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Original Article

The influence of long-term ingestion of D-allulose in hypercholesterolemia patients under statin therapy

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ABSTRACT — D-allulose is a non-caloric natural sugar with health benefits. A few clinical trials with continuous D-allulose intake have been reported; one indicated significant increase in low-density lipoprotein cholesterol (LDL-C) levels, though the study was not a randomized controlled trial. D-allulose is predicted to be widely used in the near future by various people; therefore, the influence of D-allulose on those who have high risk for LDL-C elevation needs to be determined. Here, the effects of D-allulose on LDL-C levels in patients with hypercholesterolemia under statin therapy were investigated in a randomized controlled trial. Twenty subjects were randomly assigned to two groups: 15 g D-allulose/day or 15 g erythritol/day (placebo); each subject consumed a daily test substance for 48 weeks. Clinical examinations were performed every eight weeks, from initial consumption until week 52. No significant increase in LDL-C was observed, although significant decrease was observed in high-density lipoprotein cholesterol (HDL-C) in the D-allulose group. HDL-C values stayed within the standard ranges during the consumption period, and the mechanism was reported to be anti-atherosclerotic. In terms of risk assessment, D-allulose did not affect all risk factors that were measured for atherosclerotic cardiovascular disease. Taken together, these results suggested that long-term D-allulose consumption did not affect LDL-C values and atherosclerotic cardiovascular disease risk in patients with hypercholesterolemia under statin therapy.

Key words: Atherosclerotic cardiovascular disease risk, Cholesterol metabolism, D-allulose, Hypercholesterolemia

INTRODUCTION

"Rare sugars" are defined as monosaccharides and their derivatives by the International Society of Rare Sugars, which are present in limited quantities in nature. D-allulose, one of the rare sugars, is a C-3 epimer of fructose and a non-caloric sweetener. D-allulose is absorbed in the small intestine via glucose transporter type 5 (Kishida *et al.*, 2019), thereafter roughly 70% of ingested D-allulose is excreted into the urine (Iida *et al.*, 2010); the remaining 30% is passed into the feces. D-allulose has potential to help diabetes patients and obese people by its health benefits and its feature of producing no calories with sweetness. Many previous studies have demonstrated that D-allulose inhibits blood glucose elevation after meals (Hayashi *et al.*, 2010; Iida *et al.*, 2008) by the suppression of α-glucosidase activity in the gut (Matsuo and Izumori, 2006) and facilitation of glucokinase trans-

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location in the liver (Hossain *et al.*, 2011). Long-term D-allulose consumption improves glucose metabolism in patients with borderline diabetes (Tanaka *et al.*, 2020). D-allulose also declines fat mass accumulation and increases fat oxidation in humans (Han *et al.*, 2018; Kimura *et al.*, 2017; Yamaguchi *et al.*, 2019). Some mechanisms related to fat metabolism are proposed to suppress lipogenesis, and enhance energy expenditure (Han *et al.*, 2016; Matsuo *et al.*, 2001; Nagata *et al.*, 2015; Ochiai *et al.*, 2014). D-allulose augments energy production derived from fat through downregulation of carbohydrate oxidation, which occurs as a consequence of the suppression of α-glucosidase activity (Kimura *et al.*, 2017). Glucagon like peptide-1 (GLP-1), which regulates glucose and lipid metabolism, may be also involved in these mechanisms since D-allulose facilitates secretion of GLP-1 (Hayakawa *et al.*, 2018; Iwasaki *et al.*, 2018).

For safety evaluation, toxicity studies in animals (Matsuo *et al.*, 2002; Yagi and Matsuo, 2009) and clinical studies in normal subjects (Hayashi *et al.*, 2010) have been conducted and have confirmed that there are no abnormal effects or clinical problems with ingestion of D-allulose. D-allulose is generally recognized as safe by the Food and Drug Administration in the USA and is divided into food categories in Japan. D-allulose is now starting to be used as a sweetener in some countries, such as the USA, Korea, Mexico, and Japan. Previously, slight increase in low-density lipoprotein cholesterol (LDL-C) after continuous consumption of D-allulose for 12 weeks is observed in patients with borderline diabetes and type 2 diabetes (Tanaka *et al.*, 2019). The degree of change was similar to a seasonal fluctuation or the effects by other food ingredients reported to increase LDL-C levels, such as n-3 fatty acid and the coffee diterpene cafestol (Harris, 1996; Mori *et al.*, 2000; Urgert *et al.*, 1997). However, because this study was an open trial without a placebo group, thereafter, a randomized controlled trial was conducted for 48 weeks in patients with borderline hyper-LDL cholesterolemia to clarify the influence of D-allulose on LDL-C (Tanaka *et al.*, 2020). There were no significant differences in LDL-C levels among the groups. Additionally, D-allulose did not influence all tested atherosclerotic cardiovascular disease (ASCVD) risk factors, which were simultaneously assessed. Therefore, it was concluded that D-allulose intake was considered safe in subjects with borderline hyper-LDL cholesterolemia. In the near future, D-allulose is expected to be more widely consumed by various people, including healthy subjects and patients with disease. Thus, the effects of D-allulose need to be verified on patients with hypercholesterolemia who have high risk for increased LDL-C. In this study, the influence of long-term D-allulose consumption on LDL-C was evaluated in hypercholesterolemia patients undergoing statin therapy.

MATERIALS AND METHODS

Ethical considerations

This experiment conformed to the Helsinki Declaration (adopted in 1964 and amended in 2013) and the Ethical Guidelines for Epidemiological Research in Japan, referring to "good clinical practice". The protocol was approved by the ethical committee of Chiyoda Paramedical Care Clinic (approval date October 20, 2016; approval no. 15000088). Subjects were fully informed about the importance, purpose, and content of the experiments. Informed consent was obtained from all individual subjects included in this study. This study was registered in the Japan Medical Association Center Clinical Trials Registry (registration ID: JMA-IIA000275).

Subjects

Adult men and women aged 20-64 years were recruited by CPCC Co., Ltd. (Tokyo, Japan). Inclusion criteria were as follows: (1) subjects who were diagnosed with hypercholesterolemia and were taking statins, (2) subjects who could come to the designated institution on the scheduled test date, and (3) subjects who could undergo self-judgement and voluntarily provided written informed consent. Exclusion criteria were as follows: (1) subjects with LDL-C levels > 160 mg/dL, (2) subjects who took anti-hypercholesterolemia drugs other than statins, (3) women who were pregnant or breastfeeding, or expecting pregnancy, (4) subjects who had donated > 200 mL blood or blood components within 4 weeks prior to the beginning of the examination, (5) men who had donated > 400 mL blood within 12 weeks prior to the beginning of the examination, (6) women who had donated > 400 mL blood within 16 weeks prior to the beginning of the examination, (7) men who had donated > 1200 mL blood, at any institution, within 12 months prior to the beginning of the examination when the planned blood sampling amount of this study was added, (8) women who had donated > 800 mL blood, at any institution, within 12 months prior to beginning of the examination when the planned blood sampling amount of this study was added, (9) subjects who were participating in other clinical studies or who were within 4 weeks of finishing another study, (10) subjects who consumed excessive amounts of alcohol (> 60 g/day) or smoked heavily (> 21 cigarettes/day), (11) subjects who had extremely random dietary habits, (12) rotating shift workers, (13)

midnight workers, (14) subjects who had liver disease, kidney disease, or heart disease, including complications from other diseases, (15) subjects with a medical history of circulatory diseases, (16) patients with diabetes, (17) subjects with an allergy to the test substance, and (18) subjects who were judged to be unsuitable for the study by the principal physician. Twenty subjects were selected by the screening examination and were randomly assigned into two groups, matched by body mass index, LDL-C, and high-density lipoprotein cholesterol (HDL-C) levels. Each group had similar characteristics regarding the physical examination, blood data, urine data, age, and gender.

Materials and Schedule

The test substance used in this study was 15 g D-allulose powder (3 × 5 g of D-allulose packed in aluminum foil). The same quantity of erythritol powder was used as the placebo. Erythritol is a sugar alcohol, which has almost zero calories, and approximately 90% of consumed erythritol is excreted into the urine without being metabolized (Munro *et al.*, 1998).

A randomized, double-blind, placebo-controlled study was conducted from February 2017 to February 2018, for 52 weeks in total, and consisted of a 48-week consumption period and a 4-week observation period, after consumption (the follow-up period). Subjects consumed the test substance or placebo once with breakfast (i.e., with coffee, tea, or yoghurt) every day for the 48-week consumption period. Prior to each examination, subjects finished dinner by 21:00 on the previous day and subsequently were not allowed to eat and drink anything other than water. On examination day, fasting morning urine and blood were collected, physical measurements were taken by the clinic's medical staff, and interviews were conducted by the principal physician. These examinations were performed the day before starting consumption, the first day of consumption, and then every eight weeks (weeks 8, 16, 24, 32, 40, and 48) after starting consumption, and after the follow-up period. On examination days, the subjects took the test substance after finishing the examination.

Clinical Examination

Physical examination, blood and urine analysis, instrument-based measurements, and interviews by the principal investigator were conducted in the clinic. Physical examinations, routine blood biochemical marker analysis (proteins, lipids, saccharides, electrolytes, hepatic function, renal function, and an inflammatory marker), hematological parameter measurements, and urine analysis were performed at each examination time point. Apolipoproteins (ApoA-I, ApoA-II, ApoB48, ApoB100, ApoC-II, ApoC-III, and ApoE), free fatty acid (FFA), cholesterol synthesis markers (desmosterol and lathosterol), and cholesterol absorption markers (campesterol and β-sitosterol) in blood were measured at weeks 0, 8, and 48, and at the follow-up period. Tumor necrosis factor-α (TNF-α), glycerol, cholesterol, and triglyceride (TG) in lipoproteins (chylomicron (CM), very low density lipoprotein (VLDL), LDL, HDL) in the blood were also measured at weeks 0, 8, and 48. Total plasminogen activator inhibitor 1 (t-PAI-1), interleukin-6 (IL-6), and malondialdehyde-modified low density lipoprotein (MDA-LDL) in the blood were measured at weeks 0 and 48. ApoB48, apoB100, cholesterol, and TG in lipoproteins (CM, VLDL, LDL, HDL), and desmosterol, lathosterol, campesterol, β-sitosterol, FFA, and glycerol were measured by Skylight Biotech Inc. (Akita, Japan). Lipoprotein subfractions are defined by the average particle diameters, which have been shown in a previous report (Okazaki *et al.*, 2005). Small dense LDL (sdLDL) is defined as LDL with a particle size < 25.5 nm (Austin *et al.*, 1988). The other parameters were measured by LSI Medience Corporation (Tokyo, Japan). Additionally, ultrasonography of the carotid artery and the liver was conducted at weeks 0 and 48 using LOGIQe (General Electric Company, Boston, MA, USA). Carotid intima-media thickness (IMT) measurements were taken from both right and left common carotid arteries. Maximum IMT (Max IMT) was measured in the posterolateral far walls in the most thickened area of each vessel, except in the carotid sinus. When the subject had plaques defined as a focal thickening lesion with an IMT ≥ 1.1 mm, the thickness of the largest plaque was measured instead of the Max IMT. The fatty liver grade was evaluated by echogenicity as follows: (-): no steatosis, (±): mild steatosis, (+): moderate steatosis, and (++): severe steatosis. The absolute risk of ASCVD was calculated and subjects were divided into three groups (Low risk, Moderate risk, and High risk), according to the 2017 Japan Atherosclerosis Society (JAS) guidelines (Kinoshita *et al.*, 2018). The principal physician interviewed each subject about living habits, abdominal symptoms, defecation conditions, occurrence of subjective symptoms in the physical condition, and adverse events.

Statistical Analysis

Each measured value is expressed as the mean ± standard deviation (S.D.). Dunnett's test was used for the actual measured values to statistically compare the data in each time point with the data in week 0, except for max

Table 1. Subject characteristics.

Item	Placebo	D-allulose
Subjects (men/women)	5/4	5/4
Age (years old)	52.6 ± 12.3	53.4 ± 8.5
Height (cm)	164.7 ± 10.2	163.2 ± 9.6
Body weight (kg)	64.0 ± 10.3	62.4 ± 15.3
BMI	23.6 ± 3.1	23.2 ± 4.1
Body fat percentage (%)	27.7 ± 6.8	25.5 ± 7.8
Systolic blood pressure (mmHg)	121.7 ± 14.9	115.1 ± 13.6
Diastolic blood pressure (mmHg)	72.2 ± 16.3	71.8 ± 9.1
Pulse (beats/min)	71.9 ± 9.5	72.6 ± 10.0
Total cholesterol (mg/dL)	201.1 ± 19.7	207.1 ± 32.7
LDL-C (mg/dL)	117.2 ± 19.6	113.8 ± 27.7
HDL-C (mg/dL)	63.7 ± 17.4	68.8 ± 23.9
AST (U/L)	23.0 ± 5.3	21.7 ± 6.1
ALT (U/L)	24.4 ± 9.9	20.3 ± 8.3
ALP (U/L)	188.6 ± 60.7	224.1 ± 50.8
γ-GTP (U/L)	24.6 ± 14.0	24.0 ± 12.1
TG (mg/dL)	86.4 ± 30.5	119.7 ± 82.8
Glucose (mg/dL)	84.6 ± 8.3	87.6 ± 8.3

Each value is the mean ± S.D.
No significant differences compared with the placebo group, as determined by an unpaired *t*-test.

IMT, IL-6, MDA-LDL, and t-PAI-1. For these parameters, a paired *t*-test was used to compare the data between weeks 0 and 48. An unpaired *t*-test was used for differential values to statistically compare the D-allulose and placebo groups. To evaluate urine qualitative analysis, absolute risk of ASCVD, and fatty liver, a Wilcoxon signed-rank test was used to compare the data in each time point with the week 0 data, and a Wilcoxon two-sample test was used to compare the data in each time point with the placebo. The χ^2 test was used to compare the incidence of adverse events between the D-allulose and the placebo groups. The software used for statistical analyses was SPSS version 13.0 J (SPSS Japan Inc., Tokyo, Japan), and the level of significance was set at under 5% ($p < 0.05$) using a two-sided test.

RESULTS AND DISCUSSION

The influence of D-allulose intake on LDL-C levels was assessed for 48 weeks in patients with hypercholesterolemia undergoing statin therapy. The study flowchart and subject characteristics are shown in Fig. 1 and Table 1, respectively. One subject declined to participate because of a personal reason. Another subject deviated from the inclusion criteria by discontinuing statin use after starting this study. Therefore, two subjects were excluded, and the data for 18 subjects were analyzed. Some adverse events were observed during the study: 14 episodes in 5 subjects (the placebo group), 17 episodes in 8 subjects (the D-allulose group). No significant difference in the incidence of adverse events was found between the placebo and the D-allulose groups, and all adverse events were judged to not be related to the test substance by the principal physician.

Changes in cholesterol levels are shown in Table 2. No significant changes in LDL-C levels were observed in the D-allulose group, and the ΔLDL-C values in the D-allulose group indicated low levels compared to those in the placebo group. Although statin therapy is regarded as the gold standard to lower LDL-C, recent studies have pointed out residual risks and have recommended combination of statins with other drugs (Davidson, 2005; Robinson *et al.*, 2014). Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have currently drawn attention as a drug used with statin because high doses of statin increases both LDL receptors and PCSK9, which degrades the LDL receptors (Mabuchi and Nohara, 2015). In previous studies, D-allulose reduces blood PCSK9 levels (Kanasaki *et al.*, 2019; Tanaka *et al.*, 2020); therefore, the combination of statin and D-allulose may contribute to lowering LDL-C levels. Meanwhile, significant decreases in HDL-C were observed in the D-allulose group compared to those in both week 0 and the placebo group (Table 2). ApoA-I and ApoA-II levels also declined with the fluc-

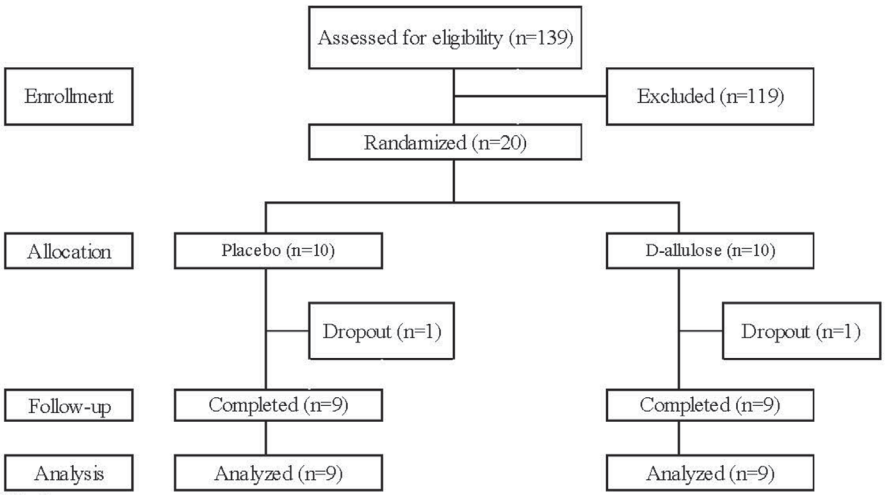

Fig. 1. Flowchart of participant screening and allocation.

Table 2. Cholesterol levels (total cholesterol, LDL-C, and HDL-C) during the D-allulose consumption period and in the follow-up period.

Treatment	Week	Total cholesterol (mg/dL)	Δ Total cholesterol (mg/dL)	LDL-C (mg/dL)	ΔLDL-C (mg/dL)	HDL-C (mg/dL)	ΔHDL-C (mg/dL)
Placebo	week 0	191.2 ± 29.4		108.3 ± 27.6		60.2 ± 16.9	
	week 8	197.0 ± 29.3	5.8 ± 8.1	114.3 ± 24.2	6.0 ± 9.2	56.7 ± 14.2	-3.6 ± 3.6
	week 16	205.8 ± 25.9*	14.6 ± 15.9	121.4 ± 23.4*	13.1 ± 16.5	57.9 ± 13.5	-2.3 ± 6.6
	week 24	195.7 ± 22.1	4.4 ± 10.0	113.2 ± 21.1	4.9 ± 9.8	58.2 ± 13.8	-2.0 ± 4.4
	week 32	200.7 ± 21.8	9.4 ± 9.1	114.6 ± 22.6	6.2 ± 9.4	58.2 ± 16.9	-2.0 ± 3.4
	week 40	192.6 ± 28.2	1.3 ± 17.5	109.0 ± 25.2	0.7 ± 11.5	59.4 ± 14.7	-0.8 ± 9.0
	week 48	203.0 ± 23.9	11.8 ± 16.5	119.7 ± 23.2*	11.3 ± 13.6	62.1 ± 12.7	1.9 ± 7.1
	Follow-up period	193.7 ± 26.8	2.4 ± 12.9	112.6 ± 25.2	4.2 ± 11.5	60.4 ± 16.5	0.2 ± 4.5
D-allulose	week 0	200.4 ± 24.4		110.9 ± 16.7		69.6 ± 23.0	
	week 8	195.0 ± 26.7	-5.4 ± 11.6#	106.9 ± 19.3	-4.0 ± 11.9	59.6 ± 19.1**	-10.0 ± 6.8#
	week 16	214.7 ± 51.9	14.2 ± 40.6	110.7 ± 36.3	-0.2 ± 26.5	58.8 ± 20.9**	-10.8 ± 7.9#
	week 24	188.4 ± 18.7	-12.0 ± 16.0#	104.1 ± 14.1	-6.8 ± 15.7	58.4 ± 17.1**	-11.1 ± 13.7
	week 32	193.9 ± 27.6	-6.6 ± 17.5#	102.4 ± 21.6	-8.4 ± 17.9#	58.3 ± 19.9**	-11.2 ± 10.0#
	week 40	209.9 ± 42.4	9.4 ± 29.3	121.4 ± 31.7	10.6 ± 30.2	60.4 ± 20.3**	-9.1 ± 10.4
	week 48	211.2 ± 32.8	10.8 ± 22.7	120.3 ± 24.0	9.4 ± 22.7	62.9 ± 20.5*	-6.7 ± 10.6
	Follow-up period	216.0 ± 39.4	15.6 ± 29.2	122.8 ± 29.5	11.9 ± 23.8	67.9 ± 22.1	-1.7 ± 9.1

Each value is the mean ± S.D.
Significant differences from the week 0 value, as determined by the Dunnett's test (*$p < 0.05$, **$p < 0.01$).
Significant differences compared to the placebo group, as determined by an unpaired *t*-test (#$p < 0.05$).

Table 3. Risk factors and markers for ASCVD during the D-allulose consumption period and in the follow-up period.

(a) sdLDL-C and TNF-α

	Treatment	sdLDL-C (mg/dL)			TNF-α (pg/mL)		
		week 0	week 8	week 48	week 0	week 8	week 48
Measured value	Placebo	26.6 ± 8.1	29.2 ± 7.9*	29.4 ± 9.4	2.49 ± 1.09	2.15 ± 0.82*	2.23 ± 0.90
	D-allulose	26.4 ± 6.0	24.8 ± 5.1	28.9 ± 4.9	1.84 ± 0.52	1.69 ± 0.36	1.92 ± 0.38
Changes from week 0	Placebo		2.59 ± 2.42	2.79 ± 4.03		-0.35 ± 0.32	-0.26 ± 0.51
	D-allulose		-1.58 ± 3.16##	2.55 ± 5.77		-0.15 ± 0.33	0.08 ± 0.38

(b) MDA-LDL, IL-6, and t-PAI-1

	Treatment	MDA-LDL (U/L)		IL-6 (pg/mL)		t-PAI-1 (ng/mL)	
		week 0	week 48	week 0	week 48	week 0	week 48
Measured value	Placebo	107.3 ± 19.6	111.4 ± 32.8	1.34 ± 1.17	0.92 ± 0.33	13.9 ± 5.2	15.7 ± 9.2
	D-allulose	113.6 ± 26.7	123.4 ± 27.7	2.00 ± 2.40	1.51 ± 1.42	18.7 ± 10.6	13.8 ± 5.4
Changes from week 0	Placebo		4.11 ± 19.9		-0.42 ± 1.15		1.78 ± 6.44
	D-allulose		9.89 ± 24.7		-0.48 ± 1.17		-4.89 ± 7.30

Each value is the mean ± S.D.
Significant differences from the week 0 value, as determined by the Dunnett's test in sdLDL-C and TNF-α (*$p < 0.05$).
No significant differences from the week 0 value, as determined by a paired *t*-test in MDA-LDL, IL-6, and t-PAI-1.
Significant differences compared to the placebo group, as determined by an unpaired *t*-test (##$p < 0.01$).

tuation of HDL-C. However, the HDL-C levels stayed within the standard range (males: 40-85 mg/dL; females: 40-95 mg/dL) during the study, and gradually increased after week 32. The declines from the initial values eventually disappeared in the follow-up period. HDL-C reductions with D-allulose intake have also been observed in previous studies (Tanaka *et al.*, 2019, 2020). Though low HDL-C levels are generally one of the risk factors for ASCVD, some reports indicate that low HDL-C does not necessarily lead to coronary heart disease risk (Rader *et al.*, 1993; Hirata *et al.*, 2016). For example, probucol, a drug used for hyperlipidemia, reduces blood LDL-C levels, while it decreases blood HDL-C levels; one of the mechanisms is thought to be promotion of hepatic scavenger receptor class B type 1 (SR-B1) activity, which is an HDL receptor (Hirano *et al.*, 2005). Because the activation of SR-B1 facilitates reverse cholesterol transport (RCT), this mechanism is regarded as anti-atherosclerotic, despite reductions in blood HDL-C levels (Kasai *et al.*, 2012). Additionally, decreases in HDL-C by D-allulose consumption is also a result of increased HDL-C intake by hepatocytes through the activation of SR-B1 (Kanasaki *et al.*, 2020). Accordingly, D-allulose accelerated RCT, which was considered to be anti-atherosclerotic. In fact, all the ASCVD risk factors and markers that were measured in this study showed no significant increase in the D-allulose group (Table 3). The ΔsdLDL-C, which is more strongly associated with ASCVD than LDL-C among Japanese populations (Kinoshita *et al.*, 2018), significantly decreases at week 8 in the D-allulose group compared to that in the placebo group. Additionally, the remnant-like particles cholesterol and high-sensitivity C-reactive protein also showed no significant changes during treatment. Max IMT, reflecting the degree of systemic arteriosclerosis, showed significant increase at week 48 in both test groups, while the Δmax IMT was significantly reduced only in the D-allulose group (Table 4). Since ASCVD is a disease associated with several complicating factors, evaluation of individual risk factors and comprehensive risk assessments have been recently emphasized, therefore, JAS recommends the evaluation of absolute risk predicting the probability of coronary artery disease within the next 10 years (Kinoshita *et al.*, 2018). The influence of long-term D-allulose consumption was assessed on absolute risk, consequently no significant changes were observed between the D-allulose and the placebo groups (Table 5). The other parameters related to lipid metabolism (ApoB48, ApoB100, ApoC-II, ApoC-III, ApoE, cholesterol synthesis and absorption markers, FFA, glycerol, cholesterol, and TG in lipoprotein subfractions) indicated no clinically significant variations.

Improvement of hepatic function by D-allulose has been reported in previous studies (Hayashi *et al.*, 2010; Itoh *et al.*, 2015; Tanaka *et al.*, 2019, 2020). In this study, all indicators associated with hepatic function (aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase [γ-GTP] and alkaline phosphatase [ALP])

Table 4. The Max IMT before and after long-term D-allulose consumption.

Treatment	Week	Right (mm)		Left (mm)	
		Max IMT	ΔMax IMT	Max IMT	ΔMax IMT
Placebo	week 0	0.40 ± 0.07		0.50 ± 0.11	
	week 48	0.64 ± 0.12**	0.24 ± 0.07	0.57 ± 0.09	0.07 ± 0.12
D-allulose	week 0	0.52 ± 0.20		0.52 ± 0.18	
	week 48	0.62 ± 0.08	0.10 ± 0.17#	0.67 ± 0.11*	0.14 ± 0.14

Each value is the mean ± S.D.
Significant differences from the week 0 value, as determined by a paired *t*-test (*$p < 0.05$, **$p < 0.01$).
Significant differences compared to the placebo group, as determined by an unpaired *t*-test (#$p < 0.05$).

Table 5. Changes in absolute risk of ASCVD with D-allulose consumption for 48 weeks.

Treatment	Risk category	week 0	week 48	Follow-up period
Placebo	Low	7	4	6
	Moderate	2	4 *	2
	High	0	1	1
D-allulose	Low	9	6	6
	Moderate	0	3	3
	High	0	0	0

Each value represents the number of people.
Significant difference from week 0 value, as determined by a Wilcoxon signed-rank test (*$p < 0.05$).
No significant differences compared to the placebo group, as determined by a Wilcoxon two-sample test.

Table 6. Changes in biliary enzymes (γ-GTP and ALP) during the D-allulose consumption period and in the follow-up period.

Treatment	Week	γ -GTP (U/L)	Δγ-GTP (U/L)	ALP (U/L)	ΔALP (U/L)
Placebo	week 0	22.8 ± 11.3		180.0 ± 55.9	
	week 8	23.1 ± 11.8	0.3 ± 1.7	188.2 ± 56.7	8.2 ± 16.6
	week 16	27.7 ± 16.4*	4.9 ± 8.6	191.2 ± 55.2	11.2 ± 13.9
	week 24	25.8 ± 15.2	3.0 ± 5.0	189.6 ± 49.9	9.6 ± 26.5
	week 32	26.8 ± 14.8	4.0 ± 4.7	185.2 ± 48.8	5.2 ± 12.6
	week 40	25.6 ± 13.3	2.8 ± 3.5	188.4 ± 50.8	8.4 ± 16.8
	week 48	25.9 ± 14.5	3.1 ± 4.9	190.3 ± 51.6	10.3 ± 12.7
	Follow-up period	25.1 ± 11.9	2.3 ± 3.8	182.1 ± 48.3	2.1 ± 19.9
D-allulose	week 0	23.0 ± 11.0		214.8 ± 50.2	
	week 8	17.4 ± 8.2**	-5.6 ± 4.6##	180.7 ± 45.2**	-34.1 ± 13.8##
	week 16	20.4 ± 12.6	-2.6 ± 8.0	188.6 ± 48.1**	-26.2 ± 36.5#
	week 24	17.9 ± 8.3*	-5.1 ± 6.3##	182.8 ± 43.8**	-32.0 ± 18.7##
	week 32	17.6 ± 9.4**	-5.4 ± 6.6##	181.9 ± 41.4**	-32.9 ± 26.3##
	week 40	17.7 ± 7.5**	-5.3 ± 5.9##	188.9 ± 37.1**	-25.9 ± 22.3##
	week 48	17.6 ± 8.1**	-5.4 ± 6.2##	192.4 ± 40.1*	-22.3 ± 15.6##
	Follow-up period	22.0 ± 8.9	-1.0 ± 7.1	210.0 ± 34.9	-4.8 ± 29.9

Each value is the mean ± S.D.
Significant differences from the week 0 value, as determined by the Dunnett's test (*$p < 0.05$, **$p < 0.01$).
Significant differences compared to the placebo group, as determined by an unpaired *t*-test (#$p < 0.05$, ##$p < 0.01$).

showed within the standard ranges, and significant decreases in γ-GTP and ALP were observed in the D-allulose group (Table 6). γ-GTP and ALP are generally recognized as biliary enzymes, and Kanasaki *et al.* (2021) have described that D-allulose modulates bile acid metabolism in the hepatic metabolomics profile of the rat. It is meaningful to conduct further researches to examine the improvement in biliary system under D-allulose administration. In addition, the systolic blood pressure level at week 24 significantly declined in the D-allulose

group compared to that in the placebo group (placebo: 120.9 ± 11.5 mmHg, D-allulose: 107.0 ± 15.2 mmHg, $p < 0.05$). Hypertension is an important risk factor for cardiovascular diseases (CVD), especially as an elevation of blood pressure levels that exceed the optimal level (< 120/80 mmHg) raises the CVD risk (Kinoshita *et al.*, 2018). Further studies in subjects with hypertension are required to reveal these effects of D-allulose because the blood pressure levels of the subjects in this study were within the optimal range. Although a few values of other parameters (anthropometric indicators, general blood biochemical markers [proteins, electrolytes, glucose metabolism, and renal function], hematological parameters, urinalysis parameters, and ultrasonography in the liver) deviated from the reference values, these deviations were not clinically significant.

In conclusion, this study assessed the long-term effect of D-allulose intake for 48 weeks on LDL-C levels in patients with hypercholesterolemia undergoing statin therapy. LDL-C levels did not significantly increase with long-term D-allulose intake compared to those in the placebo group, although decrease in HDL-C within standard ranges was observed. The mechanism of low HDL-C by D-allulose consumption was anti-atherosclerotic, and the ASCVD risks that were evaluated using various risk factors demonstrated no clinically significant changes. D-allulose is a non-caloric sweetener with health utility, and these findings encourage the wide use of D-allulose in the near future.

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