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Oat β-glucan depresses SGLT1- and GLUT2-mediated glucose transport in intestinal epithelial cells (IEC-6)☆

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ABSTRACT

Oat β-glucan consumption is linked to reduced risk factors associated with diabetes and obesity by lowering glycemic response and serum level of low-density lipoproteins. The purpose of this study was to identify the mechanism of action of oat β-glucan at the interface between the gut wall and the lumen responsible for attenuating glucose levels. We proposed that viscous oat β-glucan acts as a physical barrier to glucose uptake in normally absorptive gut epithelial cells IEC-6 by affecting the expression of intestinal glucose transporters. Concentration and time-dependent changes in glucose uptake were established by using a nonmetabolizable glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose. The effectiveness of nutrient transport in IEC-6 cells was shown by significant differences in glucose uptake and corresponding transporter expression. The expressions of glucose transporters sodium-glucose–linked transport protein 1 (SGLT1) and glucose transporter 2 (GLUT2) increased with time (0-60 minutes) and glucose levels (5-25 mmol/L). The suppression of glucose uptake and SGLT1 and GLUT2 expression by increasing concentrations (4-8 mg/mL) of oat β-glucan demonstrated a direct effect of the physical properties of oat β-glucan on glucose transport. These results affirmed oat β-glucan as a dietary agent for minimizing postprandial glucose and showed that modulating the activity of the key intestinal glucose transporters with oat β-glucan could be an effective way of lowering blood glucose levels in patients with diabetes.

1. Introduction

Diabetes is characterized by chronic hyperglycemia [1]. Presently, management of diabetes relies on the use of hypoglycemic agents that act to lower blood glucose levels through dietary changes [2]. Controlling the intestinal absorption of glucose in the brush-border membrane of the gut would help to improve levels of glucose in the blood. It has

Abbreviations: 2-NBDG, 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose; GLUT2, glucose transporter 2; IEC-6, rat small intestine epithelial cell line; SGLT1, sodium-glucose–linked transport protein -1.

☆ Competing interests: The authors have declared that no competing interests exist.

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been previously shown that sodium-glucose–linked transport protein 1 (SGLT1) and glucose transporter 2 (GLUT2) are part of the mechanism that regulates glucose uptake in response to extracellular glucose levels [3]. Changes in glucose absorption by enterocytes are a result of increased activity and expression of SGLT1 and GLUT2 [4,5]. Modulating the activity of these key transporters would seem to be an effective way of lowering blood glucose levels in patients with diabetes [6].

Oat β-glucan appears to provide opportunities for managing blood glucose levels based on clinical studies showing a moderation of glycemic response [7,8] and lowering of serum level of low-density lipoprotein cholesterol [9] with consumption of oat β-glucan. Oat β-glucan delays absorption of glucose and significantly reduces postprandial blood glucose levels. However, there does not seem to be a clear understanding of how oat β-glucan affects the interface between the gut wall and the lumen which leads to lowered glucose uptake.

To investigate this, we used the small intestine epithelial cell line IEC-6 as a model to study the effect of oat β-glucan treatments on glucose transport. We hypothesized that treatment with viscous oat β-glucan may reduce glucose uptake and expression of intestinal glucose transporters by reducing nutrient diffusion. We first established the responsiveness of IEC-6 cells to various glucose concentrations and evaluated the time-dependent changes in glucose uptake and expression of intestinal glucose transporters SGLT1 and GLUT2. We treated the cells with viscous oat β-glucan to assess whether there was any effect on glucose uptake and transporter expressions. Herein, we report that oat β-glucan reduces the key intestinal glucose transporters and it could represent an effective way of preventing intestinal glucose absorption in patients with diabetes.

2. Methods and materials

2.1. Cell culture procedures

The nontransformed rat small intestine epithelial cell line IEC-6 [10] was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells from passages 5 to 18 were used in this study, and normal growth rate and cell morphology were observed throughout this period. Cells were grown in 94-mm Petri dishes (Invitrogen/Thermo Fisher Scientific, Burlington, Ontario, Canada) at 37°C under 10% CO_2. The complete growth medium used was Dulbecco modified Eagle medium (DMEM; Sigma-Aldrich, St Louis, MO, USA) with the addition of 10% fetal bovine serum (PAA, Toronto, Canada), 100 mg/mL insulin (Invitrogen, Grand Island, NY, USA), and 10 mL/L penicillin-streptomycin (Sigma-Aldrich). Cultured cells were split when they reached 85% to 90% confluence by treatment with 0.25% trypsin (Invitrogen) followed by resuspension in fresh medium. Cells (9×10^5) were seeded for 48 hours prior to start of the experiment. The experiments were performed on confluent cells.

2.2. Experimental design

To establish the viability of IEC-6 cells as a model for studying glucose transport, the effect of different glucose concentrations on glucose uptake was investigated using a stable fluorescent D-glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG). SGLT1 and GLUT2 glucose transporter expression was also determined as a function of time (10-60 minutes) and glucose concentration (0-25 mmol/L). The responsiveness of glucose transport to oat β-glucan was determined by treating cells with a single dose (8 mg/mL) of a highly viscous oat β-glucan. The dose-response effect of oat β-glucan on glucose uptake and transporters was examined by incubating cells with different concentrations of β-glucan (4, 6, or 8 mg/mL β-glucan) and 25 mmol/L glucose.

2.3. Glucose starvation procedure

IEC-6 cells were grown in normal growth media (25 mmol/L glucose) and then starved of glucose for 1 hour in glucose-free DMEM immediately prior to evaluation of glucose uptake and glucose transporters expression at different glucose concentrations (5-25 mmol/L) and oat β-glucan viscosities (4-8 mg/mL) for different durations of exposure (10-60 minutes) [11,12], as described below.

2.4. Measurement of glucose uptake

The absorption of fluorescent D-glucose analog 2-NBDG by the cells was used as an indicator of glucose uptake. 2-NBDG is a derivative of glucose that is incorporated but not metabolized in living cells [11]. The experiments were performed as described by Blodgett et al [12] with minor modifications: 5 mg of 2-NBDG (Invitrogen) was dissolved in 10 mmol/L of phosphate-buffered saline (PBS; 138 mmol/L NaCl, 2.7 mmol/L of KCl at pH 7.4 at 25°C) to obtain a stock solution. The 2-NBDG stock solution was diluted with glucose-free DMEM and high-glucose DMEM to yield final glucose concentrations of 0, 5, 8, 15, and 25 mmol/L and a final 2-NBDG concentration of 100 μM. After an hour of incubation in glucose-free DMEM, the medium was removed and cells were rinsed twice with PBS. Cells were then placed in media containing 100 μM 2-NBDG and 0-25 mmol/L glucose for 0, 10, 30, or 60 minutes. The cells were subsequently lysed with 1 mL of cell lysis buffer (1% sodium deoxycholate, 40 mmol/L KCl, and 20 mmol/L Tris [pH 7.4]) for 10 minutes and homogenized by 10-second sonication (Sonics Vibracell model VCX130; Sonics & Materials Inc, Newtown, CT, USA). This step was found necessary in order to minimize the background noise that was otherwise evident in measuring the fluorescence intensity of the glucose analog in suspensions of intact cells. The homogenates were centrifuged at 12000*g* for 10 minutes at 4°C, and the fluorescence of 3 aliquots of 200 μL of the supernatant was measured at excitation and emission wavelengths of 485 and 528 nm, respectively, using a VICTOR^3 multiplate reader (PerkinElmer, Waltham, MA, USA). Fluorescence of PBS alone was taken as a control to account for variations in background signal.

In order to correct for any variations in the number of the lysed cells, the amount of DNA in the homogenates was measured. Aliquots from cell homogenates were diluted in the lysis buffer and 1 μg/mL Hoechst 33258 was added. Fluorescence of 50 μL Hoechst 33258 was measured at 350-nm excitation and 460-nm emissions in the VICTOR^3 multiplate reader. The DNA content was quantified by comparison of fluorescent intensity

against a standard curve constructed using calf thymus DNA (Sigma) [12]. 2-NBDG uptake in each lysed sample was then normalized with its DNA content and compared at various glucose concentrations.

2.5. Measurement of glucose transporters SGLT1 and GLUT2 by real-time polymerase chain reaction

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed to quantify the expressions of the glucose transporters SGLT1 and GLUT2. Total RNA was extracted from the control and treated IEC-6 cells with the Qiagen RNeasy kit (QIAGEN, Mississauga, Ontario, Canada) as described in the manufacturer's instructions. RNA concentration was determined using a NanoDrop spectrophotometer (ND-1000; Nano Drop Technologies, Wilmington, DE, USA), and RNA integrity was assessed using the Agilent 2000 Bioanalyzer (Agilent Technologies Inc, Palo Alto, CA, USA). RNA used had a 260/280 range between 1.9 and 2.1 and RNA integrity number values within a range of 8.5 to 10.0. Primers appropriate for RT-PCR were designed using Primer Express software v. 2.0 (Applied Biosystems, Forster City, CA, USA). Primer sequences and accession numbers are given in Table 1.

The first strand of cDNA was synthesized from 500 ng of purified RNA by reverse transcriptase using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Mississauga, Ontario, Canada). The real-time PCR reaction was performed in triplicate using StepOnePlus from the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Reaction conditions for the first step were 25°C for 10 minutes; the second step was run at 37°C for 120 minutes, the third step was run at 85°C for 5 minutes, and the last step at 4°C was run for an indefinite amount of time.

2.6. Oat β-glucan preparation

Purified oat β-glucan was obtained from Megazyme (Bray, County Wicklow, Ireland). The concentration of oat β-glucan was determined using a standard AOAC protocol (AOAC 995.16) and the molecular weight was determined as previously described [13]. The molecular weight for the oat β-glucan was 580000 g/mol. The oat β-glucan was dissolved by following the protocol provided by Megazyme with slight modifications for cell culture study. To dilute the oat β-glucan, 100 mL of PBS was heated for 30 minutes at 100°C and 2 g of oat β-glucan was added to the PBS and stirred for 3 hours by a magnetic stirrer. This oat β-glucan stock solution was cooled to room temperature for storage. The stock solution was diluted with complete DMEM to suitable concentrations before use.

2.7. Statistical analyses

All experiments were performed in duplicates and repeated 3 times. The values obtained for the control and treated samples at various time points were normalized by the basal, zero time value represented as 1. The expression of glucose transporters was normalized against α-actin and compared as a fold change relative to the basal values. Results are represented as means ± SE. Statistical significances were assessed using 3-way analysis of variance (ANOVA) and general linear means, followed by Bonferroni post hoc test and unpaired Student *t* tests using SAS software (SAS Institute, Cary, NC, USA). Differences were considered to be statistically significant at $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

3. Results

3.1. IEC-6 intestinal cells are responsive to glucose and oat β-glucan

The absorption of the nonmetabolizable glucose analog 2-NBDG into intestinal IEC-6 cells as a function of glucose concentration in the DMEM medium after incubation for 10, 30, or 60 minutes is shown in Fig. 1 (white bars). At 5 mmol/L of glucose in the medium, there was a 70% decrease in the level of 2-NBDG within the cells at 60 minutes vs 10 minutes. Because 2-NBDG is a nonmetabolizable glucose analog, its lower levels at 60 minutes suggested that at 5 mmol/L of glucose, some amounts of the analog were released from the cells at longer times. At 8 and 15 mmol/L of glucose, the accumulated 2-NBDG levels were not significantly different at all time intervals, whereas at 25 mmol/L of glucose, there was a 56% (10-60 minutes; $P < .0001$) increase in accumulated 2-NBDG.

To evaluate whether the glucose uptake is related to changes in glucose transport systems, the expression of SGLT1 and GLUT2 messenger RNAS (mRNAs) were assessed using RT-PCR (Figs. 2 and 3). As shown in Fig. 2, SGLT1 expression was significantly down-regulated by incubation of the cells in 5 mmol/L of glucose (10-60 minutes; $P < .05$). SGLT1 expression with incubation in 8 mmol/L of glucose initially increased (10-30 minutes) and then decreased between 30 and 60 minutes. The cells treated with 15 mmol/L glucose showed a mild 7% decrease (10-60 minutes; $P < .0001$) in the expression of SGLT1. The cells exposed to high glucose (25 mol/L) demonstrated a dramatic 47% increase (10-60 minutes; $P < .0001$) in SGLT1 expression. The SGLT1 expression with respect to glucose concentration and time mimicked the pattern of 2-

Table 1 – List of primers used in quantification of glucose transporter expression

Target gene	GenBank no.	Orientation	Primer sequence (5′ → 3′)
β-Actin	NM 031144	Sense	CGTGAAAAGATGACCCAGATCAC
		Antisense	CAGCCTGGATGGCTACGT
Glut2	NM 012879.2	Sense	CTGTCTGTGTCCAGCTTTGCACCC
		Antisense	AAGCCACCCACCAA
SGLT1	NM 013033.2	Sense	GAGTTTGCCTACGGAACTGGAAG
		Antisense	GACCCCGCAGATGATCTT

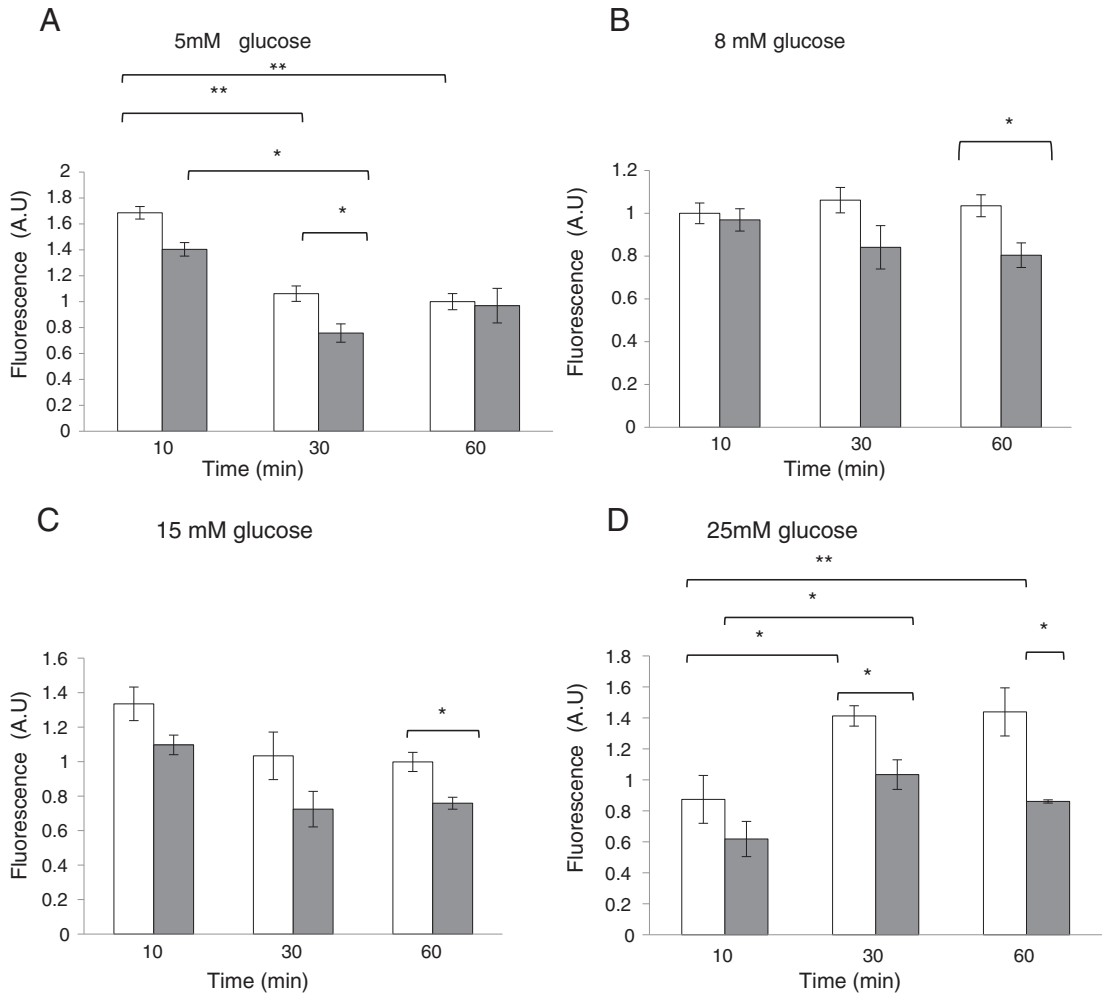

Fig. 1 – Intestinal epithelial cells as a responsive model for glucose uptake and oat β-glucan studies. The IEC-6 cells were treated for a period of 10, 30, and 60 minutes with 5 mmol/L (A), 8 mmol/L (B), 15 mmol/L (C), and 25 mmol/L (C) glucose in the presence (gray bars) or absence (white bars) of oat β-glucan (8 mg/mL). Glucose uptake was measured by absorption of the fluorescent analog 2-NBDG. The data obtained were normalized by cell DNA content and presented as a relative fold change. Values are reported as mean ± SE of triplicates, each repeated 2 times (n = 6). Differences are judged to be significant at *P < .05, **P < .01, and ***P < .001 (2- and 3-way ANOVA followed by Student unpaired *t* test) compared with respective control groups.

NBDG uptake shown in Fig. 1. Therefore, the fluctuation and initial decline in SGLT1 expression at lower glucose concentrations (5, 8, and 15 mmol/L) and its increase at the highest glucose concentration (25 mmol/L) appear to be directly related to glucose uptake. An important observation is that SGLT1 expression was reduced at all glucose concentrations when oat β-glucan was present ($P < .0004$; Fig. 2A-D). This implies that the rate of SGLT1 expression was decreased by the extracellular presence of oat β-glucan, presumably because of a decreased availability of glucose at the cell surface.

Results for GLUT2 mRNA expression are shown in Fig. 3. At the lowest glucose concentration in the medium (5 mmol/L), the GLUT2 expression was reduced by 23% (10-60 minutes; $P > .05$). The extracellular presence of 8 mmol/L of glucose did not significantly change the expression of glucose transporters, but in 15 mmol/L of glucose, GLUT2 mRNA declined by 8% ($P > .05$; Fig. 3B). Exposure of cells to the highest glucose concentration (25 mmol/L) increased GLUT2 expression by 38% (10-60 minutes; $P > .05$) and in a similar manner to SGLT1 expression. GLUT2 mRNA was also significantly reduced at all glucose concentrations in the presence of oat β-glucan ($P < .0001$). GLUT2 expression also followed the same trend with respect to glucose concentration that was observed with 2-NBDG (Fig. 1).

The suitability of intestinal epithelial IEC-6 cells for investigating the effects of oat β-glucan on glucose uptake (Figs. 1-3) was confirmed by a thorough statistical analysis (3-way ANOVA, Table 2) of the variations in glucose uptake and transporter expressions in the cells in response to varying concentrations of glucose, and in the presence or absence of oat β-glucan in the media. As shown in Table 2, fold changes in the uptake of the glucose analog (2-NBDG, $P = .0181$) and expression of SGLT1 ($P < .0004$) and GLUT2 ($P < .0001$) varied significantly with both glucose amount and the presence of oat β-glucan. SGLT1 was more responsive than GLUT2 and its expression constantly and significantly varied with time. A significant interaction ($P < .0001$) between the effects of glucose concentration and time was observed for all 3 parameters (2-NBDG, SGLT1, and GLUT2), showing that the

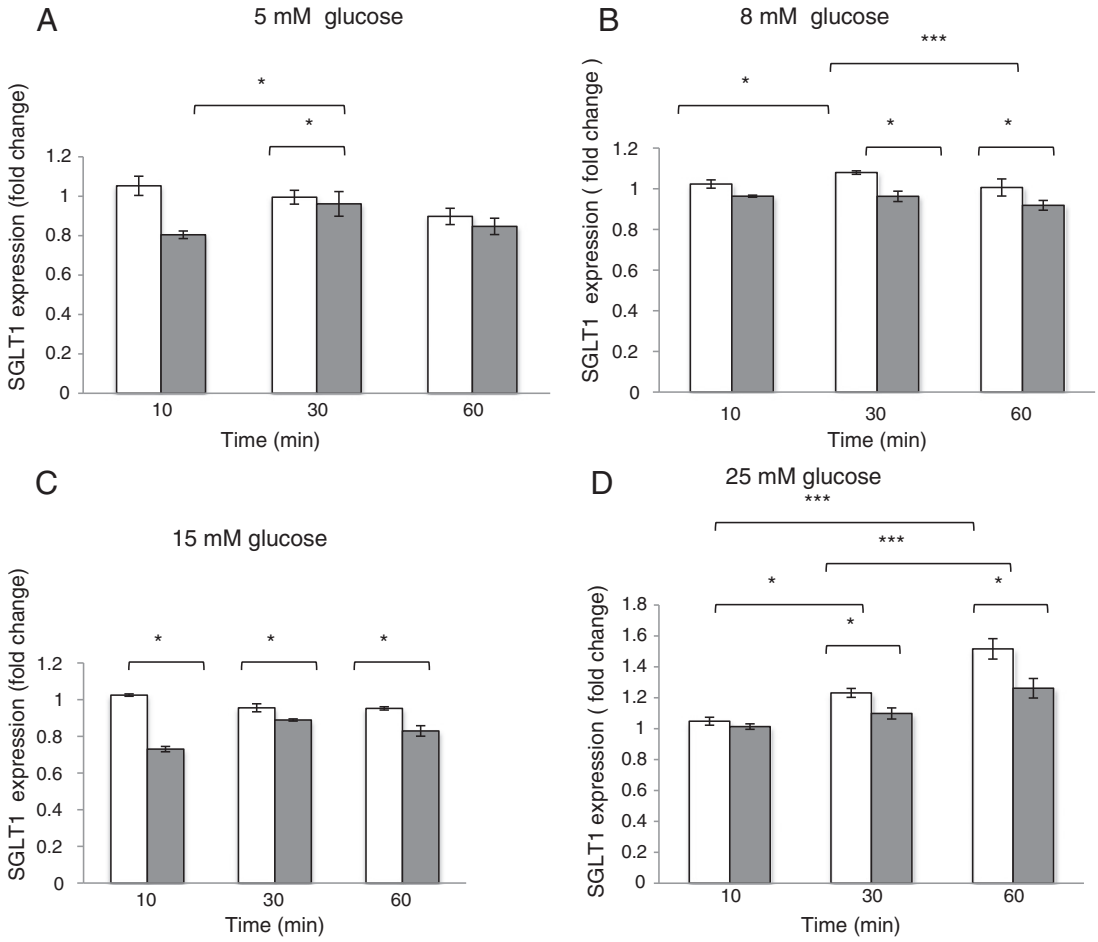

Fig. 2 – Regulation of SGLT1 transporter by glucose and oat β-glucan. IEC-6 cells were exposed to 5 mmol/L (A), 8 mmol/L (B), 15 mmol/L (C), or 25 mmol/L (D) glucose with (gray bars) or without (white bars) oat β-glucan (8 mg/mL). The cells were exposed to glucose and/or β-glucan (8 mg/mL) for a period of 10, 30, and 60 minutes. The SGLT1 mRNA expression is normalized relative to β-actin and presented as a fold change under various conditions. The values are presented as means ± SE of triplicates, each repeated 3 times. Differences are judged to be significant at *P < .05, **P < .01, and ***P < .001 (2- and 3-way ANOVA followed by Student unpaired *t* test) compared with respective control groups.

amount of glucose in the medium influenced the rate of glucose uptake and glucose transporter expressions.

3.2. *Oat β-glucan inhibits glucose absorption by modifying viscosity of the medium and transporter expression*

The second sets of experiments were designed to test if oat β-glucan delayed the glucose uptake by raising the viscosity of the cell culture medium. The viscosity of oat β-glucan solution can vary by changing either the molecular weight or the concentration of oat β-glucan, and in this study, we decided to use variations in the concentration of oat β-glucan as a means of modifying the viscosity of DMEM media containing 25 mmol/L of glucose.

Fig. 4 summarizes the effects of 4-8 mg/mL of oat β-glucan on the uptake of the glucose analog 2-NBDG. As expected, cells exposed to 25 mmol/L of glucose but no oat β-glucan (controls) showed a progressive accumulation of 2-NBDG with time (10-60 minutes). The initial 10-minute response to a challenge of 4, 6, or 8 mg/mL oat β-glucan was an 86% increase, a 28% decrease, and a 26% decrease in glucose uptake, respectively. The presence of oat β-glucan generally reduced the accumulation of the glucose analog at longer times. After 30 minutes, the treatments with 4 mg/mL of oat β-glucan resulted in a 35% ($P < .05$) decrease in glucose uptake relative to controls, and at 8 mg/mL of oat β-glucan, a 38% ($P < .05$) decrease was observed. After 60 minutes, the reduction in glucose accumulation was 40% to 60% for all β-glucan levels. Taken together, these results establish that increases in the viscosity of the media due to increasing concentrations of oat β-glucan progressively inhibited the glucose uptake in the cells.

The effects of increasing viscosity in the medium by increasing concentration (0-8 mg/mL) of oat β-glucan on SGLT1 and GLUT2 expression showed an intriguing pattern because cells adjusted the expression of both glucose transporters according to the levels of available glucose (Figs. 5 and 6). In the medium with 4 mg/mL of oat β-glucan, SGLT1 mRNA significantly increased relative to control levels at 10 minutes, and then mildly decreased at 30 and 60 minutes ($P < .0001$). SGLT1 expression in cells exposed to 6 mg/mL of oat β-glucan varied slightly at 10 to 30 minutes, whereas there was the highest reduction in the transporter expression in cells exposed to the

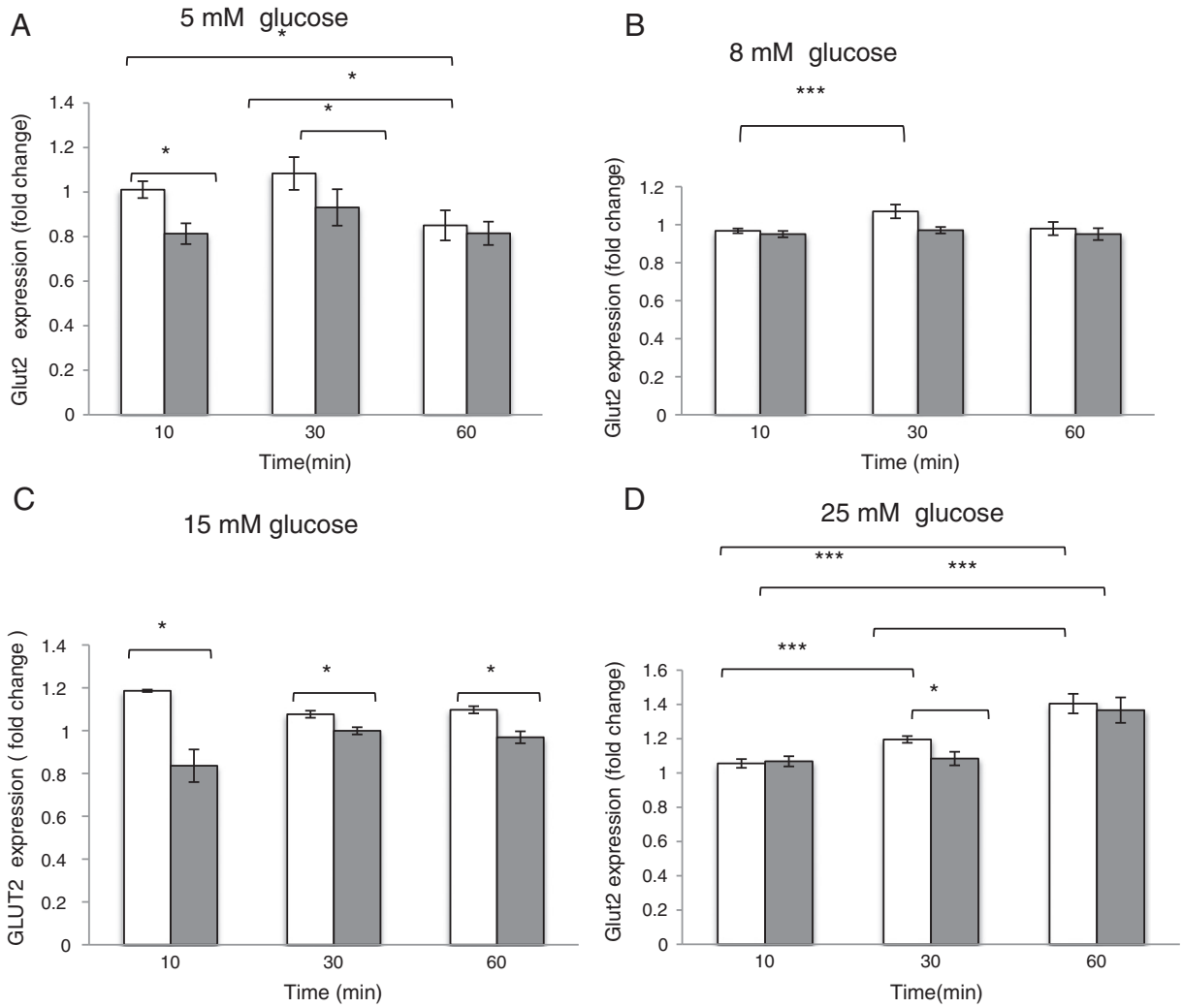

Fig. 3 – Regulation of GLUT2 transporter by glucose and oat β-glucan. IEC-6 cells were exposed to 5 mmol/L (A), 8 mmol/L (B), 15 mmol/L (C), or 25 mmol/L (D) glucose with (gray bars) or without (white bars) oat β-glucan (8 mg/mL). The cells were exposed to glucose and/or β-glucan for a period of 10, 30, and 60 minutes. The SGLT1 mRNA expression is normalized relative to β-actin and presented as a relative fold change under different conditions. The values are presented as means ± SE of triplicates, each repeated 3 times. Differences are judged to be significant at *P < .05, **P < .01, and ***P < .001(2- and 3-way ANOVA followed by Student unpaired t test) compared with respective control groups.

Table 2 – Data analysis for the effects of glucose on glucose transport in IEC-6 cells

	ANOVA [a], P value		
	2-NBDG	SGLT1	GLUT2
Control vs treatment	<.0001	.0004	<.0001
[Glucose]	.0181	<.0001	<.0001
Time	NS	.0003	NS
Treatment * [glucose]	NS	NS	NS
Treatment * time	NS	NS	NS
[Glucose] * time	<.0001	<.0001	<.0001

[a] Three-way ANOVA showing a highly significant effect (P < .05) of glucose concentration and time of sampling on glucose uptake (2-NBDG) and glucose transporter (SGLT1 and GLUT2) expression. Values of P > .05 are taken as nonsignificant (NS).

highest level (8 mg/mL) of oat β-glucan; that is, expression was 13% and 25% lower at 30 and 60 minutes, respectively (Fig. 5). GLUT2 expression by cells in higher viscosities of medium, (corresponding to higher concentrations of 6 and 8 mg/mL of oat β-glucan) showed the greatest significant reduction at 30 minutes, by 6% and 11%, respectively (Fig. 6). By 60 minutes, the GLUT2 expression was lowest in cells exposed to 4 and 8 mg/mL of oat β-glucan. GLUT2 mRNA expression in cells incubated in medium of lower viscosity (4 mg/mL of oat β-glucan) slightly increased by 4% (P < .0001) at 10 minutes and then modestly decreased at 30 and 60 minutes (P < .0001; Fig. 6).

The summary data of the statistical analysis by 3-way ANOVA are shown in Table 3. Overall, there was a significant effect of the addition of β-glucan on glucose uptake (P = .0003), SGLT1 (P = .0071), and GLUT2 (P = .015) expression. Oat β-glucan significantly affected 2-NBDG uptake (P = .0007) and

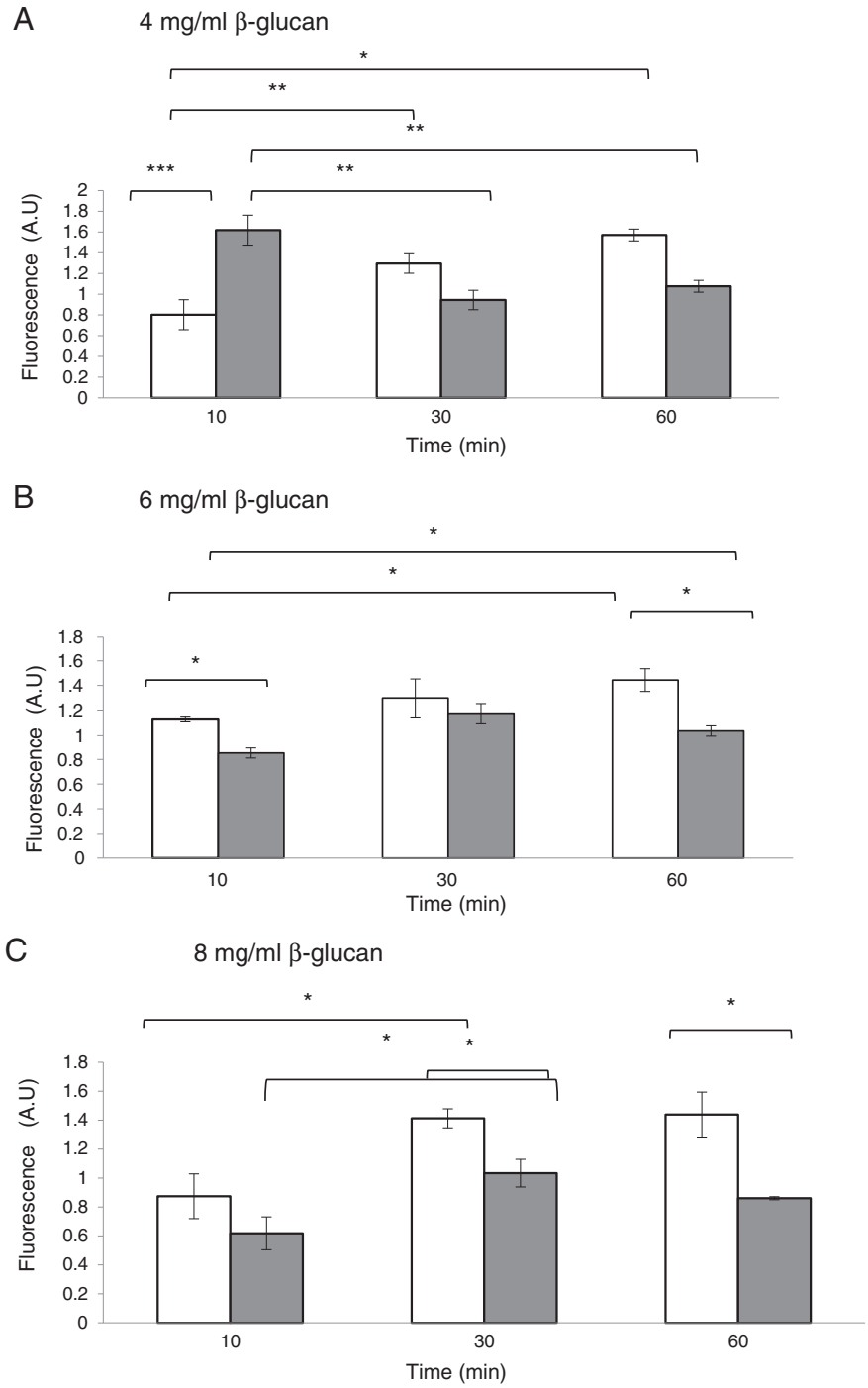

Fig. 4 – Effect of oat β-glucan viscosity on glucose uptake. The cells were exposed to constant glucose (25 mmol/L) and various media viscosities of 4 mg/mL (A), 6 mg/mL (B), and 8 mg/mL (C) of oat β-glucan. Glucose uptake was monitored by 2-NBDG fluorescence at different time intervals (10-60 minutes) after treatments. The white bars represent glucose-only treatments (nonviscous control), and gray bars represent glucose + oat β-glucan treatments (different viscosities). Data are reported as means ± SE of triplicates, each repeated 3 times. 2-NBDG results are presented as a relative fold change as described in Methods and materials. Differences are judged to be significant at *P < .05, **P < .01, and ***P < .001 (2- and 3-way ANOVA followed by Student unpaired t test) compared with respective control groups.

SGLT1 and GLUT2 expression ($P < .0001$) at the 3 time points studied. A significant interaction was observed for "treatment * time" for 2-NBDG uptake ($P = .0002$) and GLUT2 expression ($P = .015$), showing that the rate of GLUT2 expression was influenced by the presence of oat β-glucan. Oat β-glucan concentration also influenced the rate of 2-NBDG uptake ($P = .0497$) and SGLT1 and GLUT2 expression ($P < .001$).

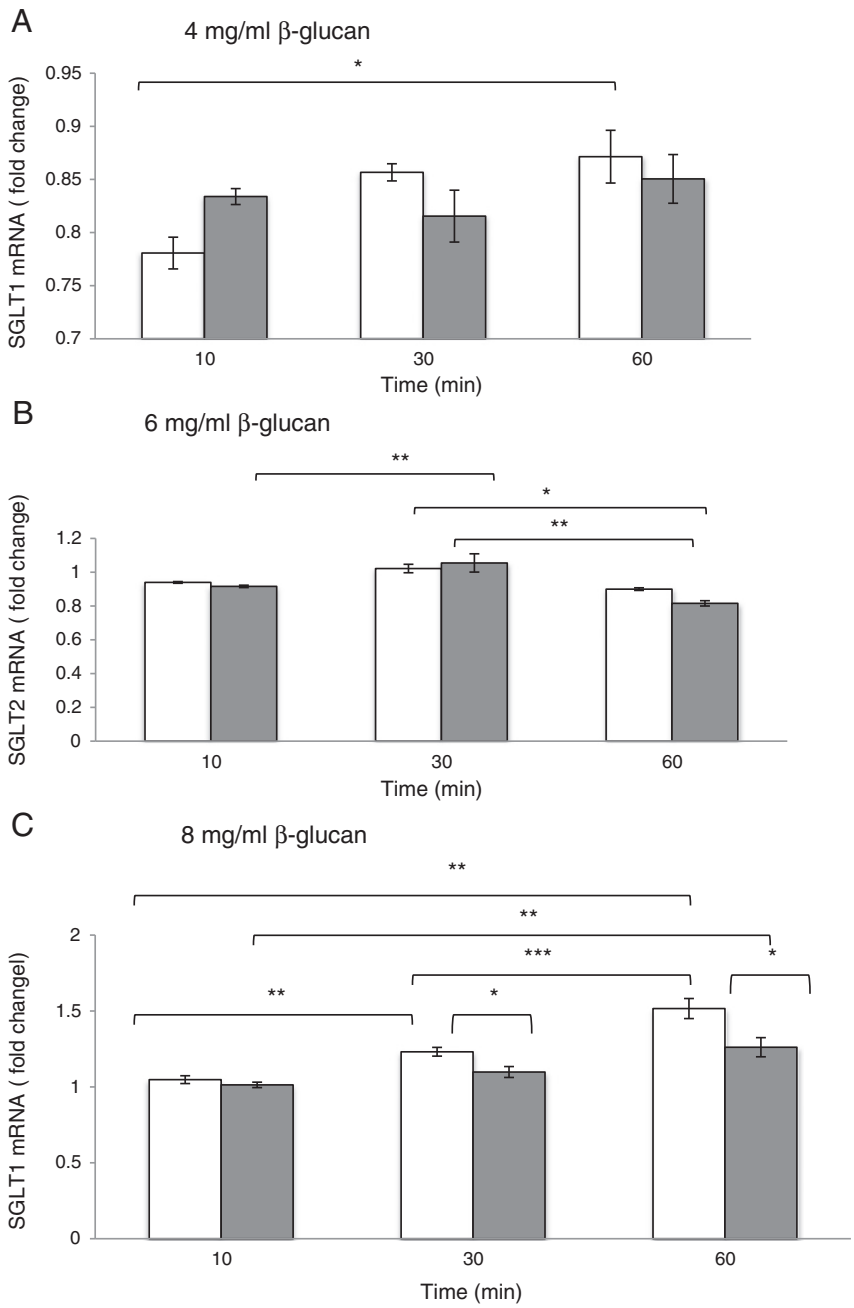

Fig. 5 – Effect of viscosity of oat β-glucan on SGLT1 transporter expression. IEC-6 cell were exposed to constant glucose (25 mmol/L) and various media viscosities of 4 mg/mL (A), 6 mg/mL (B), and 8 mg/mL (C) oat β-glucan for a period of 10, 30, and 60 minutes. The white bars represent glucose-only controls, and gray bars represent glucose + oat β-glucan treatments (different viscosities). Data are shown as a relative fold change in SGLT1 mRNA levels as described in Methods and materials. The values are presented as means ± SE of triplicates, each repeated 3 times. Differences are judged to be significant at *P < .05, **P < .01, and ***P < .001 (2- and 3-way ANOVA followed by Student unpaired *t* test) compared with respective control groups.

The data suggest that a changed viscosity of oat β-glucan acts as a physical barrier for diffusion, hence limiting the availability of glucose at the cell surface to act as the primary signal for GLUT2 and SGLT1 expression. A low concentration of the viscous oat β-glucan (4 mg/mL) coincides with high glucose availability and increased expression of glucose transporters. Higher concentrations of the viscous oat β-glucan (6 and 8 mg/mL) decreased glucose availability for absorption and caused a decrease in SGLT1 and GLUT2 expression.

4. Discussion

Lowering the rate of intestinal glucose uptake is a valuable therapeutic strategy for the management of blood glucose

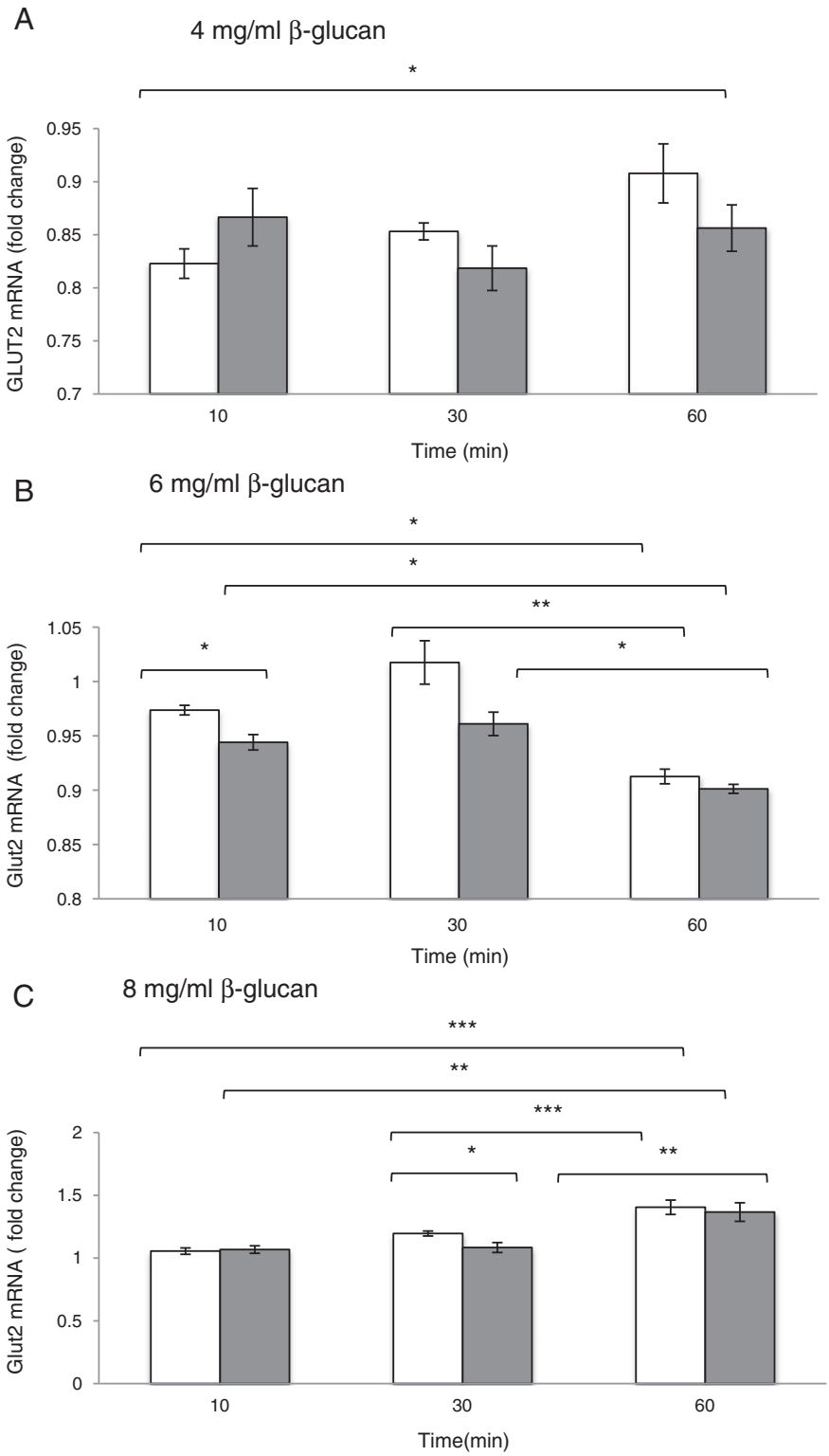

Fig. 6 – Effect of viscosity of oat β-glucan on GLUT2 transporter expression. IEC-6 cells were exposed to constant glucose (25 mmol/L) and various media viscosities of 4 mg/mL (A), 6 mg/mL (B), and 8 mg/mL (C) oat β-glucan for a period of 10, 30, and 60 minutes. The white bars represent glucose-only controls, and gray bars represent glucose + oat β-glucan treatments (different viscosities). Data are shown as a relative fold change in GLUT2 mRNA as described in Methods and materials. The values are presented as means ± SE of triplicates, each repeated 3 times. Differences are judged to be significant at *P < .05, **P < .01, and ***P < .001 (2- and 3-way ANOVA followed by Student unpaired *t* test) compared with respective control groups.

Table 3 – Data analysis for the effects of oat β-glucan on glucose transport in IEC-6 cells

	ANOVA [a], P value		
	2-NBDG	SGLT1	GLUT2
Treatment	.0003	.0071	.015
[β-Glucan]	NS	<.0001	<.0001
Time	.0007	<.0001	<.0001
Treatment * [β-glucan]	.0105	.0475	.007
Treatment * time	.0002	NS	.015
[β-Glucan] * time	.0497	<.0001	<.0001

[a] Three-way ANOVA showing that oat β-glucan concentration (viscosity of the medium) and time of sampling had highly significant effects ($P < .05$) on glucose uptake and glucose transporter expression. Values of $P > .05$ are taken as nonsignificant (NS).

levels. Recent clinical evidence shows that oat and barley β-glucans ameliorate blood glucose levels after meals [13]. This functionality is associated with the development of viscosity in the upper intestine [13,15]. The purpose of the current study was, firstly, to confirm the suitability of intestinal IEC-6 cells as a model to study glucose absorption and transport and, secondly, to investigate the effect of oat β-glucan on these processes.

Caco-2 cells are the most frequent cell line used to study nutrient transport, as their functionality resembles the human intestinal epithelium when grown under standard conditions [16]. However, Caco-2 cells are adenocarcinoma-derived cells that have abnormally tight lateral connections, as evident by very high transepithelial electrical resistance when compared with the epithelial cells of the normal human gut, and are also known to only weakly express the cytochrome P450-3A (CYP3A) gene, which is involved in the synthesis of cholesterol and other lipids [17]. In contrast, IEC-6 cells have a villous enterocyte phenotype and previous studies showed IEC-6 cells to be an effective model for iron uptake in the gut [18].

In the present study, we showed that IEC-6 cells were an appropriate cell model for the study of glucose uptake and expression of glucose transporters. We found that at low glucose concentrations (5 mmol/L), the greatest amount of glucose analog uptake was at shorter times (10 minutes), whereas at 25 mmol/L of glucose, the greatest amount of glucose analog uptake was at longer times (60 minutes). These results are consistent with the earlier studies reporting that glucose present in the lumen modulates its own absorptive capacity [3]. Glucose absorption occurs by a combination of SGLT1 and GLUT2 activities in enterocytes [19,20]. Some debate exists about the relative role of SGLT1 and GLUT2 and their localization in the cells. The traditional explanation is that increased osmotic pressure of high glucose and/or high salt extracellular concentrations leads to transport of glucose into epithelial cells at both the apical brush border surface of the cells and paracellularly through the lateral tight junctions [21] by SGLT1, and that increased GLUT2 expression is related to its role as an effluent transporter at the basal surface of the cell where it transports glucose into the bloodstream. Later studies [22–29] suggested that GLUT2 might also be distributed at the apical brush border when high extracellular glucose levels are experienced. However, a more recent study by Röder et al [27] demonstrates that GLUT2 is normally functional only at the basolateral cell surface, an interpretation supported by Naftalin [28]. It is important to note that some of the results of Grefner et al [23] were obtained on Caco-2 cell models, where paracellular transport may be atypical because of their abnormally strong tight junctions, as discussed above.

A relevant finding in the present study was the responsiveness of both glucose transporters in the IEC-6 cells. SGLT1 expression highly correlated with media glucose and uptake levels. SGLT1, which is present in the apical membrane, had an enhanced expression because it constantly transports glucose to the enterocytes. The cells also responded to the high amount of glucose by increasing the levels of GLUT2. This was most evident at 25 mmol/L of glucose when there was a 38% increase in GLUT2 expression over time. These results are in agreement with previous studies showing that the presence of high glucose in the lumen is causing GLUT2 translocation to the apical membrane [25].

The observed changes in both glucose transport and transporter expression over time confirm the hypothesis that changes in viscosity oat β-glucan acted as a barrier to glucose transport. This was demonstrated by the effect of various amounts of oat β-glucan on modifying of both glucose uptake and glucose transporter activities. In the presence of oat β-glucan, the cells showed a dramatic (38%-58%) decrease in glucose uptake at longer times. At the same time, oat β-glucan significantly decreased SGLT1 and GLUT2 expression, clearly indicating that an active glucose transport system responsive to oat β-glucan and glucose was present in IEC-6 cells.

Limitations to this in vitro cell model for absorption studies do exist. For instance, mucin plays a crucial role in the absorption of nutrients [30]. Caco-2 and HT-29 co-cultures are commonly used to provide more physiological environments resembling the conditions presented in vivo. Thus, co-culturing IEC-6 and HT-29 cells is an essential step to be considered for future studies. However, it is certain from the current findings that IEC-6 cells possess the appropriate glucose transporters necessary for a glucose uptake study. Epithelial cells maintain associations with their surrounding neighbors and seal the gut with tight junctions [31]. This creates physical barrier for regulating passage of molecules from the lumen to the enterocytes. This experiment reveals that such a physiological barrier was present in our system, as is appropriate for studying glucose transport. These findings further supported the usefulness of IEC-6 as a readily available cellular model for nutrient absorptions. Overall, unlike the commonly used Caco-2 cell line, IEC-6 is not derived from carcinoma and hence is representative of noncancerous gut epithelial cells.

Oat β-glucan has been reported to delay absorption of glucose and significantly reduce the glycemic peak response to a carbohydrate-rich meal [13]. Findings from clinical studies point to an inverse relationship between viscosity and glucose absorption [14,15]. The current study is the first in vitro study to examine the underlying mechanism responsible for the modulation of the uptake of glucose by oat β-glucan. We showed that both glucose uptake and transporters expressions were maximally reduced with more viscous oat β-glucan concentrations.

Several studies suggest that the regulation of SGLT1, which includes transcriptional regulation, is caused by availability of

extracellular glucose provided through the diet [32,33]. Diabetic animal models with prolonged feeding of high-carbohydrate diets increased their intestinal glucose transporter levels [28–31]. Low extracellular glucose reduces and high extracellular glucose increases the SGLT1-mediated glucose uptake expressions [34]. In addition to the action of SGLT1 as an active transporter, the involvement of GLUT2 allows for diffusional transport of glucose. Our findings revealed both SGLT1 and GLUT2 mRNAs were significantly decreased in cells exposed to high-glucose media after oat β-glucan treatment. This was interesting because it suggested the potential beneficial effects of oat β-glucan on the regulation of intestinal glucose transporters. The presence of high-viscosity oat β-glucan adjacent to the epithelial cell layer lining the intestine may act as a physical barrier limiting diffusion of high glucose to the cells apical surfaces in a viscosity-dependent manner. Certainly, the results reported here support the hypothesis that the viscosity of oat β-glucan lowered the expression of glucose transporters and hence reduced glucose absorption levels.

GLUT 2 is considered to provide basolateral glucose exit, and there is considerable debate surrounding the assertion that apical GLUT2 mediates intestinal glucose absorption [3]. GLUT2 is present in the brush-border membrane vesicles prepared from rat jejunum [35] and detected only in the basolateral membranes of the mouse jejunum [3]. Previous pharmacologic work [26] did not detect GLUT2 in the rat intestinal cells, but we detected GLUT2 in the same cells using more sensitive methodology, RT-PCR. We were able to demonstrate that the variations in GLUT2 expression significantly related to glucose uptake. Although the physical barrier was postulated to be the primary reason for the effects observed, the possibility that some other signaling pathways were also modified due to oat β-glucan treatments cannot be ignored. In Caco-2 and RIE-1 cells, a high luminal glucose concentration activated PKCβII, which facilitates the movement of intracellular GLUT2 vesicles to the apical membranes [26]. The presence of oat β-glucan could change the GLUT2 transporter movement, in addition to minimizing the glucose diffusion, by retaining high luminal glucose content due to limited diffusion. Therefore, there may be a significant contribution of direct effects of the viscosity of oat β-glucan on cellular mechanisms of uptake in enterocytes to the overall physiological effect of lowering glycemic response.

Author contributions

Conceived and designed the experiments: N.N.A. and P.P.P. Performed the experiments: N.N.A. Analyzed the data: N.N.A., P.P.P., S.M.T., M.B. Wrote the paper: N.N.A., P.P.P., S.M.T., M.B.

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