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Effects of Crystalline Lactose on Expression of Sodium-dependent Glucose Transporter (SGLT)-1 mRNA

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Effects of Crystalline Lactose on Expression of Sodium-dependent Glucose Transporter (SGLT)-1 mRNA

Supplementation of crystalline lactose increases SGLT-1 mRNA relative abundance, glucose transport to the basolateral compartment, and reduces the LPS-mediated inhibitory effect on glucose transport in IPEC-J2 cells.

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Summary

A preliminary experiment was conducted to evaluate the effects of crystalline lactose on the relative abundance of sodium-dependent glucose transporter (SGLT)-1 mRNA in vitro in model porcine jejunal epithelial cells (IPEC-J2). Cells were treated with low (28 mM) or high (56 mM) concentrations of lactose alone or in combination with lipopolysaccharide (LPS; 10 ng/mL). Total RNA and culture media samples from both apical and basolateral compartments were harvested at 3, 6, 12, and 24 hours following the addition of respective treatments. With respect to relative abundance of SGLT-1 mRNA, there were no interactions of lactose, LPS, and time; however, a main effect of lactose ($P < 0.01$) was observed. Cells treated with a high concentration of lactose had greater SGLT-1 mRNA expression compared to the control cells ($P = 0.003$) and low concentration lactose ($P = 0.02$) treated cells. With respect to glucose quantification, polarization of glucose in the basolateral rather than in the apical compartment ($P = 0.04$) was observed in a time-dependent manner ($P < 0.001$). Supplementation of lactose in LPS-treated cells reduced LPS-mediated inhibitory effect on glucose transport from the apical to basolateral compartment ($P = 0.07$).

Introduction

Previous research demonstrated that including lactose in diets for nursery pigs can improve growth performance and gut health (i.e., stimulating epithelial cell proliferation and mediating beneficial bacteria colonization in the gastrointestinal tract (GIT). However, molecular mechanisms for how lactose affects pig growth remain unclear. In the small intestine, lactose is hydrolyzed and absorbed as glucose and galactose via the action of lactase present along the brush border membrane. The absorption of glucose and galactose involves two steps mediated by the sodium-dependent glucose transporter (SGLT)-1 and glucose transporter (GLUT)-2. First, sugar transport is coupled to sodium and electrical gradients across the epithelial cell membrane providing energy for the influx of the sugars. This step is facilitated by SGLT-1 (an integral protein in the brush border membrane). Second, glucose and galactose are transported across the basolateral membrane of enterocytes to the blood via GLUT-2. The dissociation of Na^+ at the cytosolic surface is the rate-limiting step of SGLT-1 transport. Thus, SGLT-1 plays an important role in sugar uptake from the GIT lumen to enterocytes. Research studies have investigated effects of different sugars, fiber, and sodium on SGLT-1 gene expression; however, a mechanism for how lactose affects expression of SGLT-1 mRNA in cultured porcine epithelial cells does not exist. Thus, a preliminary experiment was conducted to evaluate the effects of supplementation of crystalline lactose on glucose transport and expression of mRNA encoding for sodium-dependent glucose transporter (SGLT)-1 *in vitro* in model porcine jejunal epithelial cells (IPEC-J2).

Materials and Methods

IPEC-J2 Cell Cultures and Treatment Design

Porcine jejunal intestinal epithelial cells have been characterized previously and are non-transformed, jejunal epithelial cells derived from neonatal pigs, and are maintained as a continuous culture. Cell cultures were maintained in DMEM-F12 growth medium supplemented with insulin/transferin/Na selenite media supplement, epidermal growth factor, antibiotic, and fetal bovine serum. For experimentation, IPEC-J2 cells were seeded onto 12-well transwell cell culture plates and maintained in the above media. The cells were incubated for 24 hours before being washed and re-fed every other day for seven days to form a model of the gut epithelium. Twenty-four hours before experimentation, cells were washed and re-fed media devoid of antibiotics.

There were six treatments (2×3 factorial) included in this experiment: 1) control (CTL; growth media devoid of antibiotics); 2) CTL + Lipopolysac-

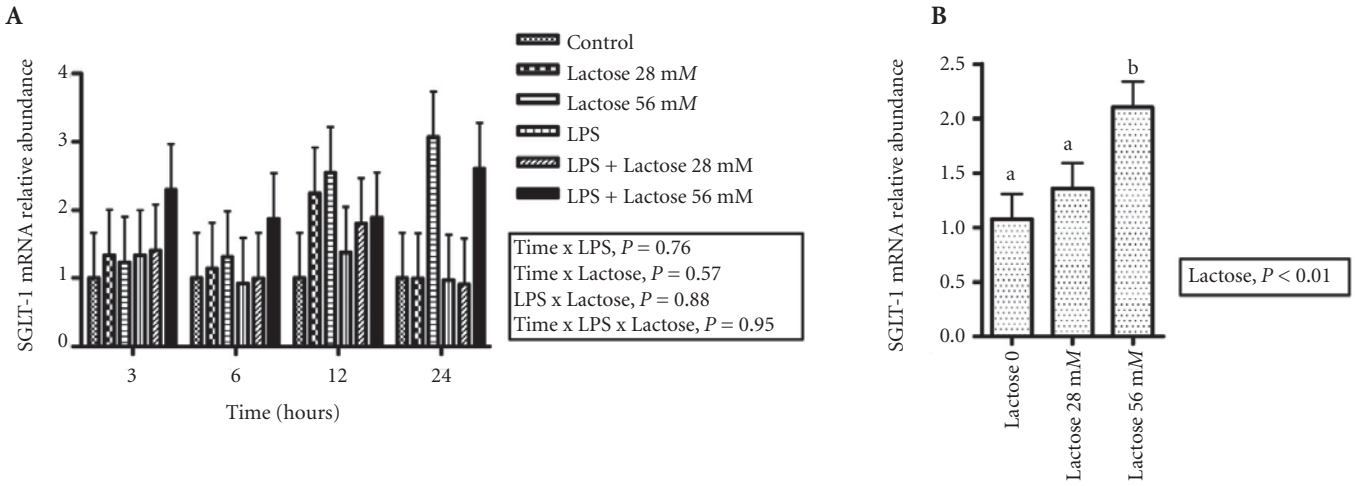

Figure 1. Effects of supplementation of crystalline lactose on relative abundance of SGLT-1 mRNA after 3, 6, 12, and 24 hours following treatment additions. Each bar represents the least-squares mean (± SEM) of three replications. Panel A represents the interactive time × LPS × lactose ($P = 0.95$) means. Panel B represents the main effects of lactose ($P < 0.01$).

charide (LPS; 10 ng/mL); 3) CTL + low lactose (28 m*M*); 4) CTL + high lactose (56 m*M*); 5) CTL + LPS + low lactose (28 m*M*); and 6) CTL + LPS + high lactose (56 m*M*).

Total RNA and cell culture media samples from both apical and basolateral compartments were harvested at 3, 6, 12, and 24 hours following the addition of treatments.

RNA Isolation and Quantitative Real-Time PCR Analysis

Total RNA was extracted and contaminating genomic DNA was removed from all RNA samples. Samples were reconstituted in nuclease-free water and frozen for further analysis. The quality of RNA was assessed by agarose gel electrophoresis and visulization of 28S and 18S rRNA bands. The quantity of RNA was determined by spectrophotometry (OD 260 nm). Complementary DNA (cDNA) was synthesized by reverse transcription (RT) from 1.0 µg of RNA. Reverse transcription reagents included 25 m*M* $MgCl_2$, 500 µ*M* dNTP's, 2.5 µ*M* random hexamers, 0.4 U/µL Rnase inhibitor, 50 U/µL MultiScribe reverse transcriptase, and TaqMan RT buffer. The RT mixture was incubated at 25°C for 10 min, heated to 37°C for 60 min, and inactivated at 95°C for 5 min. Synthesized cDNA was used as a template for real-time quantitative polymerase reaction (q-PCR) to quantify SGLT-1 mRNA relative to the quantity of the endogenous control (18S rRNA). The q-PCR reaction was conducted in 384-well plate with the SGLT-1 specific primers (TaqMan Gene Expression Assays, Applied Biosystems; Assay ID No. Ss03394307_m1). Commercially available eukaryotic 18S rRNA primers and a probe were used as an endogenous control. The PCR reactions, run in triplicate wells, were carried out with the Applied Biosystems 7900HT Fast Detection System using 40 cycles of amplification with alternating 15 sec, 95°C denaturation and 1 min, 60°C anneal/extension cycles.

Circulating Glucose Quantification

Media samples were used to quantify glucose concentration (Enzy-Chrom™ Glucose Assay Kit, Bioassay Systems) which were based on glucose oxidase and color reactions. The optical density of samples was read at OD 570 nm.

Statistical Analysis

Relative abundance of SGLT-1 mRNA in IPEC-J2 cells was calculated by ΔΔCT method using the average CT values of cells from control wells as the reference expression. These ΔΔCT values were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, N.C.) to determine effects of SGLT-1 relative gene expression. The model consisted of the main effects of LPS, lactose, time, and their interactions. For glucose concentration quantification, the statistical model included effects of LPS, lactose, direction, time, and their interactions.

Results and Discussion

SGLT-1 mRNA Gene Expression in IPEC-J2 Cells

Relative abundance SGLT-1 mRNA of each treatment was shown in comparison with the control wells which have relative abundance SGLT-1 mRNA equal to one. There were no interactions of LPS × lactose × time (Figure 1A) or their two-way interactions. However, a main effect of lactose on increasing relative abundance of SGLT-1 mRNA ($P < 0.01$) in IPEC-J2 cells was detected when averaged across all time points (Figure 1B). Cells treated with a high concentration of lactose had greater SGLT-1 mRNA compared to the control ($P = 0.003$) and low lactose concentration ($P = 0.02$). This result agrees with previous research that reported feeding high glucose and galactose increased SGLT-1 mRNA abundance in mice. In addition, research also indicated that mRNA expression of SGLT-1 may be affected within hours, to one to three days after feeding a high-carbohydrate diet. Lastly, there were no differences

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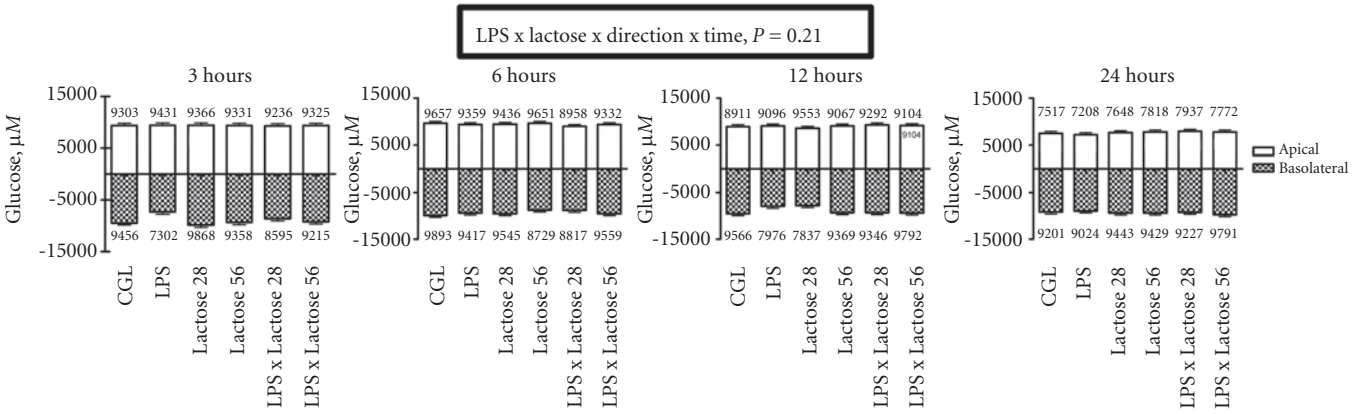

Figure 2. Effects of supplementation of low and high concentration of crystalline lactose on glucose concentration in IPEC-J2 cell culture media at 3, 6, 12, 24 hours following addition of treatments ($P = 0.21$). Each bar represents the least-squares means (± SEM) of three replications.

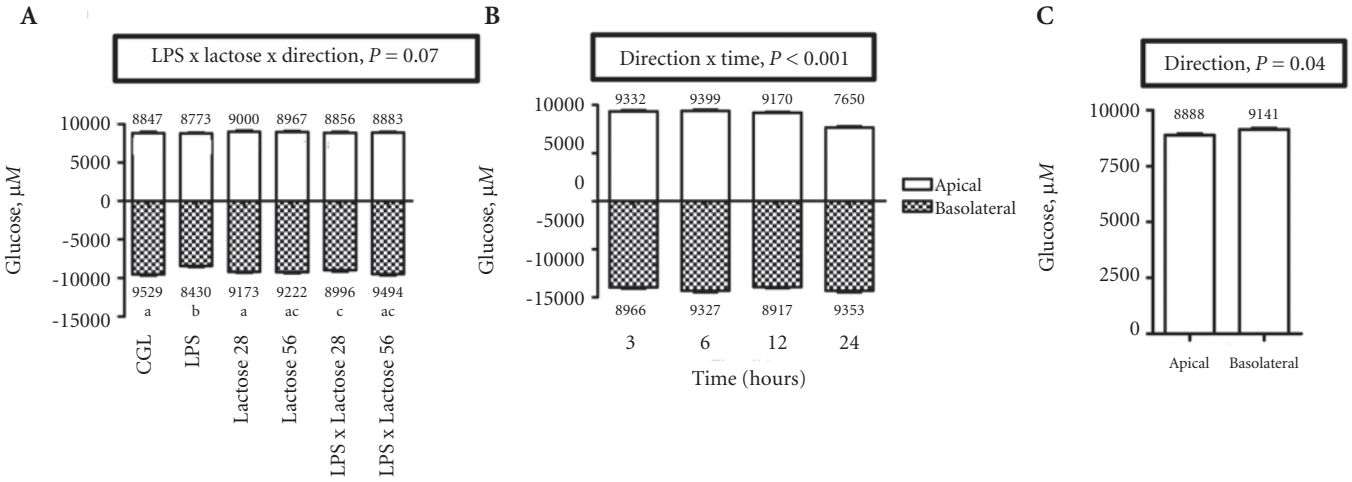

Figure 3. Effects of supplementation of low and high concentrations of crystalline lactose on glucose concentration in IPEC-J2 cells. Panel A represents interactive LPS × lactose × direction ($P = 0.07$) means. Panel B represents interactive direction x time ($P < 0.001$) means. Panel C represents polarized glucose transport (direction) among all timepoints and treatments ($P = 0.04$).

between control cells and cells treated with low lactose concentration on relative abundance of SGLT-1 mRNA.

Glucose Concentration in IPEC-J2 Cell Culture Media

Figures 2 and 3 illustrate the effects of supplementation of low and high concentration of crystalline lactose on glucose concentration in IPEC-J2 cell culture media at 3, 6, 12 and 24 hours following the addition of the respective treatments. There was no LPS × lactose × direction × time interaction (Figure 2). However, there was a LPS × lactose × direction interaction tendency ($P = 0.07$) when averaged across all time points ($P = 0.07$; Figure 3A). Lipopolysaccharide-treatment tended to inhibit glucose transport to the basolateral compartment compared to control wells. However, supplementation of lactose in LPS-treated cells tended to reduce ($P = 0.07$) the LPS-mediated inhibitory effect of glucose transport from the apical to the basolateral compartment.

As expected, glucose transport in IPEC-J2 was polarized basolaterally rather than apically (9,141vs. 8,888 μ*M; P* = 0.04; Figure 3C). The polarization of glucose transport basolaterally, followed a time-dependent pattern (direction × time, $P < 0.001$; Figure 3B). At 24 hours, glucose in the apical media (7,650 μ*M*) was significantly decreased compared to the basolateral (9,353 μ*M*) media and compared to 3, 6, and 12 hour apical media. In addition, glucose in the basolateral media at 24 hours was significantly greater compared to basolateral media at other timepoints.

Conclusions

This research indicates that crystalline lactose affects the expression of SGLT-1 mRNA, polarized glucose transport, and may restore LPS-induced reduction of basolateral glucose transport *in vitro* in model porcine jejunal epithelial cells.

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