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

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Original Research

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

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β- … β- … β- … -6 … 2- N-(7- -2- -1,3- -4-) -2- … -6 … 1 (1) … 2 (2) … (0-60) … (5-25 /) … 1 … 2 … (4- /) … β- … β- … β- … β- … 2016

1. Introduction

… [1] … [2] …

Abbreviations: 2- , 2- N-(7- -2- -1,3- -4-) -2- … 2, … 2 -6, … 1, … -1.

☆

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1 (G, 1) 2 (G, 2)

3. G

G, 1 G, 2 4,5.

6.

β-

7,

β- β-

β-

β-

C-6 β-

β-

C-6

G, 1 G, 2.

β-

β-

2. Methods and materials

2.1. Cell culture procedures

C-6

10 CC (, , ,). C C C 5 1

C 4-

C (/ , 37 C 10% C 2.

10%

G (, , C), 100 / (G, , C), 10 /

(-). C

5% 0% 0.25%

C ( ) (10^5) 4

2.2. Experimental design

C-6

2- N-(7- -2- -1,3- -4-) -2- (2- G,). G, 1 G, 2

(10-60)

(0-25 /).

β-

(/) β-

β-

β- (4, 6, / β-) 25 /

2.3. Glucose starvation procedure

C-6 (25 /) 1

(5-25 /) β- (4- /) (10-60) 11,12,

2.4. Measurement of glucose uptake

2- G, G 2- ,

11.

12 5

2- G, ( ) 10 C, 2.7 /

C 7.4 25 C) (13 / G 2- ,

0, 5, 15, 25 / 2- G, 100 μ.

C 100 μ 2- G,

0-25 / 0, 10, 30, 60

1 (1%

, 40 / C, 20 /

7.4) 10 10-

(C 130 &

G, ,).

3 12 000g 10 4 C, 200 μ

4 5 52,

C 3 (

, ,).

1 μ / 3325

50 μ 3325 350-

460- C 3

( ) 12. 2-

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

(-1000), 2000 ( ). 260/2 0 1. 2.1 .5 10.0. 2.0 ( ). 1. 500 ( ). 7000 ( ). 25 10 37 120 5 5 4

2.6. Oat β-glucan preparation

β- (,). β- (5.16) 13. β- 5 0000 / β- β- , 100 30 100 2 β- 3 β-

2.7. Statistical analyses

3 1. α- 3- ( ) t (, ,). *P .05, **P .01, ***P .001.

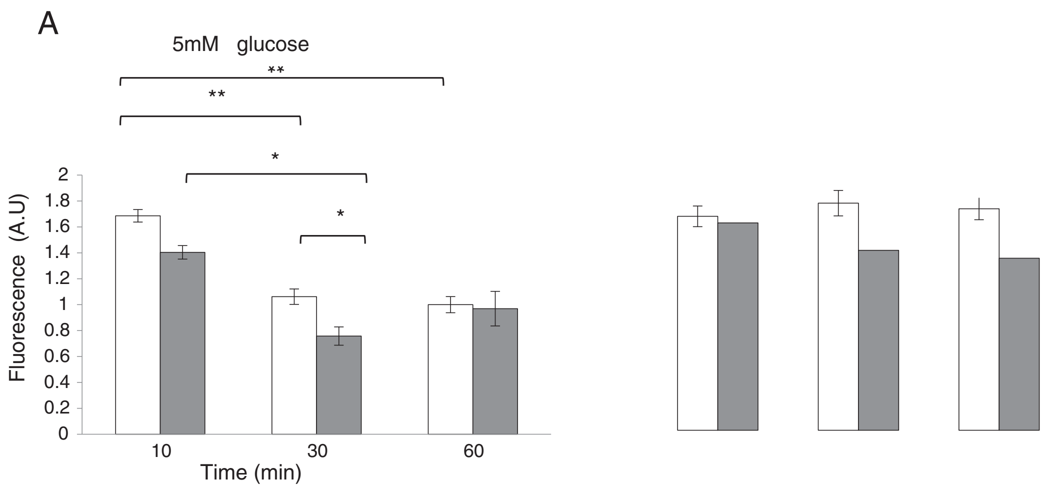
3. Results

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

2- -6 10, 30, 60 . 1 ( ). 5 / 70% 2- 60 10 2- 60 5 / 15 / 2- 25 / 56% (10-60 P .0001) 2- 1 2 ( ) (. 2 3). . 2, 1 5 / (10-60 P .05). 1 / (10-30) 30 60 . 15 / 7% (10-60 P .0001) 1. (25 /) 47% (10-60 P .0001) 1 1 2-

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

		(5′ → 3′)
β-Actin	031144	[illegible]
Glut2	012 7 .2	[illegible]
SGLT1	013033.2	[illegible]



G , . 1. , -
G , . 1 β- (P .0001). G , . 2
(5, , 15 /)
(25 /) G 2- . , (. 1).
. G , . 1 C -6
β-
β- (P .0004 . 2 -). (. 1-3) (3-
G , . 1 , . 2)
β- ,
. ,
G , . 2 . 3. β- . . 2,
(5 /), (2- G , , P .01 1)
G , . 2 23% (10-60 P .05). G , . 1 (P .0004) G , . 2 (P .0001).
/
, β- G , . 1 G , . 2
15 / G , , . 2 % (P .05 .
. 3). (P .0001)
(25 /) G , . 2 G 3 % (10-60 3
P .05) G , . 1 G , . 2 (2- G , G , . 1, G , . 2),

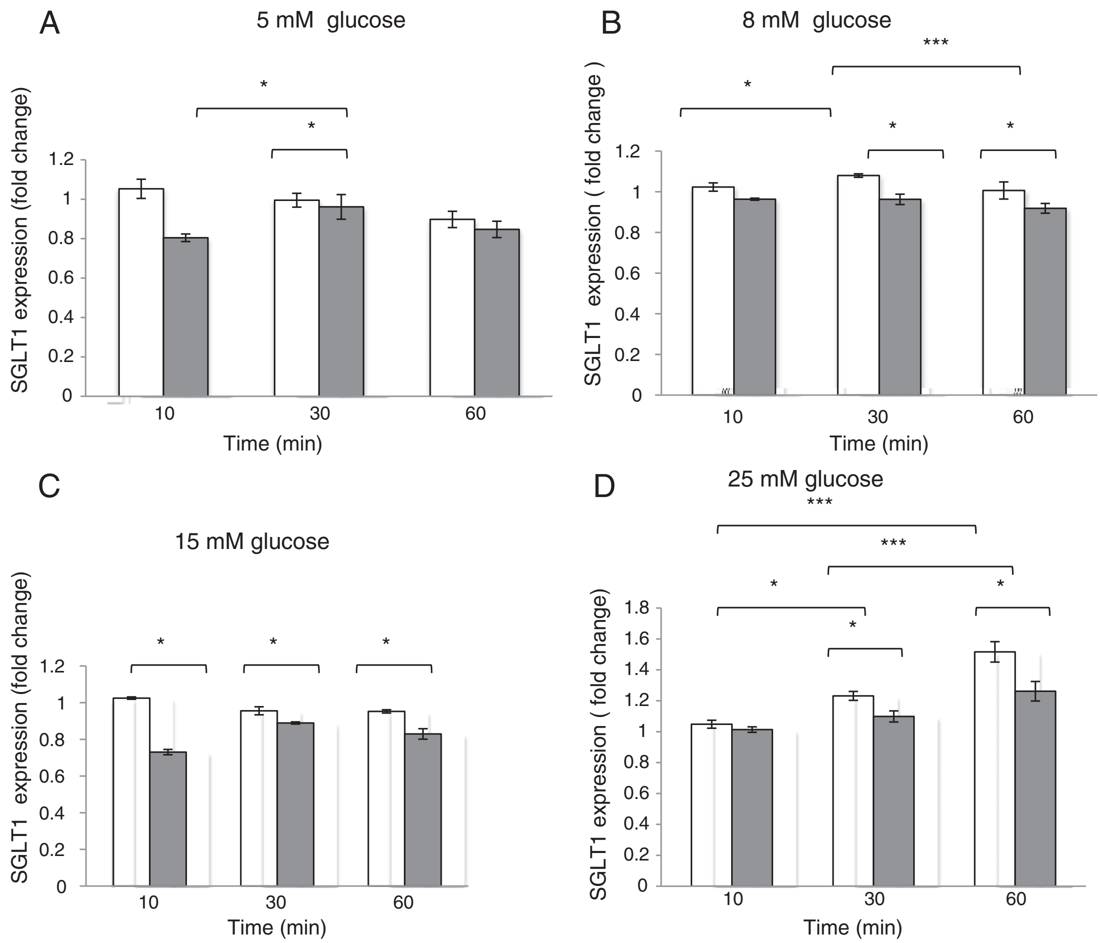


Fig. 2 – Regulation of SGLT1 transporter by glucose and oat β-glucan. IEC-6 cells were exposed to 5 mmol/L (A), 8 mmol/L (B), 15 mmol/L (C), or 25 mmol/L (D) glucose with (gray bars) or without (white bars) oat β-glucan (8 mg/mL). The cells were exposed to glucose and/or β-glucan (8 mg/mL) for a period 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.

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

β- β- β- , , β- 25 / . 4 4- / β- 2- , 25 / β- ( ) 2- , (10-60). 10- 4, 6, / β- 6% , 2 % , 26%

, β- . 30 , 4 / β- 35% (P .05) , / β- , 3 % (P .05) 60 , 40% 60% β- , β- (0- /) β- , 1 , 2 (. 5 6). 4 / β- , , 1 10 , 30 60 (P .0001). , 1 6 / β- 10 30 ,

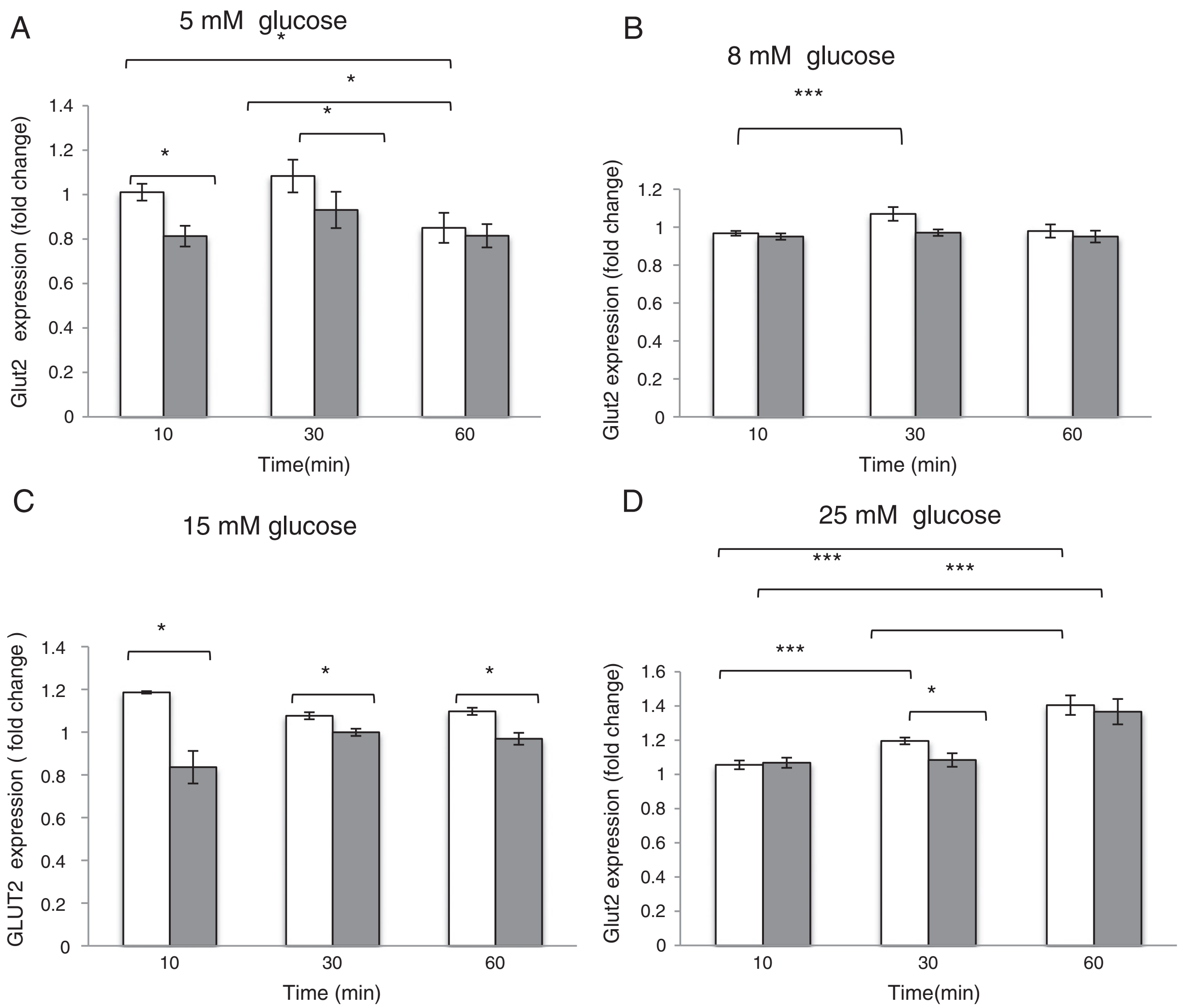


Fig. 3 – Regulation of GLUT2 transporter by glucose and oat β-glucan. IEC-6 cells were exposed to 5 mmol/L (A), 8 mmol/L (B), 15 mmol/L (C), or 25 mmol/L (D) glucose with (gray bars) or without (white bars) oat β-glucan (8 mg/mL). The cells were exposed to glucose and/or β-glucan for a period of 10, 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

	, P.		
	2- . ,	, . 1	, . 2
	.0001	.0004	.0001
	.01 1	.0001	.0001
		.0003	
*			
*			
*	.0001	.0001	.0001

(P .05) (2- (, . 1 , . 2) P .05 ( ).

(/) β- , 13% 25% 30 60 , (. 5). , . 2 , (6 / β-) 30 , 6% 11%, (. 6). 60 , , . 2 4 / β- . , . 2 (4 / β-) 4% (P .0001) 10 30 60 (P .0001 . 6). 3- 3. , β- (P .0003), , . 1 (P .0071), , . 2 (P .015) β- 2- . , (P .0007)

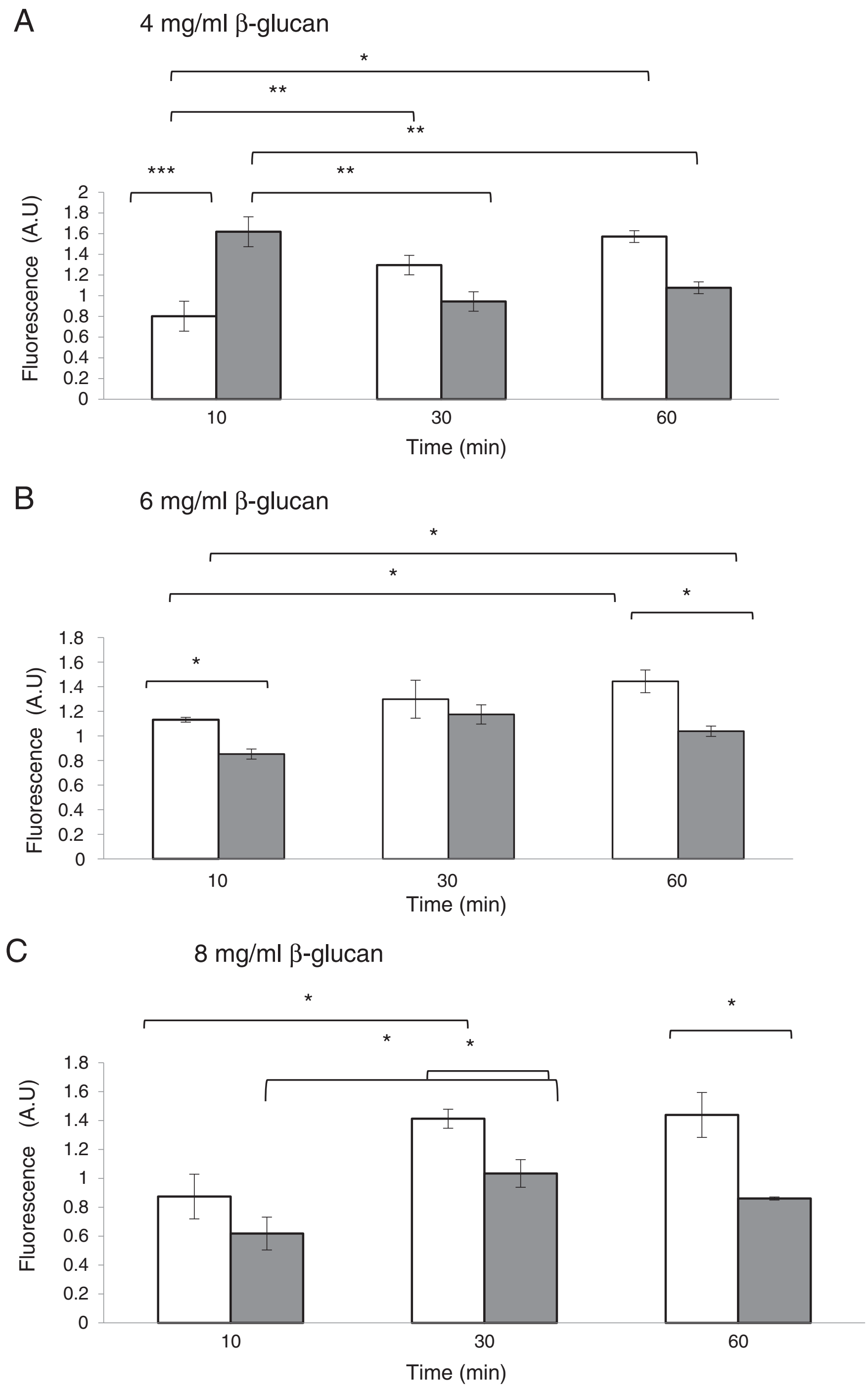


Fig. 4 – Effect of oat β-glucan viscosity on glucose uptake. The cells were exposed to constant glucose (25 mmol/L) and various media viscosities of 4 mg/mL (A), 6 mg/mL (B), and 8 mg/mL (C) of oat β-glucan. Glucose uptake was monitored by 2-NBDG fluorescence at different time intervals (10-60 minutes) 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.

G , . 1 G , . 2 . . (P .0001) 3 . . . . β- .
. " - β- . 2-
* " . . 2- . G , (P .0002) G . 2 . , G , (P .04 7) G , . 1 G , . 2 . . (P
. . (P .015), . . . G , . 2 .001).

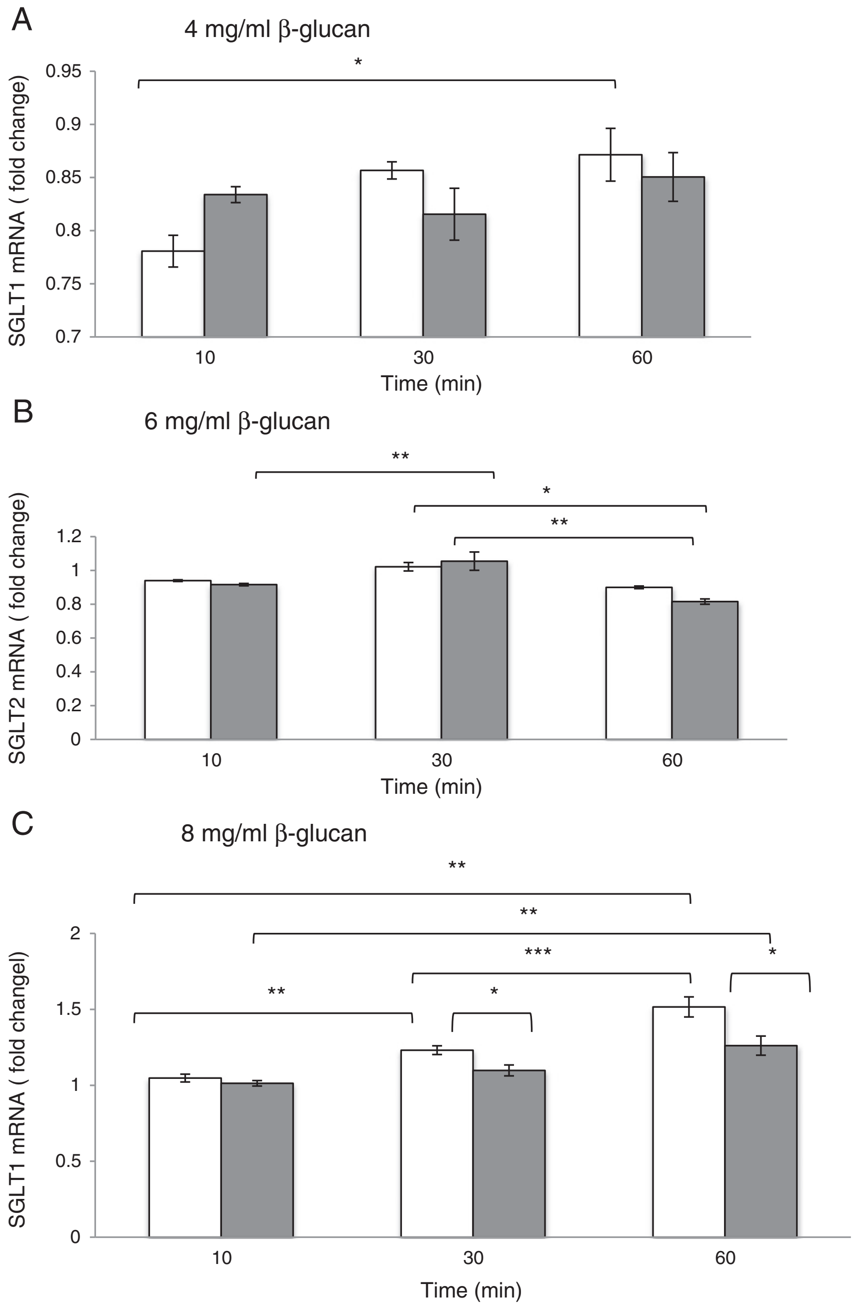


Fig. 5 – Effect of viscosity of oat β-glucan on SGLT1 transporter expression. IEC-6 cell were exposed to constant glucose (25 mmol/L) and various media viscosities of 4 mg/mL (A), 6 mg/mL (B), and 8 mg/mL (C) oat β-glucan for a period of 10, 30, and 60 minutes. The white bars represent glucose-only controls, 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.

4. Discussion

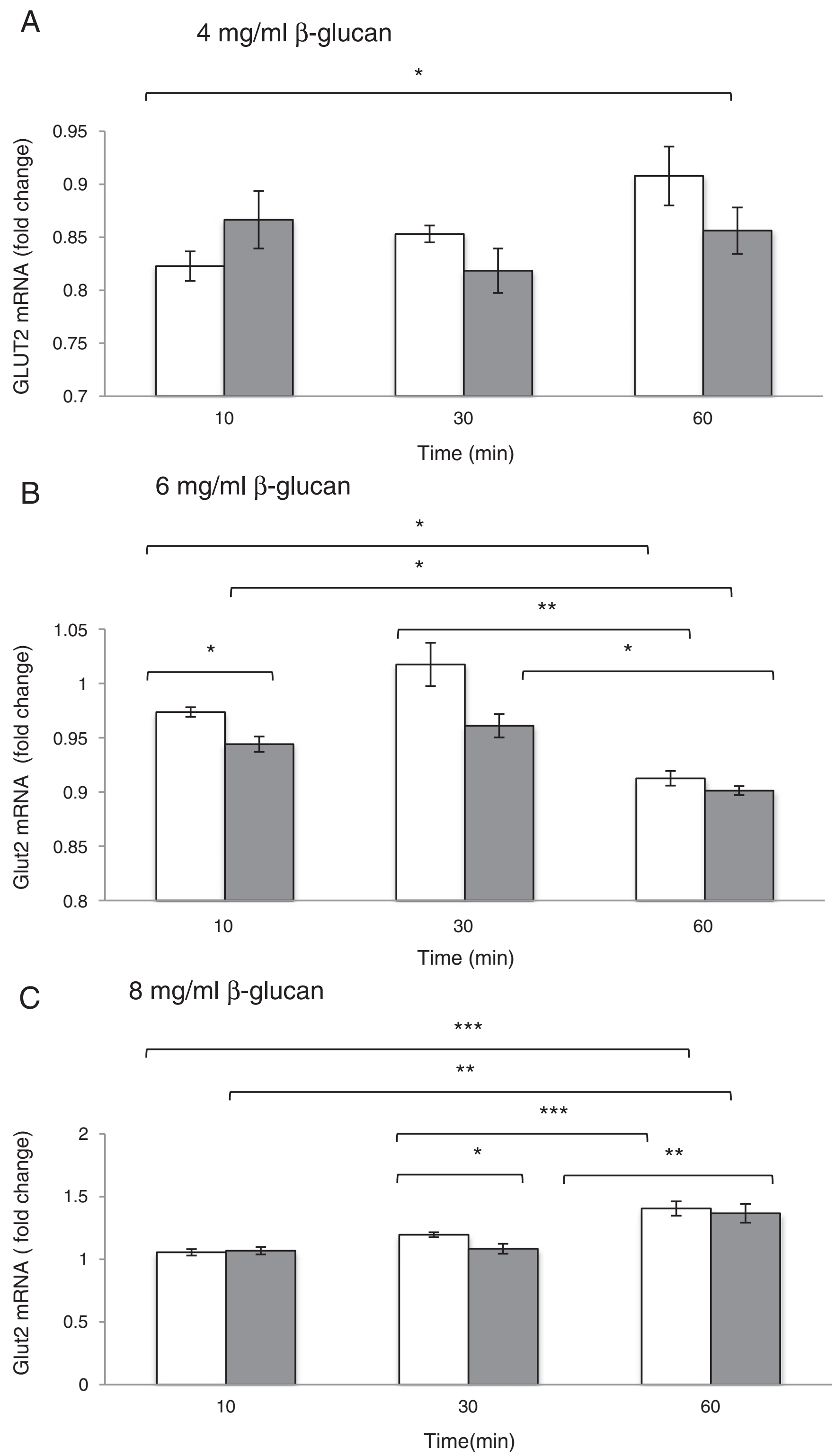


Fig. 6 – Effect of viscosity of oat β-glucan on GLUT2 transporter expression. IEC-6 cells were exposed to constant glucose (25 mmol/L) and various media viscosities of 4 mg/mL (A), 6 mg/mL (B), and 8 mg/mL (C) oat β-glucan for a period of 10, 30, and 60 minutes. The white bars represent glucose-only 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

	, P		
	2- G ,	G , 1	G , 2
G β-,	.0003	.0071	.015
		.0001	.0001
	.0007	.0001	.0001
* β-	.0105	.0475	.007
*	.0002		.015
G β-, *	.04 7	.0001	.0001

- β- ( ) (P .05) P .05 ( ).

β- 13 .

13,15 . C-6

, , β-

C -2

16 . C -2

450-3 (C 3)

17 . C-6 C-6

1 C-6

(5 /), (10), 25 / (60).

3 G G , 1 G , 2 1 ,20 . G , 1 G , 2

/ .

G , 1, G , 2 21

22–2 G , 2

27 G , 2

2 .
23 C -2 G ,

C-6 G , 1

G , 1,

G , 2.

25 / 3 % G , 2

G , 2 25 .

β-

β-

β- , (3 %-5 %) β-

G , 1 G , 2

β- C-6 .

30 . C -2 -2

C-6 -2

C-6

31 .

C-6

C -2 , C-6

β-

13 .

14,15 .

β-

β- G , 1,

32,33.

2 –31. 1- 34. 1 2 1 2

β- β- β-

β-

2

2 3. 2 35

3. 26 2

2

2

β- -2 -1 C_β

2. 26. β- 2

β-

Author contributions

Acknowledgment

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16 2007 26 63 –44.

17 G , , , , ,
. —
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1 G G , , , , , , , ,
46.
2001 2 64 –6 .
1