Note

Comparison of Anti-Obesity Effect between Two Types of Syrup Containing Rare Sugars in Wistar Rats

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Summary D-Allulose-containing rare sugar sweeteners have been categorized into two types, rare sugar syrup (RSS), consisting of 4 rare monosaccharides, and modified glucose syrup (MGS), rich in D-allulose, which was previously referred to D-psicose. The anti-obesity effect of RSS and D-allulose has been already clarified, but that of rare monosaccharides other than D-allulose in RSS has not yet been well understood. Here, we investigated and compared the anti-obesity effect of RSS and MGS in rats. Male Wistar rats were divided into 4 dietary groups: a high-sucrose control diet group (S), a high-fructose corn syrup diet group (HFCS), an RSS diet group (RSS), and an MGS diet group (MGS). RSS significantly suppressed abdominal adipose tissue weight and total body fat accumulation in comparison to sucrose. On the other hand, MGS reduced body weight gain, but not abdominal fat accumulation, relative to sucrose. The weight of the liver and kidneys was significantly higher in the RSS and MGS groups than in the S and HFCS groups, but serum biochemical parameters and hepatic lipids contents were not significantly different among the groups. The present study shows that two types of D-allulose-containing rare sugar sweeteners can suppress body fat accumulation or weight gain in a different manner and that RSS could be used as more effective sweeteners in place of sucrose and HFCS to maintain healthy body weight.

Key Words rare sugar sweetener, modified glucose syrup, D-allulose, fat accumulation, rat

High fructose corn syrup (HFCS), which is made by isomerizing D-glucose to D-fructose, has been used in a wide range of food and beverage. The rise in HFCS consumption has been continuously reported to be associated with the growing risk of obesity and diabetes in the United States (1, 2). In 2015, the World Health Organization (WHO) recommended that adults and children should reduce their daily intake of free sugars to less than 5% of their total energy intake (3). Therefore, much more attention has recently been paid to non- or low-calorie sweeteners to maintain healthy body weight and prevent obesity (4). On the other hand, studies have reported that some conventional non- or low-calorie sweeteners such as aspartame and saccharin increase body weight and food consumption, and induce lower thermogenesis and glucose intolerance as adverse effects (5–7).

In order to avoid the adverse effects described above, we have developed rare sugar sweeteners. Among rare sugar sweeteners, D-allulose (D-ribo-2-hexulose), which was previously referred to D-psicose, has recently received much attention as a potential anti-diabetic and anti-obesity ingredient (8–15), and has not shown adverse effects that are observed in conventional non- or

low-calorie sweeteners.

There have been two methods for producing D-allulose-containing rare sugar sweetener from D-fructose through an isomerization process, using alkali hydrolysis or a microbially-derived isomerizing enzyme. In the latter method, D-glucose derived from cornstarch is isomerized to D-fructose by xylose isomerase, and then is further epimerized to D-allulose by D-allulose 3-epimerase, which has greater substrate specificity to D-allulose than D-tagatose 3-epimerase (14). The enzymatic method converts D-fructose to D-allulose at a high conversion rate (~25%), resulting in pure and costly D-allulose. In contrast, D-allulose produced from HFCS by alkali hydrolysis is present at the level of ~6% in rare sugar syrup (RSS), which has been sold as a less expensive sweetener in local markets. We have reported that RSS shows no adverse health effects in rats or humans (16) and suppresses body fat accumulation in rats (17). We also showed that RSS reduced abdominal fat accumulation and serum glucose, insulin, and leptin levels in rats compared to HFCS (18). In our previous study, D-allulose suppressed lipogenic enzymatic activities in the liver compared to glucose or fructose in rats (19, 20). Thus, RSS containing D-allulose seem to be effective in preventing body fat accumulation and obesity partly via lowered lipogenic enzymatic activities. However, D-allulose may not be the sole monosaccharide in

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Fig. 1. Production method of RSS and MGS and their sugar compositions. Rare sugar syrup (RSS) and modified glucose syrup (MGS) are categorized as rare sugar sweeteners. RSS is produced by alkali-isomerization from high-fructose corn syrup (HFCS) as described in Iida et al. (18), and contains D-allulose, D-sorbose, D-tagatose, and D-allose as a rare monosaccharide. MGS is produced by the enzymatic reaction (xylose isomerase and D-allulose 3-epimerase) from glucose syrup, and contains mainly D-allulose as a rare saccharide. These two enzymes were successfully reacted in a single step to produce the MGS from glucose syrup more effectively in the present study.

RSS to reduce body fat accumulation because other rare monosaccharides in addition to D-allulose are also present in RSS.

In the present study, we prepared a new rare sugar sweetener rich in D-allulose with the modified enzymatic reaction using glucose syrup as described above, and referred to it as a new modified glucose syrup (MGS). Then, we compared the anti-obesity property of the two types of D-allulose-containing rare sugar sweeteners (RSS and MGS) in rats.

Materials and Methods

Preparation of MGS and RSS. Both RSS and MGS are categorized as rare sugar sweeteners. MGS was produced from glucose syrup by the patented method developed by Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan) as described in Fig. 1. Briefly, RSS is produced by alkali-isomerization from HFCS as described by Iida et al. (18), and contains D-allulose, D-sorbose, D-tagatose, and D-allose as rare monosaccharides. MGS is produced by the enzymatic reaction (xylose isomerase and D-allulose 3-epimerase) from glucose syrup, and contains mainly D-allulose as its rare monosaccharide. Two enzymatic steps for producing D-allulose from glucose were combined to one step by mixing two enzymes. HFCS, RSS, and MGS were supplied by Matsutani Chemical Industry Co., Ltd. The solid concentration of HFCS, RSS, and MGS used in the present study was 69, 75, and 75% (g/100 g), respectively. MGS mainly consists of D-glucose, D-fructose, D-allulose, and oligosaccharides while RSS mainly consists of D-glucose, D-fructose, D-allulose, D-mannose, D-sorbose, D-tagatose, D-allose, and oligosaccharides. HFCS contains D-glucose, D-fructose, and oligosaccharides.

Reagents. Chemical reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Nacalai tesque, Inc. (Kyoto, Japan). Powdered fat (Magic

fat 200) was donated from Miyoshi Oil & Fat Co., Ltd. (Tokyo, Japan). A mineral mixture and vitamin mixture were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Casein, sucrose, cellulose, and cornstarch were purchased from Fonterra Co. (Auckland, New Zealand), Mitsui Sugar Co., Ltd. (Tokyo, Japan), Nippon Paper Industries Co., Ltd. (Tokyo, Japan), and Oji Cornstarch Co., Ltd. (Tokyo, Japan), respectively.

Animal treatment. Animal experiments were approved by the Experimental Animal Care Committee of Kagawa University (Approval No. 2014-30). Thirty-four male Wistar rats (3 wk of age) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Rats were individually housed in stainless steel cages and acclimatized at $22 \pm 2^\circ\text{C}$ under 12 h light/dark cycle (lights on from 08:00 to 20:00). Rats were given water and an MF diet (Oriental Yeast Co., Ltd.) ad libitum for 7 d. Rats were then divided into four dietary groups: rats fed a high-sucrose control diet (S, $n=8$), an HFCS diet (HFCS, $n=8$), an RSS diet (RSS, $n=8$), and an MGS diet (MGS, $n=10$). The composition of the diets is shown in Table 1. The levels of RSS and MGS were within the range that does not cause diarrhea, based on our previous experiment (Misaki et al., unpublished data). Rats were fed the diets for 8 wk, and body weight and dietary food intake were monitored daily. At the end of the feeding period, rats were fasted for 12 h and subsequently sacrificed by decapitation. Serum, liver, kidney, abdominal adipose tissues, and muscles were stored at -80°C until biochemical analyses. Other organs, tissues, head, legs, and tail were removed, and remaining parts were defined as the carcass. Carcass samples were also stored at -20°C until analyses.

Biochemical analysis. The levels of serum glucose, triacylglycerol (TG), and total cholesterol were measured using commercial kits (Wako Pure Chemical Industries, Ltd.). The serum insulin level was measured using

Table 1. Composition of experimental diet (g/kg diet).

	S	HFCS	RSS	MGS
Ingredients				
Casein	200.0	200.0	200.0	200.0
DL-Methionine	3.0	3.0	3.0	3.0
Cornstarch	390.0	390.0	390.0	390.0
Sucrose	259.9			
HFCS		374.8 ¹		
RSS			346.1 ¹	
MGS				346.5 ¹
Powdered fat ²	50.0	50.0	50.0	50.0
AIN-76 mineral mixture	35.0	35.0	35.0	35.0
AIN-76A vitamin mixture	10.0	10.0	10.0	10.0
Cellulose	50.0	50.0	50.0	50.0
Choline chloride	2.0	2.0	2.0	2.0
Butylhydroxytoluene	0.1	0.1	0.1	0.1
Total				
Solid weight	1,000.0	1,000.0	1,000.0	1,000.0
Total weight including moisture	1,000.0	1,114.9	1,086.2	1,086.6
Rare monosaccharide (g/kg diet)				
D-Allulose ³	0	0	14.6	33.3
D-Sorbose ⁴	0	0	29.4	0.0
D-Tagatose/D-Allose ⁵	0	0	10.9	0.0
Nutritional composition (g/kg diet)				
Carbohydrate ⁶	674.6	674.6	634.5	609.1
Fat	36.5	36.5	36.5	36.5
Protein	203.0	203.0	203.0	203.0
Energy (kcal/kg) ⁷	3,802.4	3,802.3	3,642.2	3,540.5

¹ Solid concentration of HFCS, RSS, and MGS is 70%, 75%, and 75%, respectively. Solid content of carbohydrate in each diet is equalized.

² Powdered fat is composed of 21.5% carbohydrate, 73.0% fat, and others.

³ D-Allulose accounts for 0% of sucrose and HFCS, 5.62% of solid RSS, and 12.83% of solid MGS as shown in Fig. 1.

⁴ Sum of D-sorbose and D-mannose accounts for 11.31% of solid RSS and 0% of sucrose, HFCS, and MGS as shown in Fig. 1.

⁵ D-Tagatose or D-allose accounts for 4.18% of solid RSS and 0% of sucrose, HFCS, and MGS as shown in Fig. 1.

⁶ Carbohydrate present cornstarch, sucrose, 21.5% of powdered fat, 11.8% of mineral mixture, and 98.1% of vitamin mixture.

⁷ Carbohydrate, fat, and protein provide energy at 4, 9, and 4 kcal per gram, respectively.

a commercial kit (Shibayagi Co., Ltd., Gunma, Japan). Total lipids in the liver were extracted as described by Folch et al. (21). The levels of hepatic TG and cholesterol were measured as described in serum analyses. The fat contents of the carcass and total body were measured and calculated as described previously (12). Briefly, a carcass sample softened by autoclave treatment (121°C, 90 min) was ground using a food mixer, and debris of bone was fully removed in order to obtain a carcass meat sample. Crude lipids in the carcass meat sample were extracted by petroleum ether (70°C) with a Soxhlet extractor, and then extracted crude lipids were weighed to quantify the carcass fat weight.

Statistical analysis. Data are expressed as means ± SE (n=8–10). Statistical analysis of differences among the groups was performed by one-way ANOVA with post-hoc Tukey-Kramer tests. A difference at $p < 0.05$ was considered to be statistically significant. Statistical analyses were carried out using a commercially available statistical package (Excel Statistics 2015, SSRI, Tokyo,

Japan).

Results and Discussion

As shown in Table 2, abdominal adipose tissues weight and total body fat percentage were significantly lower in the RSS group, but not the MGS group, than in the S group. These results were consistent with our previous study (18) indicating that dietary RSS suppressed fat accumulation in abdominal adipose tissues of rats. RSS contains D-allulose, D-tagatose, D-sorbose, and D-allose as rare monosaccharides while MGS consists of D-allulose. Thus, the reason why RSS, but not MGS, induced the anti-obese action is probably that each rare sugar other than D-allulose in RSS has a different function, such as fermentability and the effects on glucokinase described below.

RSS contains D-sorbose, D-tagatose, and D-allose as rare monosaccharides in addition to D-allulose. D-Sorbose has been reported to inhibit intestinal α -glucosidase activity and postprandial hyperglycemia in rats

Table 2. Body weight, food intake, tissue weights, serum biochemical parameter, and hepatic lipids content.

Group	S	HFCS	RSS	MGS
D-Allulose content in diet (%)	0	0	1.5	3.3
Number of rats	8	8	8	10
Body weight and food intake				
Body weight (initial) (g)	67.4±1.0	67.2±1.1	67.4±1.3	67.4±1.4
Body weight (final) (g)	281±6.2	268±6.6	267±5.7	254±7.3*
Food intake (g/rat/d) ¹	16.4±0.3	16.2±0.6	16.5±0.4	16.0±0.4
Food efficiency (g BW/g)	0.23±0.00	0.22±0.00	0.21±0.00*	0.20±0.00***
Weight of tissues and organs, and fat weight				
Liver (mg/g BW)	32.3±0.4	31.1±0.5	34.8±0.7####	34.9±0.4####
Kidney (mg/g BW)	6.1±0.1	6.3±0.1	7.1±0.1####	7.9±0.1####SSS
Abdominal adipose tissues (mg/g BW)				
Perirenal	28.3±1.5	26.8±1.7	21.8±1.3*	24.9±1.5
Epididymal	34.1±1.0	31.6±1.7	25.7±1.5**	30.4±1.5
Mesenteric	24.6±1.0	21.8±1.3	18.5±1.4*	20.9±1.2
Total	86.9±3.3	80.2±4.5	66.0±4.1**	76.2±4.1
Muscles (mg/g BW)				
Soleus	0.72±0.02	0.73±0.02	0.72±0.01	0.71±0.02
Plantaris	1.86±0.03	1.94±0.03	1.95±0.04	1.94±0.03
Gastrocnemius	8.88±0.14	9.16±0.12	9.34±0.17	9.36±0.18
Total	11.5±0.17	11.8±0.16	12.0±0.21	12.0±0.22
Carcass fat weight (%)	16.7±0.7	16.9±1.6	13.3±0.8	15.5±1.0
Total body fat weight (%)	16.4±0.6	15.8±1.1	12.6±0.7*	14.7±0.8
Serum biochemical component				
Triacylglycerol (mg/100 mL)	197±19.0	137±9.3	172±15.2	183±16.4
Total cholesterol (mg/100 mL)	93.5±5.4	95.5±4.6	94.3±5.1	93.8±2.9
Glucose (mg/100 mL)	137±5.8	134±4.6	135±5.6	139±4.9
Insulin (ng/mL)	1.9±0.4	1.7±0.2	2.2±0.3	1.9±0.2
Liver lipid contents				
Triacylglycerol (mg/g tissue)	73.2±8.3	92.4±8.1	67.5±7.2	70.6±6.8
Cholesterol (mg/g tissue)	12.7±0.9	13.1±0.2	15.1±0.6	14.1±0.7

Data represent mean±SE for 8–10 rats per group.

Statistically significant differences were evaluated by one-way ANOVA and the Tukey-Kramer test. A difference of $p<0.05$ was considered to be statistically significant. *, **, *** $p<0.05$, 0.01, 0.001 compared with the S group, respectively. ##, ### $p<0.01$, 0.001 compared with the HFCS group, respectively. SSS $p<0.001$ compared with the RSS group.

S, sucrose; HFCS, high-fructose corn syrup; RSS, rare sugar syrup; MGS, modified glucose syrup. ¹ Food intake is shown as solid weight.

(22). Our previous study (23) has shown that D-sorbose improves serum insulin level and increases cecum weight in rats. However, the anti-obesity effects of D-sorbose have not yet been reported. D-Tagatose prevented diabetes and obesity (24). In the review (24), D-tagatose-1-phosphate, a D-tagatose metabolite, has been reported to induce the translocation of glucokinase and inhibit glycogen phosphorylase, which in turn promotes hepatic glycogen synthesis. Bär (25) also suggested that tagatose-induced hepatic hypertrophy was accompanied with glycogen deposition, but not lipid deposition. In addition, D-tagatose has been shown to exert an inhibitory activity against intestinal α -glucosidase, improving postprandial hyperglycemia (24). However, the contribution of dietary D-tagatose (at most around 1% in the RSS diet) to the anti-obesity effect induced by RSS is not clear. On the other hand, D-allose, another rare monosaccharide in RSS, has not been studied for its anti-obesity effect although it has various physiological

functions such as anti-cancer and anti-oxidative functions (26). Thus, how each rare monosaccharide in RSS contributes to the anti-obese effect should be addressed in future studies.

On the other hand, MGS had a smaller effect on abdominal fat compared to RSS (Table 2), although MGS contains twice more D-allulose than RSS in each diet (3.3% vs. 1.5%). In animal studies investigating the anti-obesity effects of D-allulose, D-allulose content in the diet was mainly set at the level of 3% (27) or 5% (11, 12, 15, 28), although the carbohydrate source in the diets and the animal model varied depending on studies. Our previous study (28) reported that a 5% D-allulose diet for 8 wk significantly suppressed body fat accumulation and increased energy expenditure in rats fed a sucrose-based diet. Nagata et al. (27) reported that a 3% D-allulose diet for 4 wk favorably altered fat metabolism by increasing energy expenditure and fat oxidation in rats fed a starch-based diet. Considering the

previous findings, D-allulose content (3.3%) in the MGS diet is supposed to be sufficient to improve fat metabolism. Although reasons why the anti-obese effects of MGS are not clear at present, as mentioned above, additional effect of rare sugars other than D-allulose in RSS may be anticipated. Thus, several kinds of rare monosaccharides in RSS may function mutually, resulting in suppressed body fat accumulation in the RSS group as also shown in our previous study (18).

Final body weight was significantly lower in the MGS group, but not in the RSS group, than in the S group. Food intake was not different among the groups, while food efficiency was significantly lower in the RSS and MGS groups than in the S group. Muscle weight was comparable among the groups. Thus, MGS suppressed body weight gain without decreasing food intake or muscle mass. Reasons for lower food efficiency in the MGS group without a change in abdominal adipose tissue weight should be addressed in future studies.

Serum biochemical parameters and hepatic lipid contents were not significantly different among the groups although RSS and MGS lowered the abdominal fat percentage and body weight, respectively. Since RSS and MGS are prepared from HFCS and glucose, respectively, and are high in glucose and fructose, these non-rare sugar monosaccharides might have lessened the lipid-lowering effect of D-allulose and other rare monosaccharides. When compared to the starch-based diet group, RSS supplementation for 8 wk reduced hepatic TG accumulation as well as intra-abdominal fat accumulation in rats (18). MGS, which contains D-allulose as its sole rare sugar, therefore could reduce hepatic lipids accumulation when a starch-based diet is supplied, which requires further studies.

Regarding its safety as a food additive, D-allulose was approved as generally recognized as safe (GRAS Notice No. GRN 498) and is allowed to be used as a form of RSS in a variety of foods and dietary supplements. As shown in Table 2, liver and kidney weight was significantly higher in the RSS and MGS groups than in the S and HFCS groups. In addition, kidney weight was significantly higher in the MGS group than in the RSS group. In our previous sub-chronic toxicity study using rats, administration of D-allulose at the level of 3% in the diet also showed weight gain of the liver and kidneys, but did not show any adverse health effects (29). Since, similar to D-tagatose, hepatic glycogen accumulation induced by D-allulose has been reported (10), enlarged liver is likely to be due to increased glycogen content. As Tsukamoto et al. (30) revealed that about 37% of administered D-allulose was excreted via urine at 120 min after administration, a higher load of D-allulose on kidney function would be also expected in rats fed RSS or MGS, probably resulting in kidney weight gain.

In conclusion, MGS suppressed body weight gain and RSS suppressed abdominal tissue weight and total fat accumulation, indicating that RSS exerts an anti-obesity effect. RSS appears to be a well-balanced rare sugar sweetener to reduce body fat weight more and shows less kidney weight gain compared to MGS. In addition

to the fact that RSS is less expensive than MGS, these findings indicate that RSS could be used as a low-calorie sweetener to provide an anti-obesity effect in place of sucrose and HFCS.

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