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Original Article

Recovery of increased weights of the liver and kidneys by cessation of D-allulose feeding in Wistar rats

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ABSTRACT — Rare sugar D-allulose prevents obesity; however, an excessive and continuous intake of D-allulose may induce weight increases in the liver and kidney without apparent pathological and functional abnormalities. Conversely, there has not been reported about how these parameters will change after cessation of D-allulose intake. In this study, effects of a 10-week D-allulose cessation on liver and kidneys weights and biomarkers were investigated in rats previously fed a D-allulose containing diet for 4 weeks. Wistar rats were fed a control diet (C, n=16) or a 3% D-allulose diet (DA, n=16) for 4 weeks, and then the half of rats in the C and DA subgroups were dissected, while the other half of rats were fed the control diet for 10 weeks (C-C and DA-C, n=8, respectively). At the end of the first 4 weeks period, halves of rats in each diet group were euthanized, and the serum, urine, liver, and kidneys were used for pathological and biochemical analyses. The remaining rats were also similarly treated at the end of latter 10 weeks treatment. At week 4, the relative weights of the liver and kidneys were higher in the DA group than in the C group, but these differences were disappeared by cessation of D-allulose. No abnormal parameters related to liver and kidneys functions were observed in the serum and urine. These findings suggested that D-allulose-induced increases in the liver and kidneys weights could be recovered to the normal levels by D-allulose cessation without accompanying functional and pathological abnormalities.

Key words: D-allulose, Weight gain, Liver, Kidney, Cessation, Wistar rat

INTRODUCTION

D-Allulose (D-*ribo*-2-hexulose), a C-3 epimer of D-fructose, is one of the rare sugars that are present in limited quantity in nature. D-Allulose has 70% of sweetness relative to sucrose, and when allulose and sucrose were blended in a 1:1 mixture, this blend achieved a near identical dose-response curve to sucrose (Wee *et al.*, 2018). In humans, approximately 70% of the ingested D-allulose is absorbed in the small intestine and excreted into the urine without being further metabolized (Iida *et al.*, 2010). Residual D-allulose that is not absorbed in the small intestine moves into the large intestine and is excreted into feces without practically being fermented by intestinal bacteria (Iida *et al.*, 2010). Based on these properties, D-allulose is used as a zero-calorie sugar substitute and has been recently reported to prevent hyperglycemia, obesity and diabetes in rats (Matsuo *et al.*, 2002; Baek *et al.*, 2010; Hossain *et al.*, 2015a, 2015b; Han *et al.*, 2016).

However, D-allulose in diets has been reported to induce weight increases in the liver and kidneys of rats (Yagi and Matsuo, 2009; Matsuo *et al.*, 2012; Chung *et al.*, 2012). A previous study showed that feeding a 3% D-allulose diet for 3, 12, and 18 months in rats results in the weight increases without obvious toxicological altera-

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tions, as assessed by blood and histological analyses.

Increases in the organ weights seem to occur specifically in rodents (Hall *et al.*, 2012) under various conditions, including feeding with carbohydrates such as sucrose, D-fructose and D-tagatose, the C-3, 4 epimer of D-allulose to rats (Bär *et al.*, 1999). In contrast, D-tagatose-induced increases in the liver weight were not observed in humans (Boesch *et al.*, 2001). These phenomena could be due to enhanced workloads or metabolic demands and are considered as a physiological adaptation.

In particular, increases in the liver and kidneys weights that we previously observed after the excessive and long-term intake of D-allulose most likely reflect a physiological adaptation, because no biochemical or histopathological alterations were observed in blood, liver or kidneys (Yagi and Matsuo, 2009; Chung *et al.*, 2012). One possible reason for the D-allulose-induced weight increase in the liver is the increase in glycogen storage. In fact, D-allulose promotes glucokinase translocation from the nucleus to the cytoplasm, leading to increased glycogen storage in the liver (Toyoda *et al.*, 2010; Hossain *et al.*, 2011), as previously observed for D-tagatose and D-fructose (Lu *et al.*, 2008). In addition to glycogen storage, D-tagatose was reported to induce an increase in the total levels of hepatic lipids, proteins and DNA, as well as a significant increment of liver weight, without any adverse effects (Bär *et al.*, 1999). Similarly, increase in the liver weight induced by a high-sucrose diet was not associated with the hepatic histological changes (Bär, 1999; Bär *et al.*, 1999).

Regarding kidneys, feeding erythritol, which is a zero-calorie and renal excretory sweetener similar to D-allulose, was reported to induce the organ weight increases without impairing renal functions (Noda *et al.*, 1993). This phenomenon was thus considered as a physiological response due to increased kidneys workload in response to the diuretic effects of erythritol (Munro *et al.*, 1998). Increase in the organ weights induced by D-allulose intake may therefore be similar to those elicited by D-tagatose, sucrose and erythritol. The mechanisms underlying these effects have been extensively investigated.

Conversely, the reversibility of D-allulose-induced increase in the liver and kidneys weights, the possible occurrence of accompanying pathological abnormalities and the impact on these events by the D-allulose cessation, have not yet been examined. Notably in a preliminary study using male Sprague-Dawley rats (4 week-old) fed either a 3% D-allulose diet or a control diet for 4 weeks, we did not observe liver weight increases. SD rats appear to be insensitive to D-allulose-induced effects, and there are no data regarding D-allulose safety in these rats. Therefore, in the present study, effects of a 10-week D-allulose cessation, following a 4-week D-allulose diet, on hepatic and renal pathophysiology were investigated in Wistar rats.

MATERIALS AND METHODS

D-Allulose and diet

D-Allulose was supplied by International Institute of Rare Sugar Research and Education (Kagawa, Japan). The purity of D-allulose was higher than 98%, as assessed by HPLC analysis. Mineral and vitamin mixtures (AIN93G) were purchased from Oriental Yeast Co., Ltd., (Tokyo, Japan). The other ingredients used for the diet (casein, sucrose, cellulose, cornstarch, and soybean oil) were food-grade. Other chemical reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Nacalai tesque, Ltd. (Kyoto, Japan). The control diet comprised 20.0% (w/w) casein, 0.3% L-cystine, 10.0% sucrose, 52.9% cornstarch, 5.0% cellulose, 7.0% soybean oil, 3.5% mineral mixture, 1.0% vitamin mixture, 0.25% choline chloride, and 0.014% tert-butylhydroquinone. In the D-allulose diet, cornstarch was substituted with 3% D-allulose.

Animal treatment

Animal experiments were approved by the Experimental Animal Care Committee of Kagawa University (Approval No. 2014-30). Thirty-two male Wistar rats (7 week-old) were purchased from Japan SLC (Shizuoka, Japan). Rats were individually housed in stainless steel cages and acclimatized to 22 ± 1°C and 55 ± 5% in humidity under 12 hr light/dark cycles (lights on from 08:00 to 20:00). Rats were given tap water and a powdered MF diet (Oriental Yeast Co., Ltd.) *ad libitum* for 3 days. Next, they were divided into two diet groups: control diet (C, n=16) and 3% D-allulose diet (DA, n=16). Each diet was supplied for 4 weeks, and body weight and dietary food intake were monitored daily. At the last week of the 4-weeks feeding period, urine was collected and stored at -80°C. Then, half of the rats in each diet group (n=8) were fasted for 12 hr in order to exclude possible effects of feeding and euthanized by decapitation. The blood was centrifuged at 1,500 × g for 15 min to obtain serum. Liver and kidney samples were fixed in 10% neutral buffered formalin for pathological examination. Serum and liver samples were stored at -80°C until analysis. After removal of head, legs and tail from body, a carcass sample was also stored at -80°C. The other half of rats in each diet group (n=8) were then fed the con-

trol diet (C-C and DA-C) for 10 weeks. At the end of the treatment period, rats were euthanized by decapitation, and samples were collected and stored at -80°C as described above.

Biochemical analysis

The serum levels of glucose (GLU), triacylglycerol (TG) and total cholesterol (TC) were measured using commercial kits from Wako Pure Chemicals Co. Ltd., and serum insulin (INS) level was measured using an ELISA kit (Type-T, Shibayagi Co., Gunma, Japan). Analysis of biochemical parameters in the serum and urine, i.e., the serum levels of total bilirubin (BIL), urea nitrogen (BUN), creatinine (CRE), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and γ-glutamyltranspeptidase (γ-GTP), and the urea levels of N-acetyl-β-D-glucosaminidase (NAG) and CRE were performed by Fujifilm Monolith Co. Ltd. (Tokyo). Extraction of the total liver lipids and measurement of liver TG and cholesterol levels were performed as previously described (Ochiai *et al.*, 2013). The glycogen content in the liver was determined as described by Hassid and Abraham (1957).

Pathological analysis

The pathological analysis was performed by the Biopathology Institute Co., Ltd. (Oita, Japan). The liver and the kidney were fixed in 10% neutral buffered formalin and embedded in paraffin. Next, 3 μm thick sections were cut, mounted on a glass slide, stained with hematoxylin and eosin (HE) and observed under a microscope. Cavitation of perilobular hepatic cells, inflammatory cell infiltration, formation of microgranuloma in the liver, and deposition of acidophilic corpuscles and hyaline droplets in the epithelium of proximal convoluted kidney tubules were measured in each sample. For each liver and kidneys, the pathological abnormalities were quantified as follows: no, 0; slight, 1; mild, 2; intermediate, 3; severe, 4.

Statistical analysis

Data were expressed as means ± SE (n=8). The statistical analysis of differences in the two paired comparisons (C and DA, C-C and DA-C) was performed by Student's *t*-test. A difference with $p < 0.05$ was considered to be statistically significant. Statistical analyses were carried out using a commercially available statistical package (Excel Statistics 2015, SSRI, Tokyo).

RESULTS AND DISCUSSION

Effect of D-allulose intake cessation on body weight, food intake, and tissues weight

Since the body weight of young rats increases as they grow, and adult rats exhibit significant individual differences, it may be difficult to assess the effect of a specific treatment on organ weight. In addition, our previous experiments have shown that the body weight tends to be lower, compared to the control rats, after the feeding of D-allulose diet because of the anti-obesity effect of D-allulose. Chung *et al.* (2012) also showed that 5% D-allulose in diet increased the liver weight, while the body and adipose tissue weight were reduced in mice.

The Society of Toxicologic Pathology recommends that organ weight is expressed as an organ-to-body weight to avoid the influence of large variations in body weight, although in the present study, body weight, food intake and food efficiency were not significantly different between the two diet groups, both at week 4 and at week 14 (Table 1). Relative abdominal adipose tissue weight as well as carcass and body fat percentage were not significantly altered by the D-allulose diet. Anti-obesity effects of D-allulose have been observed only when a high-fat or high-sucrose diet was supplied or when obese animal models are used (Matsuo *et al.*, 2012; Hossain *et al.*, 2011; Ochiai *et al.*, 2013). Nagata *et al.* (2015, 2018) also reported that a 3% D-allulose-containing starch-based normal diet favorably improved lipid metabolism but did not significantly suppress adipose fat accumulation. Moreover, Matsuo *et al.* (2001) showed that a D-allulose-containing starch-based diet caused no differences in the body weight and carcass fat accumulation. Our results are therefore consistent with previous findings.

The relative weights of liver and kidneys were significantly higher in D-allulose-fed rats at week 4 (C *vs.* DA, $p < 0.05$, Fig. 1). Cessation of D-allulose for 10 weeks abolished the differences in the relative weights of liver and kidneys (C-C *vs.* DA-C, $p > 0.05$). Serum levels of AST, ALT and γ-GTP, which are biomarkers of liver function, were not altered by either D-allulose diet or cessation (Table 3). Serum levels of CRE and BUN, which are biomarkers of renal function, were not different between the C-C and the DA-C group. Consistently, in clinical trials conducted by Hayashi *et al.* (2010), hepatic and renal biomarkers were not significantly affected in D-allulose-treated subjects (15 g/day, for three months) compared to the placebo group.

Tsukamoto *et al.* (2014) demonstrated that most of the D-allulose orally administered to rats passes through the liver and kidney and approximately 5% of the radio-la-

Table 1. Body weight gain, food intake, and tissues weight of rats.

Diet period		4 weeks		14 weeks	
		C	DA	C-C	DA-C
Initial body weight	(g)	174 ± 2	173 ± 3	175 ± 2	176 ± 2
Final body weight	(g)	279 ± 6	273 ± 5	368 ± 7	380 ± 8
Food intake	(g/day)	17.2 ± 0.5	17.0 ± 0.4	15.6 ± 0.2	16.4 ± 0.3
Liver	(g)	7.1 ± 0.3	8.0 ± 0.3	9.1 ± 0.3	9.4 ± 0.3
Kidneys	(g)	1.6 ± 0.1	1.8 ± 0.1*	1.9 ± 0.1	2.1 ± 0.1
Adipose tissue[1]	(g)	15.7 ± 0.8	15.2 ± 1.1	31.2 ± 1.2	31.4 ± 1.6
	(mg/g)	55.9 ± 1.6	55.5 ± 3.6	84.6 ± 2.9	82.3 ± 3.0
Carcass[2]					
Carcass fat	(%)	11.8 ± 0.9	12.1 ± 0.4	14.0 ± 0.7	13.9 ± 1.4
Total body fat	(%)	9.5 ± 0.6	9.4 ± 0.4	13.0 ± 0.6	12.3 ± 0.7

Data are the mean ± SE (n=8 rats per group). Statistically significant differences for each score were evaluated by Student's t-test (*$p < 0.05$, C *vs*. DA, C-C *vs*. DA-C). C, group of rats fed a control diet for 4 weeks; DA, group of rats fed a D-allulose diet for 4 weeks; C-C, group of rats fed a control diet throughout the experimental period (14 weeks); DA-C, group of rats fed a D-allulose diet for 4 weeks, followed by a control diet for 10 weeks. 1, sum of perirenal, mesenteric, and epididymal adipose tissues. 2, carcass fat analysis was carried out according to our previous study (Ochiai *et al*. 2013).

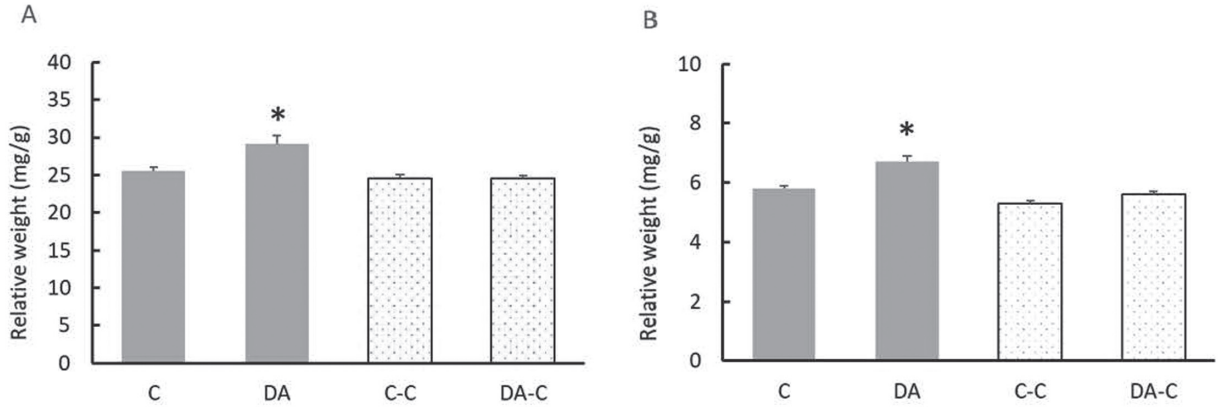

Fig. 1. Relative weight of the liver and kidneys before and after the D-allulose cessation. A: Liver, B: Kidneys. The data of 8 rats per group are presented as mean ± SE. Statistically significant differences in each score were evaluated by Student's t-test (*$p < 0.05$, C *vs*. DA, C-C *vs*. DA-C). C, rats fed a control diet for 4 weeks; DA, rats fed with a D-allulose diet for 4 weeks; C-C, rats fed with a control diet throughout the experimental period (14 weeks); DA-C, rats fed with a D-allulose diet for 4 weeks, followed by the control diet for 10 weeks.

beled D-allulose remains in these organs for up to 2 hr. Approximately 37% of the ingested ^{14}C-labeled D-allulose was excreted into the urine within 2 hr after the oral administration, and almost D-allulose was not detected in the urine at 7 days after the administration (Tsukamoto *et al.*, 2014). Moreover, Matsuo *et al.* (2003) observed that D-allulose was entirely excreted into the urine within 24 hr after the oral administration. Furthermore, Whistler *et al.* (1974) reported that approximately 98% of intravenously administered D-allulose is excreted into the urine within 6 hr. Most of the orally administered D-allulose that is absorbed from the small intestine is believed to be temporarily passed through the liver and kidneys, and then finally excreted into the urine without being metabolized. The interactions of D-allulose on organs were transient, and therefore unlikely to cause serious adverse events.

Effect of D-allulose intake cessation on hepatic and renal pathological parameters

Inflammatory cell infiltration in the liver was significantly lower in the DA group than in the C group

Table 2. Pathological score of liver and kidney of rats after the 4 weeks-treatment period.

		C (4 weeks)						DA (4 weeks)					
		0	1	2	3	4	Mean ± SE	0	1	2	3	4	Mean ± SE
Liver		The number of rats with pathological score						The number of rats with pathological score					
	Cavitation of perilobular hepatic cells	1	1	5	1	0	1.8 ± 0.3	0	3	5	0	0	1.6 ± 0.2
	Inflammatory cell infiltration	0	3	5	0	0	1.6 ± 0.2	4	4	0	0	0	0.5 ± 0.2*
	Formation of microgranuloma	5	3	0	0	0	0.4 ± 0.2	4	4	0	0	0	0.5 ± 0.2
Kidney													
	Deposition of acidophilic corpuscle in epithelium of proximal convoluted tubule	1	2	3	2	0	1.8 ± 0.4	1	1	6	0	0	1.6 ± 0.3
	Deposition of hyaline droplet in epithelium of proximal convoluted tubule	0	0	3	5	0	2.6 ± 0.2	0	1	2	5	0	2.5 ± 0.3

Data represent liver and kidneys pathological scores in each group of 8 rats. The number of rats presenting pathological scores (0 ~ 4) and the average score are indicated. Statistically significant differences for each score were evaluated by Student's t-test. Means with letters are significantly different ($p < 0.05$); C, rats fed a control diet for 4 weeks; DA, rats fed with a D-allulose diet for 4 weeks. The pathological analysis was performed by the Biopathology Institute Co., Ltd. (Oita, Japan), and the image is described in Fig. 1. Cavitation of perilobular hepatic cells, inflammatory cell infiltration, formation of microgranuloma in each liver, and deposition of acidophilic corpuscles and hyaline droplets in the epithelium of proximal convoluted tubules in each kidney were evaluated. The pathological findings quantified as follows: no alterations, 0; slight alterations, 1; mild alterations, 2; intermediate alterations, 3; severe alterations, 4.

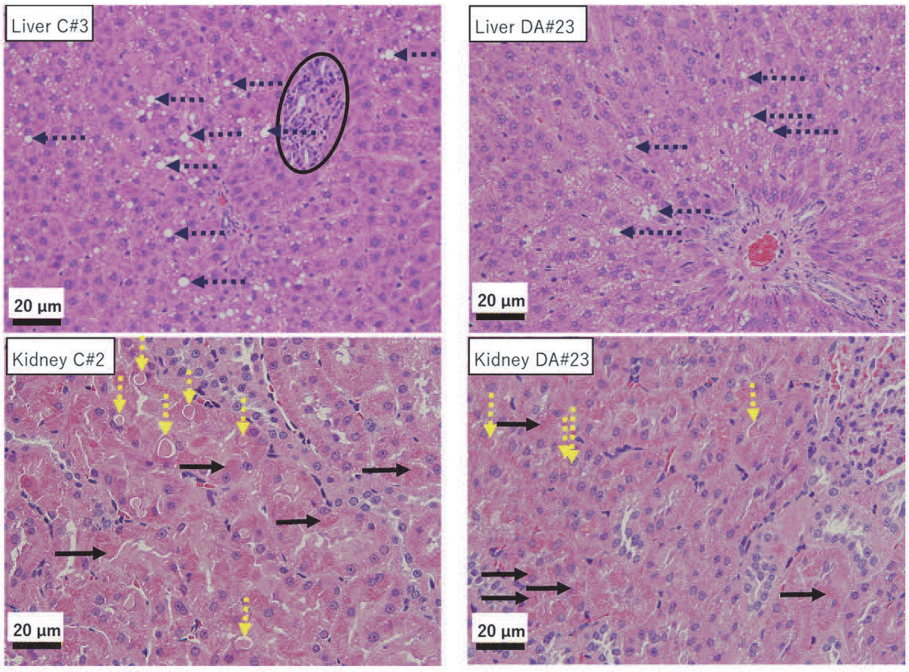

Fig. 2. Representative liver and kidneys sections of rats after the 4 weeks-treatment period in each group (scale bar = 20 µm). The liver and the kidneys were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (3 µm) were stained with hematoxylin and eosin (HE). Cavitation of perilobular hepatic cells, inflammatory cell infiltration, formation of microgranuloma in the liver, and deposition of acidophilic corpuscles and hyaline droplets in the epithelium of proximal convoluted kidney tubules were measured in each sample. The dotted left blue arrow represents the cavitation of perilobular hepatic cells in the liver. The black circle represents inflammatory cell infiltration in the liver. The dotted down yellow arrow and right straight black arrow represent the deposition of acidophilic corpuscles and hyaline droplets, respectively, in the epithelium of proximal convoluted renal tubules. The liver and kidneys sections in the images were from the rat in each group at the average score in the pathological analysis (see Table 2).

Table 3. Plasma components, lipids, and glycogen accumulation in the liver of rats.

Diet period		4 weeks		14 weeks	
		C	DA	C-C	DA-C
Plasma					
GLU	(mg/100 mL)	114.5 ± 2.8	122.3 ± 3.2	139.5 ± 4.3	118.8 ± 4.1**
TG	(mg/100 mL)	267 ± 12	265 ± 11	363 ± 21	347 ± 12
TC	(mg/100 mL)	173 ± 6	183 ± 7	236 ± 13	241 ± 11
HDL-C	(mg/100 mL)	102 ± 3	99 ± 2	89 ± 2	89 ± 2
NEFA	(mEq/L)	2.0 ± 0.2	2.0 ± 0.2	1.2 ± 0.1	1.0 ± 0.1
INS	(ng/mL)	0.53 ± 0.02	0.39 ± 0.01***	1.79 ± 0.19	1.80 ± 0.36
AST	(IU/L)	228 ± 10	230 ± 13	250 ± 13	254 ± 14
ALT	(IU/L)	53.6 ± 2.7	52.5 ± 1.5	61.9 ± 2.4	68.6 ± 4.3
γ-GTP	(IU/L)	0.6 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
BIL	(mg/100 mL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
ALB	(g/100 mL)	3.9 ± 0.0	4.1 ± 0.1**	4.0 ± 0.0	4.2 ± 0.0*
BUN	(mg/100 mL)	11.8 ± 0.6	11.6 ± 0.5	12.1 ± 0.4	12.9 ± 0.5
CRE	(mg/100 mL)	0.30 ± 0.0	0.25 ± 0.0*	0.35 ± 0.0	0.33 ± 0.0
Urine					
NAG	(IU/L)	5.0 ± 1.3	6.3 ± 1.5		
CRE	(mg/100 mL)	44.3 ± 5.7	52.2 ± 6.3		
Liver					
TG	(mg/g)	31.9 ± 5.0	35.0 ± 3.8	29.8 ± 4.3	31.8 ± 3.6
TC	(mg/g)	4.6 ± 0.2	5.8 ± 0.3**	4.2 ± 0.2	4.3 ± 0.2
Glycogen	(mg/g)	1.5 ± 0.3	6.5 ± 1.4**	5.4 ± 1.5	2.4 ± 0.6

Data are the mean ± SE (n=8 rats per group). Statistically significant differences for each score were evaluated by Student's t-test (*, **, ***$p < 0.05$, 0.01, 0.001, respectively, C *vs*. DA, C-C *vs*. DA-C). C, rats fed a control diet for 4 weeks; DA, rats fed a D-allulose diet for 4 weeks; C-C, rats fed a control diet throughout the experimental period (14 weeks); DA-C, rats fed a D-allulose diet for 4 weeks, followed by a control diet for 10 weeks. GLU, glucose; TG, triacylglycerol; TC, total cholesterol; HDL-C, high-density-lipoprotein-cholesterol; NEFA, non-esterified fatty acid; INS, insulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ- glutamyltransferase; BIL, bilirubin; ALB, albumin; BUN, blood urea nitrogen; CRE, creatinine; NAG, N-acetyl-β-D-glucosaminidase.

(Table 2, Fig. 2). Recently, it was reported that D-allulose ingestion suppressed macrophage infiltration in adipose tissues and normalized inflammatory cell infiltration in pancreatic islets of diabetic rats (Hossain *et al.*, 2015b). Hossain *et al.* (2015b) showed that the serum levels of tumor necrosis factor-alpha and interleukin-6, which are inflammatory markers, were also suppressed by D-allulose supplementation in drinking water. These results suggest that D-allulose might suppress inflammatory cell infiltration in the liver and kidneys. In this study, other hepatic and renal pathological parameters were not different between the C and the DA groups. Cavitation of perilobular hepatic cells, which is an indicative of TG deposition, was not significantly different between the groups. Chung *et al.* (2012) reported that a D-allulose diet produced no pathological changes in the liver of diet-induced obese mice, suggesting that D-allulose does not trigger hepatic toxicity. Some reports showed that D-allulose suppressed the cavitation of perilobular hepatic cells and hepatic steatosis in obese mice (Itoh *et al.*, 2015; Iwasaki *et al.*, 2018). These inconsistent results might be due to differences in mouse models or employed diets. A low extent of microgranuloma formation, an index of chronic inflammation, was not statistically significant between the groups. Deposition of acidophilic corpuscles and hyaline droplets in the epithelium of proximal convoluted kidney tubules were not different between the two groups, suggesting that D-allulose did not influence renal functions. Pathological analyses after the cessation of D-allulose were not performed, because alterations of pathological parameters were unlikely to occur after the D-allulose cessation.

Effect of D-allulose intake cessation on plasma, urine, and liver components

Serum levels of INS, TG and TC were increased age-dependently regardless of the presence of D-allulose (Table 3). Significant differences in INS and CRE levels in the serum and TC and glycogen contents in the liver were observed between the C and the DA diet group

but disappeared after the cessation of D-allulose. Serum ALB level was significantly higher in the DA than in the C diet group, and a significant difference remained after the D-allulose cessation. However, serum ALB level was within the normal range in all samples, according to the data in 1998 from the Charles River Laboratories (19-21-week old Wistar rats: 3.3-4.7 g/100 mL) (Charles River Laboratories). D-Allulose is a renal excretory monosaccharide, hence the ALB increment was thought to be due to diuretic effects. However, the persistence of ALB increment after the D-allulose cessation spoke against this hypothesis. Serum ALB is an indicator of the nutritional status and functions as a transporter of fatty acids (Kuwahata, 2011), whereas D-allulose facilitates fatty acid metabolism (Kimura, 2017). D-Allulose-induced slight increment in the serum ALB level might suggest the facilitation of lipid metabolism or an improved nutritional status in the liver. The significant decrease in the serum glucose level in the DA-C diet group may reflect that changes in lipid or carbohydrate metabolism have been continued even after the cessation of D-allulose. Matsuo *et al.* (2012) showed that a D-allulose diet for 3-months in rats increased the serum ALB and total protein levels. Contrarily, Yagi and Matsuo (2009) reported that a D-allulose diet for 12-months in rats did not significantly affect the serum ALB level, as well as other serum biochemical parameters. The ALB increment might not be detectable after the longer treatments with D-allulose. Other serum parameters were not different between the two groups at week 4 or 14.

Our experiment has indicated that the D-allulose-induced increase in organ weights of rats was not due to irreversible changes in inflammatory response or pathophysiological functions and, therefore, liver and kidneys could be slowly restored to the normal size after the D-allulose cessation.

In conclusion, feeding a 3% D-allulose-containing diet to rats for 4 weeks induced weight increases in the liver and kidneys without any pathological impairment, and a 10-week D-allulose cessation disappeared these effects. Further research is needed to cover some limitations in this report, such as the use of young rats and the lack of measured parameters. Consistently, D-allulose has been recognized by the US Food and Drug Administration (GRAS Notice No. GRN 498). Thus, the results of the present study further demonstrate that D-allulose is a safe and suitable zero-calorie sugar substitute.

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Conflict of interest---- M.N. T.Y. and T.I. are employees of Matsutani Chemical Industry Co. Ltd. (Itami, Japan). There are no other patents. All the other authors have no conflicts of interest to declare.

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