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# $Na^+$/glucose co-transporter abundance and activity in the small intestine of lambs: enhancement by abomasal infusion of casein

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The purpose of the present study was to determine the effect of abomasal casein infusion on glucose uptake and abundance of the $Na^+$/glucose co-transporter (SGLT1) 1 in the ovine small intestine. Lambs (body weight 35 (SEM 1·0) kg) were surgically fitted with abomasal infusion catheters and were fed diets containing equal portions of wheat hay and cracked maize. Lambs were infused with either 500 g water/d or with 500 g water containing 35 g casein/d. The infusion period lasted 10 d, after which lambs were killed, exsanguinated and eviscerated. Brush border membrane vesicles (BBMV) were prepared using mucosa from different small intestinal regions. Intake and total tract digestibility of nutrients were similar between treatments and averaged 1134, 1142 and 486 g/d and 67, 70, and 94 % for DM, organic matter and non-structural carbohydrates respectively. Crude protein (N×6·25) digestibility was 15 % greater in the casein-infused than control lambs. Glucose uptake to BBMV ranged from 101 to 337 pmol/mg protein per s along the small intestine and was greatest in the mid-section of the small intestine. In the mid-jejunum, glucose uptake was greater ($P<0·07$) in lambs infused with casein and averaged 120 pmol/mg protein per s compared with 68 pmol/mg protein per s in the control group. SGLT1 affinity was similar between treatments and averaged 104 μM in the different segments of the small intestine of lambs. However, lambs infused with casein exhibited similar values along the small intestine and affinity averaged 106 μM, while in the control group a greater affinity (85 μM) was measured in the mid-jejunum. SGLT1 protein abundance was correlated with glucose uptake in the BBMV in the casein-treated lambs, but not in the control group. These results suggest that glucose uptake along the small intestine of lambs is influenced by casein or its derivatives in the small intestine via SGLT1 affinity and activity at the brush border membrane, and that SGLT1 activity may be regulated by post-translational events affected by amino acids and peptides.

**Starch digestion: Glucose transporter: Sheep**

Grains are a major carbohydrate (CHO) source in ruminant animal production for meat and milk. Starch is the predominant CHO in grains and supplies substrate both for the rumen microflora and for energy utilized by the host animal. In diets containing high proportions of grains, some starch escapes rumen fermentation and passes intact to the small intestine (Ørskov *et al.* 1970). Starch fermentability in the rumen differs between grains; up to 42 % of dietary starch from maize and sorghum fed to cattle reaches the small intestine, where 47–88 % is digested (Owens *et al.* 1986). However, digestion of starch is not always complete. This was first reported by Larson *et al.* (1956) and Huber *et al.* (1961), who showed that abomasal starch infusion failed to elevate blood glucose concentration to the same degree as glucose, lactose or maltose infusions. As starch flow to the small intestine increases, starch is digested with decreasing efficiency, resulting in a loss of potential metabolizable energy (Huntington, 1997). When the starch reaches the small intestine, digestion commences with α-amylase hydrolysis and the final stages of digestion are affected by oligosaccharidases located in the brush border membrane of the enterocyte. Absorption of glucose units is predominantly via the $Na^+$/glucose co-transporter (SGLT) 1 (Harmon, 1993). It was concluded that inadequate pancreatic α-amylase activity is the primary reason for incomplete

**Abbreviations:** BBMV, brush border membrane vesicle; CHO, carbohydrate; CP, crude protein (N×6·25); SGLT, $Na^+$/glucose co-transporter; $V_{max}$, maximal velocity.

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starch digestion in the small intestine of ruminant animals (Huntington, 1997). Pancreatic α-amylase secretion and activity were negatively affected by post-rumen starch concentration (Kreikemeier *et al.* 1990; Walker & Harmon, 1995; Swanson *et al.* 1998). It has also been observed that duodenal starch content had no influence on disaccharidase and SGLT1 activities in steers (Bauer, 1992).

Protein and protein derivatives infused into the duodenum affect pancreatic secretions, including that of α-amylase in single-stomached animals (Shimizu *et al.* 1994). Recently, it was suggested that dietary regulation of pancreatic α-amylase expression is complex, and probably regulated by both transcriptional and post-transcriptional events (Swanson *et al.* 2000).

The abundance of SGLT1 mRNA in lambs changes with age; it was 4-fold greater in the pre-ruminant stage and activity of SGLT1 was 150-fold greater than the ruminant stage when animals consumed grass pasture (Lescale-Matys *et al.* 1993). Glucose infusion into the small intestine was found to increase both activity and expression of SGLT1 in lambs (Allison & Shirazi-Beechey, 1997; Dyer *et al.* 1997). Despite these studies, little information is available concerning the connection between SGLT1 expression and activity and quantitative glucose absorption in the small intestine of ruminant animals.

The purpose of the present study was to investigate the relationship between SGLT1 abundance and activity, and in addition, to determine the effect of duodenal casein infusion on SGLT1 activity and glucose uptake along the small intestine. This was determined in lambs fed diets rich in maize starch, which is not completely fermented in the rumen. We hypothesized that casein infusion would enhance starch hydrolysis by increasing α-amylase activity, thus increasing glucose availability, which would then upregulate expression and activity of SGLT1.

## Methods

### *Animals and experimental design*

All procedures were approved by the Animal Care and Ethics Committee of the Hebrew University of Jerusalem. Twelve lambs (body weight 35·1 (SEM 1·0) kg) were surgically fitted with abomasal infusion catheters as previously described (Gross *et al.* 1990). Lambs were fed diets containing equal portions of wheat hay and cracked maize grain. Diets contained (g/kg DM): crude protein (CP, N×6·25) 93, neutral-detergent fibre 385, acid-detergent fibre 220, non-structural CHO 400; the diets provided 11·88 kJ (2·84 kcal)/kg DM digestible energy. Diets were formulated to maintain moderate growth of at least 50 g/d during the experimental period. Lambs were group-fed for 10 d before the beginning of the experiment and then placed in individual metabolism crates in an environmentally controlled room at 22°C with 70 % relative humidity and 16 h light–8 h dark cycle. Crates were equipped with automatic feeders to provide twelve equal meals and water was available *ad libitum*. Lambs were randomly allotted to two treatment groups (*n* 6 per group), where the control group received an aqueous abomasal infusion (500 g/d) and the casein group an infusion of 35 g casein dissolved in 500 g water/d, provided by a peristaltic pump (Minipuls 2; Gilson, Villieres le Bel, France). The infusion period lasted 10 d. During the last 8 d, Co-EDTA was added to the infusate as a non-absorbable reference substance (Uden *et al.* 1980). Faeces and urine (acidified with $H_2SO_4$ (100 ml/l)) were collected during the last 5 d of the infusion period, composited within animal across the sampling days and stored at −20°C for later analysis. Jugular blood samples were withdrawn by venepuncture on the last day of the experimental period before tissue collection, and centrifuged at 3000 ***g*** for 10 min at 4°C to separate plasma; this was stored at −20°C for later analysis.

### *Tissue harvesting and sample collection*

Lambs were killed, exsanguinated and eviscerated. The small intestine was detached and freed from the mesentery, and small intestinal segments were immediately separated by tightening a cotton thread around the tissue to prevent mixing of digesta from the different sections. The duodenum and the next four 1 m segments were removed and the remaining intestine to the ileo–caecal junction were removed as 2 m segments. Duodenum, and segments 4 and 10 m post-duodenum were referred as duodenum, mid-jejunum and distal ileum respectively. Digesta was gently removed from each section into marked and pre-weighed containers, and segments were placed on Al sheets placed on an iced-water bath. Tissue samples were removed from the midpoint of each section and immediately frozen in liquid $N_2$ and stored at −80°C for further analysis. The rest of the section was opened longitudinally and flushed with cold saline (9 g NaCl/l) to remove residual digesta; the mucosal layer was removed by gentle scraping with a Teflon slide into marked containers, frozen in liquid $N_2$ and stored at −80°C for further preparation.

### *Preparation of brush border membrane vesicles*

Brush border membrane vesicles (BBMV) were prepared using $MgCl_2$ precipitation and sequential centrifugation as described by Zhao *et al.* (1998). The final protein concentration in BBMV was 10–20 mg/ml. Portions (100–200 μl) of BBMV were frozen in liquid $N_2$ and stored at −80°C until use. Purity of BBMV was determined by the enrichment in specific activity of γ-glutamyl transpeptidase, which was 14–22-fold greater than in the corresponding homogenates.

### *Assay of glucose uptake in brush border membrane vesicles*

SGLT1 uptake activity was determined at 37°C as described by Shirazi-Beechey *et al.* (1991). Briefly, a suspension of 10 μl BBMV was added into 190 μl solution containing either 150 mM-NaCl or -KCl and 30 μM-D-glucose containing D-[6-$^3$H(n)]glucose (37·00 kBq/ml; Sigma Chemical, St Louis, MO, USA) The reaction was stopped after 3 s by addition of 2 ml ice-cold solution containing 150 mM-NaCl and 0·25 mM-phlorizin (Lescale-Matys *et al.* 1993). Uptake was measured in duplicate in each BBMV for each

lamb within treatments. The variation in uptake was $<2\%$ in multiple assays of the same preparation. The SGLT1 kinetics were measured in pooled BBMV of the six lambs in each treatment in three segments; duodenum, mid-jejunum and distal ileum. D-Glucose concentrations ranged from 1 to 200 μM prepared in 150 mM-NaCl or -KCl. $Na^+$-dependent uptake activity was calculated as the difference between uptakes measured in the presence and absence of $Na^+$. Lineweaver–Burke plots were utilized to calculate the kinetic variables $K_m$ and maximal velocity ($V_{max}$).

### *Western blot analysis*

BBMV from duodenum, mid-jejunum and distal ileum from each individual sheep were subjected to immunoblot analysis. Samples (21 μg protein) were separated by SDS-PAGE on 10% gels under reducing conditions (Laemmli, 1970). Following electrophoresis, proteins were transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). After blocking with TBS-T (50 mM-Tris-HCl pH 7·5, 150 mM-NaCl and Tween 20 (5 ml/l)) containing bovine serum albumin (30 g/l), membranes were incubated overnight at 4°C with rabbit-anti SGLT1 (1:50; Biogenesis, Poole, Dorset, UK). Membranes were then washed with TBS-T and visualized using enhanced chemiluminescence by incubating for 1 h with horseradish (*Amoracia rusticana*) peroxidase-conjugated donkey secondary anti-rabbit at room temperature (1:50 000; Biogenesis).

### *Chemical analyses and calculations*

CP (N×6·25), DM and organic matter in feeds and faeces were determined in accordance with the procedures of the Association of Official Analytical Chemists (1990). Non-structural CHO was determined as described by Smith (1981) using ferrocyanide as the colorimetric indicator. The neutral-detergent and acid-detergent fibre fractions were measured according to the method of Van Soest *et al.* (1991). Digestible energy of the diet was calculated from National Research Council (1989). Protein content of BBMV was determined using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). The concentrations of Co in digesta were measured by inductively-coupled plasma atomic emission spectrometry (Spectro Gmbh, Kleve, Germany), following wet digestion with concentrated HCl. Total tract digestibility (%) was calculated for each nutrient (Nt) as follow:

$$\text{digestibility}_{Nt}\ (\%) = 100 \times (\text{intake}_{Nt}\ (g) - \text{faecal output}_{Nt}\ (g))/\text{intake}_{Nt}\ (g).$$

Net disappearance of nutrients at the small intestine was calculated as the difference between duodenal and ileal flow. Nutrient flow was calculated from the infusion rate and the concentration of Co in the corresponding digesta:

$$\text{flow}_{Nt}\ (g/d) = I_{Co}\ (mg/d)/\text{Co in digesta}\ (mg/g\ DM),$$

where I is the infusion rate.

Digital images of the autoradiographic film from the immunoblot analyses were scanned (HP DeskScan II; Hewlett Packard, Palo Alto, CA, USA) and the intensities of bands were determined using the UN–SCANIT software program (Silk Scientific; Orem, UT, USA). Data were normalized to protein content and are reported as arbitrary units per mg protein.

### *Statistical analysis*

Data were analysed as using ANOVA to compare treatment effects by the GLM procedures of SAS (*SAS® User's Guide: Statistics*, version 5, 1985; SAS Inst. Inc., Cary, NC, USA). The linear model included the effect of treatment and the residual error term. Two–way ANOVA was also conducted to test the effect of treatment and intestinal region on SGLT1 activity along the small intestine. Means were separated using Student's *t* test and differences were considered significant when $P<0{\cdot}05$; tendency was declared at $0{\cdot}05>P>0{\cdot}10$. Results are reported as least squares mean values with their standard errors.

## Results

### *Feed intake and digestibility*

Feed intake, total gastrointestinal tract and small intestinal digestibility of nutrients are shown in Table 1. In general, nutrient intake and total gastrointestinal tract digestibility were similar between treatments and averaged 1134 and 486 g/d, and 68 and 94%, for DM and non-structural CHO respectively. CP (N×6·25) intakes, corrected for casein infusion and digestibility, were 28 and 15% greater in lambs infused with casein than in the control group respectively. Small intestinal DM flow and net disappearance were similar for both treatments and averaged 611 and 278 g/d respectively. Small intestinal net disappearance of non-structural CHO was greater in the casein-infused lambs by 22 g/d compared with the control group, which resulted in an 18% increase in digestibility. In addition, net disappearance (g/d) of CP was greater by 54% in the casein-infused lambs compared with the control group ($P<0{\cdot}06$): this was a 13% increase in the CP digestibility.

### *$Na^+$/glucose co-transporter activity and kinetic measurements along the small intestine*

Glucose uptake (pmol/mg protein per s) was measured in BBMV along the small intestine and similar trends with intestinal site were observed in both treatments (Fig. 1). Furthermore, the uptake profile for glucose along the small intestine was similar in both treatments. In the duodenum, and segments 2, 3, 6, 8, 10 and 14 m post-duodenum, glucose uptake was moderate ($P<0{\cdot}05$) compared with distal sections and averaged 63 pmol/mg protein per s, while in the proximal section of the jejunum (1 m post-duodenum), the mid-jejunum (4 m post-duodenum), and 12 and 16 m post-duodenum, glucose uptake was greater ($P<0{\cdot}05$) and averaged 100 pmol/mg protein per s. Comparing uptake between the two treatments indicated that in the mid-jejunum glucose uptake tended to be greater

**Table 1.** Feed intake, flow to small intestine and apparent total gastrointestinal tract digestibility of nutrients by sheep abomasally infused with water (500 g/d) or casein (35 g dissolved in 500 g water/d)*

(Mean values for six sheep per group)

| | Treatment | | | |
|---|---|---|---|---|
| | Water | Casein | SEM | Statistical significance of effect: *P* |
| Intake (g/d) | | | | |
| DM | 1127 | 1140 | 60·8 | 0·890 |
| Crude protein†‡ | 123 | 157 | 5·84 | 0·002 |
| Non-structural CHO | 480 | 492 | 28·6 | 0·780 |
| Flow to small intestine (g/d) | | | | |
| DM | 593 | 628 | 3·10 | 0·447 |
| Crude protein‡ | 146 | 201 | 18·0 | 0·06 |
| Non-structural CHO | 64·8 | 82·9 | 7·38 | 0·121 |
| Net disappearance at small intestine (g/d) | | | | |
| DM | 261 | 295 | 3·78 | 0·547 |
| Crude protein‡ | 107 | 165 | 18·3 | 0·056 |
| Non-structural CHO | 42·8 | 64·3 | 5·34 | 0·021 |
| Digestibility (%) | | | | |
| Total GIT | | | | |
| DM | 65·0 | 70·0 | 1·62 | 0·400 |
| Crude protein‡ | 61·8 | 70·9 | 2·38 | 0·023 |
| Non-structural CHO | 94·1 | 94·6 | 0·58 | 0·673 |
| Small intestine§ | | | | |
| DM | 43·7 | 46·3 | 4·72 | 0·704 |
| Crude protein‡ | 72·2 | 81·3 | 3·37 | 0·094 |
| Non-structural CHO | 66·2 | 77·9 | 1·64 | 0·001 |

CHO, carbohydrate; GIT, gastrointestinal tract.
* For details of diets and procedures, see p. 574.
† Casein infusion was added to crude protein intake.
‡ Crude protein = N × 6·25.
§ % Duodenal flow.

($P<0·07$) in lambs infused with casein compared with those with water only infusion (120 *v.* 68 pmol/mg protein per s respectively). The overall small intestinal glucose net uptake estimated from the weighted means of the individual segments along the small intestine was 909 pmol/mg protein per s in the casein-infused lambs compared with 767 pmol/mg protein per s in the control group (SEM 84·1, $P<0·06$).

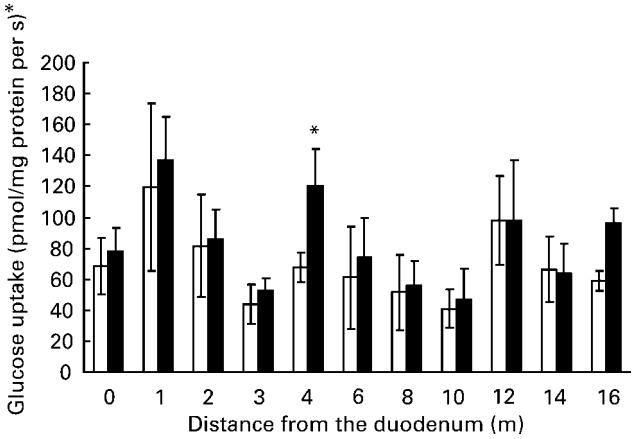


**Fig. 1.** Glucose uptake profile along the small intestine of lambs infused with 500 g water/d (□) or 500 g water plus 35 g casein/d (■). Uptake was measured in brush border membrane vesicles and values on the *x* axis describe distance from the duodenum. Sections 1, 4, 12 and 16 m post-duodenum exhibited high glucose uptake (mean value 100 pmol/mg protein per s, $P<0·05$) compared with low uptake in all other sections (mean value 63 (SEM 16·5) pmol/mg protein per s). For details of procedures, see p. 574. Values are means with standard errors shown by vertical bars. Mean values were significantly different from those of the control group: *$P<0·07$.

The Lineweaver–Burke plots were made for the three segments describing kinetic variables for SGLT1. These were all significant linear regressions ($P<0·001$) with high correlation coefficients ($R^2$ 0·98–0·99). The corresponding variables calculated from these regressions are summarized in Table 2. The $V_{max}$ values ranged from 117 to 337 pmol/mg protein per s, and were significantly greater ($P<0·032$) for the casein treatment in the mid-jejunal section. The maximal activity profiles had a similar pattern in both treatments with activity greatest in the duodenum, intermediate in the distal ileum and least at the mid-jejunum. The transporter affinity in BBMV in the small intestine of lambs was similar between treatments and averaged 104 μM. The $K_m$ values along the small intestine of casein infused lambs was similar and averaged 106 μM. However, in lambs infused with water, greater affinity (smaller $K_m$) was measured in the mid-jejunum (85 μM), intermediate in distal ileum (93 μM) and smaller in the duodenum (131 μM).

### *Western blot analysis*

Western blot analysis with rabbit-anti SGLT1 detected a protein of 75 kDa (Fig. 2 (A)) in all BBMV prepared from the small intestine segments where kinetic measurements were made. The densitometric analysis, corrected for protein content, indicated that in the duodenum 2·7-fold more

**Table 2.** Kinetic variables of Na+/glucose co-transporter (SGLT) 1 along the small intestine of sheep abomasally infused with water (500 g/d) or casein (35 g dissolved in 500 g water/d)*

| | Treatment | | | |
|---|---|---|---|---|
| | Water | Casein | SEM† | Statistical significance of effect: *P* |
| $V_{max}$ (pmol/mg protein per s) | | | | |
| Duodenum | 274·0[a] | 336·7[a] | 23·9 | 0·205 |
| Mid-jejunum | 116·6[c] | 164·5[b] | 6·17 | 0·032 |
| Distal ileum | 220·0[b] | 256·3[ab] | 17·6 | 0·280 |
| SEM‡ | 5·27 | 23·8 | | |
| $K_m$ (μM) | | | | |
| Duodenum | 130·6[a] | 115·8[a] | 9·68 | 0·392 |
| Mid-jejunum | 84·8[b] | 96·40[a] | 4·03 | 0·181 |
| Distal ileum | 93·0[ab] | 104·4[a] | 9·61 | 0·491 |
| SEM‡ | 6·82 | 9·44 | | |

$V_{max}$, maximal velocity.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different. ($P<0·05$).
* For details of diets and procedures, see p. 574.
† SEM between treatment.
‡ SEM within segments between treatments.

($P<0·05$) SGLT1 protein was found in BBMV from lambs infused with casein compared with the control group. In the mid-jejunum, this pattern was reversed, with SGLT1 abundance greater ($P<0·05$) by 1·6-fold in BBMV in the control group compared with the casein-infused group. Similar SGLT1 protein abundance was found in both treatments in the distal ileum (Fig. 2 (B)).

The relationship between $V_{max}$ and SGLT1 abundance was examined in the two treatments in Fig. 3 (A and B). There was no significant linear correlation between $V_{max}$ and SGLT1 abundance in BBMV from the small intestine of the control group (Fig. 3 (A)); in contrast, a significant linear correlation (Fig. 3 (B), $R^2$ 0·99) was observed for lambs on the casein treatment.

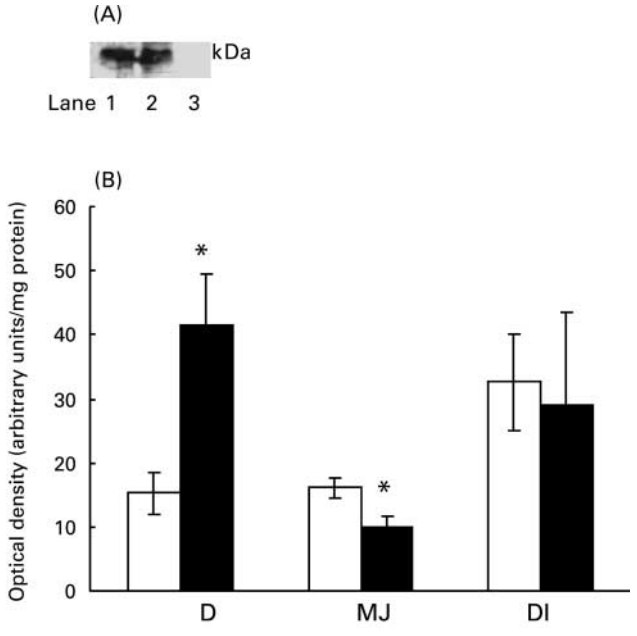


**Fig. 2.** (A), immunoblot of Na+/glucose co-transporter (SGLT) 1 in brush border membrane vesicles (BBMV). Lanes 1 and 2 contain 21 μg protein from BBMV of two lambs and lane 3 contains molecular mass standards. (B), optical density of SGLT1 in the duodenum (D), mid-jejunum (MJ) and distal ileum (DI) in lambs infused with 500 g water/d (□) or 500 g water plus 35 g casein/d (■). For details of procedures, see p. 574. Values are means with standard errors shown by vertical bars. Mean values were significantly different between treatments: *$P<0·05$.

## Discussion

The present study investigated the association between SGLT1 abundance and activity and the mechanisms by which enhanced levels of protein in the ovine duodenum influence small intestinal digestion and absorption of CHO.

Nutrient intake in the current study was not affected by the protein infusion. Digestion of non-structural CHO, mostly starch, was 94 % over the whole gastrointestinal

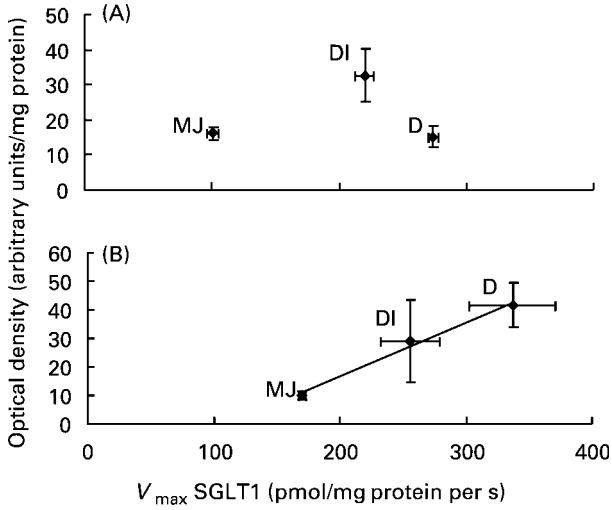


**Fig. 3.** The relationship between maximal activity ($V_{max}$) of Na+/glucose co-transporter (SGLT) 1 in brush border membrane vesicles and optical density in the duodenum (D), mid-jejunum (MJ) and distal ileum (DI) in (A) lambs infused with 500 g water/d or (B) 500 g water plus 35 g casein/d ($R^2$ 0·9897). For details of procedures, see p. 574. Values are means with standard errors.

tract. Similar values of high starch digestibilities in the whole gastrointestinal tract in lambs have been reported when diets containing a high concentration of starch were consumed at 1·2 and 1·8 × maintenance requirements (Swanson *et al.* 2000) and in diets containing 65 % concentrated feeds (Hussein *et al.* 1991). These values parallel starch digestibility in high-producing dairy cows fed high levels of maize in the diet (Knowlton *et al.* 1998; Shabi *et al.* 1999) or infused with maize starch (Arieli *et al.* 2000), and in beef steers fed lucerne hay (Taniguchi *et al.* 1995).

The flow of non-structural CHO to the duodenum tended towards slightly greater values (NS) in the casein-infused lambs; the reason for this is not clear, as abomasal infusion would not be expected to influence rumen activity. Apparent small intestinal digestibility and net intestinal disappearance of non-structural CHO were low compared with total gastrointestinal tract digestibility. This is the normal pattern reported for ruminant animals (Huntington *et al.* 1997) and parallels high-producing dairy cows (Shabi *et al.* 1999; Arieli *et al.* 2000). However, abomasal casein infusion in the current study increased the digestibility and apparent intestinal net disappearance of non-structural CHO in the small intestine by 18 and 50 % respectively.

Apparent total gastrointestinal tract digestibility of CP (N × 6·25) indicated that almost complete post-rumen digestion (calculated by difference) of the infused casein took place. Net disappearance of CP along the small intestine indicated that casein net absorption was complete by the proximal ileum. In the casein-infused lambs, an additional 58 g CP disappeared from the small intestine compared with the control group. This amount is greater than the original casein infused (35 g/d). This difference is probably due to more endogenous CP secreted to the duodenum when casein was infused. This possibly includes additional secretion of pancreatic enzymes to the intestinal lumen (see later for discussion).

The SGLT1 affinity for glucose ($K_m$) in different regions of the small intestine were within the ranges previously reported for chickens (25–150 μM (Gal-Garber *et al.* 2000)), cattle (100 and 63 μM (Zhao *et al.* 1998; Bauer *et al.* 2001)), and rats, rabbits and human subjects (13–490 μM (Freeman & Quamme, 1986; Panayotova-Heiermann *et al.* 1996)). However, the current values are greater than the range reported by Shirazi-Beechey *et al.* (1991) in sheep (36–40 μM). This could be due differences in the diets, species of sheep, or to the adaptation the sheep underwent in the different experiments. Results of SGLT1 activity (e.g. glucose absorption; $V_{max}$) in the current study suggested maximal absorption in the proximal small intestine. A similar pattern for starch digestion was observed in steers for the proximal half of the small intestine (Krehbeil *et al.* 1996). This parallels the main site of nutrient absorption for lipids, minerals and protein along the small intestine of sheep (Sklan & Halevy, 1985; Sklan & Hurwitz, 1985; Sklan *et al.* 1985).

SGLT1 activity in the proximal jejunum, and in the whole small intestine, was increased in lambs infused with casein. This paralleled the greater apparent non-structural CHO disappearance. Richards *et al.* (1998) reported that abomasal casein infusion in steers resulted in a linear increase in pancreatic α-amylase secretion and activity, and this was also reported in calves fed diets based on soyabean meal (Le Drean *et al.* 1995). Similarly, casein infusion in lambs resulted in an increase in plasma glucose concentration (Barry *et al.* 1982). In the current study, greater amylase activity would produce more glucose in the proximal small intestine in lambs infused with casein and thus influence uptake.

Determination of the relationship between abundance of SGLT1 protein and SGLT1 activity indicated a linear relationship in casein-infused lambs, but not in the control group. This suggests a response to the infused casein either of SGLT1 expression, activity or turnover. Lambs infused with casein in the current study exhibited constant transporter affinity along the small intestine, as compared with changing affinity in the control group. This could be due to casein itself or its hydrolytic products, possibly peptides or amino acids. However, it has been observed that dietary CHO entering the small intestine regulates SGLT1 gene expression and activity in small intestine of ruminant animals (Shirazi-Beechy *et al.* 1994, 1996). It has been suggested that such regulation by lumen sugars is primarily controlled at both transcriptional and post-transcriptional level in sheep enterocytes (Freeman *et al.* 1993; Lescale-Matys *et al.* 1993). Although increased CHO entered the small intestine in the present study, it is possible that SGLT1 activity may also be controlled by events that may require peptides and amino acids. However, other factors have been shown to modulate SGLT1 activity. In the pig and rabbit it was shown that the RS1 protein influences SGLT1 transport activity in different tissues (Veyhl *et al.* 1993; Reinhardt *et al.* 1999). The regulatory action of RS1 and its interaction with membrane transporters is not yet fully understood. Results from the present study suggest that amino acids and peptides might provide some extra or intracellular signal to activate SGLT1 protein. In the avian jejunum, nutritional status (fasting *v.* re-feeding) was shown to affect expression, activity and affinity of SGLT1 (Gal-Garber *et al.* 2000). Additional factors have also been shown to regulate SGLT1 transporter activity, both directly and indirectly, in different species and cell lines. Protein kinase C downregulates rabbit SGLT1 activity in COS-7 cells by a direct effect on the transporter (Vayro & Silverman, 1999). The effect described in that study was due to a reduction in $V_{max}$ with no change in the number of cell surface SGLT1; this caused a 2-fold reduction of the transport turnover rate. In small and large intestine of human subjects, synthetic endothelin-1 significantly inhibited glucose absorption via reduction of SGLT1 activity *in vitro* (Kuhn *et al.* 1997). The current experiment was designed to measure the effect of the diet on the active transportation of glucose from the intestinal lumen, which was measured *in vitro* in BBMV. The diffusive mechanism responsible for intestinal glucose absorption, however, was not investigated; its contribution might be a major route *in vivo*, as was shown recently in rat jejunum (Kellett & Helliwell, 2000). This route is believed to be via the glucose transporter 2 system at the brush border membrane level and controlled by the transport of glucose by SGLT1. Hence, the results of

the present study should be limited within the context of the experimental design described herein.

Clearly, SGLT1 activity is susceptible to a wide range of modifiers and modulators. The present study suggests casein should be added to that list, although whether the effect is direct or a consequence of metabolic perturbation induced by the additional casein supply remains to be elucidated.

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