读书报告

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2016年08月13日

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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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Introduction

Diabetes is characterized by chronic hyperglycemia.

Controlling the intestinal absorption of glucose in the **brush-border membrane** of the gut → Improve levels of glucose in the blood

↓

SGLT1 + GLUT2

Oat β -glucan →

- delays absorption of glucose
- significantly reduces postprandial blood glucose levels

Methods and materials

1. Cell culture procedures — IEC-6

2. $C_{2\text{-NBDG}}$ = 100 μM ; $C_{\text{Oat β-glucan}}$ = 8 mg/ml

   a. $C_{glucose}$ = 5 ; 8 ; 15 ; 25 mM

   b. Time = 10 ; 30 ; 60 minutes

3. $C_{glucose}$ = 25 mM

   $C_{\text{Oat β-glucan}}$ = 4 ; 6 ; 8 mg/ml

Cell lysis buffer 1 ml ; 10 min

↓

Sonication ; 10 sec

↓

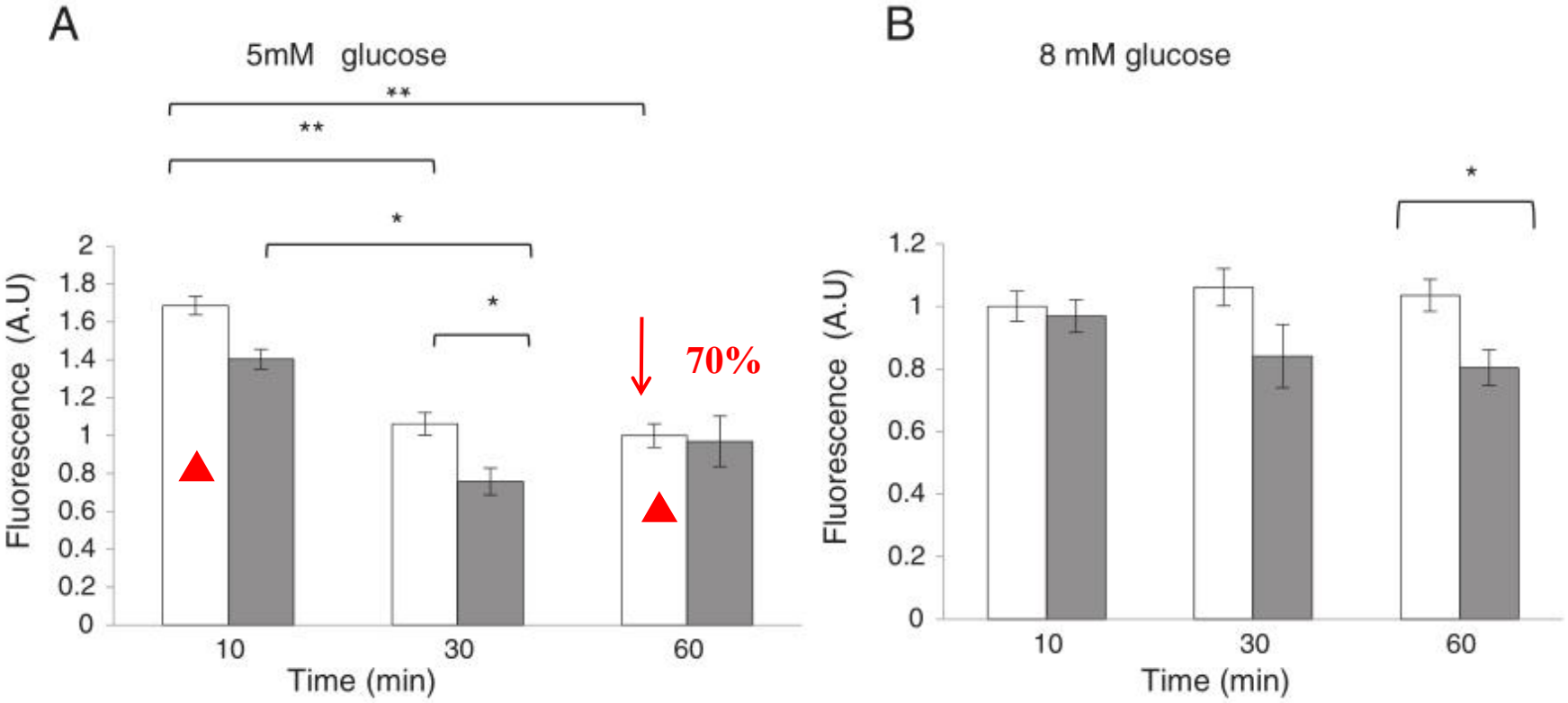
12000 g ; 4 ℃ ; 10 min

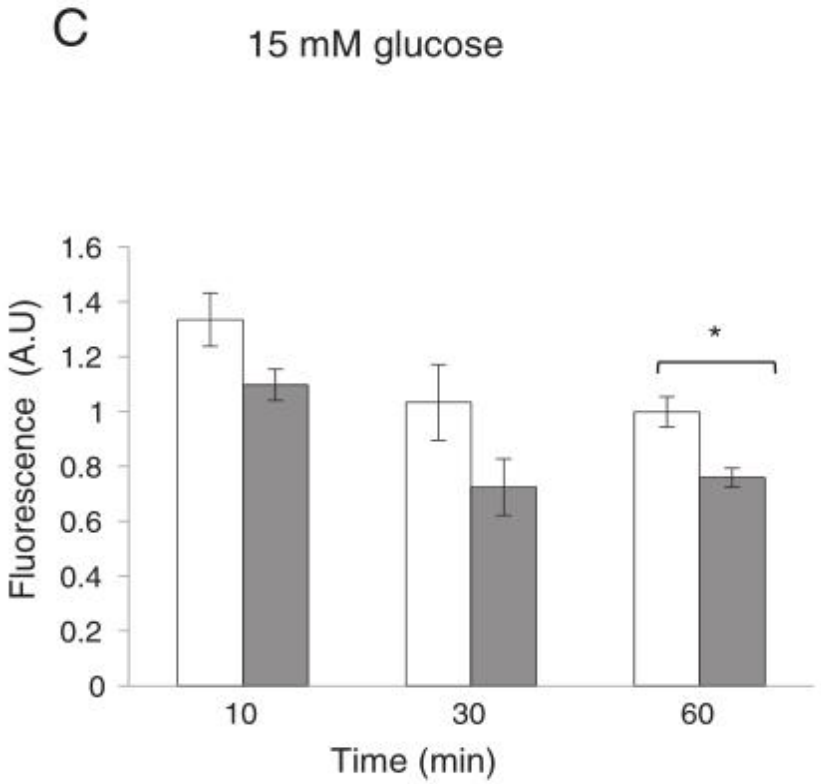
↓

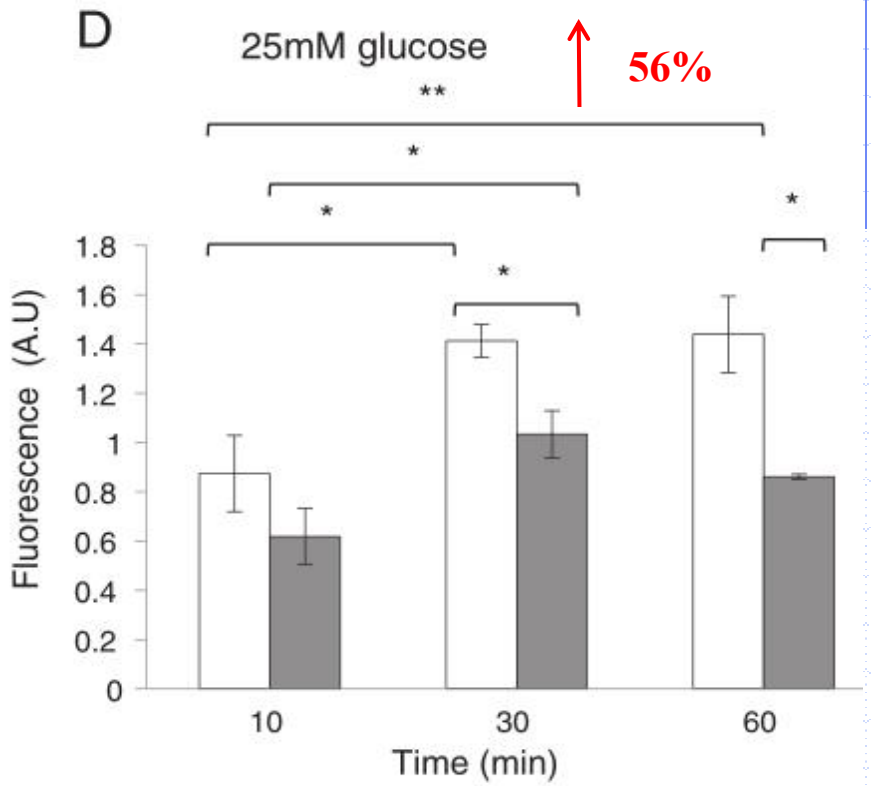
3 aliquots ; 200 μl

注：构建标准曲线

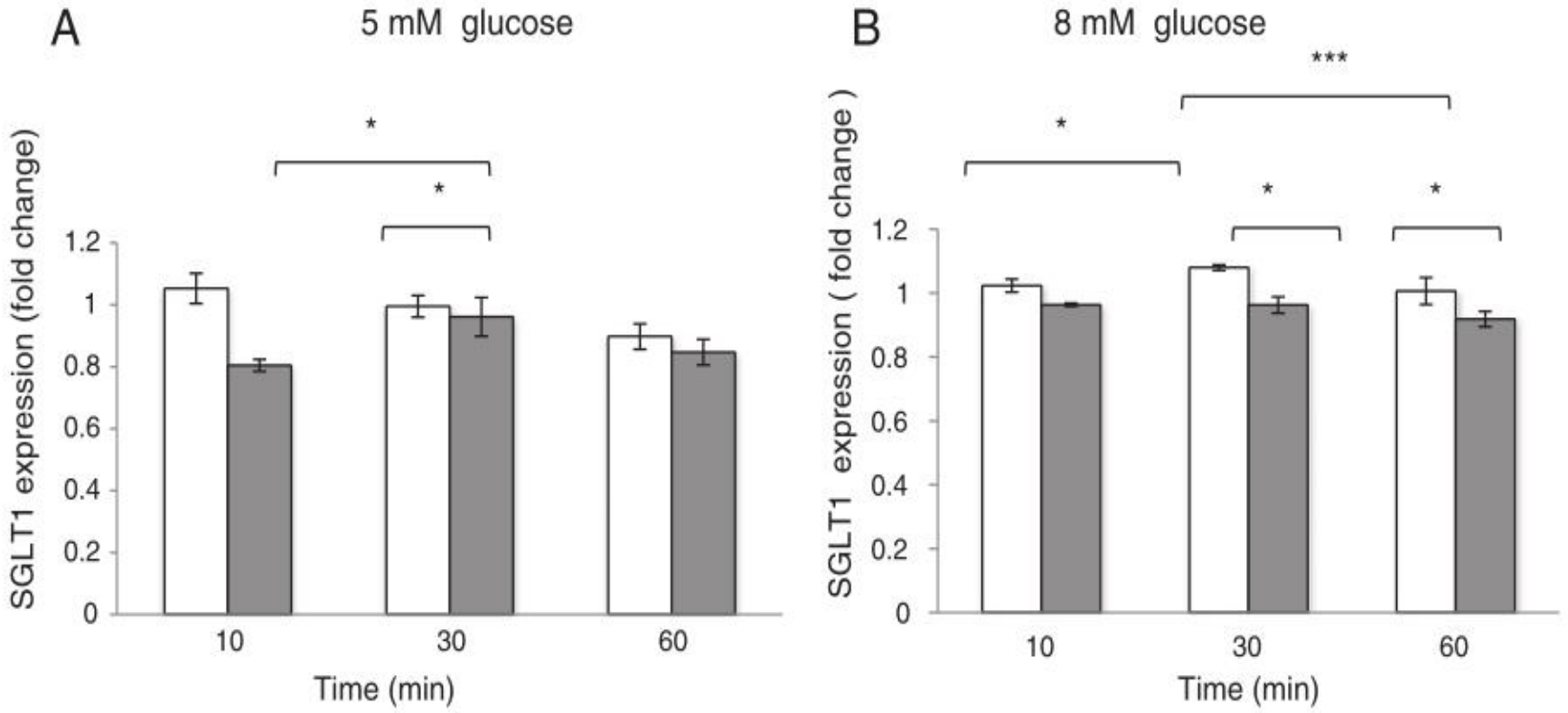
1. IEC-6 intestinal cells are responsive to glucose and oat β -glucan



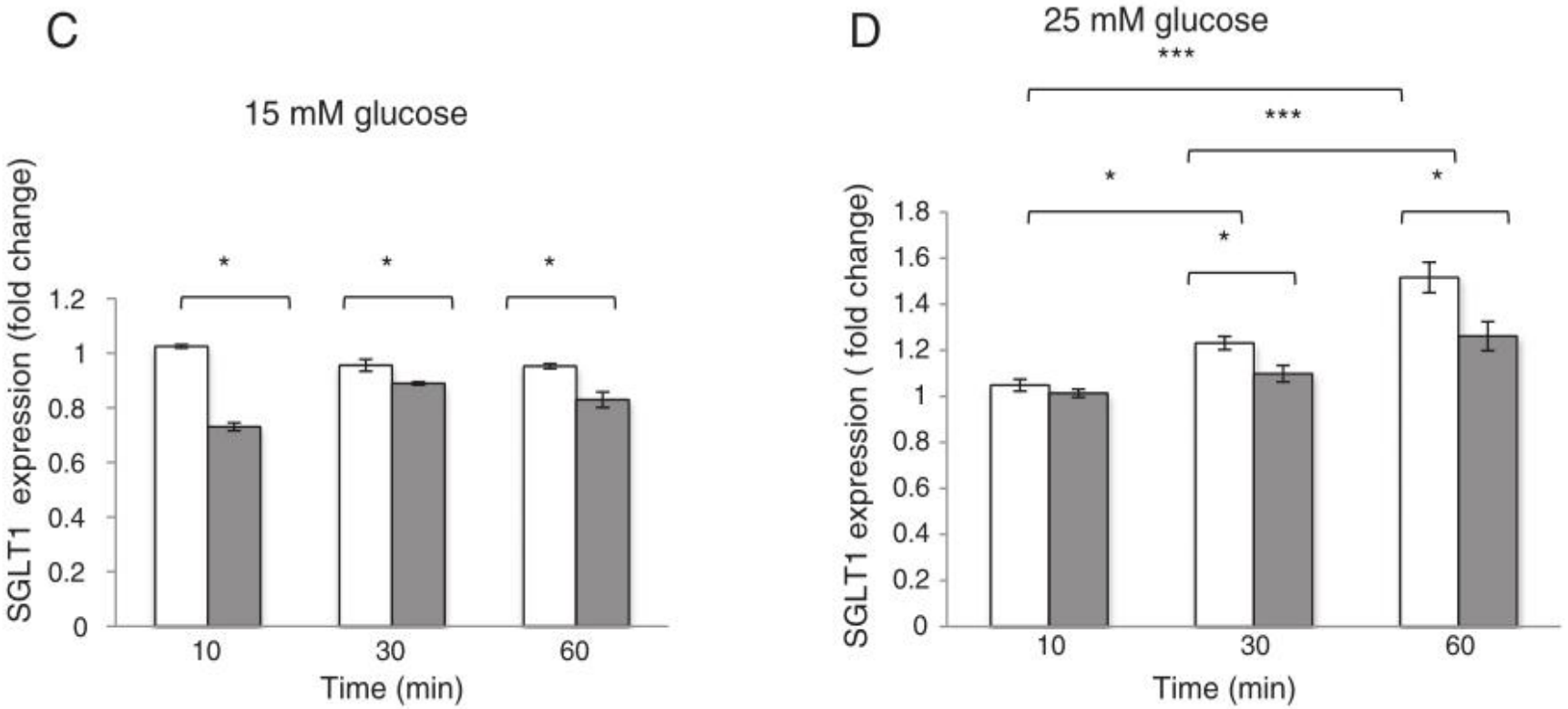

C
15 mM glucose
Fluorescence (A.U)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
10
30
60
Time (min)


D
25mM glucose
56%
**
*
*
*
*
Fluorescence (A.U)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
30
60
Time (min)

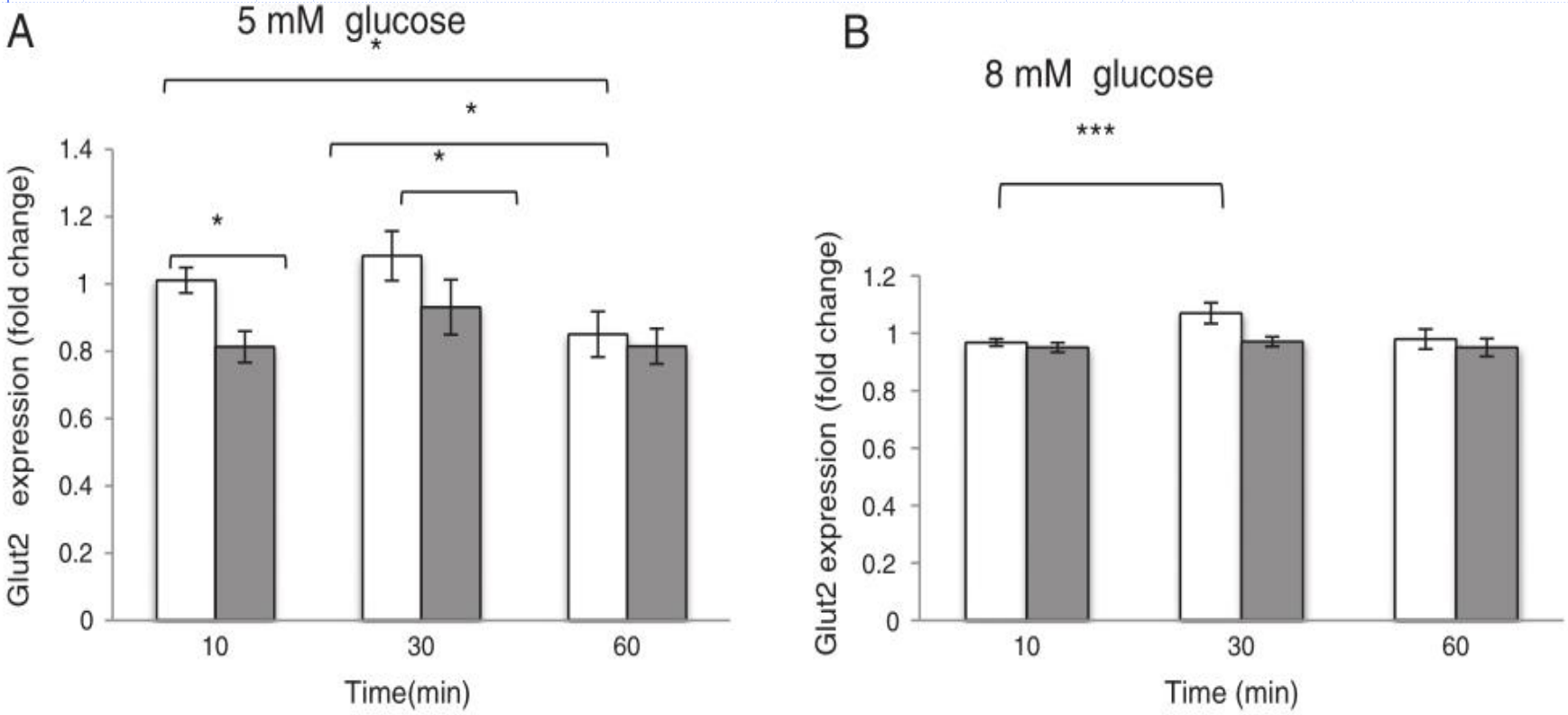
SGLT1


A
5 mM glucose
SGLT1 expression (fold change)
1.2
1
0.8
0.6
0.4
0.2
0
*
*
10
30
60
Time (min)
B
8 mM glucose
SGLT1 expression (fold change)
1.2
1
0.8
0.6
0.4
0.2
0
*
***
*
*
10
30
60
Time (min)

SGLT1


C
15 mM glucose
SGLT1 expression (fold change)
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
10
30
60
Time (min)
D
25 mM glucose
***
***
*
*
*
SGLT1 expression (fold change)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
30
60
Time (min)

GLUT2


A
5 mM glucose
Glut2 expression (fold change)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
30
60
Time(min)
*
*
*
*
B
8 mM glucose
***
Glut2 expression (fold change)
1.2
1
0.8
0.6
0.4
0.2
0
10
30
60
Time (min)

GLUT2

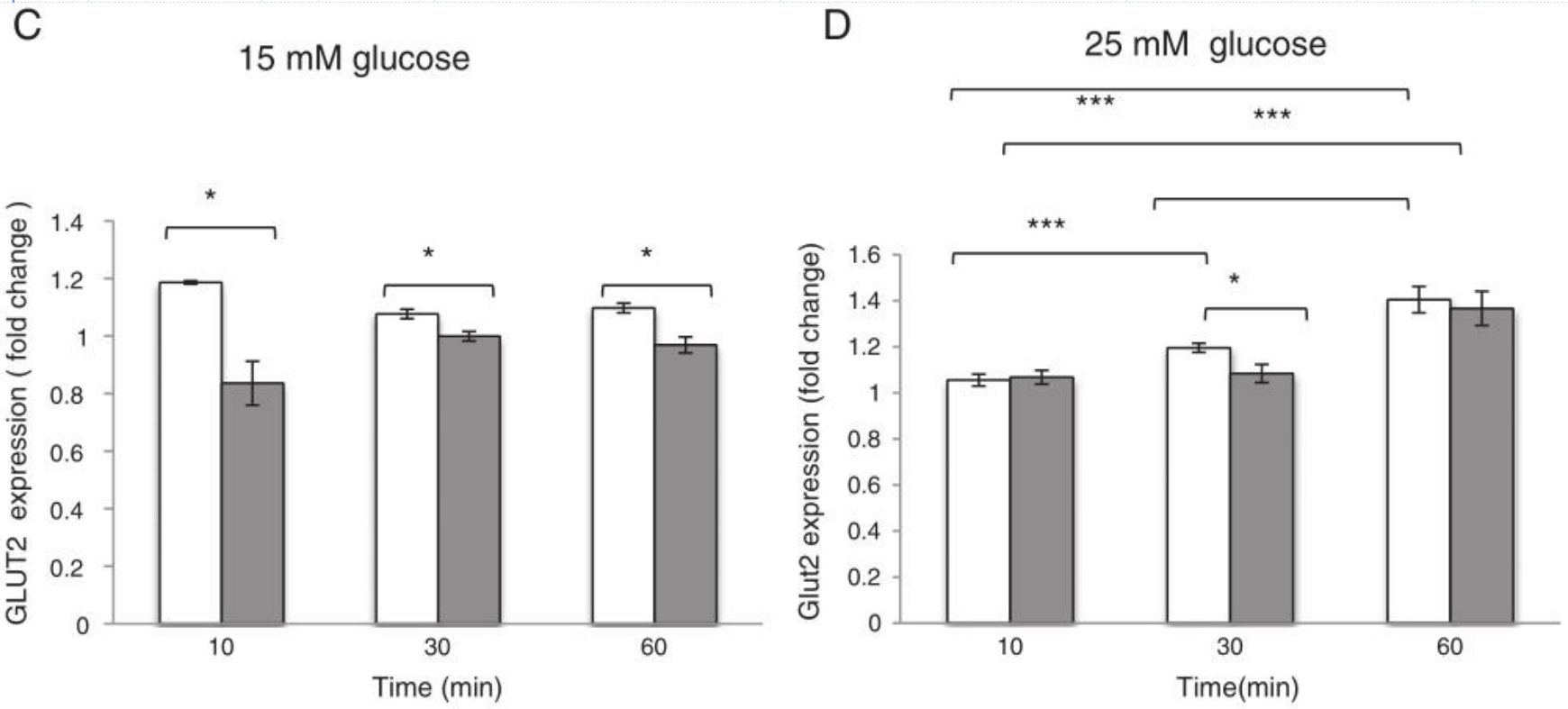
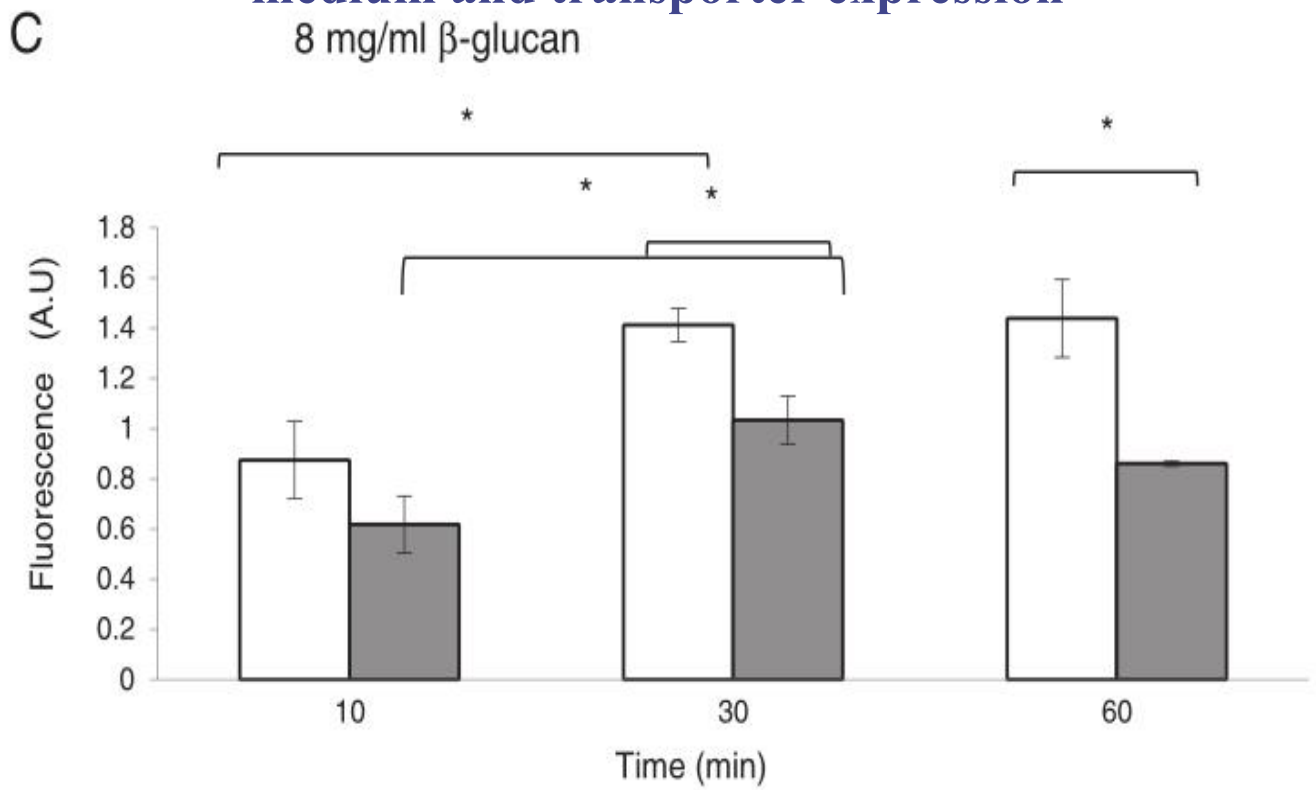

C
15 mM glucose
GLUT2 expression (fold change)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
10
30
60
Time (min)
D
25 mM glucose
***
***
***
*
Glut2 expression (fold change)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
30
60
Time(min)

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

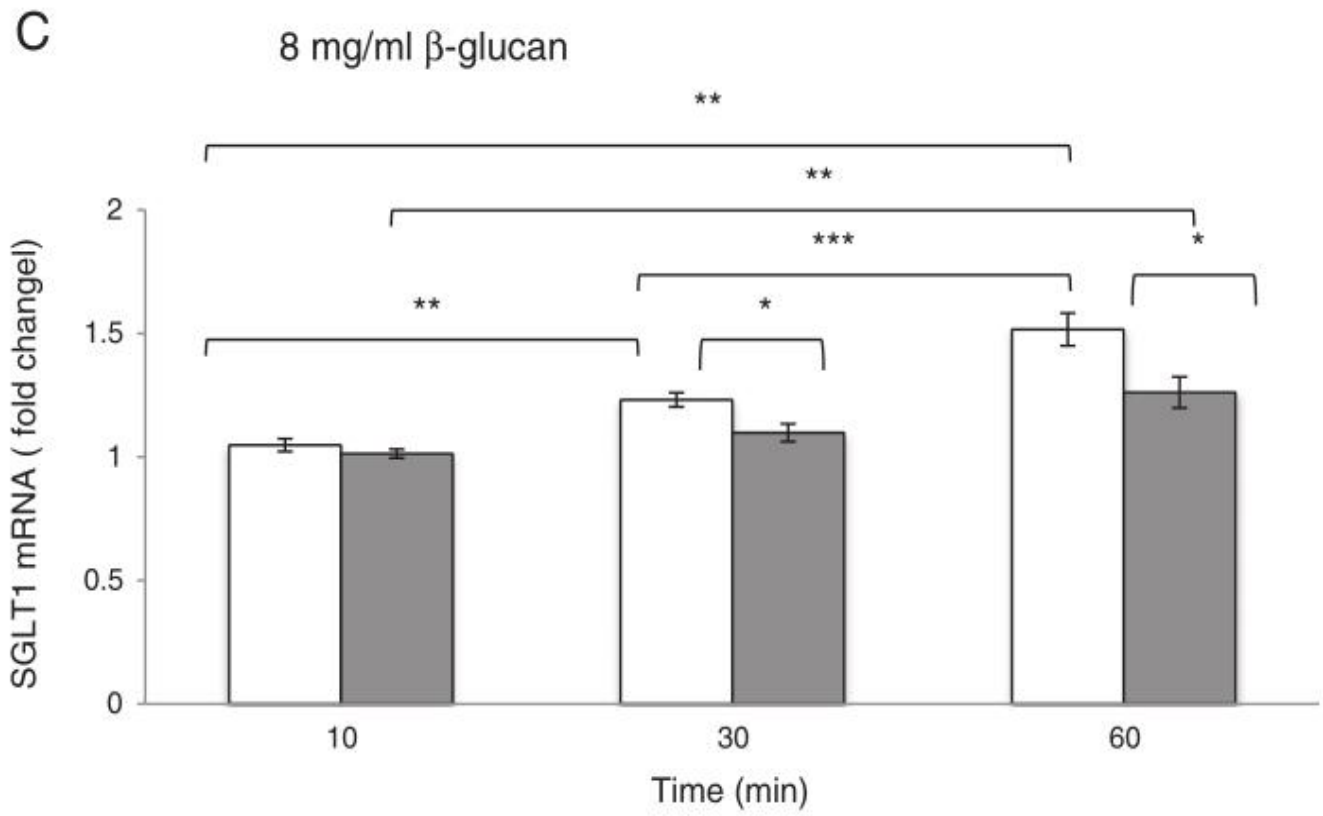
	ANOVA [a], P value		
	2-NBDG	SGLT1	GLUT2
Control vs treatment	<.0001	.0004	<.0001
[Glucose]	.0181	<.0001	<.0001
Time	NS	.0003	NS
Treatment * [glucose]	NS	NS	NS
Treatment * time	NS	NS	NS
[Glucose] * time	<.0001	<.0001	<.0001

[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).

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



SGLT1


C
8 mg/ml β-glucan
**
**
***
*
**
*
SGLT1 mRNA (fold changel)
2
1.5
1
0.5
0
10
30
60
Time (min)

GLUT2

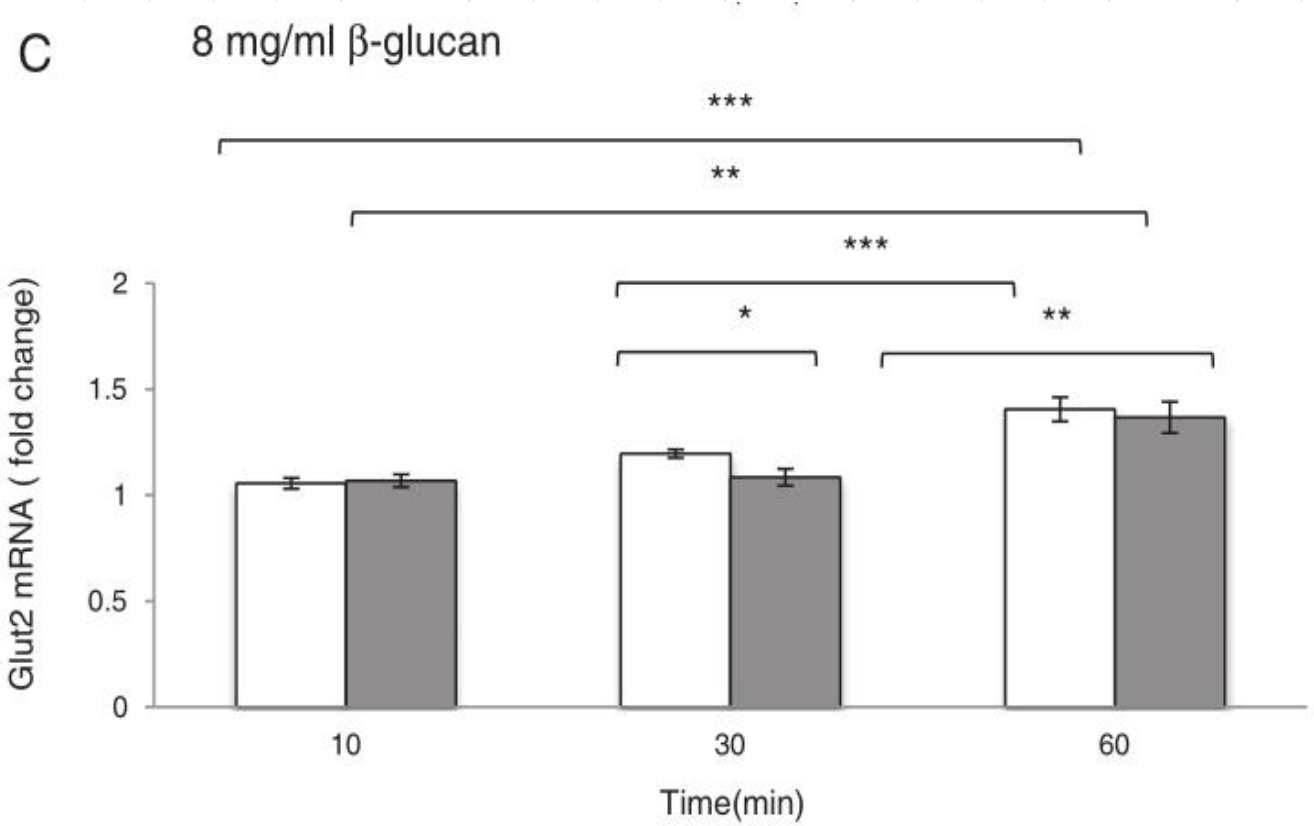

C
8 mg/ml β-glucan
***
**
***
*
**
Glut2 mRNA (fold change)
2
1.5
1
0.5
0
10
30
60
Time(min)

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).

This study affirmed oat β-glucan as a dietary agent for minimizing postprandial glucose and showed that modulating the activityof the key intestinal glucose transporters with oat β-glucan could be an effective way of lowering blood glucose levels in patients with diabetes.

感悟

Experimental design

Discussion

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Nutrients. 2014 Oct; 6(10): 4165–4177.
Published online 2014 Oct 13. doi: 10.3390/nu6104165
PMCID: PMC4210911

The Role of Sodium-Dependent Glucose Transporter 1 and Glucose Transporter 2 in the Absorption of Cyanidin-3-*O*-β-Glucoside in Caco-2 Cells

Tang-Bin Zou,[1,2] Dan Feng,[2] Gang Song,[1] Hua-Wen Li,[1] Huan-Wen Tang,[1] and Wen-Hua Ling[2,*]

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This study examined the absorption mechanism of Cy-3-G in the small intestine, with respect to the role of the glucose carrier SGLT1 and GLUT2 in the transportation of cyanidin glycosides across the intestinal brush border membrane using a Caco-2 cell model.

A better understanding of the mechanisms of anthocyanin absorption would be helpful in optimizing the application of nutrients in oxidative-induced diseases.

Methods

RNA Interference

To knockdown SGLT1 and GLUT2 expression, we performed transfection of human SGLT1 and GLUT2 small interfering RNA (sc-61538, sc-35495) with Caco-2 cells on Day 18 after differentiation, respectively.

One-point-eight microliters of transfection reagents were added to 2.0 mL of DMEM serum-free medium containing 2 nmol/L of each siRNA oligo, incubated for 20 min and then added to the 12-well Transwell containing 1.0 mL fresh medium.

A nonrelated, scrambled siRNA (sc-37007) was used as a control.

Transfection reagent and all siRNA oligos were designed and synthesized by Santa Cruz.

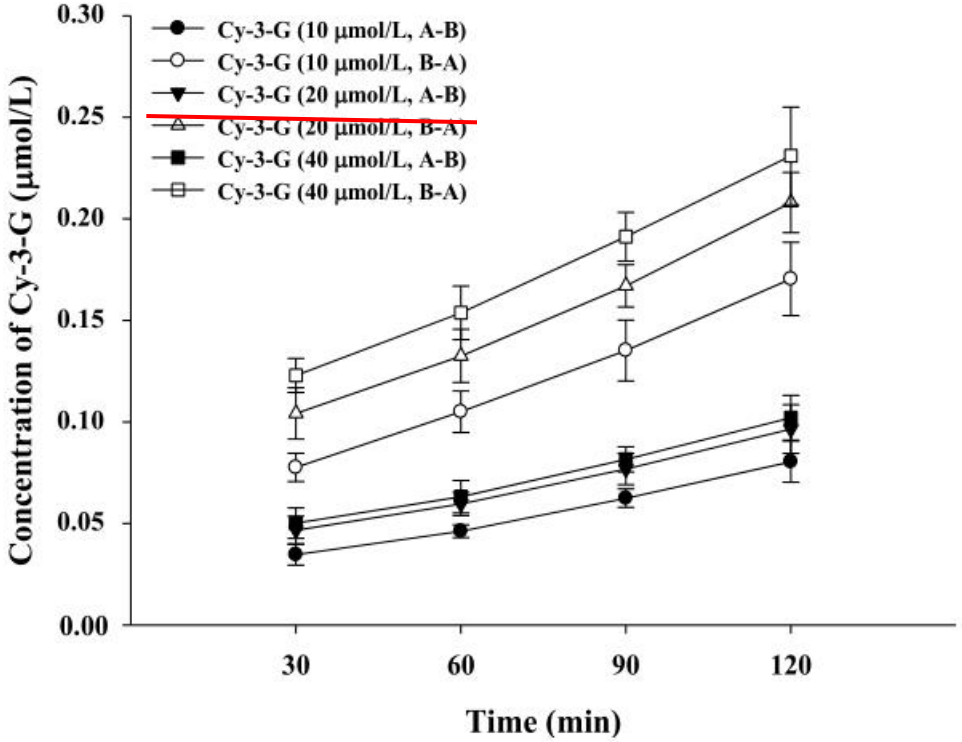
Twenty four, 48 and 72 h post-transfection, western blotting and real-time PCR were used to measure intracellular SGLT1 and GLUT2 levels.

1. Transport of Cy-3-G in Caco-2 Cell Monolayer

Table 1. Transport parameters of Cy-3-G across Caco-2 cell monolayer ($n = 6$).

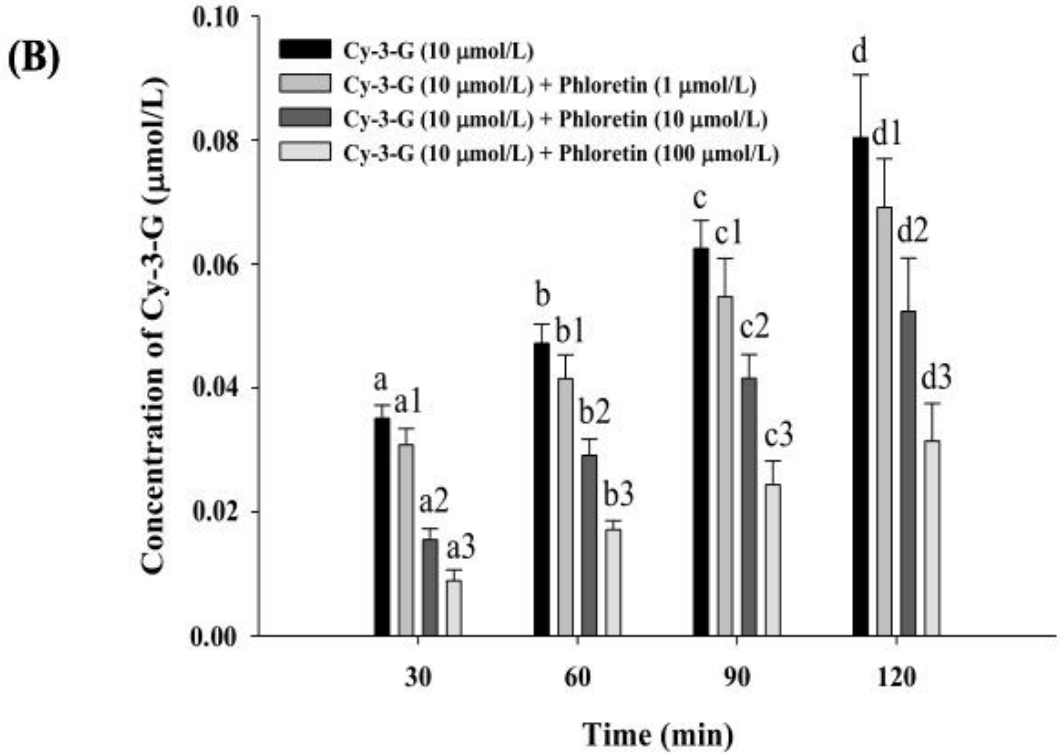
Cy-3-G (μmol/L)	P_{app} ($\times 10^{-7}$ cm/s)		Efflux Ratio	Transport Efficiency (%)
	B → A	A → B		
10	10.57 ± 1.12	14.96 ± 1.88	0.71	2.41
20	6.45 ± 0.46	8.98 ± 1.12	0.72	1.45
40	3.58 ± 0.37	4.75 ± 0.51	0.75	0.76

Figure 1. Transport of Cy-3-G in Caco-2 cells; samples were collected from 30 to 120 min.

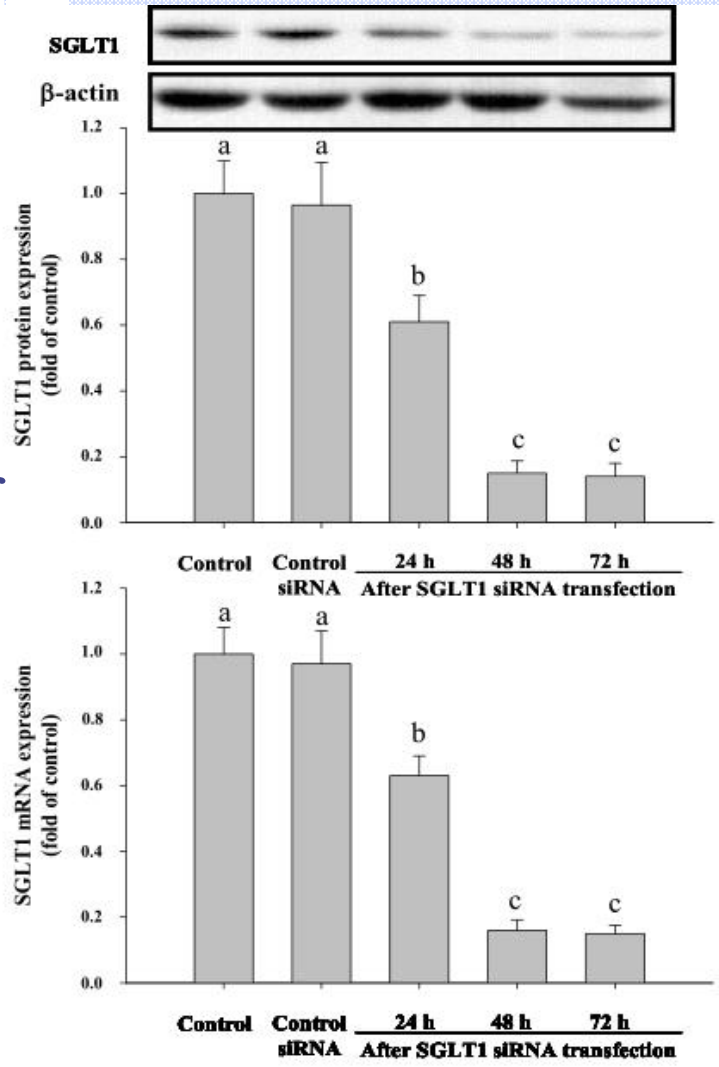


2. Absorption of Cy-3-G in the Presence of either Phloridzin or Phloretin

Figure 2. Effects of phloridzin and phloretin on Cy-3-G absorption. The cell monolayer was treated with phloridzin (**A**) or phloretin (**B**) for 0.5 h before the assays were carried out. Values without a common letter differ, $p < 0.05$.



4. SGLT1 and GLUT2 Involve the Absorption of Cy-3-G in Caco-2 Cells



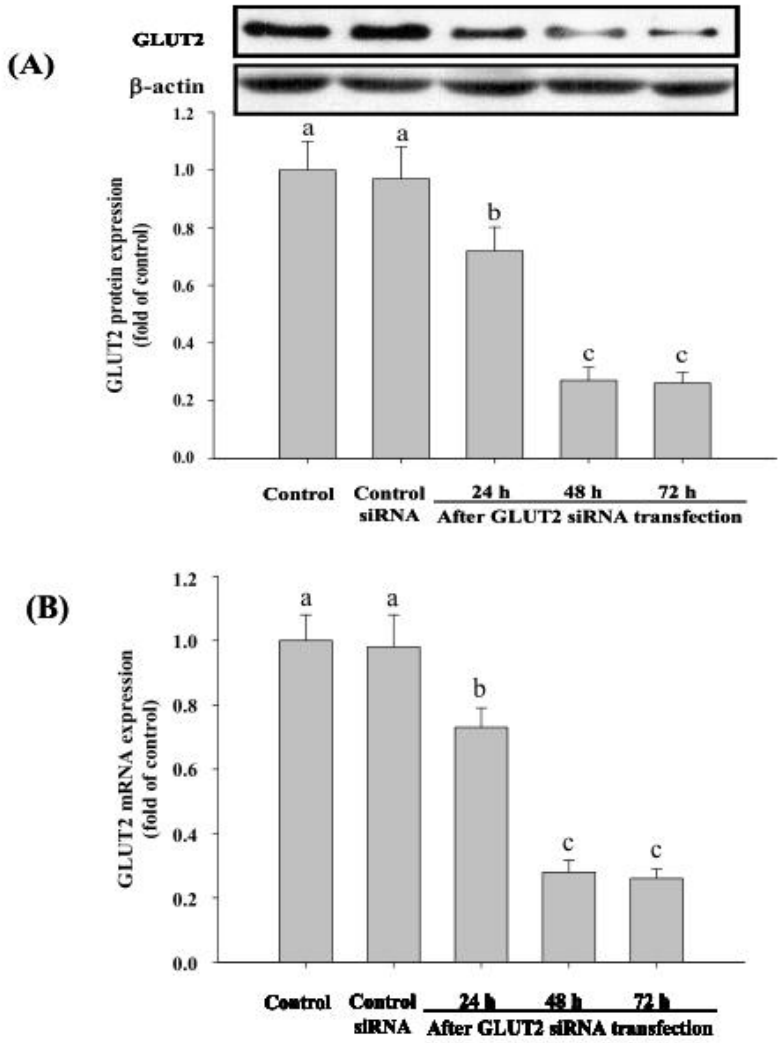
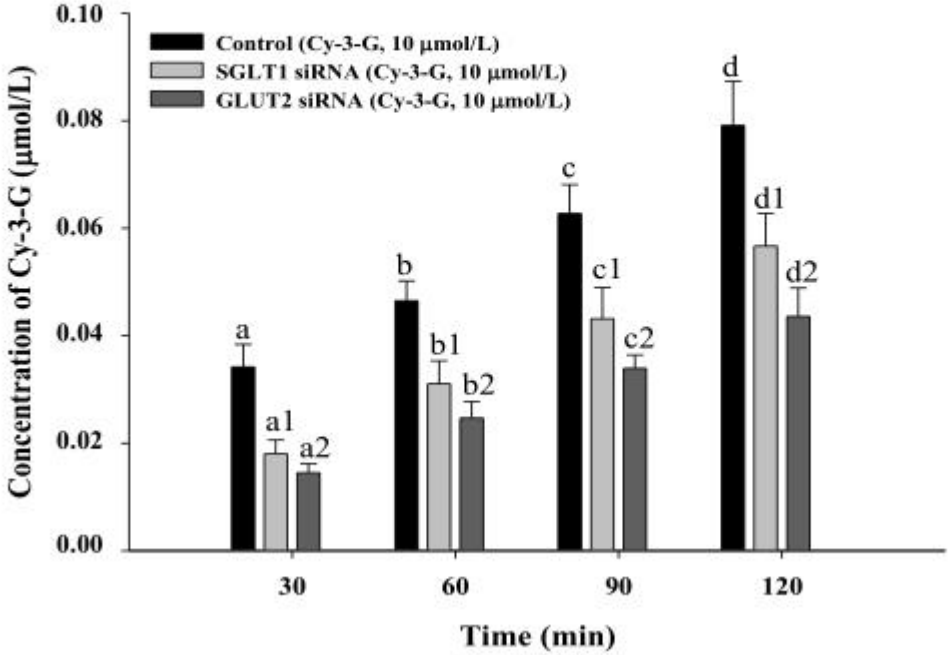

(A)
GLUT2
β-actin
GLUT2 protein expression (fold of control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
c
c
Control
Control siRNA
24 h
48 h
72 h
After GLUT2 siRNA transfection
(B)
GLUT2 mRNA expression (fold of control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
c
c
Control
Control siRNA
24 h
48 h
72 h
After GLUT2 siRNA transfection

Figure 5. Absorption of Cy-3-G in Caco-2 cells after siRNA transfection. After transfection for 48 h, Cy-3-G absorption was measured. Values without a common letter differ, $p < 0.05$.



These findings suggest that Cy-3-G absorption is dependent on the activities of SGLT1 and GLUT2 in the small intestine and that SGLT1 and GLUT2 could be a limiting step for the bioavailability of Cy-3-G.

SANTA CRUZ BIOTECHNOLOGY, INC.

SGLT-1 siRNA (h): sc-61538

BACKGROUND

Glucose is the main source of energy for mammalian cells and its entry is mediated by various transporters. Seven facilitative (GLUT-1 to -7) and 2 concentrative glucose transporters (SGLT-1 and -2) are identified. The Na^+/glucose cotransporter gene SGLT-1 encodes the primary carrier protein responsible for the uptake of the dietary sugars glucose and galactose from the intestinal lumen. The glycoprotein is localized in the brush border of the intestinal epithelium and contains 12 membrane spans. SGLT-1 uses the electrochemical gradient of two sodium ions to transport one glucose molecule. Both the sodium glucose co-transporters SGLT-1 and -2 are also expressed in kidneys. The mRNA of SGLTs increases steadily from the fetal period to maturity along with the increase in their functional activity, i.e., glucose uptake. The interaction between a nucleocytoplasmic protein and a regulatory uridine-rich sequence in the 3'-UTR is important for cAMP-mediated SGLT-1 message stabilization. Defects in SGLT-1 cause Glucose-Galactose Malabsorption (GGM), resulting in neonatal onset of diarrhea, which results in death unless sugars are removed from the diet.

REFERENCES

1. Turk, E., Klisak, I., Bacallao, R., Sparkes, R.S. and Wright, E.M. 1993. Assignment of the human Na^+/glucose cotransporter gene SGLT-1 to chromsome 22q13.1. Genomics 17: 752-754.
2. Martin, M.G., Turk, E., Lostao, M.P., Kerner, C. and Wright, E.M. 1996. Defects in Na^+/glucose cotransporter (SGLT-1) trafficking and function

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SGLT-1 siRNA (h) is recommended for the inhibition of SGLT-1 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

THANKS