Original Research

Characterization of D-Glucose Transport across Equine Jejunal Brush Border Membrane Using the Pig as an Efficient Model of Jejunal Glucose Uptake

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\textbf{ABSTRACT}

To test the hypotheses that glucose transport capacity across the brush border membrane (BBM) of the large colon is lower than that of the small intestine in equids, and that small intestinal transport capacity in equids is lower than suids. D-glucose transport capacity (V_{\text{max}}) and affinity (K_M) across the BBM of the distal jejunum (DJ) and proximal large colon (PLC) of the pony and pig were measured. Mucosa was collected from the DJ and PLC of ponies (n = 4) and pigs (n = 3), flash-frozen in liquid nitrogen, and stored at −80°C. Jejunal and colonic BBM vesicles were manufactured, and D-glucose transport was determined. There was no detectable active uptake of glucose in the equine PLC. Compared with the pig DJ, D-glucose transport capacity was lower (2595 ± 633 vs. 655 ± 286 pmol mg\(^{-1}\) protein\(^{-1}\) s\(^{-1}\), respectively, P < .01) and transport affinity tended to be lower (0.09 ± 0.07 vs. 0.27 ± 0.06 mM, respectively, P = .11) in the pony DJ. Compared with the pig DJ, D-glucose transport capacity (2595 ± 331 vs. 571 ± 331 pmol mg\(^{-1}\) protein\(^{-1}\) s\(^{-1}\), respectively, P < .001) and transport affinity (0.09 ± 0.07 vs. 0.54 ± 0.07 mM, respectively, P < .001) in the pig PLC were lower. Results show there is negligible D-glucose uptake across pony PLC, and capacity for D-glucose absorption across DJ BBM is fourfold lower in the pony compared with the pig. Results further exemplify, at a physiological level, the limited capacity of the equid small intestine to transport glucose relative to an omnivorous mammal, and the likely evolutionary adaptation of equids to low dietary levels of nonstructural carbohydrates.

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1. Introduction

Equids are obligate hindgut fermenters that rely on dietary forages and thus are equipped with a large cecum and voluminous proximal colon for microbial fermentation of plant cell wall fiber components. Ingestion of nonstructural carbohydrates (NSC) in excess of small intestinal absorptive capacity of monosaccharide units increases NSC flow to the large intestine, and is associated with a shift in large intestinal microbiota in favor of lactobacilli growth and an increased risk for colic and development of laminitis [1]. Although the microbial events surrounding equine NSC-induced laminitis have been chronicled [2], the physiological basis for higher susceptibility to NSC-induced laminitis in equids compared with other hindgut fermenters remains unclear. For instance, consumption of NSC-rich diets in suids, which share similar gastrointestinal
anatomical features with equids, is not associated with large intestinal disturbances. Suids are also hindgut fermenters; however, because they have evolved consuming highly diverse diets, they are also classified as omnivores and thus are likely to have higher small intestinal capacity for NSC absorption than equids, which have strictly evolved as grazers on low-quality forages [3]. Indeed, it is well documented that absorption of hexoses (glucose, fructose, galactose) by the small intestine of mammalian omnivores is an efficient process to minimize flow of nutrients into the colon [4].

Small intestinal capacity for glucose transport in horses has been reported previously [5], but the contribution of the large colon to glucose absorption is unknown. The majority of dietary glucose is purportedly absorbed from the small intestine, although no study has characterized glucose transport kinetics across both small and large intestinal segments in equids or suids. We have recently demonstrated that, compared with the equine jejunum, the large colon has limited transcript abundance for the genes encoding glucose transporters (GLUTs), in particular those of high capacity and low affinity (ie, GLUT2 and GLUT5), but a remarkably higher transcript abundance of the gene encoding GLUT1 [6]; it remains unknown whether these transcripts, especially those encoding GLUT1, are functionally expressed in the apical epithelial membrane of the horse. It is possible the GLUT1 expression is localized to the basolateral membrane of the mucosa in the horse, as reported in the mouse by Yoshiwaka et al. [7], perhaps playing a role in the in situ utilization of glucose by the arterially supplied mucosa.

With these notions in mind, we hypothesized that glucose transport capacity across the apical membrane of the large colon is lower than that of the small intestine in the pony. Using a porcine model of small intestinal glucose transport capacity, we further hypothesized that capacity for glucose transport across the apical membrane of the small intestinal mucosa of the pony is lower than that of the pig. In this study, the pig colonic mucosa was used as a negative control. The objective of this study was to measure maximal glucose transport capacity ($V_{\text{max}}$) and affinity ($K_M$) across the brush border membrane (BBM) of the distal jejunum (DJ) and proximal large colon (PLC) mucosa of the pony and the pig.

2. Materials and Methods

All methods were approved by the Institutional Animal Care and Use Committee.

2.1. Animals and Collection of Tissue

Four ponies (mixed breeds, 210 ± 27 kg) and three market pigs (Yorkshire crossbred, 122.5 ± 3.9 kg) were used. Ponies were of mature age and had been maintained on low-quality grass hay before euthanasia. Ponies were provided with their last meal at 6 PM and euthanized between 10 AM and 2 PM the next day. Ponies were euthanized for reasons other than history of gastrointestinal problems. After euthanasia, a 2.5-m incision was made along the ventral midline to expose the entire gastrointestinal tract. Approximately 20-cm-long sections were sampled from the DJ, located 12 m distal to the duodenal colic ligament, and from the left ventral colon, representing the PLC, located between the sternal and pelvic flexures at the lateral band. Pigs were group-housed at the MSU Swine Teaching and Research Center and provided ad libitum access to a corn—soybean meal-based diet containing 14% crude protein. Pigs were transported to the Michigan State University Meat Laboratory at 4 PM and provided with free access to water and no access to feed. Pigs were humanely killed at 6 AM the next day, and the entire intestinal tract was immediately removed. Approximately 20-cm-long sections were obtained from the DJ, 2 m distal to the stomach, and from the ascending colon, representing the PLC, 30 cm distal to the cecum. For both species, intestinal segments were rinsed thoroughly in a 0.9% ice-cold NaCl solution and opened lengthwise to expose the mucosa. The mucosal layer was scraped from the serosal layer using a glass microscope slide, and scrapings were transferred to conical tubes until approximately 12 g was obtained. Samples were flash-frozen in liquid nitrogen and stored at −80°C.

2.2. Preparation of BBM Vesicles

BBM vesicles (BBMVs) were prepared as described by Fan et al. [8,9], with the exception that mucosal scraping instead of whole mucosa tissue was used. Briefly, mucosal scrapings (2.6 g) were homogenized over ice in a buffer solution (52 mL; 50 mM -mannitol, 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES); pH 7.4. Incubation buffer contained 150 mM sodium thiocyanate (NaSCN), 5 mM HEPES, pH 7.4, and mannitol:glucose at 9.99 mM o-mannitol:0.001 mM o-glucose, including 0.8 μM o-[3H]glucose. Each point represents the average of triplicate observations of six animals in BBMVs manufactured from porcine jejunal (n = 3), colonic (n = 1), and pony jejunal (n = 2) mucosal scrapings.

Fig. 1. Initial rate (J) of α-glucose uptake at a concentration of 0.001 mM using Na+-gradient uptake buffer. Brush border membrane vesicles (BBMVs) were preloaded with a buffer containing 150 mM potassium thiocyanate (KSCN), 10 mM mannitol, 5 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES); pH 7.4. Incubation buffer contained 150 mM sodium thiocyanate (NaSCN), 5 mM HEPES, pH 7.4, and mannitol:glucose at 9.99 mM o-mannitol:0.001 mM o-glucose, including 0.8 μM o-[3H]glucose. Each point represents the average of triplicate observations of six animals in BBMVs manufactured from porcine jejunal (n = 3), colonic (n = 1), and pony jejunal (n = 2) mucosal scrapings.
resulting homogenate was collected for protein and enzyme analysis, and the remaining homogenate was centrifuged at 2,000 × g for 15 minutes at 4°C. The supernatant was collected, and the pellet discarded. A 200-mM MgCl₂ solution was added to the supernatant and mixed to a final concentration of 10 mM MgCl₂, gently shaken over ice for 15 minutes, and centrifuged at 2,400 × g for 15 minutes at 4°C. Again, the supernatant was collected and the pellet discarded. The supernatant was divided into ultracentrifuge tubes and centrifuged at 19,000 × g for 30 minutes at 4°C. The resulting supernatant was discarded, and the remaining pellet representing the crude BBM was suspended in vesicle preloading buffer (150 mM potassium thiocyanate, 10 mM mannitol, 5 mM HEPES; pH 7.4) using a Pasteur pipette. Samples were centrifuged for an additional 30 minutes at 39,000 × g and 4°C. The resulting supernatant was discarded, and the final BBM pellet was suspended in 1 mL of vesicle preloading buffer. A subsample of the final BBMV suspension was collected for determination of protein concentration and enzyme activity. The remainder of the BBMV suspension was used for uptake experiments, as described later in the text.

2.3. Protein Concentration and Alkaline Phosphatase Enzyme Measurements

Protein concentration of the initial homogenate and the final BBMV suspension was determined with the Lowry assay (Bio-Rad Laboratories, Hercules, CA), according to manufacturer’s instructions and using bovine serum albumin as standard. Purity of BBMVs was tested using alkaline phosphatase as a marker. Alkaline phosphatase was measured according to Fan et al. [8] using p-nitrophenyl phosphate as substrate.

2.4. Measurement of Total (Na⁺-Dependent and Independent) β-Glucose Uptake into BBMVs

Freshly prepared BBMV suspension was diluted with vesicle preloading buffer to contain 6-10 mg protein mL⁻¹. The final BBMV suspension remained on ice until uptake experiments were performed (not more than 6 hours after final suspension). Uptake experiments were carried out using the rapid filtration procedure, as outlined by Fan et al. [8,9]. Uptake buffer (50 μL; 150 mM sodium thiocyanate, 10 mM mannitol, 5 mM HEPES; pH = 7.4) containing

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**Fig. 2.** Kinetics of β-glucose uptake into pony jejunal (A) and colonic (B) BBMVs. The initial rates of β-glucose uptake (J) into BBMVs were measured and calculated at each glucose concentration, and were regressed against each respective glucose concentration. BBMVs were preloaded with a buffer containing 150 mM KSCN, 10 mM mannitol, 5 mM HEPES; pH 7.4. Incubation buffer contained 150 mM NaSCN, 5 mM HEPES; pH 7.4, 0.8 μM β-[³²P]glucose, and nonlabelled β-glucose at 0.1, 0.25, 0.5, 1.0, and 2.5 mM. β-mannitol was added to maintain osmolarity. Each point represents the mean ± standard error of the mean (average data) of uptake experiments (triplicates) using BBMVs manufactured from the pony (n = 4) jejunal and colonic mucosal scrapings. The total uptake is represented by the average data point and the saturable and diffusion component curves depicted.
[3H]-D-glucose was first loaded into polystyrene tubes and allowed to warm to room temperature (22-24°C). Two separate 5-µL droplets of BBMV suspension were added along the side of the tube using a Microman pipette (Gibson S.A.S., Villiers-le-Bel, France). After warming to room temperature for 10 seconds, uptake was initiated by a foot-switch-activated vibromixer attached to an electronic timer (Timer model 545, GraLab Corporation, Centerville, OH). Uptake was terminated by immediate addition of ice-cold stop and wash buffer (1.2 mL; 150 mM potassium thiocyanate, 10 mM mannitol, 5 mM HEPES; pH 7.4). Incubation buffer contained 150 mM NaSCN, 5 mM HEPES, pH 7.4, 0.8 µM D-[3H]glucose, and nonlabelled D-glucose at 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 mM. D-Mannitol was added to maintain osmolarity. Each point represents the mean ± standard error of the mean (average data) of uptake experiments (triplicates) using BBMVs manufactured from pig (n = 3) jejunal and colonic mucosal scrapings. The total uptake is represented by the average data point and the saturable and diffusion component curves depicted.

Fig. 3. Kinetics of D-glucose uptake into pig jejunal (A) and colonic (B) BBMVs. The initial rates of D-glucose uptake (J) into BBMVs were measured and calculated at each glucose concentration, and were regressed against each respective glucose concentration. BBMVs were preloaded with a buffer containing 150 mM KSCN, 10 mM mannitol, 5 mM HEPES; pH 7.4. Incubation buffer contained 150 mM NaSCN, 5 mM HEPES, pH 7.4, 0.8 µM D-[3H]glucose, and nonlabelled D-glucose at 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 mM. D-Mannitol was added to maintain osmolarity. Each point represents the mean ± standard error of the mean (average data) of uptake experiments (triplicates) using BBMVs manufactured from pig (n = 3) jejunal and colonic mucosal scrapings. The total uptake is represented by the average data point and the saturable and diffusion component curves depicted.

2.4.1. Time Course Experiments

Time course experiments were performed to determine the incubation time needed for the transport kinetics experiments (outlined later in the text). The pony DJ and pig DJ were used under a sodium (Na)\(^+\) gradient, and [3H]-D-glucose uptake measurements were conducted using a D-glucose concentration of 0.001 mM. Uptake was terminated at 0, 2, 5, 10, 20, 30, and 60 seconds. Each uptake measurement (ie, each time point) was conducted in triplicate. Nonspecific binding of D-glucose to BBMVs and filters was corrected by subtracting the time-zero radioactivity counting.

2.4.2. Transport Kinetics Experiments

For D-glucose uptake experiments used in the determination of transport kinetics, uptake buffers consisted of...
replacing mannitol with d-glucose at 0.1, 0.25, 0.5, 1.0, and 2.5 mM for ponies and 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 mM for pigs. Each uptake experiment was conducted in triplicate. Nonspecific binding of d-glucose to filters was corrected for by measuring d-glucose uptake using buffer without the addition of BBMVs. Composition of incubation buffer is provided in detail in the figure legends.

2.5. Calculations to Determine Glucose Kinetics

To determine uptake at various time points and under Na+–dependent conditions, the following equation was used:

\[ J = (\{R_F-R_B\} \times S)/R_1)/(W \times T) \]  

Equation 1

where \( J \) is the initial rate of total glucose uptake into BBMVs (\( \text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{s}^{-1} \)); \( R_F \) is the radioactivity in disintegration per minute of filters (DPM/filter); \( R_B \) is the radioactivity for nonspecific binding to filters (DPM/filter); \( S = \text{extravesicular glucose concentrations (mM)} \); \( R_1 \) is the radioactivity in the uptake media (DPM/\( \mu \)L); \( W \) is the amount of membrane protein provided for the incubations (mg); and \( T \) is the time of incubation for initial uptake (s).

Kinetic parameters, namely, \( V_{\text{max}} \) and \( K_M \), were estimated according to the method of Wolffram et al. [10] based on derivation of a two-component equation (equation 2) composed of a saturable and a passive diffusion component, respectively, described by the Michaelis–Menten equation and \( D[S] \) as follows:

\[ J = \frac{V_{\text{max}}[S]}{K_M[S]} + D[S] \]  

Equation 2

where \( J \) is the initial rate of glucose uptake into BBMVs (\( \text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{s}^{-1} \)); \( V_{\text{max}} \) is the maximal transport rate (\( \text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{s}^{-1} \)); \( S = \text{extravesicular glucose concentrations (mM)} \); \( K_M \) is the Michaelis constant (50% saturation; mM); and \( D \) is the diffusion component.

2.6. Statistical Analysis

The initial rate of glucose uptake into BBMVs (\( J, \text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{s}^{-1} \)) at each glucose concentration as well as the kinetic parameters (i.e., \( V_{\text{max}} \) and \( K_M \)) were analyzed using the PROC MIXED procedure (SAS version 9.0, SAS Institute, Inc., Cary, NC). The model for analysis of \( J \) included the fixed effects of animal species, intestinal segment, and d-glucose concentration; the interaction between animal species and intestinal segment; and the random effect of individual animal nested within species. The model for analysis of \( V_{\text{max}} \) and \( K_M \) included the interaction between animal species and intestinal segment, and the random effect of individual animal nested within species. Results are reported as least squares means ± standard error of the mean. Because no detectable active uptake of glucose was derived from equation 2 for the pony colon, the PLC segment for the pony was omitted from statistical analysis. Significant differences and trends for differences in \( V_{\text{max}} \) and \( K_M \) were determined at \( P < .05 \) and \( P \leq .10 \), respectively.

3. Results

3.1. Membrane Purity

Comparison of alkaline phosphatase enzyme activity in the BBMVs relative to the mucosal tissue scraping crude homogenate yielded an average enrichment factor of 4.6 ± 0.4-fold, indicating the BBMVs had little contamination by the basolateral membrane. This value falls within the reported range of 2.3-fold in Aedes aegypti larvae midgut [11] to 31.1-fold in rat jejunum [12], and is close to the 5.1-fold enrichment determined in bovine jejunum and ileum [13].

3.2. Time Course of d-Glucose Uptake into BBMVs

Time course experiments of d-glucose uptake into equine and porcine DJ BBMVs indicated d-glucose uptake was linear up to 20 seconds at d-glucose concentration of 0.001 mM (Fig. 1). Because d-glucose is transported rapidly into BBMVs [5,10,12,14,15], an incubation time of 3 seconds was used to measure the rate of d-glucose uptake. This incubation time is in agreement with Wolffram et al. [10] and Dyer et al. [5] who used an incubation time of 3 seconds or less to measure glucose kinetics across jejunal BBMVs of pig and horse, respectively.

3.3. Relationship between the Initial Rate of d-Glucose Uptake into BBMVs and Glucose Concentrations

Average initial rates (\( J \)) of total d-glucose uptake in pony DJ and PLC and in pig DJ and PLC estimated at each of the d-glucose concentrations and calculated from equation 1 are presented in Figures 2 and 3, respectively, in relation to each respective d-glucose concentration. As shown in Figure 2B, there was no active uptake of glucose into the pony colonic BBMVs. At d-glucose concentration of 0.001 mM, initial rates of total d-glucose uptake into BBMVs did not differ between pony and pig DJ or between pig PLC and DJ. At d-glucose concentrations greater than 0.001 mM, initial rates of total d-glucose uptake were lower in pony DJ compared with pig DJ and lower in pig PLC compared with pig DJ (\( P < .001 \)).

3.4. Kinetics of d-Glucose Uptake into BBMVs

To characterize d-glucose transport kinetic parameters, total d-glucose uptake was divided into a saturable (Michaelis–Menten) and a diffusion component based on equation 2 [10]. Kinetic parameters \( V_{\text{max}} \) and \( K_M \) and diffusion are presented in Table 1 and the two components illustrated in Figures 2 and 3. Transport capacity, \( V_{\text{max}} \), was lower (\( P < .01 \)) in the pony compared with the pig DJ. In the pony DJ, \( K_M \) of glucose uptake was not different than that of pig (\( P = .11 \)). In the pig, \( V_{\text{max}} \) and \( K_M \) of glucose uptake were lower in the PLC compared with the DJ (\( P < .05 \)). Diffusion was lower in the pony DJ and pig PLC compared with the pig DJ (\( P < .01 \)).
Table 1
Kinetic parameters of α-glucose uptake in brush border membrane vesicles of equine and porcine jejunum and large colon*

<table>
<thead>
<tr>
<th>Species</th>
<th>Segment</th>
<th>$V_{max}$ $^*$ ($\mu$mol mg−1 protein−1 s−1)</th>
<th>$K_m$ $^*$ ($\mu$M)</th>
<th>Diffusion $^{**}$ ($\mu$mol mg−1 protein−1 s−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine</td>
<td>Jejunum</td>
<td>2,595.21 ± 330.56$^a$</td>
<td>0.09 ± 0.07$^a$</td>
<td>974.17 ± 86.90$^a$</td>
</tr>
<tr>
<td></td>
<td>Large colon</td>
<td>571.04 ± 330.56$^b$</td>
<td>0.54 ± 0.07$^b$</td>
<td>171.05 ± 86.90$^b$</td>
</tr>
<tr>
<td>Equine</td>
<td>Jejunum</td>
<td>653.37 ± 286.28$^b$</td>
<td>0.27 ± 0.06$^a$</td>
<td>NE$^{****}$</td>
</tr>
<tr>
<td></td>
<td>Large colon</td>
<td>No measurable uptake</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$Within a column, means with uncommon superscripts differ, $P < .05$.

- Data are least squares means ± standard error of the mean.
- $^*$ Animal species × intestinal segment differs, $P < .01$.
- $^{**}$ Animal species × intestinal segment differs, $P < .02$.
- $^{***}$ Animal species × intestinal segment differs, $P < .01$.
- $^{****}$ NE = nonestimable.

4. Discussion

The main objective of this work was to determine the uptake of glucose across the apical membrane of the jejunum and PLC of the pony and the pig using BBMVs after a 16-hour feed deprivation. BBMVs have been an effective tool for characterizing glucose transport kinetics in a host of animal species, including humans [14,16,17], rabbits [18,19], pig [10], sheep [10,15], cattle [15,18], chicken [20,21], and horses [5,22]. This study is the first to characterize the kinetics of glucose uptake across the apical small intestinal and colonic BBM and to compare kinetics of glucose transport across jejunal BBMVs between equids and suids. Our values of glucose transport capacity and affinity fall within the range of those reported for other monogastric and neonatal animals (Table 2), being most similar to those of the human jejunum [16,17].

Transport capacity across the pony DJ BBMVs was fourfold lower and the affinity threefold higher than that of the pig DJ. There are limited data available in the literature on the kinetics of glucose transport across equine intestinal mucosa. In studies by Dyer et al. [5,22], total glucose uptake, presumably including a diffusion component, was reported to be 698 and 1,312 μmol mg−1 protein−1 s−1 in the equine ileum and duodenum, respectively. Diffusion is likely associated with disruption of the BBM extracellular matrix during preparation, thus increasing membrane permeability [8,23]. Because it is unlikely such diffusion rates would occur in vivo, estimation of the saturable component of transport rather than the total (saturable + diffusion) transport of α-glucose is a closer reflection of in vivo processes. Nonetheless, for purpose of comparison, we calculated total glucose uptake across pony jejunal BBMVs to be 1,250 μmol mg−1 protein−1 s−1, which falls between 1,312 and 918 μmol mg−1 protein−1 s−1 for the duodenum and jejunum, of horses maintained on a grass−hay diet in the study by Dyer et al. [5].

Glucose transport kinetic values were noticeably higher in this study for the pig DJ compared with those obtained in the study by Wolfram et al. [10], who reported a Na+−gradient glucose transport capacity of 398 μmol mg−1 protein−1 s−1 and affinity of 0.69 mM. The difference in pig jejunal glucose kinetics between our study and that by Wolfram et al. [10] is unclear; however, the glucose uptake value reported by Wolffram et al. [10] was also surprisingly lower compared with those of several other studies in monogastric animals (Table 2), including the lamb [15] and the calf [15]. As expected, our glucose transport capacity estimates for the pony and pig are higher than those for ruminant animals, with bovine jejunum $V_{max}$ ranging from 0.52 to 146 μmol mg−1 protein−1 s−1 [15,18] and ovine jejunum $V_{max}$ ranging from 2.7 to 31.7 μmol mg−1 protein−1 s−1 [10,15]. Adult ruminant animals have little glucose entering the small intestine [24], hence functional expression of glucose transporters is likely to be relatively small compared with monogastric animals. In early−weaned sheep, activity and expression of small intestinal sodium−dependent glucose cotransporter 1 (SGLT1) decreased in response to transition from milk to forage intake and the increased forage use by the rumen [15]. Conversely, bypassing the rumen by adding α-glucose directly to the luminal content of the ovine small intestine reestablished expression levels of SGLT1 [15].

We recently demonstrated the equine colon has low mRNA abundance of glucose transporters SGLT1, GLUT2, and GLUT 5 and higher mRNA abundance of GLUT1 compared with the jejunum [6]. The lack of measurable active uptake of glucose across pony colon BBMVs indicates that a majority of these glucose transporter transcripts are not functionally expressed on the equine colonic apical membrane or that in vitro functional expression is dependent on previous

Table 2
Kinetic parameters of α-glucose uptake across small intestinal brush border membrane vesicles of monogastric and neonatal ruminant animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Segment</th>
<th>$V_{max}$ (μmol mg−1 protein−1 s−1)</th>
<th>$K_m$ (μM)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Jejunum</td>
<td>931</td>
<td>0.86</td>
<td>Harig et al., 1989 [16]</td>
</tr>
<tr>
<td></td>
<td>Jejunum</td>
<td>805</td>
<td>0.57</td>
<td>Malo and Berteloot, 1991 [17]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Jejunum</td>
<td>470</td>
<td>0.57</td>
<td>Ikeda et al., 1989 [19]</td>
</tr>
<tr>
<td>Pig</td>
<td>Jejunum</td>
<td>398</td>
<td>0.69</td>
<td>Wolfram et al., 1986 [10]</td>
</tr>
<tr>
<td>Calf</td>
<td>Jejunum</td>
<td>360</td>
<td>0.10</td>
<td>Wood et al., 2000 [15]</td>
</tr>
<tr>
<td>Lamb</td>
<td>Jejunum</td>
<td>620</td>
<td>0.04</td>
<td>Wood et al., 2000 [15]</td>
</tr>
<tr>
<td>Horse</td>
<td>Duodenum</td>
<td>1,312$^a$</td>
<td>0.49</td>
<td>Dyer et al., 2002 [5]</td>
</tr>
<tr>
<td>Horse</td>
<td>Jejunum</td>
<td>918$^a$</td>
<td>0.49</td>
<td>Dyer et al., 2002 [5]</td>
</tr>
<tr>
<td>Horse</td>
<td>Ileum</td>
<td>698$^a$</td>
<td>0.49</td>
<td>Dyer et al., 2002 [5]</td>
</tr>
<tr>
<td>Pony</td>
<td>Jejunum</td>
<td>655</td>
<td>0.27</td>
<td>This study</td>
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<tr>
<td>Pig</td>
<td>Jejunum</td>
<td>2,595</td>
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<tr>
<td>Pony</td>
<td>Large colon</td>
<td>No measurable uptake</td>
<td>NE</td>
<td>This study</td>
</tr>
<tr>
<td>Pig</td>
<td>Large colon</td>
<td>571</td>
<td>0.54</td>
<td>This study</td>
</tr>
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</table>

**NE = nonestimable.**

*Estimate of total (saturable + diffusion) glucose uptake.
in vivo adaptation to luminal glucose. In contrast, there was significant active glucose transport across the pig colon BBMV, indicative of the presence of functional glucose transporters on the pig colonic apical membrane. Nonetheless, the large difference in transport capacity of glucose between the jejunum and colon of the pig combined with the lack of measurable glucose transport across the pony colon is solid evidence for the sole reliance on the small intestine for prececal glucose clearance.

We acknowledge that the comparison of intestinal glucose uptake between these two animal species is confounded not only by their distinctive long-term dietary regimen (grazing vs. meal-fed) but also by the vastly different ingredients and nutrient composition profiles of their diet. Ponies obtained for this study were maintained on a forage-based diet, with little exposure to dietary NSC, whereas pigs were maintained on a corn soybean meal based diet, which is rich in NSC. To minimize this confounding factor, samples were taken from both ponies and pigs that had restricted access to feed for a minimum of 16 hours. Indeed, the remarkably large difference in glucose uptake between the pony and pig DJ found in this study is consistent with the previously reported lack of the high-capacity glucose transporter GLUT2 and the suggested sole presence of the low-capacity glucose transporter SGLT1 on the apical membrane of the small intestine of equids [22]. Conversely, in horses maintained on diets supplemented with concentrate feeds rich in NSC, the same authors [22] reported a two-fold increase in glucose transport V_{max} value across the jejunum, as high as 2.883 \text{ pmol} \cdot \text{mg protein}^{-1} \cdot \text{s}^{-1}, which is similar to that reported for the pig in this study. As discussed earlier, it appears that the diffusion component was unaccounted for, thus leading to overestimation of the actual V_{max}. In that same study [22], horses fed increasing levels of NSC during a 3-week period had increased expression of SGLT1 in the jejunal and ileal regions of the small intestine, with levels of expression eventually approaching that of the duodenum [22], clearly demonstrating that glucose uptake capacity reaches plateau, consistent with the small capacity and high-affinity properties of SGLT1 transporter. It is nevertheless possible that the higher uptake of glucose across the BBMV of the pig jejunum in this study is merely a reflection of increasing abundance of glucose transporters in response to long-term adaptation to dietary NSC; in which case, 16 hours of feed restriction would be insufficient to infer on the observed differences between ponies and pigs used in this study.

Nonetheless, the results presented herein are novel, in that we are, to the best of our knowledge, the first to report specifically on the active glucose uptake kinetics across the small and large intestine of the pony and to demonstrate the near absence of functional glucose uptake in the large intestine of the pony, despite the previously reported presence of transcripts encoding SGLT1 and GLUT1 transporters [6].

Results show that the pony jejunum has a remarkably lower transport capacity for glucose uptake compared with the pig. Furthermore, the pig colon is capable of significant glucose transport, in contrast to the pony, whose large colon is completely devoid of facilitated glucose transport. The latter supports the notion that GLUT1 is localized to the basolateral membrane [7] and not involved in luminal glucose transport, despite a marked increase in the transcript expression of the gene encoding for GLUT1 in the PLC mucosa of the horse compared with the jejunum [6]. Further research is critically needed to assess the role of glucose transport in the etiology and the increased susceptibility of equids to dietary NSC-induced large intestinal dysfunction.

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**References**


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