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D-Allulose is a substrate of glucose transporter type 5 (GLUT5) in the small intestine

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ABSTRACT

D-Allulose has been reported to have beneficial health effects. However, the transport system(s) mediating intestinal D-allulose transport has not yet been clearly identified. The aim of this study was to investigate whether intestinal D-allulose transport is mediated by glucose transporter type 5 (GLUT5). When D-allulose alone was gavaged, plasma D-allulose levels were dramatically higher in rats previously fed fructose. This suggests enhanced intestinal D-allulose absorption paralleled increases in GLUT5 expression observed only in fructose-fed rats. When D-allulose was gavaged with D-fructose, previously observed increases in plasma D-allulose levels were dampened and delayed, indicating D-fructose inhibited transepithelial D-allulose transport into plasma. Tracer D-[^{14}C]-fructose uptake rate was reduced to 54.8% in 50 mM D-allulose and to 16.4% in 50 mM D-fructose, suggesting D-allulose competed with D-[^{14}C]-fructose and the affinity of D-allulose for GLUT5 was lower than that of D-fructose. GLUT5 clearly mediates, likely at lower affinity relative to D-fructose, intestinal D-allulose transport.

1. Introduction

D-Allulose (also known as D-psicose) is widely found in daily food products in limited amounts, and therefore is considered as a rare sugar (Hossain, Yamaguchi, & Matsuo et al., 2015). D-Allulose is also produced by enzymatic or chemical isomerization methods and is now commercially available in bulk and can be used for studies with humans as well as animals. Many kinds of D-allulose-containing foods have already been marketed in Japan, Korea, and the USA. D-Allulose was approved as generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) in June 2014 (GRAS Notice No. GRN 498) and is allowed to be used as an ingredient in a variety of foods and dietary supplements (Hossain, Yamaguchi, & Matsuo et al., 2015). D-Allulose is an epimer of D-fructose that is 70% as sweet as sucrose and has been demonstrated to have antihyperglycemic, antihyperlipidemic, and antiobesity effects by various studies, including in human subjects (Baek, Park, & Lee, 2010; Chung et al., 2012; Hayashi et al., 2010; Hossain, Yamaguchi, & Hirose et al., 2015, Hossain et al., 2012; Hossain, Yamaguchi, & Matsuo et al., 2015; Hossain et al., 2011; Itoh et al., 2015; Matsuo & Izumori, 2014; Nagata, Kanasaki, Tamaru, & Tanaka, 2015; Ochiai, Nakanishi, Yamada, Iida, & Matsuo, 2013; Masaru Ochiai, Onishi, Yamada, Iida, & Matsuo, 2013). D-Allulose could thus replace more common natural sugars, such as sucrose, in diets for obese and diabetic subjects to maintain glucose levels in the blood and control healthy body weight. An interest in natural sugar substitutes has arisen because noncaloric sweeteners, like sucralose, have not been proven to be a healthy replacement for caloric sweeteners, like D-fructose, as chronic consumption of these noncaloric sugar substitutes also increased the risk for developing metabolic syndrome (Swithers, 2013).

Despite the recent remarkable surge in the number of metabolic studies on this rare sugar, the studies focusing on its absorption mechanism have not been conducted except the study using an *in vitro* Caco-2 model (Hishiike et al., 2013). D-Allulose is known to be absorbed and then eliminated rapidly *in vivo* via urine after either oral or intravenous administrations (Tsukamoto et al., 2014), suggesting that its intestinal absorption and renal excretion are mediated by transporters. It is essential to clarify the absorption mechanism of D-allulose in order to elucidate its physiological effects and apply it to food products.

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Sugar absorption is known to be affected by the amount and composition of dietary sugar because regulation of sugar transporters is highly specific (Bode, Eisenhardt, Haberich, & Bode, 1981; Ferraris, 2001; Miyamoto et al., 1993). D-glucose and D-fructose are transported across the apical membrane in the small intestine by sodium-dependent glucose cotransporter 1 (SGLT1) and glucose transporter type 5 (GLUT5), respectively, and they pass through the basolateral membrane into the hepatic portal vein via glucose transporter type 2 (GLUT2) (Ferraris, 2001). Dietary D-fructose has been reported to induce intestinal GLUT5 mRNA and protein, and enhance D-fructose transport (Bode et al., 1981; Ferraris, 2001; Miyamoto et al., 1993).

Since D-allulose is an epimer of D-fructose, we hypothesized that D-allulose is transported through GLUT5. To test this hypothesis, we compared plasma D-allulose concentrations in normal and intestinal GLUT5-induced rats after oral administration of D-allulose alone, D-allulose with D-fructose, and D-allulose with D-glucose. Tracer D-[^{14}C]-fructose and D-[^{14}C]-glucose uptake were also determined *in vitro* in the presence of D-allulose in the everted sleeve of mouse small intestine to examine whether D-allulose shares transporters with D-fructose and/or D-glucose.

2. Materials and methods

2.1. Chemicals

D-Allulose was obtained from Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan) and its purity was 99.5%. D-[^{14}C]-glucose, D-[^{14}C]-fructose and L-[^{3}H]-glucose were purchased from PerkinElmer Inc. (Waltham, MA, USA). All other chemicals were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA) or Wako Pure Chemical Industries, Ltd (Osaka, Japan).

2.2. D-Allulose oral administration experiment

Five-week-old male Sprague–Dawley rats were purchased from Kiwa Laboratory Animals Co. Ltd., (Wakayama, Japan). The rats were housed at 22 ± 3 °C, 55 ± 15% humidity, under 12 h light and dark cycles and allowed free access to a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) for a week, and then fed a high-fructose or high-glucose diet for 8 days (Table 1, n = 4, each).

The adult rats were fasted for 12 h and orally administrated D-allulose (1 g/kg body weight (BW)), D-allulose and D-fructose (1 g + 1 g/kg BW), or D-allulose and D-glucose (1 g + 1 g/kg BW). The dose of 1 g/kg BW was chosen based on our preliminary experiment to detect plasma D-allulose concentrations by HPLC. Sugars were dissolved in Milli-Q water and administered at a volume of 3 ml/kg BW. Blood samples were collected into a heparinized tube from the tail vein at 0, 30, 60, 90, 120 and 180 min after administration. To determine plasma sugar (D-allulose, D-glucose, and D-fructose) concentrations, the plasma was mixed with an equal volume of 0.6 mol/l perchloric acid, a protein-precipitating reagent. The mixture was centrifuged at 8000*g* for 10 min at 4 °C. Sugar concentrations in the supernatant were measured by a HPLC system (JASCO Corporation, Tokyo, Japan) as described previously (Taguchi et al., 2003). The HPLC system was equipped with a Finepak GEL SA-121 anion-exchange column (10 cm × 6.0 mm (i.d.), JASCO). The column was maintained at 80 °C. The injection volume was 20 µl. Elution was with a gradient of 0.25 mol/l sodium borate buffer, pH 8.0 (solvent A), and 0.6 mol/l sodium borate buffer, pH 8.0 (solvent B), as follows: gradient from 80%A/20%B to 60%A/40%B for 17 min; gradient from 60%A/40%B to 0%A/100%B for 1 min; maintenance at 0%A/100%B for 17 min; gradient from 0%A/100%B to 80%A/20%B for 2 min; and maintenance at 80%A/20%B for 20 min. The eluate from the column was mixed with a reagent consisting of 50 mmol/l guanidine hydrochloride, 0.5 mmol/l sodium metaperiodate, and 0.1 mol/l boric acid in 200 ml/l acetonitrile and adjusted to pH 11.0 with 5 mol/l NaOH. This mixture was heated at 170 °C in a reaction oven. The guanidine adducts of sugars were monitored by a fluorescence detector with excitation at 310 nm and emission at 415 nm. The flow rate of both the mobile phase and the guanidine reagent was 0.5 ml/min.

Table1
Composition of Diet (g/kg).

Ingredient	High-glucose diet	High-fructose diet
D-Glucose	629.5	0
D-Fructose	0	629.5
Casein	200	200
Purified soybean oil	70	70
Cellulose powder	50	50
AIN-93G mineral mix	35	35
AIN-93 vitamin mix	10	10
L-Cystine	3	3
Choline bitartrate	2.5	2.5
Tertiary butylhydroquinone	0.014	0.014

After administration of sugars by gavage, the rats were sacrificed by exsanguination from the abdominal aorta under isoflurane anesthesia. The small intestine was collected and immersed in RNAlater solution (Thermo Fisher Scientific, Inc. Waltham, MA, USA), and stored at −80 °C prior to RNA extraction. This study was approved by the Animal Care Committee of Kindai University, and the animals were maintained in accordance with the guidelines.

2.3. Quantitative RT-PCR

Total RNA was extracted by using Qiazol and a RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Aliquots of the total RNA samples (1 µg) were converted into cDNA by reverse transcription using iScript™ RT Supermix for RT-qPCR (BioRad, Hercules, CA, USA). To quantitatively estimate gene expression, PCR amplification was performed using a MiniOpticon Real-Time PCR Detection System (BioRad, Hercules, CA, USA). Real-time PCR amplification was carried out in a 20 µl reaction volume containing cDNA, 500 nM of gene-specific primers, and SsoFast™ EvaGreen® Supermix (BioRad, Hercules, CA, USA). Relative gene expression standardized to β-actin was calculated using the $2^{(-\Delta\Delta CT)}$ method (Livak & Schmittgen, 2001). There were no changes in results when another housekeeping gene, HPRT, was used. The sequences of the PCR primer pairs are 5′-TGCAGAGCAACGATGGAGAAA-3′ and 5′-ACAGCAGCGTC AGGGTGAAG-3′ for GLUT5, and 5′-CATTGCCATGGGTGGCTTC-3′ and 5′-GCTTGCCTCCAAATCGCTTC-3′ for SGLT1, and 5′-TTGGTGCCATCA ACATGATCTTC-3′ and 5′-AGATGGCCGTCATGCTCACATA-3′ for GLUT2, and 5′-GGAGATTACTGCCCTGGCTCCTA-3′ and 5′-GACTCATC GTACTCCTGCTTGCTG-3′ for β-actin.

2.4. D-[^{14}C]-fructose and D-[^{14}C]-glucose uptakes

To examine the effect of D-allulose on D-fructose and D-glucose uptakes in the small intestine, tracer D-[^{14}C]-fructose and D-[^{14}C]-glucose uptakes were determined by using the everted sleeve technique described in detail elsewhere (Karasov & Diamond, 1983; Shu, David, & Ferraris, 1997). Six-week-old C57BL/6 mice (Taconic Laboratories, Hudson, NY) were anesthetized with pentobarbital sodium (0.1 ml/30 g BW ip). The small intestine was isolated and gently flushed with ice-cold Ringer solution. A 1-cm jejunal segment was everted and mounted on a grooved steel rod and preincubated for 5 min at 37 °C in Ringer solution bubbled with 95% O_2-5% CO_2. The sleeve was then incubated at 37 °C in an oxygenated, stirred (1200 rpm) solution containing either D-[^{14}C]-fructose for 2 min or D-[^{14}C]-glucose for 1 min. D-[^{14}C]-fructose uptake was determined in the presence of 50 mM D-mannitol (control), 50 mM D-fructose (positive control), and 50 mM D-allulose (n = 6, each). D-[^{14}C]-glucose uptake was determined in the presence

of 50 mM D-mannitol (control), 50 mM D-glucose (positive control), and 50 mM D-allulose (n = 6, each). To reduce the radioactive label in the adherent fluid, there was a 20-s rinse in 30 ml ice-cold Ringer solution immediately after the incubation. L-[^{3}H]-glucose was used to correct for adherent fluid and passive diffusion of D-[^{14}C]-fructose and D-[^{14}C]-glucose. Relative uptake rate (100% in the presence of D-mannitol) was calculated and compared among treatments. All procedures conducted in this experiment were approved by the Institutional Animal Care and Use Committee, New Jersey Medical School (NJMS), Rutgers University.

2.5. Statistical analyses

All data were expressed as mean ± SE for each group. Statistical analysis was performed using an unpaired Student's *t*-test or a one-way ANOVA with Tukey's multiple range tests using IBM SPSS statistics software, version 19.0 (IBM Co., New York, USA). P-values < 0.05 were considered statistically significant and indicated by a single asterisk (*) or different letters.

3. Results and discussion

3.1. GLUT5 mRNA expression in small intestine.

To compare plasma D-allulose levels of normal and intestinal GLUT5-induced rats, animals were fed the high-glucose diet and high-fructose diet, respectively. Fig. 1 shows that the rats fed the high-fructose diet had significantly higher GLUT5 mRNA expression, suggesting that sugar transport mediated by GLUT5 was enhanced as reported previously (Bode et al., 1981; Ferraris, 2001; Miyamoto et al., 1993). GLUT2 and SGLT1 mRNA expression were not affected by different dietary sugars (Fig. 1).

3.2. D-Allulose transport mediated by GLUT5

Fig. 2A shows plasma D-allulose concentrations in rats administrated D-allulose alone (1 g/kg BW) after consumption of the high-glucose or -fructose diet. The fructose-fed rats showed dramatically higher plasma D-allulose levels at 30, 60, 90 min and conversely a lower (P = 0.051) D-allulose level at 180 min after administration. Cmax and Tmax values were 1.11 ± 0.05 mM and 75.0 ± 8.7 min in the glucose-fed rats and 3.47 ± 0.23 mM and 52.5 ± 7.5 min in the fructose-fed rats, respectively. These results indicate more D-allulose could be rapidly absorbed via highly expressed GLUT5 in the fructose-fed rats. Plasma D-glucose concentrations gradually decreased in parallel in both diet groups (Fig. 2B). This suggests that D-allulose did not elevate plasma D-glucose

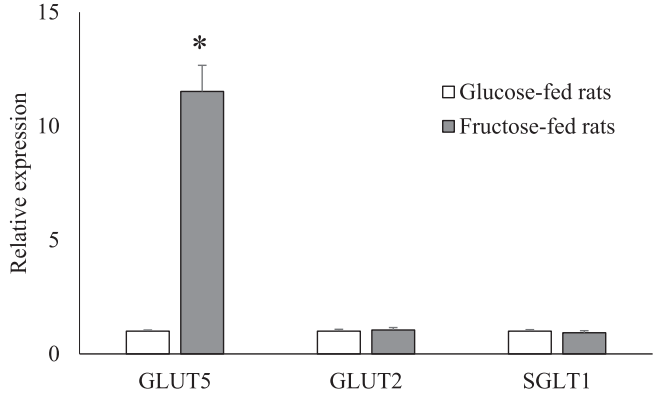

Fig. 1. mRNA expression of intestinal sugar transporters in rats fed the high-glucose or -fructose diet. Data are means ± SE (n = 4). Expression of each marker was normalized to that in the glucose-fed rats. A single asterisk (*) represents statistically significant differences ($P < 0.05$).

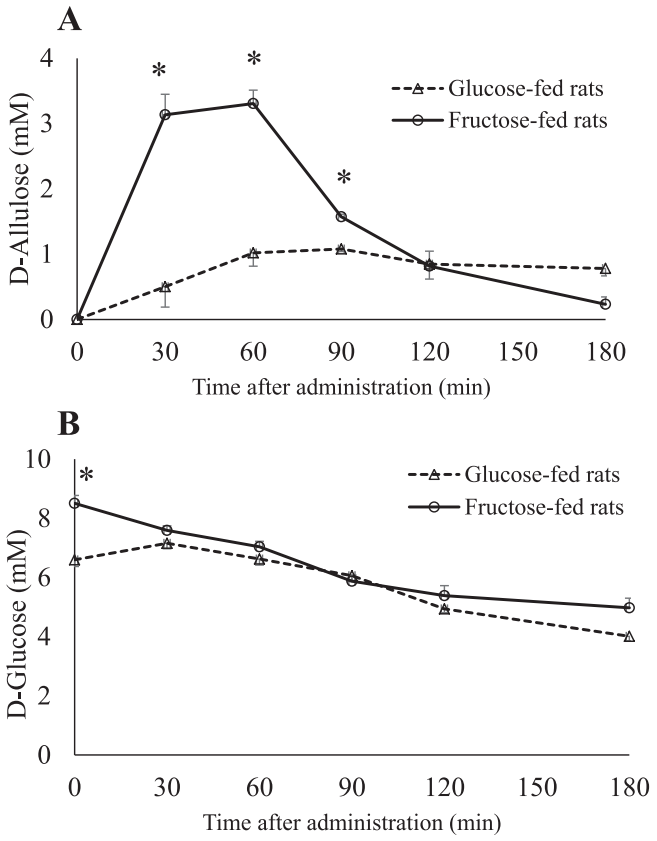

Fig. 2. Plasma D-allulose (A) and D-glucose (B) concentrations in rats gavaged with D-allulose alone (1 g/kg BW) after consumption of the high-glucose or -fructose diet. Rats were fasted for 12 h prior to gavage. Data are means ± SE (n = 4). A single asterisk (*) represents statistically significant differences ($P < 0.05$).

levels, which is in agreement with the results from human subjects (Iida et al., 2008). Plasma D-fructose levels were below detection limit throughout the experiment (0–180 min) in both diet groups, indicating that D-allulose did not affect plasma D-fructose concentrations.

We subsequently carried out the competitive experiment by coadministrating D-allulose and D-fructose or D-allulose and D-glucose to examine whether D-allulose absorption was affected in the presence of luminal D-fructose or D-glucose. Fig. 3A shows D-allulose concentrations in rats gavaged with D-allulose and D-fructose (1 g + 1 g /kg BW) simultaneously after consumption of the high- glucose or -fructose diet. The increase of plasma D-allulose (Fig. 3A, D-allulose gavaged with D-fructose) was delayed when compared to the increase in plasma D-allulose in both glucose- and fructose-fed rats (Fig. 2A, D-allulose gavaged without fructose). The levels of D-fructose peaked earlier (Fig. 3B, D-allulose gavaged with D-fructose) compared to the levels of D-allulose in both glucose- and fructose-fed rats (Fig. 3A). These results indicate that luminal D-allulose and D-fructose competed for GLUT5 to be transported and D-allulose absorption was delayed probably because of lower affinity of D-allulose for GLUT5 than that of D-fructose. Also in this experiment, the fructose-fed rats showed higher D-allulose concentrations at 30, 60, 90, and 120 min compared to the glucose-fed rats (Fig. 3A), which confirms that D-allulose transport increased with consumption of the high-fructose diet because of GLUT5 upregulation. As shown in Fig. 3, the plasma D-allulose levels were much higher than those of D-fructose even though the gavaged dose was the same, indicating that metabolism of D-allulose was much slower relative to D-fructose. This is probably because D-allulose is not catabolized into energy while it is phosphorylated by ketohexokinase (Detheux, Vandercammen, & Van Schaftingen, 1991; Iida et al., 2010; Matsuo, Suzuki, Hashiguchi, &

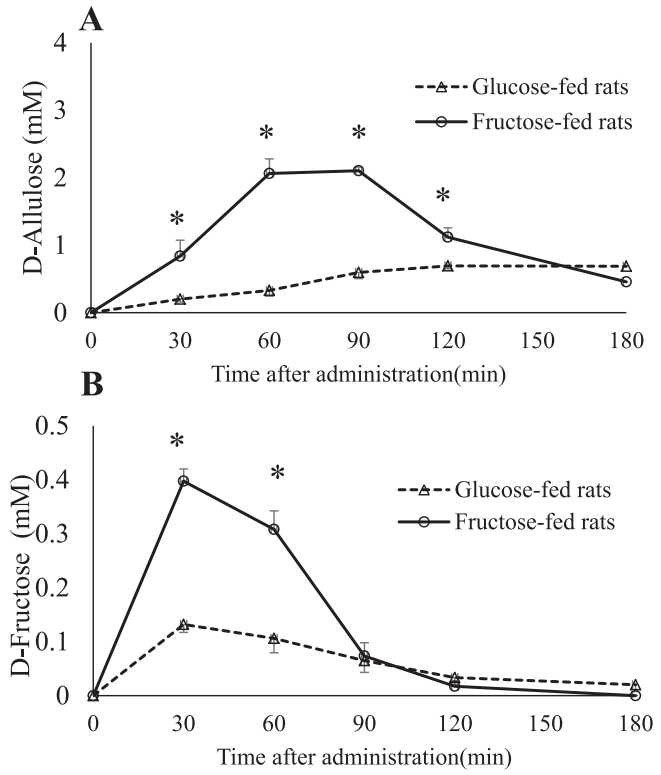

Fig. 3. Plasma D-allulose (A) and D-fructose (B) concentrations in rats simultaneously gavaged with D-allulose and D-fructose (1 g + 1 g /kg BW) after consumption of the high-glucose or -fructose diet. Rats were fasted for 12 h prior to gavage. Data are means ± SE (n = 4). A single asterisk (*) represents statistically significant differences ($P < 0.05$).

Izumori, 2002). Dietary D-fructose has been known to enhance not only D-fructose transport by GLUT5 but also D-fructose metabolism by fructokinase, aldolase B, and triokinase (i.e. fructolysis) (Bode et al., 1981; Korieh & Crouzoulon, 1991; Patel et al., 2015). Therefore, the present result shows that the degree of enhancement was higher in transport than that in metabolism (fructolysis) because higher plasma D-fructose concentrations were observed at 30 and 60 min in the fructose-fed rats (Fig. 3B).

Fig. 4 displays plasma D-allulose levels in rats gavaged with D-allulose and D-glucose (1 g + 1 g /kg BW) simultaneously after consumption of the high-glucose or -fructose diet. The fructose-fed rats showed higher D-allulose levels than the glucose-fed rats again and the D-allulose concentrations at 30 min were significantly higher compared to those shown in Fig. 3A (coadministration of D-allulose and fructose) in both groups (1.59 mM vs 0.84 mM in the fructose-fed rats, 0.30 mM vs 0.20 mM in the glucose-fed rats, respectively). These results show that intestinal D-allulose transport is inhibited more by D-fructose and probably mediated by GLUT5. However, in the presence of luminal D-glucose (Fig. 4), the plasma D-allulose curve seems to be interfered compared to that shown in Fig. 2A, suggesting that luminal D-glucose also somehow delayed D-allulose transport. This may be due to the different gavaged dose (1 g/kg BW of D-allulose vs 1 g + 1 g /kg BW of D-allulose and D-glucose). Hishiike et al. suggested that D-allulose competes with D-glucose for GLUT2 expressed on the basolateral membrane of intestinal absorptive cells by the Caco-2 cells experiment (Hishiike et al., 2013). Thus, the delay of plasma D-allulose curve in the presence of luminal D-glucose may be because of the competition for GLUT2. Even when D-allulose was gavaged with D-fructose, not only GLUT5 but also GLUT2 might have been partly involved in an inhibition of D-allulose transport. Furthermore, it should be noted that the structural diversity in anomeric forms of D-allulose, D-fructose, and D-glucose in solution could affect their affinity in ligand recognition of transporters (Ferraris, Choe, & Patel, 2018). The pyranose form is 46% for D-allulose in solution while it is dominant for D-glucose (99%) and D-fructose (75%) (Angyal, 1984). Further research is necessary to elucidate the contribution of other sugar transporters like SGLT1 and GLUT2 for *in vivo* D-allulose transport.

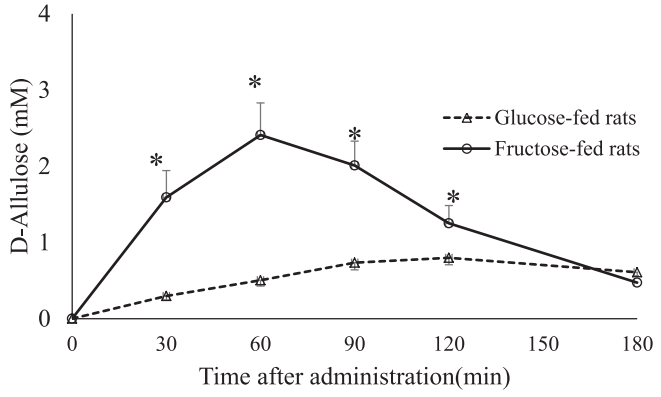

Fig. 4. Plasma D-allulose concentrations in rats simultaneously gavaged with D-allulose and D-glucose (1 g + 1 g /kg BW) after consumption of the high-glucose or -fructose diet. Data are means ± SE (n = 4). A single asterisk (*) represents statistically significant differences ($P < 0.05$).

3.3. D-[^{14}C]-fructose and D-[^{14}C]-glucose uptake in the presence of D-allulose

To examine whether D-allulose shares sugar transporters (GLUT5, SGLT1) with D-fructose and/or D-glucose and compare the affinity of D-allulose for GLUT5 and SGLT1 with that of D-fructose and D-glucose, respectively, we determined tracer D-[^{14}C]-fructose and D-[^{14}C]-glucose uptakes in the presence of D-allulose. The relative D-[^{14}C]-fructose uptake decreased to 16.4 ± 4.7% in the presence of D-fructose as D-[^{14}C]-fructose uptake was inhibited by the competition with D-fructose. In the presence of D-allulose, the relative D-[^{14}C]-fructose uptake was significantly affected and decreased to 54.8 ± 5.8% (Fig. 5A). These suggest that D-allulose competed with D-[^{14}C]-fructose for GLUT5 to be transported and the affinity of D-allulose for GLUT5 was lower than that of D-fructose. Interestingly, D-allulose significantly decreased D-[^{14}C]-glucose uptake to 51.2 ± 9.0% while D-glucose decreased D-[^{14}C]-glucose uptake to 9.8 ± 3.2% (Fig. 5B). These results suggest that D-allulose may be transported via SGLT1 as well as GLUT5. Kim et al. reported that mice had higher SGLT1 gene expression than that of GLUT5 whereas rats expressed GLUT5 more highly than SGLT1 in the small intestine, which could explain the little difference between our *in vivo* and *in vitro* results (Kim, Park, Cho, Chae, Sung, Kim et al., 2007). Further studies are needed to clarify the role of SGLT1 in D-allulose transport.

4. Conclusions

GLUT5 clearly mediates, likely at lower affinity relative to D-fructose, intestinal D-allulose transport. Our data also suggest SGLT1 may mediate D-allulose uptake and that future studies need to be done to verify whether intestinal absorption of this sugar is mediated by multiple transport systems.

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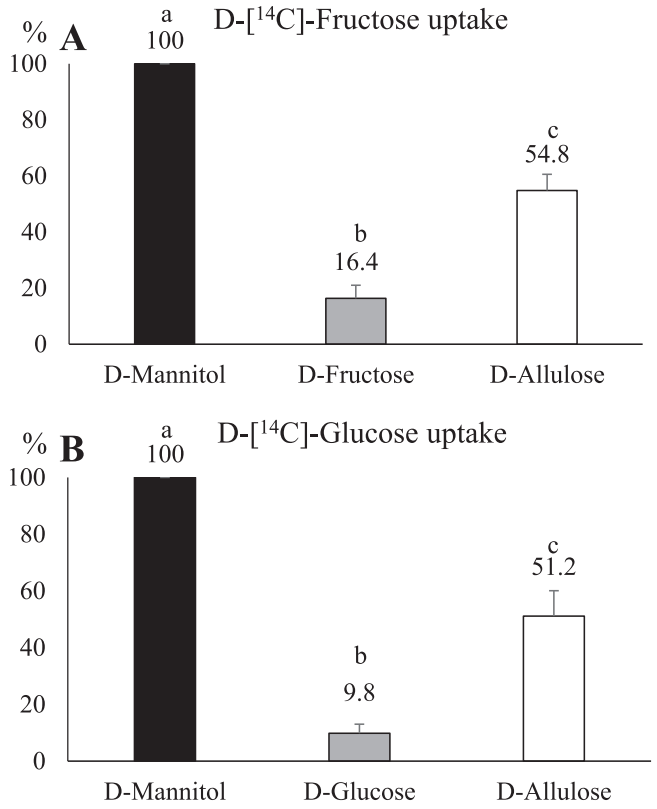

Fig. 5. Tracer D-[^{14}C]-fructose (A) and D-[^{14}C]-glucose (B) uptakes in the presence of 50 mM D-allulose in the everted sleeve of mouse small intestine. Data are means ± SE (n = 6). Different letters indicate statistically significant differences between groups ($P < 0.05$).

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Author contributions

K. Kishida and R. P. Ferraris designed research, performed experiments, analyzed data and wrote the manuscript. G. Martinez performed experiments. T. Iida and T. Yamada helped to design research and revised manuscript. Y. Toyoda helped to design research, performed experiments, analyzed data and revised manuscript.

Conflict of interest

Tetsuo Iida and Takako Yamada are employed by Matsutani Chemical Industry Co., Ltd. This work was partly supported by the funding from Matsutani Chemical Industry Co., Ltd.

References

Angyal, S. J. (1984). The composition of reducing sugars in solution. In R. S. Tipson, & D. Horton (Vol. Eds.), *Advances in Carbohydrate Chemistry and Biochemistry: vol. 42*, (pp. 15–68). Academic Press.

Baek, S. H., Park, S. J., & Lee, H. G. (2010). D-psicose, a sweet monosaccharide, ameliorate hyperglycemia, and dyslipidemia in C57BL/6J db/db mice. *Journal of Food Science, 75*(2), H49–53.

Bode, C., Eisenhardt, J. M., Haberich, F. J., & Bode, J. C. (1981). Influence of feeding fructose on fructose and glucose absorption in rat jejunum and ileum. *Research in Experimental Medicine (Berlin), 179*(2), 163–168.

Chung, Y. M., Hyun Lee, J., Youl Kim, D., Hwang, S. H., Hong, Y. H., Kim, S. B., ... Hye Park, C. (2012). Dietary D-psicose reduced visceral fat mass in high-fat diet-induced obese rats. *Journal of Food Science, 77*(2), H53–58.

Detheux, M., Vandercammen, A., & Van Schaftingen, E. (1991). Effectors of the regulatory protein acting on liver glucokinase: A kinetic investigation. *European Journal of Biochemistry, 200*(2), 553–561.

Ferraris, R. P. (2001). Dietary and developmental regulation of intestinal sugar transport. *The Biochemical Journal, 360*(Pt 2), 265–276.

Ferraris, R. P., Choe, J. Y., & Patel, C. R. (2018). Intestinal absorption of fructose. *Annual Review of Nutrition, 38*, 41–67.

Hayashi, N., Iida, T., Yamada, T., Okuma, K., Takehara, I., Yamamoto, T., ... Tokuda, M. (2010). Study on the postprandial blood glucose suppression effect of D-psicose in borderline diabetes and the safety of long-term ingestion by normal human subjects. *Bioscience, Biotechnology, and Biochemistry, 74*(3), 510–519.

Hishiike, T., Ogawa, M., Hayakawa, S., Nakajima, D., O'Charoen, S., Ooshima, H., & Sun, Y. (2013). Transepithelial transports of rare sugar D-psicose in human intestine. *Journal of Agriculture and Food Chemistry, 61*(30), 7381–7386.

Hossain, A., Yamaguchi, F., Hirose, K., Matsunaga, T., Sui, L., Hirata, Y., ... Tokuda, M. (2015b). Rare sugar D-psicose prevents progression and development of diabetes in T2DM model Otsuka Long-Evans Tokushima Fatty rats. *Drug Design, Development and Therapy,* 525.

Hossain, A., Yamaguchi, F., Matsunaga, T., Hirata, Y., Kamitori, K., Dong, Y., ... Tokuda, M. (2012). Rare sugar D-psicose protects pancreas beta-islets and thus improves insulin resistance in OLETF rats. *Biochemical and Biophysical Research Communications, 425*(4), 717–723.

Hossain, A., Yamaguchi, F., Matsuo, T., Tsukamoto, I., Toyoda, Y., Ogawa, M., ... Tokuda, M. (2015a). Rare sugar D-allulose: Potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus. *Pharmacology & Therapeutics, 155*, 49–59.

Hossain, M. A., Kitagaki, S., Nakano, D., Nishiyama, A., Funamoto, Y., Matsunaga, T., ... Tokuda, M. (2011). Rare sugar D-psicose improves insulin sensitivity and glucose tolerance in type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biochemical and Biophysical Research Communications, 405*(1), 7–12.

Iida, T., Hayashi, N., Yamada, T., Yoshikawa, Y., Miyazato, S., Kishimoto, Y., ... Izumori, K. (2010). Failure of d-psicose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans. *Metabolism, 59*(2), 206–214.

Iida, T., Kishimoto, Y., Yoshikawa, Y., Hayashi, N., Okuma, K., Tohi, M., ... Izumori, K. (2008). Acute D-psicose administration decreases the glycemic responses to an oral maltodextrin tolerance test in normal adults. *Journal of Nutritional Science and Vitaminology (Tokyo), 54*(6), 511–514.

Itoh, K., Mizuno, S., Hama, S., Oshima, W., Kawamata, M., Hossain, A., ... Tokuda, M. (2015). Beneficial effects of supplementation of the rare sugar "D-allulose" against hepatic steatosis and severe obesity in Lep(ob)/Lep(ob) Mice. *Journal of Food Science, 80*(7), H1619–1626.

Karasov, W. H., & Diamond, J. M. (1983). A simple method for measuring intestinal solute uptake in vitro. *Journal of Comparative Physiology, 152*(1), 105–116.

Kim, H.-R., Park, S.-W., Cho, H.-J., Chae, K.-A., Sung, J.-M., Kim, J.-S., ... Shin, H.-C. (2007). Comparative gene expression profiles of intestinal transporters in mice, rats and humans. *Pharmacological Research, 56*(3), 224–236.

Korieh, A., & Crouzoulon, G. (1991). Dietary regulation of fructose metabolism in the intestine and in the liver of the rat. Duration of the effects of a high fructose diet after the return to the standard diet. *Archives Internationales de Physiologie et de Biochimie, 99*(6), 455–460.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods, 25*(4), 402–408.

Matsuo, T., & Izumori, K. (2014). Effects of dietary D-psicose on diurnal variation in plasma glucose and insulin concentrations of rats. *Bioscience, Biotechnology, and Biochemistry, 70*(9), 2081–2085.

Matsuo, T., Suzuki, H., Hashiguchi, M., & Izumori, K. (2002). D-psicose is a rare sugar that provides no energy to growing rats. *Journal of Nutritional Science and Vitaminology (Tokyo), 48*(1), 77–80.

Miyamoto, K., Hase, K., Takagi, T., Fujii, T., Taketani, Y., Minami, H., ... Nakabou, Y. (1993). Differential responses of intestinal glucose transporter mRNA transcripts to levels of dietary sugars. *The Biochemical Journal, 295*(Pt 1), 211–215.

Nagata, Y., Kanasaki, A., Tamaru, S., & Tanaka, K. (2015). D-psicose, an epimer of D-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. *Journal of Agriculture and Food Chemistry, 63*(12), 3168–3176.

Ochiai, M., Nakanishi, Y., Yamada, T., Iida, T., & Matsuo, T. (2013). Inhibition by dietary D-psicose of body fat accumulation in adult rats fed a high-sucrose diet. *Bioscience, Biotechnology, and Biochemistry, 77*(5), 1123–1126.

Ochiai, M., Onishi, K., Yamada, T., Iida, T., & Matsuo, T. (2013). d-Psicose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. *International Journal of Food Sciences and Nutrition, 65*(2), 245–250.

Patel, C., Douard, V., Yu, S., Tharabenjasin, P., Gao, N., & Ferraris, R. P. (2015). Fructose-induced increases in expression of intestinal fructolytic and gluconeogenic genes are regulated by GLUT5 and KHK. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology, 309*(5), R499–509.

Shu, R., David, E. S., & Ferraris, R. P. (1997). Dietary fructose enhances intestinal fructose transport and GLUT5 expression in weaning rats. *American Journal of Physiology, 272*(3 Pt 1), G446–453.

Swithers, S. E. (2013). Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. *Trends in Endocrinology and Metabolism, 24*(9), 431–441.

Taguchi, T., Miwa, I., Mizutani, T., Nakajima, H., Fukumura, Y., Kobayashi, I., ... Miwa, I. (2003). Determination of D-mannose in plasma by HPLC. *Clinical Chemistry, 49*(1), 181–183.

Tsukamoto, I., Hossain, A., Yamaguchi, F., Hirata, Y., Dong, Y., Kamitori, K., ... Tokuda, M. (2014). Intestinal absorption, organ distribution, and urinary excretion of the rare sugar D-psicose. *Drug, Design, Development and Therapy, 8*, 1955–1964.