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Original Article

Safety and efficacy of a 48-week long-term ingestion of D-allulose in subjects with high LDL cholesterol levels

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ABSTRACT — D-allulose is one of the rare sugars with almost zero calories and several health benefits. Previous studies have reported the safety of D-allulose in normal, overweight/obese, and diabetic humans. However, one study reported significant increases in T-Cho and LDL-C after 12 weeks of D-allulose intake; this report was not a randomized controlled trial and these changes were considered to be due to seasonal variations. We, therefore, conducted a randomized, double-blind, placebo-controlled trial in 90 subjects with high LDL-C levels for 48 weeks to clarify the influence of long-term D-allulose consumption on cholesterol metabolism and efficacy. Subjects were randomly divided into 3 groups: high-dose D-allulose (15 g D-allulose/day), low-dose D-allulose (5 g D-allulose/day), and placebo group (0 g D-allulose/day); each subject consumed a daily test beverage for 48 weeks. Clinical examinations were performed every eight weeks, beginning from initial consumption until week 52. No significant increases in T-Cho and LDL-C between test groups were observed, and 48 weeks of D-allulose consumption did not change risk factors for atherosclerotic cardiovascular disease. Furthermore, no clinical problems were recognized for other parameters. Additionally, significant improvements in hepatic enzyme activities, fatty liver score, and glucose metabolism after long-term D-allulose consumption were observed. The results from our study revealed that 1) D-allulose consumption is considered safe for long-term intake up to a year, and 2) D-allulose may be effective for improving hepatic functions and glucose metabolism.

Key words: ASCVD risk, Cholesterol metabolism, D-allulose, Glucose metabolism, Hepatic function

INTRODUCTION

D-allulose is a C-3 epimer of fructose and a rare sugar, which is a general term for less abundant monosaccharides. Approximately 70% of ingested D-allulose is absorbed through glucose transporter type 5 in the small intestine (Kishida *et al.*, 2019) and is excreted into the urine without providing any energy (Iida *et al.*, 2010). The remaining 30% is passed into the feces, and only provides a trace amount of energy. Hence, D-allulose has the strong potential for use as a zero-calorie sweetener. D-allulose has some beneficial health functions, such as suppression of postprandial blood glucose augmentation (Hayashi *et al.*, 2010; Hossain *et al.*, 2011; Iida *et al.*, 2008) and reduction in fat mass accumulation (Han *et al.*, 2016, 2018; Ochiai *et al.*, 2014). The former mechanisms were elucidated by the inhibition of α-glucosidase activity in the small intestine (Matsuo and Izumori, 2006) and enhancement of glucokinase translocation in liver cells (Hossain *et al.*, 2011). Additionally, D-allulose was recently found to be a glucagon-like peptide-1 (GLP-1) releaser that acts via vagal afferents and

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thereby restricts hyperglycemia and overfeeding (Iwasaki *et al.*, 2018). The latter mechanisms have been reported to suppress hepatic enzymes of fatty acid synthesis and enhance hepatic enzymes of fatty acid oxidation in rodents (Nagata *et al.*, 2015; Ochiai *et al.*, 2014) and fat oxidation in humans (Kimura *et al.*, 2017; Yamaguchi *et al.*, 2019). As for the safety of D-allulose, an 18 month toxicity study using rats was conducted (Yagi and Matsuo, 2009), and neither any abnormal effects nor clinical problems were reported for 12 weeks of continuous ingestion in normal humans (Hayashi *et al.*, 2010) or overweight/obese humans (Han *et al.*, 2018). D-Allulose is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) in the USA and as a food category in Japan. However, an open clinical trial of D-allulose consumption for 12 weeks without the placebo group in borderline diabetics and type 2 diabetics demonstrated significant increases in total cholesterol (T-Cho) and low-density lipoprotein cholesterol (LDL-C) compared with the first day of consumption (Tanaka *et al.*, 2019). Elevations of T-Cho and LDL-C by food ingredients have also been reported in some clinical trials; for example, n-3 fatty acid (Harris, 1996; Mori *et al.*, 2000), coffee diterpenes cafestol (Urgert *et al.*, 1997), boiled coffee (Aro *et al.*, 1987), and carbohydrate-restricted diets (Foster *et al.*, 2010). Although the exact mechanisms were not indicated in these reports, a suggested conclusion was that these variations in serum cholesterol were not clinical problems because they were short-term. Changes in cholesterol levels by the consumption of D-allulose were considered to be caused as a result of a seasonal variation and not judged to be serious problems in the previous report.

Cholesterol lowering therapy, especially LDL-C, by drug-like statin is thought to be the best way to reduce atherosclerotic cardiovascular disease (ASCVD) risks. However, despite aggressive LDL-C reduction by statin therapy, some clinical studies have revealed significant residual cardiovascular risks, which has drawn attention recently (Davidson, 2005; Sampson *et al.*, 2012). Therefore, not only the quantity but also the quality of lipids and other factors such as inflammation are considered to play important roles in ASCVD. In ApoE-deficient mice fed food items such as dietary blueberries (Wu *et al.*, 2010), dried plums (prunes) (Gallaher and Gallaher, 2009), and folic acid (Carnicer *et al.*, 2007), the improvements in atherosclerosis lesions were actually observed regardless of the increase in T-Cho or LDL-C. At present, many risk factors for ASCVD are suggested; in particular, small dense LDL (sdLDL: mean diameter < 25.5 nm) is more strongly associated with the risks for coronary artery disease (CAD) (Hirano *et al.*, 2004) and the incidence of ASCVD in secondary prevention (Nishikura *et al.*, 2014) than LDL-C among Japanese populations. sdLDL-C could more easily penetrate the arterial wall and be oxidized (Packard *et al.*, 2000). Decreases in high-density lipoprotein cholesterol (HDL-C) are also known to be one of the risk factors of ASCVD because HDL has the atheroprotective effect called reverse cholesterol transport (RCT) (Relchl and Miller, 1989; Tall, 1990). Probucol, which is a drug for dyslipidemia with a high LDL-C level, is known to decrease HDL-C; one of the drug mechanisms is reported to be the activation of hepatic scavenger receptor class B type 1 (SR-B1), an HDL receptor (Hirano *et al.*, 2005). Although patients with hypercholesterolemia treated by probucol for 24 months actually responded with a reduction in HDL-C levels, no changes in carotid intima-media thickness (IMT) were recorded (Baldassarre *et al.*, 1997). This mechanism, which activates hepatic SR-B1, is accordingly considered not to be atherogenic due to promoting RCT in spite of decreases in HDL-C levels (Kasai *et al.*, 2012). Some previous studies reported reductions in HDL-C levels within the standard ranges by D-allulose intake (Hayashi *et al.*, 2010; Tanaka *et al.*, 2019), and the mechanism is estimated to enhance HDL-C uptake into the liver through HDL receptors because D-allulose was reported to suppress the decrease in the expression of CLA-1 (cluster of differentiation 36 and Lysosomal integral membrane protein-2 analogous-1: a human homologue of SR-B1) in Hep G2 cells under high glucose level conditions (Iida and Okuma, 2012). In terms of the mechanism, the decrease in HDL-C levels by D-allulose intake was not considered a serious clinical problem. According to the 2017 Japan Atherosclerosis Society (JAS) guideline, oxidized LDL, remnant lipoprotein, lipoprotein(a), postprandial hyperlipidemia, inflammatory markers, blood coagulation, and fibrinolytic factors are also suggested as risk factors for ASCVD (Kinoshita *et al.*, 2018). IMT reflects the degree of systemic arteriosclerosis and is used as an alternative assessment factor for predicting the onset of ASCVD and the risks of the complications. IMT is recommended as a standard method for assessing the degree of arteriosclerosis by the Japan Society of Ultrasonics in Medicine and the Japan Academy of Neurosonology. JAS furthermore recommends using absolute risk of ASCVD for a comprehensive risk assessment. This risk is calculated by eight items (age, gender, smoking, blood pressure, HDL-C, LDL-C, impaired glucose tolerance, and family history of premature CAD), and can predict probability of CAD within the next 10 years (Kinoshita *et al.*, 2018). Thus, comprehensive risk evaluation is regarded as an important point for risk assessment of ASCVD.

In this study, we performed a randomized, double-blinded placebo-controlled trial to mainly clarify the influence of D-allulose ingestion on cholesterol metabolism in subjects with high LDL-C levels. Moreover, by assessing the effects of D-allulose for a longer period (48 weeks) than that of the previous trial, we elucidated the influenced duration and the risks of ASCVD by measuring various risk factors. Additionally, the effects on hepatic and glucose metabolism by longer-term D-allulose intake were also evaluated.

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; the experiments were conducted under the guidance of the principal investigator. The protocol was approved by the ethical committee of Chiyoda Paramedical Care Clinic (approval date October 20, 2016; approval no. 15000088). Subjects were given full information about the importance, purpose, and contents 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-IIA000274).

Subjects

Adult men and women aged 20-65 years were recruited by CPCC Co., Ltd. (Tokyo, Japan). Inclusion criteria were as follows: (1) LDL-C concentration of 120-159 mg/dL (high LDL-C levels), and a fasting blood glucose concentration of 100-125 mg/dL or Hemoglobin A1c (HbA1c, which was measured using NGSP) of 6.0-6.5%; (2) a homeostasis model assessment as an index of insulin resistance (HOMA-IR) of beyond 2.5 with high LDL-C levels; and (3) high LDL-C levels. In subgroup analysis with borderline diabetes based on HbA1c and oral glucose tolerance test (OGTT), the subjects that satisfied criteria (1) and (2) were used. Exclusion criteria were as follows: (1) undergoing medical treatment by drugs; (2) daily intake of dietary supplements related to blood glucose or lipids; (3) pregnancy, breastfeeding, or expected pregnancy; (4) subjects who had blood collected (> 200 mL; total blood or directed donation) within 4 weeks prior to starting the examination; (5) men who had blood collected (> 400 mL) within 12 weeks prior to starting the examination; (6) women who had blood collected (> 400 mL) within 16 weeks prior to starting the examination; (7) men who had more than 1200 mL of blood collected within 12 months prior to starting the examination when the planned blood sampling amount of this study was added; (8) women who had more than 800 mL of blood collected within 12 months prior to starting the examination when planned blood sampling amount of this study was added; (9) concurrent participation in other clinical studies or within four weeks of finishing another study; (10) heavy alcohol use of more than 60 g/day or heavy smoker beyond 21 cigarettes/day; (11) individuals with extremely random dietary habits, rotating shift workers, midnight workers; (12) serious damage to the liver, the kidney, the heart; (13) subjects with a history of circulatory diseases; (14) diabetic patients; (15) allergy to the test substance; and (16) anyone judged as ineligible to participate in this study by the principal physician. Ninety subjects were selected by the screening examination and randomly assigned to three groups, as matched by LDL-C levels and visceral fat area. Each group had similar characteristics regarding the physical examination, blood data, urine data, age, and gender.

Materials

Three test beverages were used in this study: a high-dose D-allulose beverage containing 15 g D-allulose, a low-dose D-allulose beverage containing 5 g D-allulose, and the placebo beverage which contained no D-allulose. The sweetness of each beverage was equivalently adjusted by sucralose. These test beverages did not differ in flavor or color.

Methods and Schedule

A randomized, double-blinded, placebo-controlled study was conducted from November 2016 to January 2018 for 52 weeks in total, which consisted of a 48-week consumption period and a 4-week observation period after consumption (follow-up period). Subjects consumed a test beverage 30 min prior to every breakfast for 48 weeks continuously during the consumption period. Prior to each examination, subjects completely 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, and physical measurements were taken by the clinic's medical staff. Interviews were conducted by the principal physician. These examinations were performed 4 weeks before starting consumption, the first day of consumption, and then every eight weeks (week 8, 16, 24, 32, 40, and 48) after starting consumption, and after the follow-up period. A 75 g OGTT was conducted only on the first day of the consumption and 48 weeks after starting consumption. On examina-

tion days, the subjects took the test beverage after finishing the examination.

Clinical Examination

Physical examination, blood and urine analysis, instrument-based measurements, and interviews by the principal investigator were conducted in clinic. Physical examinations, routine blood biochemical marker analysis (protein, lipid, saccharide, electrolyte, hepatic function, and renal function), hematological parameter measurements, and urine analysis were performed at each examination timepoint. Proprotein convertase subtilisin/kexin type 9 (PCSK9), apolipoproteins (ApoA-I, ApoA-II, ApoB48, ApoB100, ApoC-II, ApoC-III, ApoE), free fatty acid (FFA), cholesterol synthesis markers (desmosterol, lathosterol) and, cholesterol absorption markers (campesterol, β-sitosterol) in blood were measured at week 0, 8, 48, and at the follow-up period. Tumor necrosis factor-α (TNF-α), glycerol, cholesterol, and triglyceride (TG) in lipoproteins (HDL, LDL, very low density lipoprotein (VLDL), chylomicron (CM)) in the blood were also measured at week 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 week 0 and 48. ApoB48, apoB100, cholesterol, and TG in lipoproteins (HDL, LDL, VLDL, CM), and desmosterol, lathosterol, campesterol, β-sitosterol, and glycerol were measured by Skylight Biotech Inc, (Akita, Japan). Lipoprotein subfractions were defined by the average particle diameters as follows: CM > 80 nm, 64 > VLDL > 31.3, large LDL=28.6, medium LDL=25.5, small LDL=23.0, 20.7 > very small LDL > 16.7, 15.0 > very large HDL > 13.5, large HDL=12.1, medium HDL=10.9, small HDL= 9.8, and 8.8 > very small HDL > 7.6. Data for subfractions in LDL and HDL were integrated into 2 groups, respectively: large + medium LDL, small + very small LDL (sdLDL), very large + large + medium HDL, and small + very small HDL. The other parameters were measured by LSI Medience Corporation (Tokyo, Japan). Additionally, ultrasonography (the carotid artery and the liver) was conducted at week 0 and 48 using LOGIQe (General Electric Company, Boston, MA, USA). IMT measurements were taken from both right and left common carotid arteries. Maximum IMT (Max IMT) was measured in 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 of ≥ 1.1 mm, the thickness of the largest plaque was measured instead of the Max IMT. The grade of fatty liver was evaluated by the echogenicity as follows: (-): no steatosis, (±): mild steatosis, (+): moderate steatosis, (++): severe steatosis. Abdominal fat areas were measured by computed tomography (Aquilion One Vision First Edition; Canon Medical Systems Corporation, Tochigi, Japan) on week 0 and 48. The absolute risk of ASCVD was calculated and divided into 3 groups (Low risk, Moderate risk, and High risk) according to the 2017 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 conditions, and adverse events. For the OGTT, blood samples were collected at 0, 30, 60, 90, 120 min, and plasma glucose and insulin concentration were measured by LSI Medience Corporation (Tokyo, Japan).

Statistical Analysis

Each measured value is expressed as the mean ± standard deviation (S.D.). For statistical comparisons in each group, the actual measured values in each measured point were compared with the actual measured values in week 0. For statistical comparisons between the placebo and the D-allulose groups, differential values in each measured point were used. To compare the data between week 0 and 48 of max IMT, MDA-LDL, IL-6, t-PAI-1, visceral fat area, and area under the curve (AUC) in OGTT, a paired t-test was used. To evaluate the urine qualitative analysis, absolute risk of ASCVD, and fatty liver, the Wilcoxon signed-rank test was used to compare the data in each measured point with the week 0 data, and the Wilcoxon two-sample test was used to compare between the placebo and the D-allulose groups. The χ^2 test was used to compare the incidence of adverse events between the test groups. Dunnett's test was used for the other examinations to compare the data in each measured point with the data in week 0, and the data between the placebo and the D-allulose groups. The software used for the statistical analysis was SPSS version 13.0 J (SPSS Japan Inc., Tokyo, Japan), and the level of significance was set at under 5% ($p < 0.05$) by a two-sided test.

RESULTS

The study flowchart and subject characteristics are shown in Fig. 1 and Table 1, respectively. Subject characteristics (blood glucose, HbA1c, and HOMA-IR) used in sub-group analysis on individuals with borderline diabetes based on HbA1c and OGTT were as below: the placebo group, 101.2 ± 9.2 mg/dL, 6.03 ± 0.39%, and 2.31 ± 3.26; the low-dose D-allulose group, 97.3 ± 12.6 mg/dL, 6.02 ± 0.30%, and 1.22 ± 0.65; the high-dose D-allulose group, 98.4 ± 8.7 mg/dL, 6.00 ± 0.41%, and

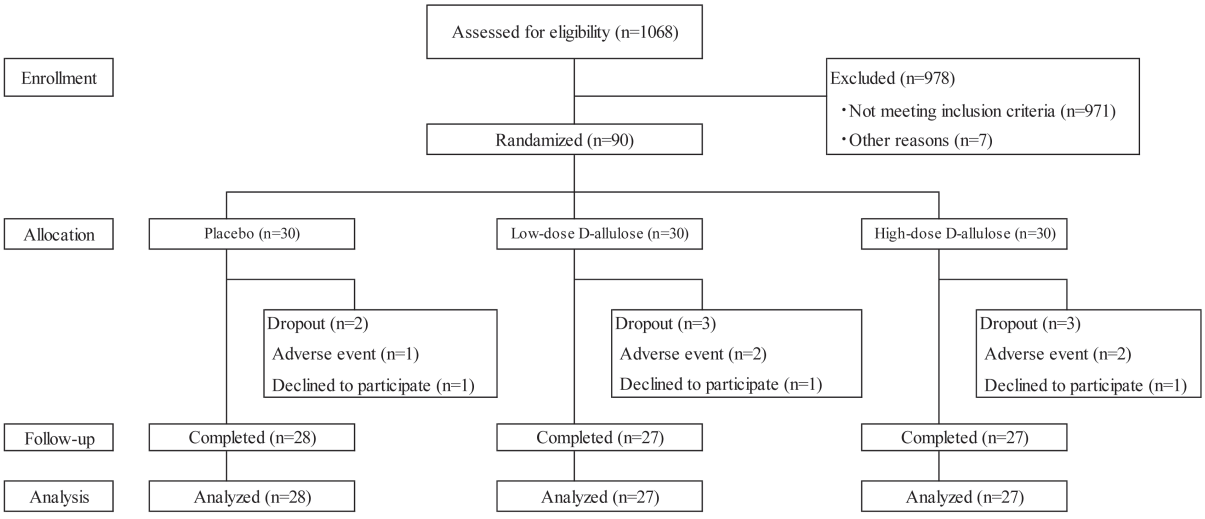

Fig. 1. The flow chart of participants screening and allocation.

Table 1. Subject characteristics.

	Placebo	Low-dose D-allulose	High-dose D-allulose
Subjects (men/women)	14/14	14/13	13/14
Age (years old)	50.5 ± 7.6	50.4 ± 7.2	51.4 ± 6.6
Height (cm)	164.6 ± 8.8	164.6 ± 7.1	163.7 ± 8.2
Body weight (kg)	64.7 ± 11.9	65.9 ± 11.7	63.2 ± 15.9
BMI	23.8 ± 3.7	24.2 ± 3.7	23.3 ± 4.4
Body fat percentage (%)	28.0 ± 7.6	29.0 ± 7.7	27.9 ± 7.9
Waist circumference (cm)	86.9 ± 9.1	86.9 ± 10.0	85.8 ± 11.9
Systolic blood pressure (mmHg)	123.2 ± 12.8	123.6 ± 10.5	120.6 ± 12.6
Diastolic blood pressure (mmHg)	78.7 ± 10.0	79.5 ± 10.6	78.6 ± 10.2
Pulse (beats/min)	70.2 ± 11.6	71.3 ± 8.4	73.2 ± 8.5
LDL-C (mg/dL)	139.3 ± 10.9	138.7 ± 11.0	138.5 ± 11.6
Visceral fat area (cm^2)	75.1 ± 43.9	72.2 ± 45.4	74.4 ± 58.9

1.33 ± 0.74, respectively. A total of eight subjects discontinued their participation. Three subjects declined to participate because of personal reasons. The other reasons why subjects discontinued participation were as follows: osteoporosis (the placebo group), anaphylactic shock (the low-dose D-allulose group), diabetes mellitus (the low-dose D-allulose group), atrophic gastritis (the high-dose D-allulose group), and rheumatoid arthritis (the high-dose D-allulose group). All events were unrelated to D-allulose consumption according to judgements by the principal physician. Therefore, eight subjects were excluded, and the data for 82 subjects aged between 32 and 64 years were analyzed.

Changes in cholesterol levels are shown in Table 2. Significant decreases in ΔT-Cho and ΔLDL-C levels were found between the placebo and the high-dose D-allulose groups only in the follow-up period. Significant increases of T-Cho and LDL-C levels were observed at week 8 in the D-allulose groups compared with week 0, although the increases were only transient. The T-Cho level at week 24 was lower than that at week 0 in the high-dose D-allulose group. The values for HDL-C in the D-allulose groups significantly decreased during the D-allulose consumption period. However, the levels stayed within the standard ranges, and then the decreases disappeared in the follow-up period. Results of the cholesterol and TG subfractions are shown in Table 3. There were no significant differences within and between groups in the CM-C and CM-TG

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

		T-Cho (mg/dL)	ΔT-Cho (mg/dL)	LDL-C (mg/dL)	ΔLDL-C (mg/dL)	HDL-C (mg/dL)	ΔHDL-C (mg/dL)
Placebo	week 0	218.4 ± 21.0	0.0 ± 0.0	137.3 ± 16.3	0.0 ± 0.0	63.1 ± 14.7	0.0 ± 0.0
	week 8	225.4 ± 22.5	7.0 ± 21.3	137.4 ± 17.9	0.1 ± 19.2	63.0 ± 15.8	-0.1 ± 5.9
	week 16	219.0 ± 23.5	0.6 ± 17.2	132.8 ± 18.4	-4.5 ± 14.6	60.6 ± 15.5	-2.5 ± 5.2
	week 24	215.6 ± 24.0	-2.9 ± 20.8	132.1 ± 21.9	-5.1 ± 21.4	60.6 ± 16.4	-2.5 ± 6.4
	week 32	218.1 ± 23.7	-0.3 ± 21.2	134.4 ± 23.7	-2.9 ± 20.8	60.3 ± 13.6	-2.8 ± 6.7
	week 40	224.2 ± 32.2	5.8 ± 22.2	139.5 ± 25.5	2.3 ± 20.7	62.1 ± 15.6	-1.0 ± 5.3
	week 48	220.7 ± 22.9	2.3 ± 17.1	138.1 ± 18.6	0.9 ± 14.8	62.0 ± 15.0	-1.1 ± 4.9
	Follow-up period	224.4 ± 29.4	6.0 ± 19.4	142.7 ± 21.1	5.4 ± 18.1	63.8 ± 16.7	0.6 ± 5.4
Low-dose D-allulose	week 0	231.1 ± 37.1	0.0 ± 0.0	146.4 ± 30.4	0.0 ± 0.0	64.3 ± 17.3	0.0 ± 0.0
	week 8	244.8 ± 26.2**	13.7 ± 29.0	156.0 ± 23.4	9.6 ± 23.9	62.0 ± 15.6	-2.2 ± 7.7
	week 16	234.6 ± 29.0	3.5 ± 23.4	148.4 ± 21.9	2.0 ± 25.7	57.2 ± 14.5**	-7.1 ± 6.5#
	week 24	231.4 ± 29.4	0.3 ± 26.8	144.2 ± 24.0	-2.2 ± 29.4	58.1 ± 14.9**	-6.2 ± 7.2
	week 32	232.9 ± 29.0	1.8 ± 21.8	147.0 ± 18.7	0.6 ± 30.8	57.7 ± 13.8**	-6.6 ± 7.9
	week 40	238.8 ± 30.2	7.7 ± 23.2	153.5 ± 20.8	7.1 ± 25.2	59.4 ± 14.6**	-4.8 ± 7.9
	week 48	227.1 ± 26.0	-3.9 ± 22.7	147.1 ± 20.9	0.7 ± 24.1	59.0 ± 14.3**	-5.3 ± 8.1
	Follow-up period	232.8 ± 25.9	1.7 ± 24.9	151.2 ± 24.8	4.9 ± 15.7	64.9 ± 16.9	0.7 ± 9.0
High-dose D-allulose	week 0	232.8 ± 19.1	0.0 ± 0.0	142.9 ± 16.1	0.0 ± 0.0	68.3 ± 19.3	0.0 ± 0.0
	week 8	242.6 ± 26.6	9.8 ± 19.9	153.7 ± 21.9*	10.9 ± 16.9	61.7 ± 19.3**	-6.5 ± 9.7##
	week 16	235.1 ± 23.1	2.3 ± 21.2	149.0 ± 16.5	6.1 ± 17.5	58.3 ± 16.5**	-10.0 ± 8.5##
	week 24	220.9 ± 23.3*	-11.9 ± 20.1	137.6 ± 18.2	-5.3 ± 17.0	56.1 ± 15.1**	-12.1 ± 9.0##
	week 32	226.1 ± 23.8	-6.6 ± 20.3	144.0 ± 17.9	1.1 ± 16.1	56.1 ± 14.2**	-12.1 ± 10.8##
	week 40	235.3 ± 26.3	2.5 ± 22.4	146.6 ± 20.0	3.7 ± 19.5	59.3 ± 16.0**	-9.0 ± 9.7##
	week 48	224.7 ± 24.8	-8.0 ± 20.4	143.0 ± 18.3	0.1 ± 18.1	61.0 ± 16.2**	-7.2 ± 9.4##
	Follow-up period	224.7 ± 24.2	-8.0 ± 19.4#	135.8 ± 20.4	-7.1 ± 17.7#	66.4 ± 18.0	-1.9 ± 10.2

Significant differences from the week 0 value, as determined by the Dunnett's test (*$p < 0.05$, **$p < 0.01$).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (#$p < 0.05$, ##$p < 0.01$).

Table 3. Cholesterol and triglyceride subfractions in lipoproteins during D-allulose consumption period.

(a) Cholesterol subfractions

	Placebo			Low-dose D-allulose			High-dose D-allulose		
(mg/dL)	week 0	week 8	week 48	week 0	week 8	week 48	week 0	week 8	week 48
CM	0.35 ± 0.32	0.66 ± 1.07	1.30 ± 4.99	0.50 ± 0.65	0.55 ± 0.72	0.81 ± 2.04	0.69 ± 1.08	0.77 ± 1.25	0.59 ± 0.84
ΔCM	0.00 ± 0.00	0.31 ± 0.94	0.95 ± 4.86	0.00 ± 0.00	0.06 ± 0.85	0.32 ± 1.60	0.00 ± 0.00	0.09 ± 0.46	-0.09 ± 0.60
VLDL	25.7 ± 7.1	35.3 ± 8.0**	32.0 ± 8.2**	30.8 ± 17.0	41.3 ± 14.7**	36.2 ± 16.7**	31.2 ± 13.3	41.0 ± 12.8**	35.3 ± 12.9*
ΔVLDL	0.0 ± 0.0	9.6 ± 7.2	6.3 ± 7.6	0.0 ± 0.0	10.5 ± 11.1	5.4 ± 5.6	0.0 ± 0.0	9.8 ± 7.6	4.0 ± 10.4
LDL									
Large + medium	97.3 ± 11.1	89.0 ± 12.1**	93.7 ± 12.7	100.6 ± 14.6	100.8 ± 13.6	101.6 ± 13.6	96.8 ± 11.8	98.8 ± 15.2	98.4 ± 13.1
ΔLarge + medium	0.0 ± 0.0	-8.3 ± 13.8	-3.6 ± 10.6	0.0 ± 0.0	0.2 ± 13.3#	1.0 ± 14.1	0.0 ± 0.0	2.0 ± 10.0#	1.6 ± 12.0
Small + very small (sdLDL)	26.4 ± 5.5	33.2 ± 6.0**	27.2 ± 7.3	28.7 ± 10.8	35.9 ± 8.4**	27.7 ± 6.7	29.1 ± 9.2	36.0 ± 8.4**	27.8 ± 7.7
ΔSmall + very small (ΔsdLDL)	0.0 ± 0.0	6.8 ± 4.3	0.9 ± 5.3	0.0 ± 0.0	7.2 ± 6.6	-1.0 ± 6.4	0.0 ± 0.0	7.0 ± 6.0	-1.3 ± 4.8
HDL									
Very large + large + medium	37.1 ± 12.8	37.7 ± 13.2	37.4 ± 12.8	37.1 ± 14.8	36.1 ± 13.4	33.6 ± 12.2**	40.9 ± 18.8	36.3 ± 16.3**	35.9 ± 15.3**
ΔVery large + large + medium	0.0 ± 0.0	0.6 ± 3.7	0.3 ± 4.2	0.0 ± 0.0	-1.0 ± 6.4	-3.5 ± 6.7	0.0 ± 0.0	-4.7 ± 7.0##	-5.1 ± 7.8#
Small + very small	21.2 ± 2.8	22.2 ± 2.6	20.4 ± 3.1	22.4 ± 3.4	22.5 ± 3.2	21.1 ± 3.5**	22.0 ± 3.1	21.8 ± 3.2	20.6 ± 3.0*
ΔSmall + very small	0.0 ± 0.0	1.0 ± 2.3	-0.9 ± 2.1	0.0 ± 0.0	0.2 ± 1.9	-1.2 ± 1.9	0.0 ± 0.0	-0.3 ± 3.2	-1.4 ± 2.5

Significant differences from the week 0 value, as determined by the Dunnett's test (*p < 0.05, **p < 0.01).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (#p < 0.05, ##p < 0.01).

(b) Triglyceride subfractions

	Placebo			Low-dose D-allulose			High-dose D-allulose		
(mg/dL)	week 0	week 8	week 48	week 0	week 8	week 48	week 0	week 8	week 48
CM	2.64 ± 2.36	4.89 ± 8.53	7.97 ± 27.89	3.54 ± 3.89	3.83 ± 4.13	4.51 ± 9.94	5.98 ± 9.72	6.14 ± 10.18	4.60 ± 6.87
ΔCM	0.00 ± 0.00	2.26 ± 7.51	5.33 ± 26.74	0.00 ± 0.00	0.29 ± 4.75	0.97 ± 7.24	0.00 ± 0.00	0.16 ± 2.76	-1.38 ± 4.79
VLDL	56.8 ± 26.9	74.0 ± 35.2*	66.2 ± 46.0	70.0 ± 50.2	72.0 ± 42.5	71.1 ± 55.1	82.3 ± 60.9	79.1 ± 66.4	76.6 ± 52.9
ΔVLDL	0.0 ± 0.0	17.2 ± 33.4	9.4 ± 42.2	0.0 ± 0.0	2.0 ± 33.9	1.0 ± 26.1	0.0 ± 0.0	-3.1 ± 27.9#	-5.7 ± 37.5
LDL									
Large + medium	16.8 ± 2.6	16.8 ± 4.0	17.3 ± 3.6	17.8 ± 4.6	18.7 ± 6.9	18.3 ± 6.0	19.5 ± 5.8	20.5 ± 5.7	20.4 ± 5.0*
ΔLarge + medium	0.00 ± 0.00	-0.04 ± 3.33	0.49 ± 3.41	0.00 ± 0.00	0.95 ± 3.43	0.56 ± 2.71	0.00 ± 0.00	0.94 ± 2.41	0.89 ± 3.32
Small + very small (sdLDL)	4.12 ± 0.93	4.94 ± 1.45	5.07 ± 2.57*	4.52 ± 1.85	5.39 ± 2.40	5.12 ± 2.68*	5.26 ± 1.80	6.01 ± 2.02	5.73 ± 1.65*
ΔSmall + very small (ΔsdLDL)	0.00 ± 0.00	0.81 ± 0.98	0.94 ± 2.32	0.00 ± 0.00	0.87 ± 1.25	0.60 ± 1.20	0.00 ± 0.00	0.75 ± 0.85	0.48 ± 1.29
HDL									
Very large + large + medium	6.99 ± 2.69	8.03 ± 2.75	8.39 ± 4.00	7.05 ± 2.81	6.88 ± 2.80	6.83 ± 3.62	9.23 ± 5.10	7.68 ± 3.69	8.04 ± 3.39
ΔVery large + large + medium	0.00 ± 0.00	1.04 ± 2.55	1.40 ± 3.62	0.00 ± 0.00	-0.16 ± 2.46	-0.22 ± 2.63	0.00 ± 0.00	-1.55 ± 2.69##	-1.19 ± 3.22#
Small + very small	3.61 ± 1.07	4.27 ± 1.48	4.14 ± 2.18	3.96 ± 1.43	4.11 ± 1.60	4.02 ± 2.02	4.67 ± 2.13	4.54 ± 1.92	4.43 ± 1.82*
ΔSmall + very small	0.00 ± 0.00	0.66 ± 1.20	0.53 ± 2.06	0.00 ± 0.00	0.14 ± 1.37	0.06 ± 1.31	0.00 ± 0.00	-0.13 ± 0.90#	-0.24 ± 1.53

Significant differences from the week 0 value, as determined by the Dunnett's test (*p < 0.05).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (#p < 0.05, ##p < 0.01).

Table 4. Markers associated with cholesterol during D-allulose consumption period and in follow-up period.

		Desmosterol (μg/mL)	ΔDesmosterol (μg/mL)	Lathosterol (μg/mL)	ΔLathosterol (μg/mL)	Campesterol (μg/mL)	ΔCampesterol (μg/mL)	β-citosterol (μg/mL)	Δβ-citosterol (μg/mL)	PCSK9 (ng/mL)	ΔPCSK9 (ng/mL)
Placebo	week 0	0.78 ± 0.21	0.00 ± 0.00	1.59 ± 0.59	0.00 ± 0.00	2.00 ± 0.88	0.00 ± 0.00	1.54 ± 0.68	0.00 ± 0.00	216.6 ± 64.0	0.0 ± 0.0
	week 8	0.63 ± 0.13**	-0.16 ± 0.19	1.44 ± 0.39	-0.15 ± 0.48	1.86 ± 0.75	-0.14 ± 0.50	1.43 ± 0.68	-0.12 ± 0.37	291.9 ± 90.3**	75.3 ± 74.3
	week 48	0.67 ± 0.33	-0.11 ± 0.32	1.38 ± 0.50*	-0.21 ± 0.43	1.80 ± 0.78	-0.20 ± 0.47	1.39 ± 0.58	-0.15 ± 0.39	214.5 ± 75.4	-2.1 ± 86.8
	Follow-up period	0.47 ± 0.17**	-0.31 ± 0.18	1.10 ± 0.46**	-0.49 ± 0.40	1.45 ± 0.73**	-0.55 ± 0.52	1.12 ± 0.51**	-0.43 ± 0.42	220.4 ± 57.7	3.8 ± 63.8
Low-dose D-allulose	week 0	0.85 ± 0.28	0.00 ± 0.00	1.70 ± 0.77	0.00 ± 0.00	1.90 ± 0.72	0.00 ± 0.00	1.55 ± 0.64	0.00 ± 0.00	274.1 ± 114.0	0.0 ± 0.0
	week 8	0.80 ± 0.20	-0.05 ± 0.18	1.73 ± 0.58	0.02 ± 0.40	1.91 ± 0.69	0.01 ± 0.48	1.51 ± 0.62	-0.04 ± 0.38	284.9 ± 103.8	10.9 ± 88.0##
	week 48	0.70 ± 0.22**	-0.15 ± 0.16	1.57 ± 0.65	-0.13 ± 0.36	1.71 ± 0.53	-0.19 ± 0.57	1.37 ± 0.45	-0.18 ± 0.49	234.9 ± 71.7	-39.1 ± 110.3
	Follow-up period	0.51 ± 0.19**	-0.34 ± 0.21	1.13 ± 0.51**	-0.57 ± 0.48	1.35 ± 0.46**	-0.55 ± 0.56	1.09 ± 0.39**	-0.46 ± 0.43	249.4 ± 80.3	-24.7 ± 94.7
High-dose D-allulose	week 0	0.83 ± 0.22	0.00 ± 0.00	1.70 ± 0.76	0.00 ± 0.00	2.12 ± 0.77	0.00 ± 0.00	1.72 ± 0.68	0.00 ± 0.00	224.5 ± 59.7	0.0 ± 0.0
	week 8	0.68 ± 0.20**	-0.14 ± 0.16	1.49 ± 0.49	-0.21 ± 0.53	2.14 ± 0.89	0.03 ± 0.36	1.69 ± 0.77	-0.03 ± 0.36	235.1 ± 57.9	10.6 ± 71.3##
	week 48	0.63 ± 0.17**	-0.20 ± 0.17	1.31 ± 0.36**	-0.39 ± 0.61	2.00 ± 0.93	-0.11 ± 0.57	1.59 ± 0.71	-0.13 ± 0.43	191.0 ± 83.8	-33.6 ± 70.2
	Follow-up period	0.49 ± 0.14**	-0.33 ± 0.15	1.11 ± 0.42**	-0.59 ± 0.62	1.52 ± 0.69**	-0.60 ± 0.47	1.24 ± 0.53**	-0.48 ± 0.40	241.2 ± 58.7	16.7 ± 72.4

Significant differences from the week 0 value, as determined by the Dunnett's test (*p < 0.05, **p < 0.01).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (##p < 0.01).

Table 5. The TG/Cho ratio in VLDL, LDL, HDL during D-allulose consumption period.

	VLDL-TG / VLDL-Cho			LDL-TG / LDL-Cho			HDL-TG / HDL-Cho		
	week 0	week 8	week 48	week 0	week 8	week 48	week 0	week 8	week 48
Placebo	2.20 ± 0.88	2.12 ± 0.87	1.98 ± 0.76	0.17 ± 0.03	0.18 ± 0.04	0.19 ± 0.06	0.19 ± 0.07	0.22 ± 0.10	0.24 ± 0.18
Low-dose D-allulose	2.23 ± 1.03	1.71 ± 0.76**	1.88 ± 0.71*	0.17 ± 0.03	0.18 ± 0.07	0.18 ± 0.07	0.20 ± 0.08	0.20 ± 0.10	0.22 ± 0.15
High-dose D-allulose	2.39 ± 1.09	1.79 ± 1.08**	2.03 ± 0.88*	0.20 ± 0.06#	0.20 ± 0.06	0.21 ± 0.05	0.24 ± 0.12	0.23 ± 0.12	0.24 ± 0.11

Significant differences from the week 0 value, as determined by the Dunnett's test (*p < 0.05, **p < 0.01).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (#p < 0.05).

Table 6. Risk factors and markers for ASCVD during D-allulose consumption period and in follow-up period

(a) Changes in TNF-α, MDA-LDL, IL-6 and t-PAI-1

	TNF-α (pg/mL)			MDA-LDL (U/L)		IL-6 (pg/mL)		t-PAI-1 (ng/mL)	
	week 0	week 8	week 48	week 0	week 48	week 0	week 48	week 0	week 48
Placebo	1.04 ± 0.44	1.67 ± 0.42**	2.27 ± 0.44**	113.5 ± 32.6	109.3 ± 35.3	2.41 ± 3.27	1.42 ± 0.71	19.2 ± 14.3	15.4 ± 7.3
Low-dose D-allulose	1.64 ± 0.77	1.90 ± 0.69*	2.34 ± 0.72**	118.1 ± 40.2	124.0 ± 54.2	1.73 ± 1.83	2.16 ± 3.06	19.5 ± 9.7	14.1 ± 7.8**
High-dose D-allulose	1.34 ± 0.68	1.73 ± 0.60**	2.30 ± 0.76**	118.4 ± 40.9	113.0 ± 47.7	1.94 ± 3.15	1.48 ± 1.51	19.5 ± 12.2	16.6 ± 9.9*

(b) Changes in RLP-C and hsCRP

(mg/dL)		week 0	week 8	week 16	week 24	week 32	week 40	week 48	Follow-up period
RLP-C	Placebo	5.21 ± 1.86	6.23 ± 2.33	5.39 ± 3.07	5.21 ± 2.30	5.35 ± 2.79	5.12 ± 2.80	5.83 ± 7.19	5.73 ± 2.00
	Low-dose D-allulose	6.68 ± 4.77	6.89 ± 3.59	7.32 ± 6.99	7.46 ± 7.84	7.52 ± 10.24	6.20 ± 7.74	5.78 ± 5.49	6.00 ± 4.14
	High-dose D-allulose	7.41 ± 4.37	7.46 ± 4.90	7.58 ± 6.64	6.64 ± 4.85	6.31 ± 4.63	6.51 ± 4.41	5.84 ± 3.38	8.00 ± 9.97
hsCRP	Placebo	0.051 ± 0.058	0.069 ± 0.127	0.071 ± 0.128	0.050 ± 0.071	0.056 ± 0.089	0.064 ± 0.105	0.065 ± 0.101	0.086 ± 0.128
	Low-dose D-allulose	0.072 ± 0.087	0.087 ± 0.127	0.078 ± 0.106	0.059 ± 0.057	0.058 ± 0.050	0.080 ± 0.093	0.087 ± 0.125	0.093 ± 0.108
	High-dose D-allulose	0.073 ± 0.110	0.078 ± 0.125	0.058 ± 0.083	0.090 ± 0.137	0.066 ± 0.092	0.084 ± 0.136	0.071 ± 0.123	0.057 ± 0.057

Significant differences from the week 0 value, as determined by the Dunnett's test in TNF-α, RLP-C, hsCRP and a paired t-test in MDA-LDL, IL-6, t-PAI-1 (*$p < 0.05$, **$p < 0.01$).

Table 7. The Max IMT before and after long-term D-allulose consumption.

		Right (mm)		Left (mm)	
		Max IMT	ΔMax IMT	Max IMT	ΔMax IMT
Placebo	week 0	0.46 ± 0.25	0.00 ± 0.00	0.58 ± 0.35	0.00 ± 0.00
	week 48	0.74 ± 0.49**	0.28 ± 0.37	0.76 ± 0.42**	0.18 ± 0.28
Low-dose D-allulose	week 0	0.47 ± 0.22	0.00 ± 0.00	0.64 ± 0.51	0.00 ± 0.00
	week 48	0.69 ± 0.46**	0.22 ± 0.32	0.81 ± 0.56*	0.17 ± 0.32
High-dose D-allulose	week 0	0.50 ± 0.24	0.00 ± 0.00	0.48 ± 0.25	0.00 ± 0.00
	week 48	0.71 ± 0.48*	0.22 ± 0.42	0.65 ± 0.25**	0.17 ± 0.19

Significant differences from the week 0 value, as determined by a paired t-test (*$p < 0.05$, **$p < 0.01$).
No significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test.

Table 8. Effects on absolute risks of ASCVD by D-allulose consumption for 48weeks.

	Risk category	week 0	week 48	Follow-up period
Placebo	Low	20	18	16
	Moderate	7	9	10 *
	High	1	1	2
Low-dose D-allulose	Low	16	16	16
	Moderate	11	11	11
	High	0	0	0
High-dose D-allulose	Low	20	18	19
	Moderate	7	9	8
	High	0	0	0

Values show the numbers of people.
Significant difference from week 0 value, as determined by the Wilcoxon signed-rank test (*$p < 0.05$).
No significant differences of the D-allulose groups compared with placebo group, as determined by the Wilcoxon two-sample.

levels. No significant differences in ΔVLDL-C between test groups were found, while ΔVLDL-TG significantly decreased in the high-dose D-allulose group at week 8. There were significant increases at week 8 in Δlarge + medium LDL-C in both the high- and low-dose D-allulose groups compared with the placebo group. On the other hand, no significant changes in ΔsdLDL-C were observed between the test groups. Both Δlarge + medium LDL-TG and ΔsdLDL-TG in the D-allulose groups did not significantly change compared with the placebo. In HDL subfractions, Δvery large + large + medium HDL-C in the high-dose D-allulose group were significantly reduced at week 8 and 48 compared with the placebo. However, Δsmall + very small HDL-C did not change. Both ΔHDL-TG subfractions significantly decreased in the high-dose D-allulose group compared with the placebo. There were no significant differences in markers indicating cholesterol synthesis (Δdesmosterol, Δlathosterol) and absorption (Δcampesterol, Δβ-sitosterol) between the placebo and the D-allulose groups, although significant reductions in all test groups were observed when compared with week 0 (Table 4). ΔPCSK9 levels in the D-allulose groups at week 8 were significantly decreased in comparison with the placebo group (Table 4). The TG/Cho ratio to evaluate lipid transfer between VLDL, LDL, and HDL showed no significant changes in LDL or HDL during the D-allulose consumption period, although the ratio in VLDL decreased in the D-allulose groups compared with week 0 (Table 5). Changes in other lipid parameters are briefly described as follows although the exact data were not shown. There were no significant changes in ΔApoB48 and ΔApoB100 between test groups. Significant decreases in ΔApoA-I and ΔApoA-II were seen in the D-allulose groups in comparison with the placebo group, and the changes were similar to the HDL-C levels. Although there were no significant changes in ΔApoC-II levels during the D-allulose consumption periods between test groups, significant reductions of ΔApoC-III levels (at week 8 and 48) and ΔApoE levels (at week 48) in the high-dose D-allulose group were observed compared with the placebo. ΔNEFA, Δglycerol, and Δcortisol did not significantly vary, but ΔTG (at week 8) and ΔPL (at week 32 and 48) indicated significant reductions in the high-dose D-allulose group compared with the placebo group.

Other risk factors and markers for ASCVD (TNF-α, MDA-LDL, IL-6, t-PAI-1, remnant-like particles cholesterol (RLP-C), and high-sensitivity C-reactive protein (hsCRP)) are shown in Table 6. Although some significant changes were observed compared with week 0, the differential values did not indicate significant increases

Table 9. Effects of long-term D-allulose consumption on glucose metabolism.

(a) HbA1c in all subjects

%		week 0	week 8	week 16	week 24	week 32	week 40	week 48	Follow-up period
Placebo	n=28	5.62 ± 0.45	5.70 ± 0.51	5.74 ± 0.51*	5.66 ± 0.51	5.69 ± 0.55	5.77 ± 0.62**	5.68 ± 0.54	5.75 ± 0.53*
Low-dose D-allulose	n=27	5.71 ± 0.39	5.75 ± 0.38	5.83 ± 0.46*	5.78 ± 0.46	5.70 ± 0.43	5.77 ± 0.43	5.69 ± 0.42	5.82 ± 0.48*
High-dose D-allulose	n=27	5.67 ± 0.42	5.73 ± 0.42	5.77 ± 0.44**	5.71 ± 0.47	5.70 ± 0.49	5.69 ± 0.47	5.69 ± 0.51	5.79 ± 0.49**

(b) HbA1c in borderline diabetes

%		week 0	week 8	week 16	week 24	week 32	week 40	week 48	Follow-up period
Placebo	n=15	5.91 ± 0.36	6.02 ± 0.45	6.07 ± 0.41	5.99 ± 0.44	6.04 ± 0.49	6.13 ± 0.61*	6.05 ± 0.43	6.10 ± 0.43
Low-dose D-allulose	n=12	6.00 ± 0.39	6.04 ± 0.37	6.18 ± 0.48	6.09 ± 0.52	6.02 ± 0.44	6.08 ± 0.45	6.03 ± 0.34	6.18 ± 0.47
High-dose D-allulose	n=14	5.93 ± 0.38	5.96 ± 0.43	6.03 ± 0.43	5.97 ± 0.48	5.98 ± 0.49	5.95 ± 0.49	5.97 ± 0.54	6.04 ± 0.51

(c) Glucose ΔAUC on OGTT

mg·hr/dL	All subjects				Borderline diabetes			
		week 0	week 48	Differential values		week 0	week 48	Differential values
Placebo	n=28	46.1 ± 28.6	47.8 ± 37.9	1.64 ± 18.8	n=15	58.3 ± 32.3	76.4 ± 27.9*	18.1 ± 26.0
Low-dose D-allulose	n=27	45.2 ± 26.4	45.9 ± 35.4	0.64 ± 21.5	n=12	58.8 ± 27.4	67.0 ± 39.1	8.3 ± 27.8
High-dose D-allulose	n=27	47.8 ± 29.1	45.6 ± 30.4	-2.22 ± 24.1	n=14	62.5 ± 27.0	57.8 ± 27.0	-4.7 ± 18.4#

Significant differences from the week 0 value, as determined by the Dunnett's test in HbA1c and a paired t-test in glucose ΔAUC (*$p < 0.05$, **$p < 0.01$).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (#$p < 0.05$).

Table 10. Effects of long-term D-allulose consumption on the hepatic function.

		AST (U/L)	ΔAST (U/L)	ALT (U/L)	ΔALT (U/L)	ALP (U/L)	ΔALP (U/L)	γ-GTP (U/L)	Δγ-GTP (U/L)
Placebo	week 0	19.6 ± 5.7	0.0 ± 0.0	16.8 ± 7.5	0.0 ± 0.0	189.9 ± 52.3	0.0 ± 0.0	27.0 ± 16.2	0.0 ± 0.0
	week 8	21.1 ± 6.1	1.6 ± 4.1	19.6 ± 9.6	2.8 ± 7.3	199.6 ± 53.7	9.8 ± 24.8	27.3 ± 12.8	0.3 ± 8.1
	week 16	19.6 ± 5.1	0.1 ± 3.9	17.2 ± 7.0	0.4 ± 5.4	193.6 ± 51.2	3.7 ± 28.6	26.8 ± 13.8	-0.2 ± 8.6
	week 24	21.0 ± 12.4	1.5 ± 11.4	17.8 ± 13.0	1.0 ± 11.2	193.2 ± 49.7	3.3 ± 24.3	27.3 ± 15.7	0.3 ± 12.5
	week 32	26.4 ± 37.0	6.9 ± 37.1	21.1 ± 27.1	4.3 ± 26.1	196.7 ± 55.3	6.8 ± 25.3	30.6 ± 23.3	3.6 ± 18.6
	week 40	26.4 ± 31.9	6.8 ± 32.0	22.4 ± 25.8	5.5 ± 24.3	199.1 ± 55.5	9.2 ± 28.1	32.5 ± 23.5	5.5 ± 17.9
	week 48	21.5 ± 6.8	1.9 ± 5.8	19.3 ± 10.1	2.5 ± 8.9	198.0 ± 49.8	8.2 ± 26.7	27.8 ± 14.1	0.8 ± 6.0
	Follow-up period	20.3 ± 5.9	0.7 ± 3.7	18.7 ± 8.3	1.9 ± 6.6	198.2 ± 52.3	8.4 ± 28.2	29.5 ± 15.6	2.5 ± 6.9
Low-dose D-allulose	week 0	22.6 ± 5.6	0.0 ± 0.0	20.9 ± 7.6	0.0 ± 0.0	216.1 ± 57.6	0.0 ± 0.0	28.8 ± 23.7	0.0 ± 0.0
	week 8	22.4 ± 5.7	-0.1 ± 4.9	19.2 ± 7.2	-1.6 ± 6.0	197.4 ± 50.7**	-18.6 ± 20.9##	25.2 ± 24.5	-3.6 ± 3.9
	week 16	20.0 ± 4.5*	-2.6 ± 4.3#	16.1 ± 5.8**	-4.7 ± 5.2##	183.5 ± 46.2**	-32.6 ± 24.3##	21.9 ± 18.9**	-6.9 ± 9.6
	week 24	19.3 ± 5.1**	-3.3 ± 4.5	15.5 ± 5.9**	-5.4 ± 4.9	182.9 ± 46.5**	-33.2 ± 21.7##	21.9 ± 18.8**	-6.9 ± 9.1
	week 32	19.7 ± 4.3*	-2.9 ± 4.4	16.5 ± 7.3**	-4.3 ± 6.4	184.4 ± 44.2**	-31.6 ± 24.0##	20.3 ± 15.3**	-8.4 ± 12.6#
	week 40	20.7 ± 5.9	-1.9 ± 5.5	18.1 ± 8.5	-2.8 ± 7.5	193.2 ± 48.5**	-22.9 ± 24.1##	23.1 ± 19.6**	-5.7 ± 11.1#
	week 48	20.0 ± 4.9*	-2.6 ± 5.4#	17.1 ± 8.5**	-3.7 ± 8.2#	187.9 ± 45.4**	-28.1 ± 31.2##	21.2 ± 19.7**	-7.6 ± 10.4#
	Follow-up period	22.3 ± 9.8	-0.3 ± 9.5	20.3 ± 8.9	-0.6 ± 9.5	208.4 ± 49.3	-7.6 ± 26.9	26.5 ± 21.7	-2.3 ± 12.6
High-dose D-allulose	week 0	22.7 ± 5.2	0.0 ± 0.0	22.9 ± 11.4	0.0 ± 0.0	220.6 ± 60.1	0.0 ± 0.0	34.8 ± 27.4	0.0 ± 0.0
	week 8	22.2 ± 5.5	-0.6 ± 5.7	20.7 ± 12.5	-2.2 ± 9.9#	198.3 ± 51.0**	-22.3 ± 23.1##	24.7 ± 22.3**	-10.0 ± 14.0##
	week 16	21.9 ± 5.3	-0.8 ± 4.8	20.1 ± 9.3	-2.9 ± 8.4	185.3 ± 47.3**	-35.4 ± 28.7##	23.6 ± 17.8**	-11.2 ± 17.0##
	week 24	21.1 ± 7.2	-1.6 ± 6.5	23.0 ± 25.1	0.1 ± 22.9	189.6 ± 48.2**	-31.1 ± 33.4##	23.9 ± 21.8**	-10.9 ± 19.9#
	week 32	21.5 ± 5.9	-1.2 ± 4.3	17.7 ± 7.8	-5.3 ± 8.3	186.8 ± 49.2**	-33.9 ± 38.1##	24.7 ± 23.9**	-10.1 ± 17.9#
	week 40	21.1 ± 5.7	-1.6 ± 4.7	17.9 ± 7.6	-5.1 ± 8.6#	199.6 ± 60.5**	-21.0 ± 36.6##	24.2 ± 20.3**	-10.6 ± 16.4##
	week 48	22.2 ± 8.6	-0.6 ± 7.3	18.6 ± 10.3	-4.4 ± 9.1##	191.7 ± 49.2**	-29.0 ± 24.5##	22.7 ± 19.5**	-12.1 ± 18.7##
	Follow-up period	21.3 ± 6.2	-1.4 ± 5.8	18.9 ± 7.7	-4.0 ± 9.3#	223.3 ± 65.2	2.7 ± 30.0	30.9 ± 27.9	-3.9 ± 16.3

Significant differences from the week 0 value, as determined by the Dunnett's test (*$p < 0.05$, **$p < 0.01$).
Significant differences of the D-allulose groups compared with the placebo group, as determined by the Dunnett's test (#$p < 0.05$, ##$p < 0.01$).

Table 11. The evaluation of fatty liver using ultrasonography before and after long-term D-allulose consumption.

	Score	week 0			week 48		
		Placebo	Low-dose D-allulose	High-dose D-allulose	Placebo	Low-dose D-allulose	High-dose D-allulose
Category	(-)	11	9	13	7	11	13
	(±)	8	5	5	6 **	2	4
	(+)	9	13	9	15	14	10
	(++)	0	0	0	0	0	0
Changes from week 0	0				0	0	0
	1				1	5	2
	2				17	19 #	22 #
	3				9	2	3
	4				1	1	0

(-): no steatosis, (±): mild steatosis, (+): moderate steatosis, (++): severe steatosis; 0: improvement, 1: slightly improvement, 2: no change, 3: slightly worse, 4: worse.
Values show the numbers of people.
Significant difference from week 0 value, as determined by the Wilcoxon signed-rank test (**$p < 0.01$).
Significant differences of the D-allulose groups compared with placebo group, as determined by the Wilcoxon two-sample test (#$p < 0.05$).

between test groups. In the results of the max IMT levels, there were no significant differences between the placebo and the D-allulose groups, while significant increases were seen in all groups between week 0 and 48 (Table 7). Additionally, no significant variations in the calculated results of absolute risk of ASCVD were shown in the D-allulose groups (Table 8).

The indicators associated with the effect of long-term D-allulose consumption on glucose metabolism are shown in Table 9. HbA1c values in all subjects significantly increased in some measured points in all test groups in comparison with week 0, although no significant differences in ΔHbA1c were observed between the D-allulose groups and the placebo (Table 9(a)). No significant changes in glucose ΔAUC on OGTT were found in all subjects (Table 9(c)). Meanwhile, in the sub-group with borderline diabetes, HbA1c values in the D-allulose groups did not vary in spite of a significant increase at week 40 in the placebo group (Table 9(b)). Glucose ΔAUC in the borderline diabetes sub-group significantly increased at week 48 only in the placebo group compared with week 0, and the differential value between week 0 and 48 significantly indicated lower values in the high-D-allulose group than in the placebo (Table 9(c)). Table 10 and 11 show changes in the hepatic functions by D-allulose consumption. Significant declines of Δaspartate aminotransferase (AST), Δalanine aminotransferase (ALT), Δalkaline phosphatase (ALP), and Δγ-glutamyl transpeptidase(γ-GTP) were found in the D-allulose groups compared with the placebo group (Table 10). Changes from week 0 in the evaluation of fatty liver significantly improved in the D-allulose groups when compared with the placebo group (Table 11).

Some values of anthropometric indicators, visceral fat area, general blood biochemical markers (protein, electrolyte, and renal function), hematological parameters, and urinalysis parameters deviated from the reference values; however, these deviations were not clinically significant (data not shown).

Lastly, adverse events were observed during the consumption period. There were forty-two episodes in the placebo, thirty-four in the low-dose D-allulose group, and thirty-eight in the high-dose D-allulose group in 19, 22, and 21 subjects, respectively. All adverse events were judged not to be related to the test substance by the principal physician. No significant differences in the incidence of adverse events were found between the placebo group and the D-allulose groups.

DISCUSSION

We assessed the safety of D-allulose intake for 48 weeks and its effect on cholesterol metabolism in subjects with high LDL-C levels. Our results indicated that there were no significant increases in T-Cho and LDL-C in the D-allulose groups compared to the placebo group (Table 2). Although a significant increase in T-Cho and LDL-C in the D-allulose groups was transiently observed only at week 8 compared with week 0, these values gradually reduced after that timepoint. These results suggested that short-term intake of D-allulose might cause a transient increase in T-Cho and LDL-C similar to that of other food ingredients such as n-3 fatty acids. We therefore evaluated various ASCVD risk factors to assess the effects of transient elevated cholesterol levels on ASCVD risks. sdLDL-C, which is an important risk factor of ASCVD

beyond LDL-C itself (Kinoshita *et al.*, 2018), did not show significant changes between the D-allulose groups and the placebo group (Table 3(a)). Although decreases in HDL-C were observed in the D-allulose groups (Table 2), the mechanism that enhances RCT by increase in HDL-C intake into hepatocytes is considered to be anti-atherosclerosis (Iida and Okuma, 2012). Other risk factors and markers also demonstrated no proatherogenic changes on D-allulose ingestion (Table 6). IMT, an indicator of the degree of arteriosclerosis, did not vary in the D-allulose groups compared with the placebo group before and after D-allulose intake for 48 weeks (Table 7). Recently, risk assessment using absolute risk is proposed to evaluate comprehensive ASCVD risks by JAS. We assessed individual absolute risk of ASCVD before and after D-allulose consumption for 48 weeks, and D-allulose consumption for 48 weeks did not affect the absolute risk of ASCVD (Table 8). These results consequently suggested that there is no evidence that transient variations in cholesterol levels through the long-term intake of D-allulose heightens ASCVD risks. The degree of the cholesterol elevations in this study did not significantly differ from the placebo group and the increases also disappeared with consecutive long-term intakes of D-allulose; therefore, they might be due to a seasonal variation like in a previous open trial (Tanaka *et al.*, 2019). However, there is a possibility that cholesterol variations are related to various metabolism changes.

In general, the main factors affecting blood LDL-C levels are as follows: the synthesis and absorption of cholesterol in the liver and the small intestine, VLDL secretion from the liver, uptake of LDL-C into the liver, and lipid transfer in the blood by cholesteryl ester transfer protein (CETP). In this study, no significant changes in cholesterol synthesis markers (desmosterol, lathosterol), cholesterol absorption markers (campesterol, β-sitosterol), or VLDL-C secretion were observed after D-allulose consumption compared with the placebo (Table 3(a), 4). Under activation of lipid transfer by CETP, cholesterol in HDL is reciprocally exchanged with TG in VLDL and LDL, and it is expected to show the decrease of TG/Cho ratio in VLDL and LDL and the increase of TG/Cho ratio in HDL. Our results demonstrated no significant changes in the TG/Cho ratio in each lipoprotein after D-allulose consumption between the D-allulose groups and the placebo group (Table 5). Our findings indicate that the synthesis and absorption of cholesterol, VLDL secretion, and lipid transfer by CETP may not be the main cause of the transient LDL-C increase; decreases in the uptake of LDL-C into the liver through the LDL-receptor is strongly presumed to cause the transient increase. The LDL receptor is regulated through sterol regulatory element-binding protein 2 (SREBP-2), which has an important role in hepatic cholesterol homeostasis (Hua *et al.*, 1993). SREBP-2 is down-regulated by AMP-activated protein kinase α (AMPKα) (Wang *et al.*, 2018), and a recent study found enhancement of AMPKα expression in D-allulose-fed rats (Chen *et al.*, 2019). These findings suggest the possibility that D-allulose might down-regulate the LDL receptor by suppression of SREBP-2 through enhancement of AMPKα expression. Moreover, LDL receptor-mediated plasma LDL increases via casein protein were reported in milk-fed rabbits (Khosla *et al.*, 1991). Researchers have hypothesized the underlying mechanisms for the decrease in plasma LDL receptor: first, cholesterol and bile acid absorption increases in the gut; after absorption, the cholesterol and bile acids are transported to the liver and lead to the increase of cholesterol and bile acid levels in the liver, thereby causing downregulation of liver LDL receptors. Although enhanced absorption of cholesterol in the intestine was not observed in our study (Table 4), it is reasonable to suppose that augmentation of cholesterol in the liver by the enhanced uptake of HDL-C into the liver with D-allulose intake, as mentioned above, causes down-regulation or no up-regulation of the LDL receptor.

Because free cholesterol from HDL-C preferentially becomes the resource of bile acids more than the free cholesterol from LDL (Schwartz *et al.*, 1978) and is excreted in the feces, this change of HDL-C metabolism might be expected to affect bile acid metabolism. A significant increase of chenodeoxycholic acid (CDCA; one of the bile acids) in feces after two months of D-allulose intake was confirmed in our unpublished data in a preliminary human trial (subjects: 15 Japanese men with borderline diabetes or high LDL-C levels, test sample: 15 g of D-allulose/day, test period: 8 weeks). Administration of CDCA was reported to downregulate hepatic LDL receptors (Laskar *et al.*, 2017), therefore changes in bile acids metabolism after D-allulose intake could also affect the uptake of LDL into the liver. It was reported that D-allulose is slightly fermented in the human large intestine (Iida *et al.*, 2010), and Matsuo *et al.* (2003) demonstrated that D-allulose was fermented by intestinal microflora and produced short-chain fatty acids in the caecum in rats. It is accordingly necessary to conduct further research about the effects of intestinal bacteria on cholesterol metabolism after the long-term intake of D-allulose.

On the other hand, decreases in LDL-C levels were observed in the D-allulose groups after week 8 in our study (Table 2), which appears to be associated with PCSK9. PCSK9 is known to degrade the LDL recep-

tor and regulate blood LDL-C levels; its inhibitors have drawn attention as new LDL-C-lowering drugs (Giugliano and Sabatine, 2015; Mombelli *et al.*, 2015). A previous report mentioned that the suppression of LDL receptors induced by CDCA treatment is counterbalanced by a reduction of PCSK9 (Laskar *et al.*, 2017). Decreases in PCSK9 by D-allulose have been reported not only in this study but also in a previous study in hamsters (Kanasaki *et al.*, 2019); changes in PCSK9 by D-allulose intake are a reliable evidence that D-allulose affects LDL uptake into the liver. These evidences suggested that a transient increase in LDL-C after D-allulose intake is caused by changes in the clearance of LDL-C through changes in cholesterol and bile acid metabolism.

Moreover, our results indicated the improvement of biliary enzyme activities (ALP and γ-GTP) by D-allulose intake (Table 10). Not only biliary enzyme activities but also liver deviance enzyme activities (AST and ALT) and the score of fatty liver improved with D-allulose intake (Table 10, 11). Similar results related to hepatic functions were reported in some previous studies (Hayashi *et al.*, 2010; Itoh *et al.*, 2015; Tanaka *et al.*, 2019). CDCA is a strong agonist for the Farnesoid X Receptor (FXR), which is a member of the nuclear receptors superfamily and regulates the metabolism of bile acid, cholesterol, lipoprotein, TG, and glucose. Consecutive changes of bile acid metabolism through FXR activation might be related to these improvements and changes in cholesterol.

D-allulose has been reported to suppress postprandial hyperglycemia (Hayashi *et al.*, 2010; Hossain *et al.*, 2011; Iida *et al.*, 2008; Matsuo and Izumori, 2006); therefore, long-term consecutive D-allulose consumption is expected to improve glucose metabolism. In this study, no significant improvements of glucose metabolism (HbA1c and glucose ΔAUC on OGTT) were found in all subjects because half of them were within normal range for glucose metabolism (Table 9(a)(c)). However, analysis in the sub-group with borderline diabetes demonstrated that the values of HbA1c, an indicator for long-term blood glucose control, did not change in the D-allulose groups even though it was significantly increased in the placebo (Table 9(b)). Moreover, the differential value of glucose ΔAUC on OGTT at week 48 in the high-dose D-allulose group significantly decreased compared with the placebo group in the same sub-group analysis (Table 9(c)). These results suggested that long-term D-allulose consumption suppressed aggravation of glucose metabolism and could improve postprandial hyperglycemia not only by a single ingestion but also by consecutive consumption.

Previous studies reported that D-allulose was a renal excretory substance (Tsukamoto *et al.*, 2014; Whistler *et al.*, 1974) and it was possible that D-allulose affects renal functions. Our results, however, demonstrated no significant changes related to renal functions (blood urea nitrogen, creatinine, uric acid, urine specific gravity, urinary microalbumin, and urine pH) by long-term and excessive D-allulose intake in both blood and urinary examinations. Other clinical studies have also reported similar results (Han *et al.*, 2018; Hayashi *et al.*, 2010; Tanaka *et al.*, 2019). Results of other examinations, including physical, blood, and urine examinations, did not show clinical problems. Although some adverse events were observed in all groups, there were no significant differences in incidence between all groups, and they were judged not to be related to the test substance by the principal physician.

In conclusion, this study assessed the long-term safety, the effect on cholesterol metabolism, and the efficacy of D-allulose intake for 48 weeks. As a result, T-Cho and LDL-C levels did not significantly change by long-term D-allulose intake compared with the placebo group although transient increases were observed similar to some other food ingredients. The ASCVD risks were evaluated by various risk factors and mechanistic perspectives, and consequently none of them demonstrated an increase in ASCVD risks with D-allulose intake for 48 weeks, even in the subjects with high LDL-C levels. We also found that long-term D-allulose consumption improved hepatic functions and glucose metabolism. D-allulose is a non-caloric sweetener with several health benefits and can be very useful for global health. Our findings would promote the daily use of D-allulose in the future.

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REFERENCES

Aro, A., Tuomilehto, J., Kostiainen, E., Uusitalo, U. and Pirjo, P. (1987): Boiled coffee increases serum low density lipoprotein concentration. Metabolism, **36**, 1027-1030.

Baldassarre, D., Franceschini, G., Peruzzotti, G., Brusoni, B. and Sirtori, C.R. (1997): Clinical Evaluation of Probucol in Hypercholesterolemia: Individual Lipoprotein Responses and Inhibito-

ry Effect on Carotid Atherosclerosis Progression. J. Cardiovasc. Pharmacol., **30**, 784-789.

Carnicer, R., Navarro, M.A., Arbones-Mainar, J.M., Acin, S., Guzman, M.A., Surra, J.C., Arnal, C., de Las Heras, M., Blanco-Vaca, F. and Osada, J. (2007): Folic acid supplementation delays atherosclerotic lesion development in apoE-deficient mice. Life Sci., **80**, 638-643.

Chen, J., Huang, W., Zhang, T., Iu, M. and Jiang, B. (2019): Anti-obesity potential of rare sugar d-psicose by regulating lipid metabolism in rats. Food Funct., 5.

Davidson, M.H. (2005): Reducing residual risk for patients on statin therapy: the potential role of combination therapy. Am. J. Cardiol., **96**, 3K-13K.

Foster, G.D., Wyatt, H.R., Hill, J.O., Makris, A.P., Rosenbaum, D.L., Brill, C., Stein, R.I., Mohammed, B.S., Miller, B., Rader, D.J., Zemel, B., Wadden, T.A., Tenhave, T., Newcomb, C.W. and Klein, S. (2010): Weight and Metabolic Outcomes After 2 Years on a Low-Carbohydrate Versus Low-Fat Diet. Ann. Intern. Med., **153**, 147-157.

Gallaher, C.M. and Gallaher, D.D. (2009): Dried plums (prunes) reduce atherosclerosis lesion area in apolipoprotein E-deficient mice. Br. J. Nutr., **101**, 233-239.

Giugliano, R.P. and Sabatine, M.S. (2015): Are PCSK9 Inhibitors the Next Breakthrough in the Cardiovascular Field? J. Am. Coll. Cardiol., **65**, 2638-2651.

Han, Y., Han, H.J., Kim, A., Choi, J., Cho, S., Park, Y.B., Jung, U.J. and Choi, M.S. (2016): D-Allulose supplementation normalized the body weight and fat-pad mass in diet-induced obese mice via the regulation of lipid metabolism under isocaloric fed condition. Mol. Nutr. Food Res., **60**, 1695-1706.

Han, Y., Kwon, E., Yu, M.K., Lee, S.J., Kim, H., Kim, S., Kim, Y.H. and Choi, M.S. (2018): A Preliminary Study for Evaluating the Dose-Dependent Effect of D-Allulose for Fat Mass Reduction in Adult Humans: A Randomized, Double-Blind, Placebo-Controlled Trial. Nutrients, **10**, 160-174.

Harris, W.S. (1996): n-3 Fatty Acids and Lipoproteins: Comparison of Results from Human and Animal Studies. Lipids, **31**, 243-252.

Hayashi, N., Iida, T., Yamada, T., Okuma, K., Takehara, I., Yamamoto, T., Yamada, K. and Tokuda, M. (2010): Study on the Postprandial Blood Glucose Suppression Effect of D -Psicose in Borderline Diabetes and the Safety of Long-Term Ingestion by Normal Human Subjects. Biosci. Biotechnol. Biochem., **74**, 510-519.

Hirano, K., Ikegami, C., Tsujii, K., Zhang, Z., Matsuura, F., Nakagawa-Toyama, Y., Koseki, M., Masuda, D., Maruyama, T., Shimomura, I., Ueda, Y. and Yamashita, S. (2005): Probucol enhances the expression of human hepatic scavenger receptor class B type I, possibly through a species-specific mechanism. Arterioscler. Thromb. Vasc. Biol., **25**, 2422-2427.

Hirano, T., Ito, Y., Koba, S., Toyoda, M., Ikejiri, A., Saegusa, H., Yamazaki, J. and Yoshino, G. (2004): Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method. Arterioscler. Thromb. Vasc. Biol., **24**, 558-563.

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

Hua, X., Yokoyama, C., Wu, J., Briggs, M.R., Brown, M.S., Goldstein, J.L. and Wang, X. (1993): SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc. Natl. Acad. Sci. USA, **90**, 11603-11607.

Iida, T., Hayashi, N., Yamada, T., Yoshikawa, Y., Miyazato, S., Kishimoto, Y., Okuma, K., Tokuda, M. and Izumori, K. (2010): Failure of D-psicose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans. Metabolism, **59**, 206-214.

Iida, T., Kishimoto, Y., Yoshikawa, Y., Hayashi, N., Okuma, K., Tohi, M., Yagi, K., Matsuo, T. and Izumori, K. (2008): Acute D-Psicose Administration Decreases the Glycemic Responses to an Oral Maltodextrin Tolerance Test in Normal Adults. J. Nutr. Sci. Vitaminol. (Tokyo), **54**, 511-514.

Iida, T. and Okuma, K. (2012): Physiological Functions of Two Rare Sugars, D-Psicose and D-Allose (in Japanese). J. Jpn. Assoc. Dietary Fiber Res., **16**, 9-17.

Itoh, K., Mizuno, S., Hama, S., Oshima, W., Kawamata, M., Hossain, A., Ishihara, Y. and Tokuda, M. (2015): Beneficial Effects of Supplementation of the Rare Sugar "D-allulose" Against Hepatic Steatosis and Severe Obesity in Lep(ob)/Lep(ob). Mice. J. Food Sci., **80**, 1619-1626.

Iwasaki, Y., Sendo, M., Dezaki, K., Hira, T., Sato, T., Nakata, M., Goswami, C., Aoki, R., Arai, T., Kumari, P., Hayakawa, M., Masuda, C., Okada, T., Hara, H., Drucker, D.J., Yamada, Y., Tokuda, M. and Yada, T. (2018): GLP-1 release and vagal afferent activation mediate the beneficial metabolic and chronotherapeutic effects of D-allulose. Nat. Commun., **9**, 1-17.

Kanasaki, A., Jiang, Z., Mizokami, T., Shirouchi, B., Iida, T., Nagata, Y. and Sato, M. (2019): Dietary d-allulose alters cholesterol metabolism in Golden Syrian hamsters partly by reducing serum PCSK9 levels. J. Funct. Foods, 60.

Kasai, T., Miyauchi, K., Kubota, N., Kajimoto, K., Amano, A. and Daida, H. (2012): Probucol therapy improves long-term (>10-year) survival after complete revascularization: a propensity analysis. Atherosclerosis, **220**, 463-469.

Khosla, P., Samman, S. and Carroll, K.K. (1991): Decreased receptor-mediated LDL catabolism in casein-fed rabbits precedes the increase in plasma cholesterol levels. J. Nutr. Biochem., **2**, 203-209.

Kimura, T., Kanasaki, A., Hayashi, N., Yamada, T., Iida, T., Nagata, Y. and Okuma, K. (2017): d-Allulose enhances postprandial fat oxidation in healthy humans. Nutrition, **43-44**, 16-20.

Kinoshita, M., Yokote, K., Arai, H., Iida, M., Ishigaki, Y., Ishibashi, S., Umemoto, S., Egusa, G., Ohmura, H., Okamura, T., Kihara, S., Koba, S., Saito, I., Shoji, T., Daida, H., Tsukamoto, K., Deguchi, J., Dohi, S., Dobashi, K., Hamaguchi, H., Hara, M., Hiro, T., Biro, S., Fujioka, Y., Maruyama, C., Miyamoto, Y., Murakami, Y., Yokode, M., Yoshida, H., Rakugi, H., Wakatsuki, A. and Yamashita, S. (2018): Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017. J. Atheroscler. Thromb., **25**, 846-984.

Kishida, K., Martinez, G., Iida, T., Yamada, T., Ferraris, R.P. and Toyoda, Y. (2019): d-Allulose is a substrate of glucose transporter type 5 (GLUT5) in the small intestine. Food Chem., **277**, 604-608.

Laskar, M.G., Eriksson, M., Rudling, M. and Angelin, B. (2017): Treatment with the natural FXR agonist chenodeoxycholic acid reduces clearance of plasma LDL whilst decreasing circulating PCSK9, lipoprotein(a) and apolipoprotein C-III. J. Intern. Med., **281**, 575-585.

Matsuo, T. and Izumori, K. (2006): D-psicose inhibits intestinal

α-glucosidase and suppresses the glycemic response after carbohydrate ingestion in rats. Tech. Bull. Fac. Agr. Kagawa Univ., **58**, 27-32.

Matsuo, T., Tanaka, T., Hashiguchi, M., Izumori, K. and Suzuki, H. (2003): Metabolic effects of D-psicose in rats: studies on faecal and urinary excretion and caecal fermentation. Asia Pac. J. Clin. Nutr., **12**, 225-231.

Mombelli, G., Pavanello, C. and Castelnuovo, S. (2015): Potential of PCSK9 as a new target for the management of LDL cholesterol. Research Reports in Clinical Cardiology, **6**, 73-86.

Mori, T.A., Watts, G.F., Burke, V., Hilme, E., Puddey, I.B. and Beilin, L.J. (2000): Docosahexaenoic Acid on Vascular Reactivity of the Forearm Microcirculation in Hyperlipidemic, Overweight Men. Circulation, **102**, 1264-1269.

Nagata, Y., Kanasaki, A., Tamaru, S. and Tanaka, K. (2015): D-Psicose, an Epimer of D-Fructose, Favorably Alters Lipid Metabolism in Sprague-Dawley Rats. J. Agric. Food Chem., **63**, 3168-3176.

Nishikura, T., Koba, S., Yokota, Y., Hirano, T., Tsunoda, F., Shoji, M., Hamazaki, Y., Suzuki, H., Itoh, Y., Katagiri, T. and Kobayashi, Y. (2014): Elevated Small Dense Low-Density Lipoprotein Cholesterol as a Predictor for Future Cardiovascular Events in Patients with Stable Coronary Artery Disease. J. Atheroscler. Thromb., **21**, 755-767.

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

Packard, C., Caslake, M. and Shepherd, J. (2000): The role of small, dense low density lipoprotein (LDL): a new look. Int. J. Cardiol., **74**, 17-22.

Relchl, D. and Miller, N.E. (1989): Pathophysiology of Reverse Cholesterol Transport; Insights from Inherited Disorders of Lipoprotein Metabolism. Arteriosclerosis, **9**, 785-797.

Sampson, U.K., Fazio, S. and Linton, M.F. (2012): Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. Curr. Atheroscler. Rep., **14**, 1-10.

Schwartz, C.C., Halloran, L.G., Vlahcevic, Z.R., Gregory, D.H. and Leon, S. (1978): Preferential Utilization of Free Cholesterol from High-Density Lipoproteins for Biliary Cholesterol Secretion in Man. Science, **200**, 62-64.

Tall, A.R. (1990): Plasma High Density Lipoproteins: Metabolism and Relationship to Atherogenesis. J. Clin. Invest., **86**, 379-384.

Tanaka, M., Hayashi, N. and Iida, T. (2019): Safety evaluation of 12-week continuous ingestion of D-allulose in borderline diabetes and type 2 diabetes. Fundam. Toxicol. Sci., **6**, 225-234.

Tsukamoto, I., Hossain, A., Yamaguchi, F., Hirata, Y., Dong, Y., Kamitori, K., Sui, L., Nonaka, M., Ueno, M., Nishimoto, K., Suda, H., Morimoto, K., Shimonishi, T., Saito, M., Song, T., Konishi, R. and Tokuda, M. (2014): Intestinal absorption, organ distribution, and urinary excretion of the rare sugar D-psicose. Drug Des. Devel. Ther., **8**, 1955-1964.

Urgert, R., Essed, N., Weg, G.d., Kosmeijer-Schuil, T.G. and Katan, M.B. (1997): Separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases. Am. J. Clin. Nutr., **65**, 519-524.

Wang, Q., Liu, S., Zhai, A., Zhang, B. and Tian, G. (2018): AMPK-mediated regulation of lipid metabolism by phosphorylation. Biol. Pharm. Bull., **41**, 985-993.

Whistler, R.L., Singh, P.P. and Lake, W.C. (1974): D-Psicose metabolism in the rat. Carbohydr. Res., **34**, 200-202.

Wu, X., Kang, J., Xie, C., Burris, R., Ferguson, M.E., Badger, T.M. and Nagarajan, S. (2010): Dietary blueberries attenuate atherosclerosis in apolipoprotein E-deficient mice by upregulating antioxidant enzyme expression. J. Nutr., **140**, 1628-1632.

Yagi, K. and Matsuo, T. (2009): The Study on Long-Term Toxicity of D-Psicose in Rats. J. Clin. Biochem. Nutr., **45**, 271-277.

Yamaguchi, Y., Kitagawa, M., Iida, T., Kishimoto, Y., Kashiwagi, S., Sugino, T. and Kajimoto, O. (2019): Effects of a Single Intake of Psicose (Allulose) on Fat Oxidation During Exercise-A Randomized, Double-blind, Placebo-controlled, Cross-over Trial-(in Japanese). Jpn. Pharmacol. Ther., **47**, 517-525.