Original paper

Dietary d-Allose Ameliorates Hepatic Inflammation in Mice with Non-alcoholic Steatohepatitis

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Nonalcoholic steatohepatitis (NASH) is characterized by excess lipid accumulation and inflammation in hepatocytes. In this study, to provide insight into the preventive effects of d-allose, a rare sugar, on the onset of NASH, we designed animal experiments using male STAM mice treated with streptozotocin and fed a high-fat diet (HFD). Experiments were initiated when the mice reached 5 weeks of age and lasted 3 weeks. After the 3-week protocol, mice fed the HFD containing d-allose exhibited significantly decreased serum alanine aminotransferase levels, hepatic lipid accumulation and inflammation, and improved nonalcoholic fatty liver disease activity score compared to mice fed HFD without d-allose (p < 0.05). Further, hepatic mRNA expression of sterol regulatory element binding protein-1 (Srebp-1) and monocyte chemotactic protein-1 (Mcp-1) was lower in mice fed d-allose. These results suggested that d-allose prevented NASH by blocking hepatic lipid accumulation and progressive inflammation.

Keywords: nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, rare sugar, d-allose

Introduction

Nonalcoholic fatty liver disease (NAFLD) is an increasingly common hepatic phenotype of metabolic syndrome not caused by chronic alcohol consumption and is characterized by lipid accumulation in hepatocytes (Williams et al., 2011). Nonalcoholic steatohepatitis (NASH), a type of NAFLD, is a significant risk factor for the development of cirrhosis and hepatocellular carcinoma (Feldstein et al., 2009; Cohen et al., 2011). The two-hit theory (Day and James, 1998) and multiple parallel hits hypothesis (Tilg et al., 2010) have been used to explain the progression of simple steatosis to NASH via lipid accumulation, inflammation, and oxidative stress in the liver, although the exact mechanisms...
have not yet been elucidated.

In the alternative medicinal food sciences, recent reports have claimed that excessive sugar intake (e.g., d-fructose and high-fructose corn syrup) is a high risk factor in the progression of NAFLD/NASH (Abdelmalek et al., 2010; Nseir et al., 2010). Meanwhile, the physiological effects of rare sugars, such as d-allose, have recently attracted attention as physiological mediators of such diseases. d-Allose is a d-glucose epimer at C3 and can be industrially obtained from d-psicose by using rhamnose isomerase (Menavuvu et al., 2006). For example, d-allose has been shown to protect against inflammatory and oxidative ischemia/reperfusion (I/R) injury in liver, retinal, and cerebral injuries in rodent models (Hossain et al., 2003; Hirooka et al., 2006; Gao et al., 2013). Previous studies have revealed the physiological benefits of d-allose, including anti-inflammatory effects resulting from reduced myeloperoxidase (MPO) activity and reduced adherent neutrophils in organs (Hossain et al., 2003), and anti-oxidant effects resulting from reduced production of H2O2 or O2· (Hirooka et al., 2006; Mizote et al., 2011). Intake of anti-inflammatory or anti-oxidant compounds can be used to prevent the development of NAFLD/NASH (Park et al., 2011; Ni et al., 2015). Thus, d-allose may have the potential to contribute to the prevention of NAFLD/NASH, as well as I/R injury, caused by excess inflammation and/or oxidative stress in the liver.

In this study, we hypothesized that dietary d-allose could prevent NAFLD/NASH. STAM mice, i.e., C57BL/6J mice treated with streptozotocin (STZ) and fed a high-fat diet (HFD) and DA-STAM (STAM mice fed a HFD containing 2% d-allose) groups. Experiments started at 5 weeks from birth and lasted for 3 weeks. STAM mice were primarily generated from C57BL/6J mice by a single subcutaneous injection of STZ on day 2 after birth, and at 4 weeks of age, they were fed with a HFD ad libitum. During breast-feeding, maternal mice were fed a normal diet (AIN93G). DA, d-allose; STZ, streptozotocin; ND, normal diet (AIN93G); HFD, high-fat diet (HFD 32)

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**Materials and Methods**

**Animals and experimental design, and sample preparation**

STAM mice were used to model NASH and were obtained according to the method described by Fujii et al. (2013). Pathogen-free, 14-day pregnant female C57BL/6J mice were purchased from Charles River Laboratories (Tokyo, Japan). To induce NASH, STAM mice were primarily obtained by treating C57BL/6J mice with a single subcutaneous injection of 200 µg STZ (Sigma-Aldrich, St. Louis, MO, USA) 2 days after birth. At 4 weeks of age, the mice were fed a HFD (HFD32; CLEA Japan, Tokyo, Japan) ad libitum for 1 week (the experimental scheme is shown in Fig. 1). At 5 weeks of age, the STAM mice with hepatic steatosis were randomly divided into two groups, the STAM group (HFD-feeding) and the DA-STAM group (HFD containing ca. 2% (precisely 1.96%) d-allose-feeding), and housed for 3 weeks. The diet compositions are shown in Table 1. d-Allose was provided by the Kagawa University Rare Sugar Research Center (Kagawa, Japan). In this study, a 2% d-allose-diet was used for the STAM mice experiment, based on a previous report demonstrating that this d-allose concentration has potential inhibitory effects on other liver diseases (hepatocarcinogenesis), whereas a lower dose (0.01 – 1.0%) has no effect on the development of liver diseases (Yokohira et al., 2008). C57BL/6J mice without STZ treatment and fed an AIN93G diet (Oriental Yeast, Tokyo, Japan) were used as a control group. All mice were housed in colony cages with a 12-hour light/dark cycle, controlled temperature (24 ± 1°C), and were given free access to food and water. At 8 weeks after birth, the mice were fasted overnight, then anesthetized with pentobarbital sodium (64.8 mg/g, Kyoritsu Seiyaku, Tokyo, Japan) and sacrificed. Blood and liver samples were collected; serum samples were stored at −30°C until use, and liver samples were immediately
D-α-Llose Ameliorates Hepatic Inflammation in NASH

Table 1. Compositions of diets.

<table>
<thead>
<tr>
<th>Ingredients (% (w/w))</th>
<th>AIN93G</th>
<th>HFD</th>
<th>HFD containing 2% D-α-Llose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>24.5</td>
<td>24.0</td>
</tr>
<tr>
<td>Egg Albumin</td>
<td>-</td>
<td>5</td>
<td>4.9</td>
</tr>
<tr>
<td>L-cystine</td>
<td>0.30</td>
<td>0.43</td>
<td>0.42</td>
</tr>
<tr>
<td>Corn starch</td>
<td>39.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>α-Corn starch</td>
<td>13.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>-</td>
<td>8.25</td>
<td>8.09</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>6.93</td>
<td>6.79</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
<td>6.75</td>
<td>6.62</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.5</td>
<td>5.4</td>
</tr>
<tr>
<td>D-α-Llose</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>7.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lard</td>
<td>-</td>
<td>15.9</td>
<td>15.6</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>-</td>
<td>20.0</td>
<td>19.6</td>
</tr>
<tr>
<td>AIN93G Vitamin mix</td>
<td>1.0</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>AIN93G Mineral mix</td>
<td>3.5</td>
<td>5.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.36</td>
<td>0.35</td>
</tr>
<tr>
<td>tert-Butylhydroquinone</td>
<td>0.0014</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Total calorie (kcal/ g) 4.16 5.53 5.53*

D-α-Llose was purchased from Oriental Yeast (Tokyo, Japan). HFD and HFD+2% D-α-Llose were purchased from CLEA Japan (Tokyo, Japan).

Serum biochemical analysis Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triacylglycerol (TG), and glucose (GLC) were measured using an Automatic Biochemistry Analyzer (Fuji Dry-Chem 7000; FUJIFILM Medical, Tokyo, Japan).

Analysis of lipid accumulation in the liver Liver samples were homogenized in ice-cold phosphate buffered saline (PBS). Liver lipids were extracted using Folch's method (Folch et al., 1957). TG levels were determined using a TG-test Wako (Wako, Osaka, Japan). Frozen liver sections fixed in 10% formalin fixative were stained with Oil Red O staining reagent (Wako). Representative images of liver sections with Oil Red O staining were obtained using a microscope at 200× magnification (Shimadzu BA210E; Shimadzu, Kyoto, Japan). Lipid accumulation was quantified using five visual fields with the image analysis software Image J (i) (Abramoff et al., 2004; Schneider et al., 2012).

Hepatic pathological diagnosis Paraffin-embedded, formalin-fixed liver sections were stained with hematoxylin and eosin (H&E), and representative images were obtained using a Shimadzu BA210E microscope at 200× magnification (Shimadzu BA210E). The magnitude of the liver injury was evaluated using NAFLD activity score (NAS), whereby the sum of the scores from three equal weighted features of steatosis (0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2) was calculated (Kleiner et al., 2005). According to the criteria outlined by Hjelkrem et al. (2011), the NAS of 4 or more, 3, and 2 or less were defined as NASH, borderline NASH, and non-NASH, respectively.

Hepatic RNA isolation and quantitative real-time polymerase chain reaction (PCR) Total RNA was isolated from liver tissues using the RNasey Plus Universal mini kit (Qiagen, Hilden, Germany). Total RNA (50 ng/μL) was reverse-transcribed into cDNA using the ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Real-time PCR was performed using a LightCycler 96 (Roche Diagnostics, Basel, Switzerland) in a final volume of 20 μL containing 0.4 μL cDNA, 10 μL of 2× SYBR Green I mixture, 0.6 μL of each primer (10 pmol/μL), and 8.4 μL PCR-grade water under the following PCR conditions: 45 cycles of 90°C for 10 s, 55°C or 58°C for 10 s, and 72°C for 10 s. Expression levels of glyceraldehyde-3-phosphate dehydrogenase (Gapdh; forward: agcttgtcatcaacgggaag, reverse: tttgatgttagtggggtctcg) as an endogenous reference were used to normalize the mRNA expression level of each target. The primers of the target genes are listed in Table 2.

Statistical analysis Results are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using JMP 11.0 (SAS Institute Inc., Cary, NC, USA). Parametric data were evaluated using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. Nonparametric data (hepatic pathological data) were analyzed using the Steel-Dwass test. Differences with p values of less than 0.05 were considered significant.

Results

Effects of D-α-Llose on body weight, liver weight, and serum biochemical parameters in mice Changes in body weight, liver weight, and serum biochemical properties of control and STAM mice fed a HFD with or without 2% D-α-Llose are summarized in Table 3. The body weights of STAM (16.6 ± 2.0 g) and DA-STAM groups (16.0 ± 1.4 g) were significantly (p < 0.05) lower than those of the control group (22.5 ± 1.3 g) at the end of the 3-week protocol. In contrast, the liver weights and liver to body weight differences in body and liver weights were observed between STAM and DA-STAM groups in this study, suggesting that administration of 2% D-α-Llose to STAM mice for 3 weeks did not affect the growth rates of the mice.

Serum AST and ALT levels in the STAM group were 2.5- to 4-fold higher, respectively, than those in the control group...
Effects of \( \alpha \)-allose on hepatic lipid accumulation in mice

Macroscopic images of liver sections were obtained from each group after the 3-week period with or without a 2% \( \alpha \)-allose diet. In the DA-STAM group, serum ALT levels were significantly reduced compared with those in the STAM group \((p < 0.05)\), suggesting that a 2% \( \alpha \)-allose-diet for 3 weeks could ameliorate the progression of hepatic inflammation. There were no significant differences in serum TG levels between mice from the control, STAM, and DA-STAM groups. Additionally, no significant difference was found in fasting GLC levels between the STAM and DA-STAM groups, although the fasting GLC levels in both groups were 7-fold higher than those in the control group.

Effects of \( \alpha \)-allose on hepatic pathology diagnosis in mice

For semiquantitative diagnostic evaluation of hepatic pathology (Kleiner et al., 2005), H&E-stained liver sections in the control, STAM, and DA-STAM groups (Fig. 2B) were scored based on the degree of steatosis, lobular inflammation, and hepatocellular ballooning and on the NAS. As shown in Fig. 3A, 3B, and 3D, the steatosis and lobular inflammation scores and NAS in the STAM group were significantly \((p < 0.05)\) higher than those in the control group, while that of the DA-STAM group was 1.4-fold lower than that of the control group, which is consistent with the results of the histological analyses of lipid accumulation in the livers from the three groups (Table 4).

Table 3. Effect of \( \alpha \)-allose on body weight, liver weight, liver to body weight ratio, serum AST, ALT, TG and GLC in mice.

<table>
<thead>
<tr>
<th>Target</th>
<th>Accession number</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ppara</td>
<td>BC016892</td>
<td>taagagatttctcattctagcatctag</td>
<td>agtggagagaggggtctg</td>
</tr>
<tr>
<td>Lxrα (Nrlh3)</td>
<td>NM_013839</td>
<td>cttctctcaggaagctcagctaaactaa</td>
<td>catgggctgggaactaaagat</td>
</tr>
<tr>
<td>Srebp-1 (Srebf1)</td>
<td>NM_011480</td>
<td>acaagatttctgagctaaacagcagcag</td>
<td>ggcgaaagacagcagatttt</td>
</tr>
<tr>
<td>Chrebp (Mlxipl)</td>
<td>NM_021455</td>
<td>atccagcagacgctagcgcag</td>
<td>gctggagagagagggtctg</td>
</tr>
<tr>
<td>Mcpl (Ccl2)</td>
<td>NM_011333</td>
<td>catccacgtttgctgcctcctcctcctcatt</td>
<td>catccacgtttgctgcctcctcctcatt</td>
</tr>
<tr>
<td>Tnf (Tnf)</td>
<td>NM_013693</td>
<td>catctctctcattgagctgcaacagcagatttatt</td>
<td>catctctctcattgagctgcaacagcagatttatt</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n = 5–8; Different superscript letters indicate significant difference \((p < 0.05)\) between groups in each parameter. AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triacylglycerol; GLC, glucose
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NASH, and non-NASH in the DA-STAM group were 0%, 12.5%, and 87.5%, respectively, which were lower than those in the STAM group (50%, 33%, and 17%, respectively).

Effects of d-allose on hepatic mRNA expression in mice

The mRNA expression levels of six target genes, i.e., the lipid metabolism-related genes \( \text{Ppar}\alpha \) (peroxisome proliferator-activated receptor alpha), \( \text{Lxr}\alpha \) (liver X receptor alpha, or \( \text{Nr}1\text{h}3 \), nuclear receptor subfamily 1, group H, member 3), \( \text{Srebp}-1 \) (sterol regulatory element binding protein-1, or \( \text{Srebf}1 \), sterol regulatory element binding transcription factor 1), and \( \text{Chrebp} \) (carbohydrate response element binding protein, or \( \text{Mlxipl} \), MLX interacting protein-like) and the inflammation-related genes \( \text{Mcp}-1 \) (monocyte chemotactic protein-1, or \( \text{Ccl}2 \), chemokine (C-C motif) ligand 2) and \( \text{Tnf}-\alpha \) (tumor necrosis factor alpha, or \( \text{Tnf} \), tumor necrosis factor) were determined in the liver of control, STAM, and DA-STAM groups by quantitative real-time PCR. The mRNA expression levels of \( \text{Srebf}-1 \) and \( \text{Mcp}-1 \) were significantly (\( p < 0.05 \)) downregulated in the DA-STAM group (0.64-fold and 0.39-fold, respectively; Fig. 4C and 4E) compared with those in the STAM group. In contrast, there were no significant differences in the expression of \( \text{Ppar} \) and \( \text{Lxr} \) between the DA-STAM and STAM groups (Fig. 4A and 4B). Finally, the mRNA levels of \( \text{Chrebp} \) (\( p = 0.052 \)) and \( \text{Tnf}-\alpha \) (\( p = 0.076 \)) showed a tendency to be downregulated (0.59-fold and 0.66-fold, respectively; Fig. 4D and 4F).

Discussion

In this study, we demonstrated that dietary d-allose has the potential to attenuate the progressive development of steatosis to NASH in STAM mice by reducing serum ALT levels, hepatic fat accumulation, and inflammation, as confirmed by histological analyses of liver sections.

We successfully generated a NAFLD/NASH model in STAM mice by treating mice with STZ and feeding them a HFD. Induction of NASH in the STAM group was confirmed by increased serum AST, ALT, and GLC levels, together with lipid accumulation and inflammation in the liver, which is consistent with features of human NASH. Although hepatic lipid accumulation was significantly increased in the STAM group compared to that in the control group, serum and hepatic TG levels did not differ between both groups. Additionally, \( \text{Ppara} \) mRNA levels were significantly decreased, whereas \( \text{Srebp}-1 \) mRNAs levels were significantly increased in the STAM group compared with those in the control group. The activities of peroxisome proliferator-activated receptor alpha (PPAR\( \alpha \)) and sterol regulatory element-binding protein 1 (SREBP-1) are associated with fatty acid oxidation and lipogenesis in the liver, respectively (Larter and Farrell, 2006). Thus, the STAM group exhibited hepatic lipid

Table 4. Effect of d-allose on hepatic lipid accumulation in mice.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STAM</th>
<th>DA-STAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>The area of Oil red O stain (%)</td>
<td>1.11 ± 0.15(^a)</td>
<td>7.78 ± 1.94(^b)</td>
<td>1.31 ± 0.44(^c)</td>
</tr>
<tr>
<td>TG (mg/g liver)</td>
<td>41.6 ± 10.0(^a)</td>
<td>25.2 ± 8.8(^ab)</td>
<td>10.6 ± 2.0(^b)</td>
</tr>
<tr>
<td>TG (mg in liver/g body weight)</td>
<td>1.62 ± 0.34</td>
<td>2.24 ± 0.90</td>
<td>0.89 ± 0.19</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 3-8; Different superscript letters indicate significant difference (\( p < 0.01 \)) between groups in each parameter.

Fig. 2. Effects of d-allose on hepatic lipid accumulation in mice. Animals were fed either AIN93G (control group), a HFD (STAM group) or a HFD containing 2% d-allose (DA-STAM group) for 3 weeks. (A) Representative image of the liver section with Oil Red O staining was obtained using a microscope with 200× magnification. (B) Representative image of liver section with H&E staining was obtained using a microscope with 200× magnification.
accumulation, as observed by histological analysis, probably owing to the repression of fatty acid oxidation and increased lipogenesis in the liver. Furthermore, the body weights of mice in the STAM group decreased compared to those in the control group. Saito et al. (2015) reported that hepatic lipid profiles showed lower TG levels in STAM mice than in HFD-fed mice. Hence, differences in hepatic TG levels are attributed to differences in body weight and hepatic lipid profile between the STAM and control groups. Alternatively, the hepatic TG detected in STAM mice may have been lower due to shedding of hepatic cells. Additionally, hypertriglyceridemia occurs in approximately 20–42% of patients with NAFLD (Hamaguchi et al., 2005), and thus serum TG levels may not necessarily increase in STAM mice.

The physiological roles of rare sugars (isomeric monosaccharides with six-carbon δ-glucose or five-carbon δ-xylose), which have low caloric content relative to more abundant sugars, have been extensively investigated in order to provide insight into their health benefits (Matsuo et al., 2002; Iga and Matsuo, 2010). Since δ-allose is rapidly excreted in the urine (91.2%) and feces (2.7%) within 24 h (Iga and Matsuo, 2010), we considered it to be a non-caloric sugar in this study. Because the body weights of mice in the STAM and DA-STAM groups were almost the same in our study, and the intake of a δ-psicose (a precursor of δ-allose)-containing diet was similar to that of a normal diet in rats (Nagata et al., 2015), the amount of food intake was assumed to be the same in both the STAM and DA-STAM groups. Furthermore, the amount of δ-allose-intake was estimated to be 1.22 – 1.53 g/kg/day based on the δ-psicose-containing diet-intake and the HFD32-intake (Nagata et al., 2015; Nishikawa et al., 2007). In the report by Iga et al., (2010) on the acute and sub-chronic toxicity study of δ-allose, no differences were found in serum biochemical parameters of rats fed the normal diet and the 3% δ-allose-containing diet, and the LD_{50} value of δ-allose was determined to be 20.5 g/kg. Thus, the 2% δ-allose-containing diet used in this study was considered to be safe.

In STAM mice from the DA-STAM group, diabetes was not alleviated, and high serum GLC levels were not reduced by δ-allose in our study, probably due to severe injury to pancreatic β-cells induced by STZ. In contrast, we found that the intake of a 2% δ-allose-containing diet for 3 weeks in STAM mice could ameliorate progressive hepatic lipid accumulation, although serum TG levels did not differ from that of STAM mice fed a HFD. In a previous report, dietary consumption of 5% δ-psicose by ob/ob mice for 15 weeks reduced the onset of simple steatosis and obesity.
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A comparison of the in vivo anti-steatosis effect of a 5% d-psicose-containing diet for 15 weeks (Itoh et al., 2015) and that of a 2% d-allose-containing diet for 3 weeks described here showed that d-allose may be more effective in preventing hepatic steatosis or NASH than d-psicose, although the experimental design and animal species used in each study differed. Dietary 3% d-psicose altered lipid metabolism via changes in Pparα but not Lxrα and Srebp-1 mRNA levels in the liver of rats (Nagata et al., 2015). Importantly, the expression of PPARα, liver X receptor alpha (LXRα), SREBP-1, and carbohydrate-responsive element-binding protein (ChREBP) is associated with lipogenesis in the liver; therefore, these proteins may be targets for the prevention and treatment of NAFLD and/or NASH (Larter and Farrell, 2006; Dentin et al., 2006; Ahmed and Byrne, 2007). We therefore evaluated the effects of d-allose on the expression of these genes in the livers of STAM mice fed a 2% d-allose-containing diet for 3 weeks. Our findings showed that Srebp-1 and Chrebp mRNA levels were reduced by d-allose, whereas no changes in Ppara and Lxra mRNA levels were observed. Additionally, hepatic TG levels in STAM mice fed a 2% d-allose-containing diet for 3 weeks decreased 0.4-fold compared to those in STAM mice fed a HFD for 3 weeks. SREBP-1 and ChREBP are major transcriptional regulators of TG synthesis in the liver (Dentin et al., 2006; Ahmed and Byrne, 2007). Thus, d-allose may reduce TG synthesis via suppression of SREBP-1 and ChREBP. Although other sugars, such as GLC and fructose, are known to induce steatosis via activation of SREBP-1 and ChREBP (Ahmed and Byrne, 2007; Samuel, 2011), d-allose repressed these targets. Thus, we conclude that structural differences in sugars may have different physiological effects on lipid metabolism in the liver.

d-Allose has been reported to alleviate hepatic I/R injury in rats through reductions in serum AST and ALT levels, hepatic MPO activity, and inflammatory cell infiltration (Hossain et al., 2003). d-Allose-induced anti-inflammatory activity has also been reported in retinal and cerebral I/R injuries in rodent models (Hirooka et al., 2006; Gao et al., 2013). In addition, Miyawaki et al. (2012) reported that d-allose attenuates cisplatin-induced toxicity, partly as a result of reduced serum and renal tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 levels. The present study also showed that d-allose could reduce hepatic Mcp-1 mRNA levels in STAM mice after consumption of a HFD for 3 weeks. It has been shown that d-allose intake can result in reduced reactive oxygen species levels in I/R injury (Hirooka et al., 2003; Mizote et al., 2011) and in the mitochondria of Neuro2A

Fig. 4. Effects of d-allose on hepatic mRNA expression in mice. Animals were fed either AIN93G (control group), a HFD (STAM group) or a HFD containing 2% d-allose (DA-STAM group) for 3 weeks. Hepatic mRNA expression of Ppara (A), Lxra (B), Srebp-1 (C), Chrebp (D), Mcp-1 (E) and Tnf-α (F) were determined by real-time PCR (normalized to GAPDH). Values are mean ± SEM, n = 5–8, * p < 0.05, ** p < 0.01
cells (Ishihara et al., 2011), and in decreased 8-hydroxy-2′-deoxyguanosine levels in the hippocampus after ischemia (Liu et al., 2014). Thus, the anti-inflammatory response to α-allose in the liver may be associated with an anti-oxidant effect in NASH. However, further experiments are needed to elucidate the mechanisms of the anti-oxidant effect of α-allose in the liver.

In conclusion, in this study, we demonstrated that α-allose attenuated lipid accumulation in the livers of STAM mice via suppression of transcription factors related to lipogenic signaling pathways, such as the SREBP-1 pathway, together with hepatic anti-inflammatory effects resulting from reduced expression levels of MCP-1. The preventive effects of α-allose on the progression of NAFLD/NASH should therefore be evaluated as a new physiological role of rare sugars without concerns associated with the risks of common sugars in NAFLD/NASH, although the mechanism needs to be further elucidated.

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