JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Rare Sugar Syrup Containing D-Allulose but Not High-Fructose Corn Syrup Maintains Glucose Tolerance and Insulin Sensitivity Partly via Hepatic Glucokinase Translocation in Wistar Rats

Tomoya Shintani,*[,†] Takako Yamada,[†] Noriko Hayashi,[†] Tetsuo Iida,[†] Yasuo Nagata,[‡] Nobuaki Ozaki,[§] and Yukiyasu Toyoda[∥]

[†]Research and Development, Matsutani Chemical Industry Company, Limited, 5-3 Kita-Itami, Itami, Hyogo 664-8508, Japan

[‡]Center for Industry, University and Government Cooperation, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-9521, Japan

[§]Japanese Red Cross Nagoya Daiichi Hospital, 3-35 Michishita, Nakamuraku-ku, Nagoya, Aichi 453-8511, Japan

[∥]Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya, Aichi468-8503, Japan

ABSTRACT: Ingestion of high-fructose corn syrup (HFCS) is associated with the risk of both diabetes and obesity. Rare sugar syrup (RSS) has been developed by alkaline isomerization of HFCS and has anti-obesity and anti-diabetic effects. However, the influence of RSS on glucose metabolism has not been explored. We investigated whether long-term administration of RSS maintains glucose tolerance and whether the underlying mechanism involves hepatic glucokinase translocation. Wistar rats were administered water, RSS, or HFCS in drinking water for 10 weeks and then evaluated for glucose tolerance, insulin tolerance, liver glycogen content, and subcellular distribution of liver glucokinase. RSS significantly suppressed body weight gain and abdominal fat mass ($p < 0.05$). The glucose tolerance test revealed significantly higher blood glucose levels in the HFCS group compared to the water group, whereas the RSS group had significantly lower blood glucose levels from 90 to 180 min ($p < 0.05$). At 30, 60, and 90 min, the levels of insulin in the RSS group were significantly lower than those in the water group ($p < 0.05$). The amount of hepatic glycogen was more than 3 times higher in the RSS group than that in the other groups. After glucose loading, the nuclear export of glucokinase was significantly increased in the RSS group compared to the water group. These results imply that RSS maintains glucose tolerance and insulin sensitivity, at least partly, by enhancing nuclear export of hepatic glucokinase.

KEYWORDS: *D-allulose, rare sugar, D-psicose, insulin sensitivity, glucokinase*

INTRODUCTION

High-fructose corn syrup (HFCS) is commercially produced by isomerizing glucose to fructose. The taste of HCFS is similar to that of sucrose, and its price is affordable. Thus, HFCS has been used in various foods, such as beverages, desserts, and confectionery and bakery products. However, the consumption of HFCS has been reported to be one of the risk factors for developing diabetes and obesity.[1] Although there is no cure for diabetes at present, lifestyle changes, such as eating healthy foods, exercising regularly, and maintaining a healthy body weight, are advised to prevent high blood sugar levels and delay the onset of complications. From a preventative point of view, it is advisible to reduce sugar intake or to replace sugar with other low-calorie sweeteners. Thus, the development of new types of sweeteners is awaiting.

D-Allulose, a C_3 epimer of D-fructose, is one of the rare sugars that are present in a limited quantity in nature. Studies have shown that D-allulose has various functions, such as reducing postprandial blood glucose elevation.[2,3] In addition, D-allulose was observed to decrease body weight gain and adipose tissue weight.[4−6] Clinical trials using a maltodextrin diet or standard meal have confirmed that D-allulose suppresses postprandial blood glucose levels.[7,8] Two mechanisms have been suggested for the glucose-lowering effect of D-allulose. One is via inhibition of α-glucosidase; D-allulose inhibits the activity of α-glucosidase *in vitro* and *in vivo*, resulting in decreased absorption of glucose.[3] The other is the effect on glycemic response in the liver; D-allulose promotes the conversion of blood glucose to glycogen in the liver via glucokinase (GK).[9,10] In addition to these mechanisms, another mechanism might be proposed: competitive inhibition of glucose absorption by D-allulose in the digestive tract delays an increment in blood sugar because glucose, fructose, and D-allulose have a common transporter, GLUT 2, at the basolateral membrane in Caco-2 cells.[11] Collectively, these mechanisms might have contributed to decreased postprandial blood glucose in humans.[7,8]

The liver plays an important role in maintaining glucose homeostasis. Glucose internalized by hepatocytes is phosphorylated to glucose 6-phosphate by GK, which is a key step of glycolysis, glycogen synthesis, and the pentose phosphate pathway.[12,13] GK (also called hexokinase IV) compared to other hexokinases (I−III) has a relatively low affinity for glucose and does not undergo an inhibition by glucose 6-phosphate.[12] Thus, GK increases the amount of glycogen in the

Received: December 19, 2016
Revised: February 16, 2017
Accepted: February 16, 2017
Published: February 16, 2017

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888−2894

liver, suppressing postprandial blood glucose elevation.[14] GK in the liver is regulated by glucokinase regulatory protein (GKRP) that inhibits the activity of GK by binding to it.[15] When the affinity of GK and GKRP is increased by fructose 6-phosphate, GK remains in the nucleus in an inactive form. However, when its affinity is reduced by fructose 1-phosphate or high glucose concentration, GK is transported from the nucleus to the cytoplasm[14−20] via an active process. GK increases glucose utilization in the liver, thus resulting in increased glycogen content or reduced blood glucose levels. D-Allulose 1-phosphate, similar to fructose 1-phosphate, reduces affinity between GKRP and GK, leading to GK activation.[2,9]

Rare sugar syrup (RSS) has been developed by alkaline isomerization of HFCS.[21] RSS is a mixed syrup containing 45% glucose, 29% fructose, 5% allulose, 5% sorbose, 2% tagatose, 1% allose, and 13% other sugars. Therefore, RSS containing D-allulose may activate GK, probably resulting in improved glycemic control.

The anti-obesity effect of RSS has also been confirmed in both humans and animals;[22,23] in a clinical trial, the daily intake of 40 g of RSS for 3 months led to a significant reduction (1.8 kg) in body weight compared to that observed after HFCS intake.[22] Furthermore, our previous study has shown that RSS inhibits the activity of intestinal α-glucosidase *in vitro* and is expected to reduce postprandial blood glucose elevation.[23] It is therefore of interest to assess whether RSS, a new sweetener derived from HFCS, maintains glucose tolerance and insulin sensitivity and, if so, whether anti-hyperglycemic action of RSS is mediated by exporting GK from the nucleus to the cytoplasm. To this end, glucose tolerance and the degree of GK translocation in rats administered water, HFCS, or RSS in drinking water were investigated in the present study.

MATERIALS AND METHODS

Experimental Animals. All animal experiments were approved by the Meijo University Faculty of Animal Care and Use Committee. Five-week-old Wistar male rats were purchased (CLEA Japan, Tokyo, Japan), fed a commercial diet (AIN93-G, Oriental Yeast Co., Ltd., Tokyo, Japan), and placed in stainless-steel cages under controlled atmospheric conditions (i.e., 22 ± 2 °C with a 12 h light/dark cycle). After 1 week of acclimation, animals were divided into three groups of 12 animals each. Rats were then fed a commercial diet *ad libitum* and administered water, HFCS (HFCS42, Nihon Shokuhin Kakou Co., Ltd., Tokyo, Japan), or RSS (Matsutani Chemical Industry Co., Ltd., Itami, Japan) in drinking water for 10 weeks. We diluted HFCS and RSS to provide 7% fructose solution, because a fructose solution of approximately 10% has generally been used in studies to induce insulin resistance.[24] Before administration to animals, we measured the concentration of total sugar and the composition of sugar by a conventional method using high-performance liquid chromatography. The total sugar concentration of the RSS and HFCS solutions was approximately 25 and 15%, respectively.

Experimental Design. At 8 weeks, the oral glucose tolerance test was carried out with 2 g of glucose/kg of body weight by oral gavage after 16 h of fasting. Blood was collected from the tail vein before and 30, 60, 90, 120, and 180 min after glucose loading. For insulin tolerance test at 8 weeks, a dose of 0.5 IU of insulin/kg of body weight (Humulin R, Eli Lilly, Indianapolis, IN, U.S.A.) was subcutaneously administered to rats. Blood was collected from the tail vein before and after 30, 60, 90, 120, and 180 min after insulin loading. The blood was then centrifuged, and the plasma was used for measurement of the levels of blood glucose by a commercial kit (C-II test Wako, Wako Pure Chemical Industries, Osaka, Japan). Insulin levels were measured using an enzyme-linked immunoassay kit (Morinaga Institute of Biological Science, Kanagawa, Japan). The area under the curve (AUC) was calculated using the trapezoidal method.

To elucidate the involvement of GK in RSS-induced anti-hyperglycemic action, an oral glucose tolerance test was carried out again at 10 weeks and GK translocation and the level of hepatic glycogen were determined, as described below. Four rats of each group were sacrificed before and 30 min after a glucose load. The liver was then dissected and used for GK translocation. For glycogen content, an additional four rats from each group underwent laparotomy under anesthesia and the rat livers were perfused through inferior vena cava with saline, excised, and stored at −80 °C before analysis.

Immunohistochemical Staining of GK. An aliquot of the excised liver was immobilized with 4% paraformaldehyde. The immobilized liver was infiltrated in 30% sucrose solution for 48 h at 4 °C. Finally, the liver tissue was infiltrated with OCT compound (Sakura Finetek, Tokyo, Japan) and frozen in liquid nitrogen. Cryosections were subjected to immunostaining using an anti-GK antibody, prepared as described previously.[17,20]

Analysis of a Distribution of Liver GK in the Nucleus and Cytoplasm. GK translocation from the nucleus to the cytoplasm was analyzed through fluorescence imaging. The intensity of fluorescent−antibody staining in the liver tissue was examined using a fluorescence microscope and confocal laser microscopy (LSM 510 META, Zeiss, Oberkochen, Germany)[25] and quantified by software ImageJ [National Institutes of Health (NIH), Bethesda, MD, U.S.A.).

Analysis of Liver Glycogen. The liver was homogenized with 0.6 M perchloric acid.[26] The homogenate was then added to 1 M potassium bicarbonate followed by 0.2 M acetate buffer to dissolve 1 mg/mL glucoamylase (Oriental Yeast Co., Ltd., Tokyo, Japan). The reaction solution was incubated for 2 h at 40 °C. Next, the enzymatic reaction was stopped by adding 500 μL of 0.6 M perchloric acid and then centrifuged. The supernatant was used for glucose measurement. The glycogen content was then calculated as glucose equivalent after converting glucose milligrams to micromolar.

Statistical Analysis. Data are expressed as the mean ± standard deviation (SD). Data were analyzed using the multiple comparisons of Fisher's protected least significant difference (PLSD) test. All analyses were performed with a statistical package (Excel Statistics 2010, SSRI, Co., Ltd., Tokyo, Japan). Values were considered to be significantly different when the p value was <0.05.

RESULTS

Body weight, calorie intake, and abdominal fat weight were examined throughout the experiment, as shown in Figure 1. There was no difference in body weight gain between the water and HFCS groups, whereas that in the RSS group was significantly suppressed throughout the experiment ($p < 0.05$) (Figure 1A). Average food intake of the HFCS group (15 ± 2 g/day) was significantly lower ($p < 0.05$) than that of the other two groups (19 ± 2 in the RSS group and 19 ± 4 g/day in the water group), while average fluid intake of the HFCS group (42 ± 6 g/day) was significantly higher ($p < 0.05$) than that of the other two groups (23 ± 3 and 19 ± 2 g/day in the water and RSS groups, respectively). Therefore, calorie intake from food and fluid was not significantly different in the HFCS and RSS groups, although the daily difference between the water and other two groups was significant from time to time (Figure 1B). The weight of the liver and kidney was not influenced by sugars at 10 weeks (data not shown). The total amount of abdominal fat, including perirenal, epididymal, and mesenteric fat, was significantly higher in the HFCS group than that in the water group, whereas it was significantly lower in the RSS group than that in the other two groups (Figure 1C).

In the oral glucose tolerance test at 8 weeks, the levels of blood glucose were determined for 180 min after a load of glucose (Figure 2A). Although no difference was observed between the blood glucose levels of the water and RSS groups before a glucose load (0 min), the levels of blood glucose in RSS rats were significantly lower than those in the HFCS

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888−2894

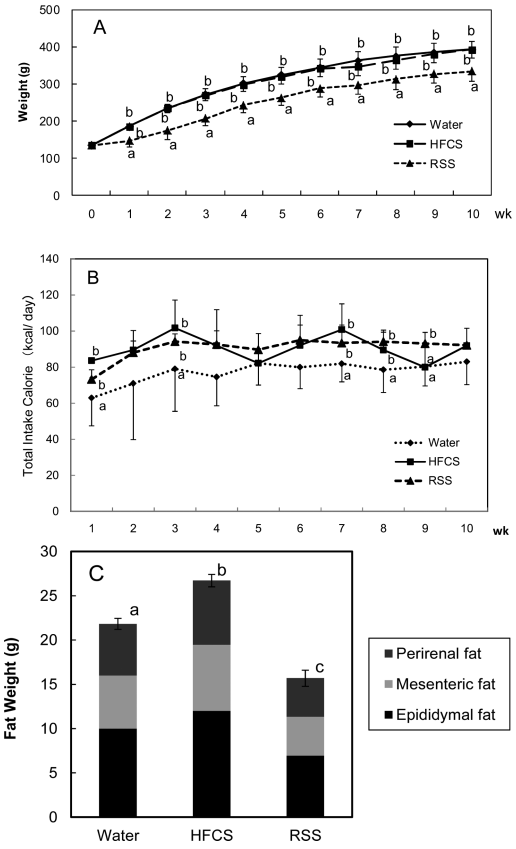

Figure 1. Changes in (A) body weight, (B) total calorie intake, and (C) abdominal fat weight in rats administrated water, RSS, and HFCS. Data are expressed as the mean ± SD (n = 12 for A and B, and n = 4–5 for C). Different letters (a, b, and c) show a significant difference at p < 0.05.

group. Blood glucose levels in the HFCS group from 0 to 180 min were higher than those in the water group, except at 90 min. Glucose levels at 90, 120, and 180 min were significantly lower in the RSS group than those in the water group. The AUC of blood glucose response in the HFCS group was higher than that in the water and RSS groups (Figure 2B).

The levels of insulin were significantly higher in the HFCS group at baseline than in the water and RSS groups. The HFCS group had higher values of insulin during the oral glucose tolerance test than those in the water group. On the other hand, insulin levels before and during the glucose tolerance test were lower in the RSS group than those in the HFCS group (Figure 2C). The AUC of insulin response was significantly lower in the RSS group than in the water and HFCS groups (Figure 2D).

The insulin tolerance test showed that the reduction in blood glucose in the RSS group was significantly higher than that in the HFCS group (Figure 3).

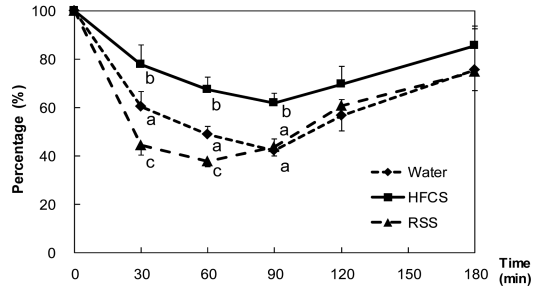

Figure 3. Effects of water, RSS, and HFCS on glucose levels during the insulin tolerance test at 8 weeks. Data are expressed as a percentage of values at 0 min. Data are expressed as the mean ± SD (n = 4). Different letters (a, b, and c) show a significant difference at p < 0.05.

Before and 30 min after a glucose load at 10 weeks, the HFCS group showed significantly higher levels of glucose than those in the water and RSS groups (Figure 4A). Similarly, insulin levels in the HFCS group were higher than those in the water and RSS groups at 0 min. At 30 min after a glucose loading, a lower insulin level was found in rats administered RSS than that in rats receiving water (Figure 4B). We also examined the hepatic glycogen content before and at 30 min after a glucose loading (Figure 4C). The glycogen content in the RSS group was 3 times higher than that in the water and HFCS groups at 0 min. After a glucose loading, the glycogen content in the HFCS group was significantly lower than in the water and RSS groups.

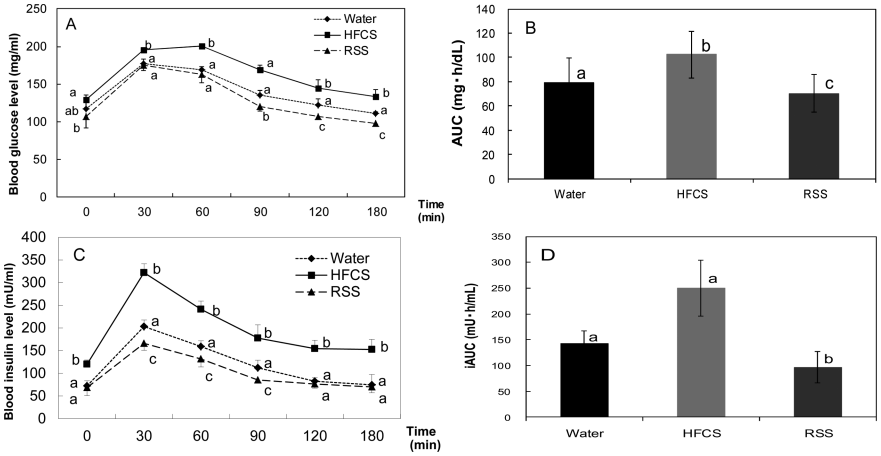

Figure 2. Effects of water, RSS, and HFCS on plasma glucose and insulin levels during the oral glucose tolerance test at 8 weeks: (A) plasma glucose, (B) AUC of plasma glucose, (C) insulin, and (D) AUC of insulin. Data are expressed as the mean ± SD (n = 4). Different letters (a, b, and c) show a significant difference at p < 0.05.

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888–2894

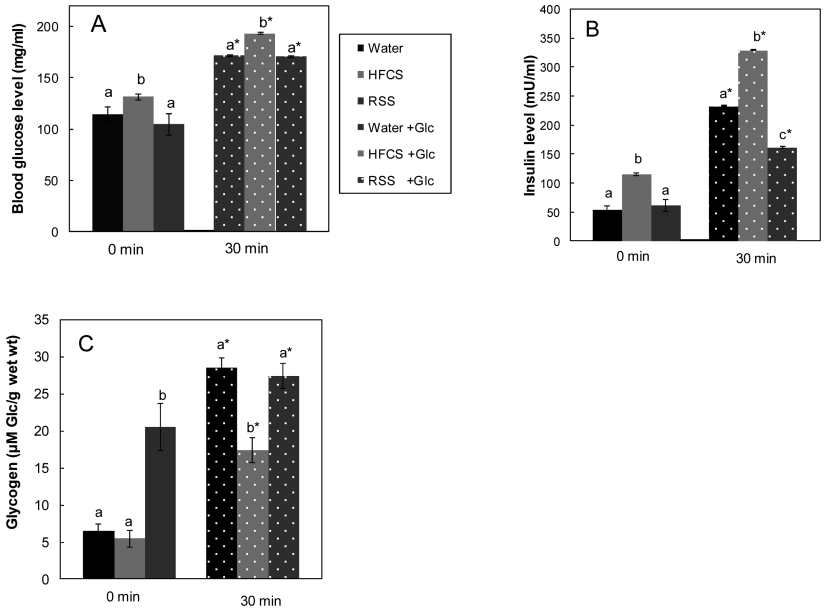

Figure 4. Effects of water, RSS, and HFCS on (A) plasma glucose levels, (B) insulin levels, and (C) hepatic glycogen levels during the oral glucose tolerance test at 10 weeks. Data are expressed as the mean ± SD (n = 4). Different letters (a, b, and c) show a significant difference at $p < 0.05$ among the three groups at 0 or 30 min. (∗) Significant difference at $p < 0.05$ compared to the corresponding group at 0 min.

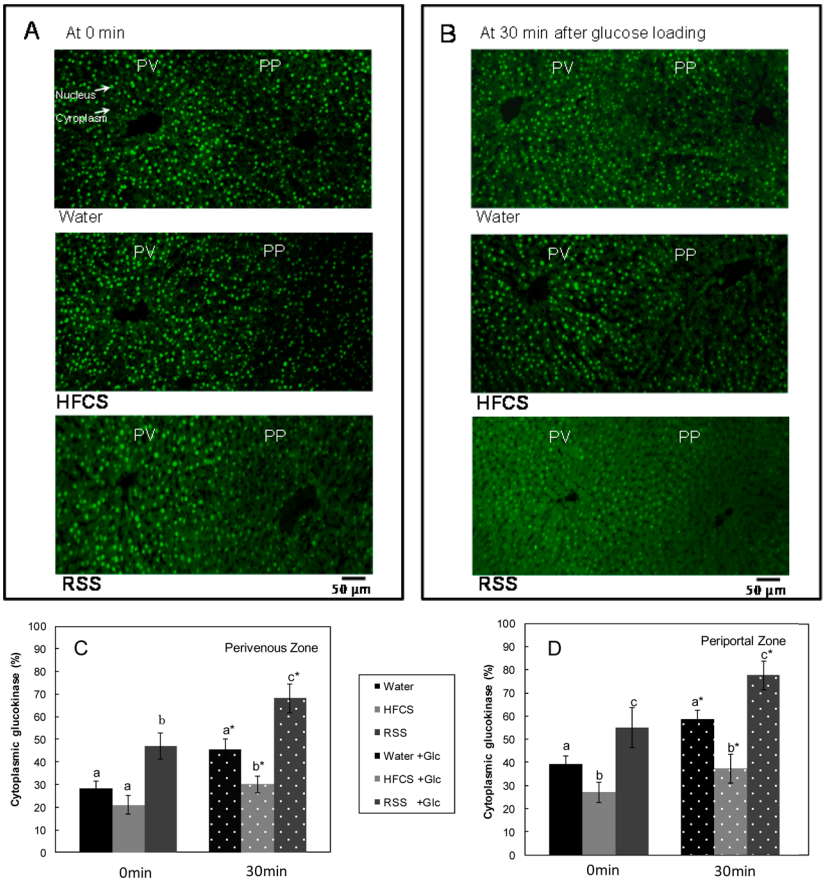

Figure 5. Effects of water, RSS, and HFCS on nuclear export of GK in the perivenous and periportal zones in the liver: (A) representative picture of immunohistochemical staining of GK in the perivenous (PV) and periportal (PP) zones from rats administered water (upper), HFCS (middle), and RSS (lower) at 0 min, (B) representative picture of immunohistochemical staining of GK in the PV and PP zones from rats administered water (upper), HFCS (middle), and RSS (lower) at 30 min after glucose loading, (C) distribution of GK in the cytoplasm in the perivenous zone at 0 and 30 min after glucose loading, and (D) distribution of GK in the cytoplasm in the periportal zone at 0 and 30 min after glucose loading. Data are expressed as the mean ± SD (n = 4). Different letters (a, b, and c) show a significant difference at $p < 0.05$ among the three groups at 0 or 30 min. (∗) Significant difference at $p < 0.05$ compared to the corresponding group at 0 min.

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888−2894

The nuclear export rate of GK in the perivenous and periportal zones in the liver before and after glucose loading was analyzed (panels A–D of Figure 5). Panels A and B of Figure 5 show that the nuclear immunofluorescence in hepatocytes from HFCS-fed rats is still evident; however, the cytoplasmic immunofluorescence in hepatocytes from RSS-fed rats is brighter than that from HFCS-fed rats. When the distribution in the perivenous zone before a glucose loading was quantified using an image analyzer, the distribution of GK in the cytoplasm in the RSS group was higher (46%) than that in the water group (28%) and that in the HFCS group (21%) (Figure 5C). After glucose loading, the distribution in the cytoplasm remained lower in the HFCS group (30%) compared to the water group (46%) and the RSS group (68%). The same tendency was observed in the periportal zone; the translocation of GK in the RSS group was higher (59%) than that in the water group (39%) and that in the HFCS group (27%) (Figure 5D). After glucose loading, the distribution in the cytoplasm was significantly lower in the HFCS group (37%) than that in the water group (55%) and that in the RSS group (78%).

DISCUSSION

We have previously administered RSS to rats and found that RSS is associated with lower body weight, abdominal fat, and fasting insulin levels in rats compared to feeding with starch and HFCS.[22] In this study, rats were administered water, RSS, or HFCS in drinking water for 10 weeks to understand the effect of RSS on glucose metabolism. We observed that glucose tolerance and insulin sensitivity in rats in the RSS group were similar or even better than in animals in the water group, whereas they deteriorated in the HFCS group, indicating that RSS may be beneficial in maintaining glucose homeostasis.

As shown in Figure 1B, no difference in total calorie intake was observed between the HFCS and RSS groups. However, the former group had a significantly higher average body weight than the latter throughout the experiment. The levels of food efficiency for the water, HFCS, and RSS groups were 0.67, 0.57, and 0.44, respectively, with the difference between the water and RSS groups being significant. In addition, administration of HFCS was associated with a greater total adipose tissue weight than that of water or RSS (Figure 1C), confirming its anti-obese action, as seen in our previous clinical study[23] and animal studies.[22] One of the rare sugars in the RSS, D-allulose, has been well-studied. The mechanisms behind the anti-obese activity of it have been proposed; D-allulose decreases hepatic lipogenic enzymes and enhances energy expenditure.[5,6] We also calculated the amount of D-allulose ingested in the RSS group as 900 mg kg^{-1} of body weight day^{-1}. It is unlikely that this amount of D-allulose affects body weight gain as a result of the findings from our previous study that 1500–3000 mg of D-allulose a day did not increase body weight gain.[27] Therefore, it is possible that other rare sugars in RSS, such as D-sorbose and D-tagatose, contributed to decreasing body weight. On the basis of these findings, we have concluded that RSS has anti-obesity activity. However, studies on the detailed mechanism of RSS-induced anti-obese action are needed in the future.

As shown in Figure 2, oral glucose tolerance testing performed at 8 weeks revealed that RSS favorably altered glucose metabolism; the levels of blood glucose in the RSS group were the lowest among the three groups. Administration of water or RSS showed a significantly lower AUC of insulin, per the results of oral glucose tolerance testing (Figure 2D), implying that RSS maintains insulin sensitivity. This finding was further supported by an insulin tolerance test, as shown in Figure 3. The decrease in glucose levels after a subcutaneous injection of insulin was markedly greater in the RSS group than in the water or HFCS group at 30 and 60 min. Matsuo et al. have also shown that, after feeding D-allulose, one of the rare sugars in RSS, to rats for 8 weeks, the levels of plasma glucose and insulin were significantly lower relative to fructose.[3] This anti-hyperglycemic activity of D-allulose was also observed in db/db mice[28] and Otsuka Long-Evans Tokushima Fatty (OLETF) rats.[10] In clinical studies, when a varying amount of D-allulose was co-administered with 75 g of maltodextrin, the AUC for glucose and insulin during glucose tolerance testing decreased in a dose-dependent manner.[7] Furthermore, subjects with a fasting plasma glucose level of 100–126 mg/dL who received a single dose of 5 g of D-allulose in tea and a standard meal containing 84.5 g of carbohydrate exhibited significantly lower plasma glucose levels at 30 and 60 min than those who received a standard meal alone.[8] Previous studies have shown that D-tagatose (one of the rare sugars in RSS) exerts anti-hyperglycemic action. The level of glucagon-like peptide-1, which is involved in secretion of insulin in the pancreas, is augmented by D-tagatose.[29] In addition, D-sorbose, one of the rare sugars in RSS, has been reported to reduce fasting plasma insulin levels.[30] One of the mechanisms underlying the anti-hyperglycemic activity of RSS may be due to an inhibitory effect of α-glucosidase because D-allulose, D-tagatose, and D-sorbose exert α-glucosidase inhibitory action.[3,21,31] Furthermore, because glucose, fructose, and D-allulose share a common transporter at the basolateral membrane in Caco-2 cells, as suggested by Hishiike et al.,[11] the competitive and suppressive effect of D-allulose in RSS on glucose absorption may provide another conceivable mechanism. On the basis of these studies, RSS containing D-allulose, D-sorbose, and D-tagatose also appears to have anti-hyperglycemic activity.

A glucose load was carried out at 10 weeks to determine the glycogen content and GK translocation. The levels of blood glucose, insulin, and hepatic glycogen were also measured before and 30 min after the glucose load. Administration of water and RSS exhibited lower glucose and insulin levels than HFCS, as shown in Figure 4, while the glycogen content in the liver was higher, which probably implies an anti-hyperglycemic action of RSS. Next, we tried to see if GK translocation is responsible for the improvement of glucose metabolism induced by RSS, because GK plays a critical role in glucose metabolism, shuttling between the nucleus and the cytoplasm and phosphorylating glucose to glucose 6-phosphate, the rate-limiting step in glycolysis.[14−20] Studies have shown that impaired GK activities in the liver may lead to hyperglycemia.[32] In addition, Toyoda et al. have found that GK translocation is malfunctioned in Goto-Kakizaki rats, which are models for diabetes.[24] Decreased GK activities were also observed in patients with impaired glucose tolerance.[33] Thus, these results indicate that GK translocation from the nucleus to the cytoplasm may be a good marker of glucose homeostasis. GK activity is 1.7 times higher in the perivenous zone than in the periportal zone.[34] In this study, the liver was sectioned into two zones, the periportal and perivenous zones, and the translocation of GK from the nucleus to the cytoplasm was measured in each zone. As shown in panels A and B of Figure 5, the area surrounding the nucleus (green spot) is brighter in RSS than in HFCS, indicating that more GK exists in the cytoplasm in the liver of RSS-fed rats. This was confirmed by

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888–2894

quantifying fluorescence intensity (panels C and D of Figure 5). The ratio of GK distributed in the cytoplasm (active form) in the RSS group was highest in both the perivenous and periportal zones among the three groups, implying that RSS intake results in a more active form of GK, regardless of the zone. Thus, glucose homeostasis modulated by RSS is at least due to stimulated translocation of GK out of the nucleus. Hossain et al. also observed that D-allulose stimulated GK translocation in OLETF rats, resulting in improved glucose tolerance and insulin sensitivity.[10] The anti-hyperglycemic action of D-tagatose has also been known to enhance the translocation of GK.[35] Interestingly, these rare sugars have been reported to increase hepatic glycogen.[4,9] Given that GK activity is related to glycogen synthesis,[14] our results suggest for the first time that RSS containing rare sugars may exert anti-hyperglycemic activity partially through activated GK and increased glycogen content. In addition, the protective effect of D-allulose on pancreas β islets, as shown in OLETF rats,[10] may also contribute to the maintenance of glucose tolerance and the enhancement of insulin sensitivity in rats fed RSS. Because GK promotes glycolysis and enhances energy metabolism when overexpressed in the liver,[36] it is of interest to investigate the relation between the mechanism underlying RSS-mediated glucose metabolism and energy metabolism, in future studies.

Although HFCS has been reported to impair glucose homeostasis and cause obesity and diabetes worldwide, reducing its consumption to prevent these lifestyle-related diseases is difficult. This report indicates that RSS, which contains a small amount of D-allulose, D-sorbose, and D-tagatose, has the potential to maintain glucose homeostasis. The underlying mechanisms could be in part due to inhibited or delayed glucose absorption in the small intestine and enhanced GK translocation in the liver. Further studies are needed to confirm the RSS role in maintaining glucose metabolism and to expand on its applicability in the food industry.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +81-72-771-2052. Fax: +81-72-771-2023. E-mail: tomoya-shintani@matsutani.co.jp.

ORCID

Tomoya Shintani: 0000-0002-8959-8875

Funding

We acknowledge funding from the Matsutani Chemical Industry Co. and Meijo University.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AUC, area under the curve; GK, glucokinase; GKRP, glucokinase regulatory protein; OLETF, Otsuka Long-Evans Tokushima Fatty; RRS, rare sugar syrup; HFCS, high-fructose corn syrup

REFERENCES

(1) Gross, S.; Li, L.; Ford, S.; Liu, S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: An ecologic assessment. *Am. J. Clin. Nutr.* **2004**, *79*, 774−779.

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

(3) Matsuo, T.; Izumori, K. D-Psicose inhibits intestinal α-glucosidase and suppresses the glycemic response after Ingestion of carbohydrates in rats. *J. Clin. Biochem. Nutr.* **2009**, *45*, 202−206.

(4) Ochiai, M.; Nakanishi, Y.; Yamada, T.; Iida, T.; Matsuo, T. Inhibition by dietary D-psicose of body fat accumulation in adult rats fed a high-sucrose diet. *Biosci., Biotechnol., Biochem.* **2013**, *77*, 1123−1126.

(5) Ochiai, M.; Onishi, K.; Yamada, T.; Iida, T.; Matsuo, T. D-Psicose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. *Int. J. Food Sci. Nutr.* **2014**, *65*, 245−250.

(6) Nagata, Y.; Kanasaki, A.; Tamaru, S.; Tanaka, K. D-Psicose, an epimer of D-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. *J. Agric. Food Chem.* **2015**, *63*, 3168−3176.

(7) Iida, T.; Kishimoto, Y.; Yoshikawa, Y.; Hayashi, N.; Okuma, K.; Tohi, M.; Yagi, K.; Matsuo, T.; Izumori, K. Acute D-psicose administration decreases the glycemic responses to an oral maltodextrin tolerance test in normal adults. *J. Nutr. Sci. Vitaminol.* **2008**, *54*, 511−514.

(8) Hayashi, N.; Iida, T.; Yamada, T.; Okuma, K.; Takehara, I.; Yamamoto, T.; Yamada, K.; Tokuda, M. 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. *Biosci., Biotechnol., Biochem.* **2010**, *74*, 510−519.

(9) Toyoda, Y.; Mori, S.; Umemura, N.; Futamura, N.; Inoue, H.; Hata, T.; Miwa, I.; Murao, K.; Nishiyama, A.; Tokuda, M. Suppression of blood glucose levels by D-psicose in glucose tolerance test in diabetic rats. *Jpn. Pharmacol. Ther.* **2010**, *38*, 261−269.

(10) Hossain, M. A.; Kitagaki, S.; Nakano, D.; Nishiyama, A.; Funamoto, Y.; Matsunaga, T.; Tsukamoto, I.; Yamaguchi, F.; Kamitori, K.; Dong, Y.; Hirata, Y.; Murao, K.; Toyoda, Y.; Tokuda, M. Rare sugar D-psicose improves insulin sensitivity and glucose tolerance in type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biochem. Biophys. Res. Commun.* **2011**, *405*, 7−12.

(11) Hishiike, T.; Ogawa, M.; Hayakawa, S.; Nakajima, D.; O'Charoen, S.; Ooshima, H.; Sun, Y. Transepithelial transports of rare sugar D-psicose in human intestine. *J. Agric. Food Chem.* **2013**, *61*, 7381−7386.

(12) Printz, R. L.; Magnuson, M. A.; Granner, D. K. Mammalian glucokinase. *Annu. Rev. Nutr.* **1993**, *13*, 463−496.

(13) Matschinsky, F. M. Assessing the potential of glucokinase activators in diabetes therapy. *Nat. Rev. Drug Discovery* **2009**, *8*, 399−416.

(14) Agius, L.; Peak, M.; Newgard, C. B.; Gomez-Foix, A. M.; Guinovart, J. J. Evidence for a role of glucose-induced translocation of glucokinase in the control of hepatic glycogen synthesis. *J. Biol. Chem.* **1996**, *271*, 30479−30486.

(15) Detheux, M.; Vandercammen, A.; Van Schaftigen, E. Effectors of the regulatory protein acting on liver glucokinase: A kinetic investigation. *Eur. J. Biochem.* **1991**, *200*, 553−561.

(16) Toyoda, Y.; Miwa, I.; Satake, S.; Anai, M.; Oka, Y. Nuclear location of the regulatory protein of glucokinase in rat liver and the translocation of the regulator to the cytoplasm response to high glucose. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 467−473.

(17) Toyoda, Y.; Miwa, I.; Kamiya, M.; Ogiso, S.; Nonogaki, T.; Aoki, S.; Okuda, J. Evidence for glucokinase translocation by glucose in rat hepatocytes. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 252−256.

(18) Toyoda, Y.; Miwa, I.; Kamiya, M.; Ogiso, S.; Nonogaki, T.; Aoki, S.; Okuda, J. Tissue and subcellular distribution of glucokinase in rat liver and their changes during fasting-refeeding. *Histochemistry* **1995**, *103*, 31−38.

(19) Toyoda, Y.; Ito, Y.; Miwa, I.; Yoshie, S. Shuttling of glucokinase between nucleus and cytoplasm in primary cultures of rat hepatocytes: Possible involvement in the regulation of the glucose metabolism. *Arch. Histol. Cytol.* **1997**, *60*, 307−316.

(20) Toyoda, Y.; Tsuchida, A.; Iwami, E.; Shironoguchi, I.; Miwa, I. Regulation of hepatic glucose metabolism by translocation of

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888−2894

glucokinase between the nucleus and the cytoplasm in hepatocytes. *Horm. Metab. Res.* **2001**, *33*, 329−336.
(21) Takamine, S.; Nakamura, M.; Iida, T.; Okuma, K.; Izumori, K. Manufacturing method of rare sugar syrup through alkali isomerization and its inhibitory effect of α-glucosidase. *Bull. Appl. Glycosci.* **2015**, *5*, 44−49.
(22) Iida, T.; Yamada, T.; Hayashi, N.; Okuma, K.; Izumori, K.; Ishii, R.; Matsuo, T. Reduction of abdominal fat accumulation in rats by 8-week ingestion of a newly developed sweetener made from high fructose corn syrup. *Food Chem.* **2013**, *138*, 781−785.
(23) Hayashi, N.; Yamada, T.; Takamine, S.; Iida, T.; Okuma, K.; Tokuda, M. Weight reducing effect and safety evaluation of rare sugar syrup by a randomized double-blind, parallel-group study in human. *J. Funct. Foods* **2014**, *11*, 152−159.
(24) Jürgens, H.; Haass, W.; Castañeda, T. R.; Schürmann, A.; Koebnick, C.; Dombrowski, F.; Otto, B.; Nawrocki, A. R.; Scherer, P. E.; Spranger, J.; Ristow, M.; Joost, H. G.; Havel, P. J.; Tschöp, M. H. Consuming fructose-sweetened beverages increases body adiposity in mice. *Obes. Res.* **2005**, *13*, 1146−56.
(25) Toyoda, Y.; Ito, Y.; Tanigawa, K.; Miwa, I. Impairment of glucokinase translocation in cultured hepatocytes from OLETF and GK rats, animal models of type 2 diabetes. *Arch. Histol. Cytol.* **2000**, *63*, 243−248.
(26) Keppler, D.; Decker, K. Glycogen. Determination with amylglucosidase. In *Methods of Enzymatic Analysis*, 2nd ed.; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 3, pp 1127−1131.
(27) Yamada, T.; Iida, T.; Hayashi, N.; Oga, H.; Okuma, K.; Izumori, K. Effects of D-psicose on Body Fat Accumulation and High Fructose Corn Syrup Diets in Rats. *Nippon Shokuhin Kagaku Kogaku Kaishi* **2010**, *57*, 263−267.
(28) Baek, S. H.; Park, S. J.; Lee, H. G. D-Psicose, a sweet monosaccharide, ameliorate hyperglycemia, and dyslipidemia in C57BL/6J db/db mice. *J. Food Sci.* **2010**, *75*, H49−53.
(29) Buemann, B.; Toubro, S.; Holst, J. J.; Rehfeld, J. F.; Bibby, B. M.; Astrup, A. D-Tagatose, a stereoisomer of D-fructose, increases blood uric acid concentration. *Metab., Clin. Exp.* **2000**, *49*, 969−976.
(30) Yamada, T.; Hayashi, N.; Iida, T.; Takamine, S.; Okuma, K.; Matsuo, T. Dietary D-sorbose decreases serum insulin levels in growing Sprague-Dawley rats. *J. Nutr. Sci. Vitaminol.* **2014**, *60*, 297−299.
(31) Oku, T.; Murata-Takenoshita, Y.; Yamazaki, Y.; Shimura, F.; Nakamura, S. D-Sorbose inhibits disaccharidase activity and demonstrates suppressive action on postprandial blood levels of glucose and insulin in the rat. *Nutr. Res. (N. Y., NY, U. S.)* **2014**, *34*, 961−967.
(32) Hawkins, M.; Gabriely, I.; Wozniak, R.; Vilcu, C.; Shamoon, H.; Rossetti, L. Fructose improves the ability of hyperglycemia per se to regulate glucose production in type 2 diabetes. *Diabetes* **2002**, *51*, 606−614.
(33) Perreault, L.; Færch, K.; Kerege, A. A.; Bacon, S. D.; Bergman, B. C. Hepatic glucose sensing is impaired, but can be normalized, in people with impaired fasting glucose. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E1154−1162.
(34) Trus, M.; Zawalich, H.; Gaynor, D.; Matschinsky, F. Hexokinase and glucokinase distribution in the liver lobule. *J. Histochem. Cytochem.* **1980**, *28*, 579−581.
(35) Agius, L. Control of glucokinase translocation in rat hepatocytes by sorbitol and the cytosolic redox state. *Biochem. J.* **1994**, *298*, 237−243.
(36) Wu, C.; Kang, J. E.; Peng, L. J.; Li, H.; Khan, S. A.; Hillard, C. J.; Okar, D. A.; Lange, A. J. Enhancing hepatic glycolysis reduces obesity: Differential effects on lipogenesis depend on site of glycolytic modulation. *Cell Metab.* **2005**, *2*, 131−140.

DOI: 10.1021/acs.jafc.6b05627
J. Agric. Food Chem. 2017, 65, 2888−2894