In sheep, having a long gestation period of 150 d, functional SGLT1 protein was detected in brush-border membrane vesicles isolated from the fetal lamb intestine as early as 80 d post-conception. There were no detectable levels of SGLT1 protein in the intestine of 60-d-old fetuses. The abundance and activity in the brush-border membranes increased progressively from 80 d post-conception, and reached a level similar to that detected in the intestinal brush-border membrane of the newborn lamb (see Fig. 5).

Notably, the apparent molecular mass of the specific immunoreactive band was 65 kDa in the intestinal brush-border membranes of 80 and 90 d fetuses, and 75 kDa in the membranes of >100 d fetuses; the latter being similar to the size of the protein detected

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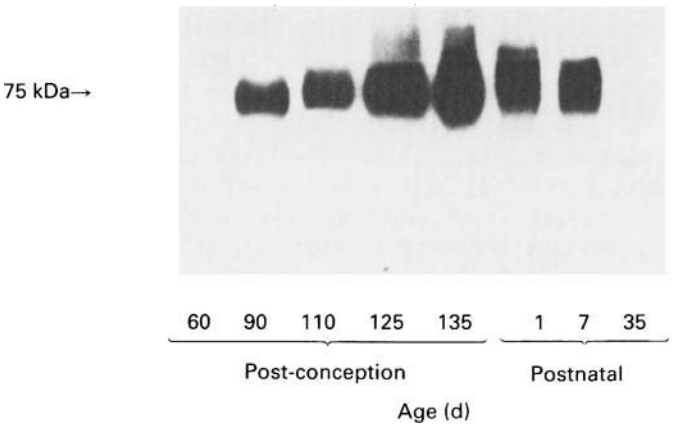

Fig. 5. The expression of sodium–D-glucose co-transporter (SGLT1) protein in brush-border membrane vesicles isolated from the intestinal tissues of fetal lambs throughout gestation. The antibody to a nonadecapeptide, amino acids 402–420 of ovine SGLT1 sequence, recognizes a specific 65–75 kDa protein in the brush-border membrane vesicles, the abundance of which, measured densitometrically, increases 40-fold from 80 to 135 d post-conception. The Na^+-dependent glucose transport activity correlates with the levels of protein at various stages. The abundance and the activity of SGLT1 protein remain constant from 135 d post-conception to birth, and are equal to the levels in newborn lamb.

postnatally. These differences in size could be due to post-translational modification of the protein. The expression and abundance of SGLT1 mRNA and protein were synchronized and matched, indicating the involvement of a transcriptional regulatory mechanism (S. P. Shirazi-Beechey and S. M. Gribble, unpublished results).

The profile of the expression of digestive activity differed from that of SGLT1. The activities of lactase and aminopeptidase N (*EC* 3.4.11.2) were detected as early as 60 d post-conception. The activities of these brush-border enzymes remained constant throughout fetal development, and were similar to those detected in the newborn lamb (for levels detected in newborn lamb, see Fig. 3). The activity and the abundance of alkaline phosphatase (*EC* 3.1.3.1) followed a profile similar to that of SGLT1 (S. P. Shirazi-Beechey and S. M. Gribble, unpublished results). This differential expression of various intestinal brush-border membrane proteins implies that they may be controlled by different regulatory mechanisms.

The role of lumen nutrients in the maintenance of intestinal structure and function during fetal development has been addressed by many workers (Grand *et al.* 1979; Phillips *et al.* 1991; Buchmiller *et al.* 1992). During its life *in utero* the fetus is supplied with most of its substrate needs via the placenta. However, it is not often appreciated that the fetus also swallows large volumes of fetal fluid, which crosses the gastrointestinal tract. The volume and the constituents of this fluid increase progressively during gestation. The fluid swallowed during the final month of gestation is as high as 750 ml/d in the human fetus (Pritchard, 1966), and 500 ml/d in the ovine fetus (Harding *et al.* 1984). It contains several nutrients, including amino acids and D-glucose in human fetal fluid (Geigy Scientific Tables, 1981), and largely D-fructose in ovine fetal fluid (Mellor & Slater, 1974). It is estimated that 10–15% of the fetal energy requirement may be normally supplied through this enteric route (Pitkin & Reynolds, 1975).

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Clinical studies have demonstrated impaired gastrointestinal function in infants with congenital defects like oesophageal artesia and intestinal artesia Cohen & Greecher, 1979), both of which restrict gastrointestinal access to swallowed input. It was also demonstrated that the oesophageal ligation of fetal rabbits caused depression of brush-border nutrient transport in the small intestine of these animals. The intra-amniotic galactose infusion to fetal rabbits caused up-regulation of SGLT1 function (Buchmiller *et al.* 1992). Galactose is not normally present in the amniotic fluid; therefore, it appears that in most mammalian species, the lumen sugar regulates the activity and the expression of intestinal SGLT1 throughout the life of the animal, postnatally as well as in the prenatal state.

CONCLUSION

The cloning and sequencing of the intestinal SGLT1 have provided molecular tools to investigate the cellular processes controlling intestinal glucose transport. Rumen development in sheep, a natural and efficient way of depriving the small intestine of monosaccharides, has presented a unique model for studying how lumen sugars regulate the expression and activity of the intestinal glucose transport.

However, the development of a suitable cell line and further work, such as: sequencing of the promoter region of the ovine SGLT1 gene, the identification of the sugar sensor, the signalling pathway, and the molecular mechanisms involved in the intracellular synthesis and processing of SGLT1, are needed to provide a complete picture of how dietary sugar regulates the intestinal sugar transport. It is clear that the young and growing field of the nutrient regulation of intestinal nutrient transporters requires much more nurturing.

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