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Original Article

Safety evaluation of 12-week continuous ingestion of D-allulose in borderline diabetes and type 2 diabetes

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ABSTRACT — D-allulose is a rare sugar with an almost zero calorie and is known to suppress postprandial hyperglycemia and fat mass accumulation. Although D-allulose has been reported to be safe in healthy subjects and overweight/obese adults, its safety in borderline diabetes and diabetes patients has not been evaluated. Therefore, we conducted an open trial aimed to investigate the long-term safety of D-allulose in borderline diabetes and type 2 diabetes. Subjects took 5 g of D-allulose with meals three times daily for 12 continuous weeks. The general blood biochemical parameters, hematological parameters, urinary parameters, and anthropometric indicators were measured at 0, 2, 4, 8, and 12 weeks of the consumption periods and 4 weeks after completing consumption. Adverse events were assessed by the principal physician on each examination day. A total of 12 and 6 subjects with borderline and type 2 diabetes, respectively, were analyzed. No serious clinical problems were found in this study, although significant cholesterol variations and the improvements of some indicators of hepatic function were observed. In conclusion, the long-term ingestion of D-allulose is safe in borderline diabetes and type 2 diabetes. D-allulose can potentially suppress postprandial hyperglycemia and fat mass accumulation, and thus might be useful in diabetes.

Key words: D-allulose, Safety, Long-term ingestion, Diabetes, Hepatic function

INTRODUCTION

The prevalence of obesity is increasing worldwide. According to The Global Burden of Disease 2015 Obesity Collaborators, a total of 108 million children and 604 million adults were obese in 2015 (The GBD 2015 Obesity Collaborators, 2017). Obesity is a risk factor for atherosclerotic cardiovascular disease (CVD) and type 2 diabetes (Grundy, 2004), and the incidence of diabetes is also increasing. The International Diabetes Federation reported an incidence of 425 million in 2017 and projects this to reach 700 million in 2045. The increasing medical costs for obesity and diabetes highlight the urgent need to manage these diseases and reduce their incidence. Among the most important strategies to prevent and improve obesity and diabetes is correcting personal habits such as diet and exercise.

D-allulose, a C-3 epimer of D-fructose, is a rare sugar (i.e., monosaccharides that are rarely found in nature) that has 70% sweetness of sucrose but an almost zero calorie. Several studies reported that D-allulose decreased body weight and fat mass accumulation in both animals and humans (Han *et al.*, 2016, 2018; Ochiai *et al.*, 2014). In addition, D-allulose has been reported to suppress elevation of postprandial blood glucose (Hayashi *et al.*, 2010; Iida *et al.*, 2008). The reported effective dose is ≥ 5 g consumed with a meal in normal adults and borderline diabetes. Previous studies have suggested that D-al-

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lulose acts by inhibiting α-glucosidase activity (Matsuo and Izumori, 2006) and increasing glucose uptake to the liver by facilitating glucokinase translocation from the nucleus to the cytoplasm in the liver (Hossain *et al.*, 2011). D-allulose has already been clarified not to cause mutagenesis nor toxicity (Matsuo *et al.*, 2002; Ochiai *et al.*, 2019). The maximum non-effective level of D-allulose in causing diarrhea in human subjects was approximately 0.50-0.60 g per kg body weight (Iida *et al.*, 2007). With respect to metabolism in the human body, 70% of ingested D-allulose is absorbed in the small intestine and excreted into the urine without providing any energy. The remaining 30% is converted into a small amount of energy then passed into the feces (Iida *et al.*, 2010). D-allulose is generally recognized as safe (GRAS) by the Food and Drug Administration. Hayashi *et al.* (2010) conducted a 12-week clinical trial in normal human subjects and reported that continuous D-allulose ingestion caused neither abnormal effects nor clinical problems. Another 12-week clinical study in overweight/obese adult humans reported no adverse effect of D-allulose (Han *et al.*, 2018). However, despite its potential to improve glucose metabolism, its long-term safety in humans with borderline diabetes and diabetes remains unclear. Therefore, we conducted an open study aimed to investigate the long-term safety of D-allulose in borderline diabetes and diabetes patients.

MATERIALS AND METHODS

Ethical considerations

This study was conducted according to the tenets of the Helsinki Declaration (adopted in 1964 and amended in 2008) and the Ethical Guidelines for Epidemiological Research in Japan. The protocol was approved by the ethical committee of the Medical Corporation Association KUNWA-KAI AIWA Clinic (July 17, 2009). The study was controlled by the principal physician in Tokyo EKIMAE-BIRU Clinic. The subjects were given full information about the importance, purpose, and contents of the experiment before obtaining written informed consent. This study was registered in the University Hospital Medical Information Network clinical trial registry (registration number: UMIN000036394).

Subjects

Adult men and women aged 20-75 years were recruited by Sugi Medical Research Co., Ltd. (Tokyo, Japan). The inclusion criteria for borderline and type 2 diabetes were fasting blood glucose concentration of 110-125 mg/dL and under diabetic drug treatment, respectively. The exclusion criteria were as follows: (1) daily intake of dietary supplement that was approved in the food for specified health uses (one of the Japanese health claim systems) related to blood glucose; (2) serious damage to the liver, the renal, the cardiac, the lung, the blood, and endocrine systems and metabolic functions; (3) currently taking medications (excluding antidiabetic agents); (4) heavy alcohol drinker and extremely random dietary habits; (5) allergies to foods and medicines; (6) abnormal values in screening examinations judged by the principal physician; (7) pregnancy, breastfeeding, or expected/planned pregnancy during the consumption period; (8) participation in other clinical studies; (9) blood donation of > 400 mL within 12 weeks or > 200 mL within 4 weeks prior to the screening examination, or directed donation within 2 weeks prior to the screening examination; (10) anyone judged as ineligible to participate in this study by the principal physician. All type 2 diabetes patients were under treatment with antidiabetic agents and diets.

Methods and schedule

We conducted a 16-week open study between July and December 2009; the study period comprised a 12-week consumption period and a 4-week observation period after the consumption (follow-up). Fasting morning urine and blood samples were collected, and blood was collected from the antecubital vein, with the subject seated, by medical staff in Tokyo EKIMAE-BIRU Clinic in the morning. Physical measurements were also taken by medical staff, and interviews were conducted by the principal physician. These measurements were conducted 2 weeks before starting consumption; on the first day of consumption; 2, 4, 8, and 12 weeks after starting consumption; and 4 weeks after completing consumption. We used 5 g of D-allulose with over 98% purity, packed in aluminum foil, from Izumoring Co., Ltd. (Kagawa, Japan). The subjects took one packed test substance with each meal, three times daily for 12 weeks continuously during the consumption period. All subjects finished dinner by 21:00 in the previous day of the examination and were subsequently not allowed to drink anything other than water.

Clinical examination

Physical examinations, blood examinations, urine analysis, and interviews by the principal physician were conducted along with dietary surveys for 3 days before the examination day. Exercise surveys were conducted every day during the consumption period. All blood and urine samples were measured by BML, Inc. (Tokyo, Japan). There were 8 parameters in the general physical examination: height, body weight, body mass index (BMI),

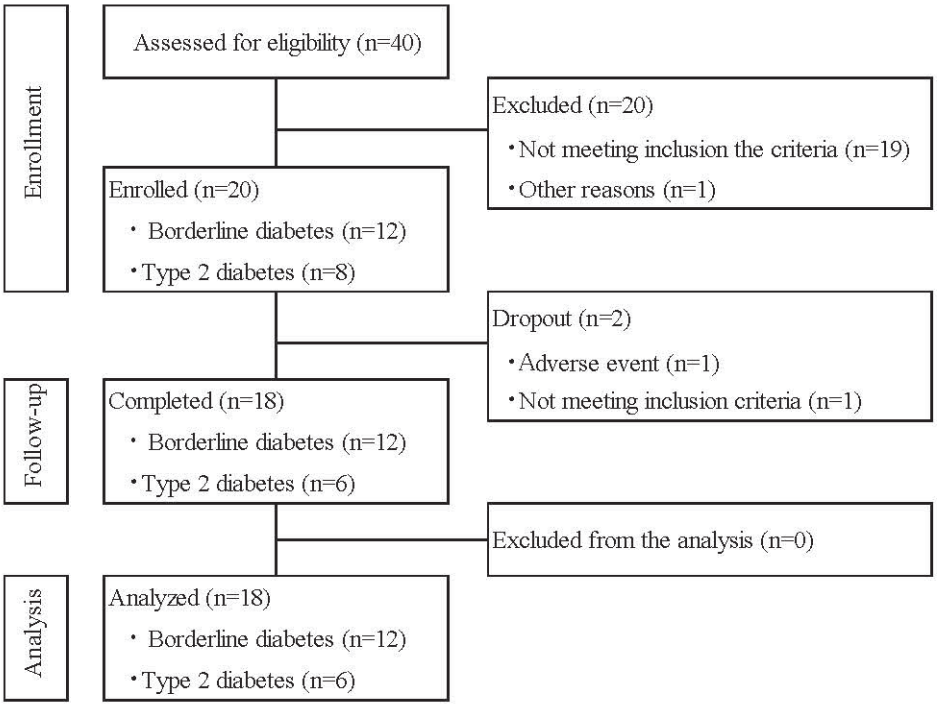

Fig. 1. Subject enrollment flow chart.

body fat percentage, waist circumference, systolic blood pressure, diastolic blood pressure, and pulse. Height and BMI were measured only before starting consumption. We measured 45 general biochemical and hematological parameters in the blood examination. Six parameters were included in the general urine analysis: urine protein, urine sugar, urine urobilinogen, occult blood, urine specific gravity, and urinary microalbumin. These parameters were measured at all of the examination days. Another 16 parameters were measured at the first day of consumption and 12 weeks after starting consumption: apolipoproteins (apoA-I, apoA-II, apoB, apoC-II, apoC-III, apoE), low-density lipoprotein (LDL-C)/apoB, apoC-II/apoC-III, β2-microgrobulin, cystatin-C, non-specific immunoglobulin-E (total IgE), growth hormone, somatomedin-C, leptin and adiponectin in the blood, and N-acetyl-β-D-glucosaminidase activity in the urine. The principal physician interviewed each subject about living habits, abdominal symptoms, defecation conditions, occurrence of subjective symptoms in the physical conditions, and adverse events. Each subject recorded their meals consumed within 3 days before each examination day. The nutritional components (energy, protein, fat, and carbohydrate) were calculated by dietitians. Each subject recorded the number of steps walked every day, which was measured by a pedometer, during the consumption period.

Statistical Analysis

Each measured value was expressed as the mean ± standard deviation (S.D.). Dunnett's test was used to compare between data on physical examinations, blood examinations, urine specific gravity, urinary microalbumin, survey of the nutritional components in the diet, and degree of exercise obtained on the first day of consumption and each examination day. A paired *t*-test was used to compare data on other parameters obtained at 12 weeks after starting consumption and at the first day of consumption. For the urine qualitative analysis, the Wilcoxon signed-rank test was used to compare between data obtained on the first day of consumption and on the other experimental days. All statistical analyses were performed using SPSS version 13.0 J (SPSS, Tokyo, Japan), and the level of significance was set at a two-sided value of under 5%.

RESULTS

In total, 12 borderline diabetes and 8 type 2 diabetes subjects were enrolled. The study flowchart and subject characteristics are shown in Fig. 1 and Table 1, respectively. One woman was terminated because she was lat-

Table 1. Subject characteristics.

Item	
Subjects (men/women)	9/9
Age (years old)	57.9 ± 7.4
Height (cm)	160.9 ± 6.9
Body weight (kg)	71.3 ± 15.8
BMI	27.5 ± 5.5
Body fat percentage (%)	31.4 ± 9.8
Waist circumference (cm)	92.9 ± 11.9
Systolic blood pressure (mmHg)	119.3 ± 13.1
Diastolic blood pressure (mmHg)	73.0 ± 9.8
Pulse (beats/min)	69.7 ± 11.0

er found to be ineligible after 2 weeks of consumption. Another man was also terminated. He showed increased hepatic markers (e.g., total bilirubin (T-Bil), 1.70 mg/dL; direct bilirubin (D-Bil), 0.600 mg/dL; aspartate aminotransferase (AST), 318 U/L; alanine aminotransferase (ALT), 1158 U/L; and γ-glutamyl transpeptidase (γ-GTP), 130 U/L) after 4 weeks of consumption, but this decreased after 5 weeks of consumption (T-Bil, 1.00 mg/dL; D-Bil, 0.300 mg/dL; AST, 51 U/L; ALT, 248 U/L; and γ-GTP 92, U/L) and recovered to standard ranges after 9 weeks of consumption. These changes in the hepatic parameters were accordingly decided to be a transient increase that was not related to the intake of D-allulose. The subject, however, was terminated from the study as decided by the principal physician of this study. Finally, data of 18 subjects (12 borderline diabetes and 6 type 2 diabetes) aged between 42 and 70 years were analyzed. Of them, 2 were taking thiazolidinedione; 2, sulfonylurea; 1, biguanide; and 1, insulin preparation. There were no significant differences in nutritional intake (energy, protein, fat, and carbohydrate) and degree of exercise during the consumption period. Changes in the general blood biochemical parameters are shown in Table 2. Total cholesterol (T-Cho), LDL-C, remnant like particles cholesterol (RLP-C), haemoglobinA1c (HbA1c), and 3-hydroxybutyric acid (BOH) were significantly higher during the consumption period compared with that on the first day of consumption ($p < 0.05$). Although the values of total protein (TP), albumin (Alb), albumin globulin ratio (A/G), AST, alkaline phosphatase (ALP), lactase dehydrogenase (LDH), cholinesterase (CHE), γ-GTP, high-density lipoprotein (HDL-C), phospholipids (PL), uric acid (UA), sodium (Na), potassium (K), chlorine (Cl), inorganic phosphate (IP), magnesium (Mg), total ketone body, and acetoacetic acid (AcAc) differed significantly during the consumption period compared with those on the first day of consumption ($p < 0.05$), they remained within standard ranges. As for the hematological parameters, there were significant changes in white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) ($p < 0.05$), but they also remained within the standard range (Table 3).

The results of the urine analysis are shown in Table 4. The urine specific gravity significantly decreased between the first day of consumption and after 8 weeks of consumption ($p < 0.05$), but it remained within the standard range. No positive reactions were noted in urine urobilinogen during and after the consumption period. No significant changes were also observed in urine protein, sugar, and occult blood between the consumption periods and the values on the first day of consumption.

Table 5 shows the results of the physical examinations. Body fat percentage was significantly higher at 12 weeks and follow-up compared with the first day of consumption. The apoB, apoC-II/apoC-III, and β2-microgrobulin values varied significantly between after 12 weeks of consumption and the first day of consumption (Table 6). Meanwhile, there were no significant changes in total IgE, hormones (growth hormone and somatomedin-C), and adipocytokines (leptin and adiponectin) (data not shown).

Table 7 shows the adverse events during the consumption period. Twenty-nine episodes of adverse events occurred in 10 subjects: gastrointestinal symptoms (stomachache, stodginess, loose feces, abdominal pain, and constipation), infections (influenza, chill, common cold, pharyngeal pain, and nasal obstruction), musculoskeletal system and connective tissue disorders (tendovaginitis and low back pain), increases in blood biochemical parameters (ALP and ALT), and other symptoms (toothache, urticarial, menstrual pain, spinal canal stenosis, and cystitis). Although increases in ALP and ALT were observed at follow-up in one subject, these values reverted to standard ranges in a re-examination at 13 days after the follow-up. All adverse events, except for one episode of constipation, were evaluated to be unrelated to the test substance by the principal physician because these symptoms were transient and disappeared during D-allulose consumption period. The causal relationship between this constipation episode and the test substance was unclear.

DISCUSSION

This open study evaluated the safety of D-allulose in borderline diabetes and type 2 diabetes subjects. Participants ingested D-allulose for 12 weeks, and the safety profile was evaluated according to blood and urine parameters, physical examinations, and subjective symp-

Table 2. Changes in the general blood biochemical parameters after the long-term intake of D-allulose.

Item	Standard range	week 0	week 2	week 4	week 8	week 12	Follow-up
TP (g/dL)	6.5 ~ 8.2	7.23 ± 0.38	7.30 ± 0.30	7.28 ± 0.32	7.28 ± 0.35	7.36 ± 0.36	7.43 ± 0.33**
Alb (g/dL)	3.7 ~ 5.5	4.29 ± 0.31	4.39 ± 0.24*	4.38 ± 0.24	4.35 ± 0.24	4.39 ± 0.26	4.42 ± 0.23*
A/G	1.30 ~ 2.00	1.48 ± 0.20	1.53 ± 0.18	1.53 ± 0.20*	1.50 ± 0.19	1.50 ± 0.20	1.48 ± 0.18
T-Bil (mg/dL)	0.3 ~ 1.2	0.889 ± 0.557	0.939 ± 0.597	0.870 ± 0.490	0.910 ± 0.540	0.810 ± 0.480	0.800 ± 0.570
D-Bil (mg/dL)	0.4 >	0.320 ± 0.430	0.350 ± 0.468	0.306 ± 0.409	0.328 ± 0.398	0.294 ± 0.380	0.317 ± 0.474
I-Bil (mg/dL)	-	0.570 ± 0.230	0.589 ± 0.222	0.570 ± 0.190	0.583 ± 0.215	0.511 ± 0.149	0.483 ± 0.130
AST(U/L)	10 ~ 40	22.1 ± 8.6	22.9 ± 7.3	24.3 ± 7.3	23.9 ± 8.1	23.5 ± 9.6	25.3 ± 11.7*
ALT (U/L)	5 ~ 45	22.2 ± 9.1	22.5 ± 7.8	22.7 ± 8.1	21.7 ± 7.8	22.4 ± 10.9	25.4 ± 15.0
ALP (U/L)	104 ~ 338	226 ± 57	207 ± 52*	202 ± 48**	197 ± 48**	195 ± 45**	227 ± 72
LDH (U/L)	120 ~ 245	175 ± 30	177 ± 31	182 ± 32	182 ± 33	179 ± 36	188 ± 38**
CHE (U/L)	M245 ~ 495 W198 ~ 452	337 ± 65	330 ± 71	322 ± 67*	314 ± 64**	320 ± 65**	339 ± 68
γ-GTP (U/L)	M79 > W48 >	39.3 ± 18.0	36.7 ± 18.3	33.7 ± 18.3	29.6 ± 14.6*	30.9 ± 17.4*	39.1 ± 23.6
CPK (U/L)	M50 ~ 230 W50 ~ 210	184 ± 290	167 ± 249	182 ± 309	168 ± 289	194 ± 395	198 ± 398
T-Cho (mg/dL)	150 ~ 219	226 ± 37	237 ± 39	239 ± 44*	239 ± 40*	248 ± 45**	236 ± 36
HDL-C (mg/dL)	M40 ~ 80 W40 ~ 90	52.1 ± 10.6	49.4 ± 10.2	48.4 ± 10.9*	47.6 ± 9.9**	48.5 ± 9.2*	53.9 ± 10.1
LDL-C (mg/dL)	70 ~ 139	149 ± 29	162 ± 32**	165 ± 35**	167 ± 33**	171 ± 35**	157 ± 31
TG (mg/dL)	50 ~ 149	125 ± 48	129 ± 57	135 ± 65	124 ± 55	134 ± 50	135 ± 59
FFA (mEq/L)	0.10 ~ 0.81	0.510 ± 0.145	0.540 ± 0.210	0.503 ± 0.170	0.492 ± 0.206	0.460 ± 0.212	0.510 ± 0.210
RLP-C (mg/dL)	7.5 ≥	7.68 ± 3.96	8.42 ± 5.09	9.57 ± 5.98	8.92 ± 5.32	9.96 ± 5.38*	9.11 ± 5.14
PL (mg/dL)	150 ~ 250	224 ± 26	233 ± 28	237 ± 35*	229 ± 31	237 ± 34*	237 ± 27*
UA (mg/dL)	M3.6 ~ 7.0 W2.7 ~ 7.0	5.61 ± 1.19	5.26 ± 1.14*	5.24 ± 1.00*	5.07 ± 1.03**	5.23 ± 0.93*	5.18 ± 1.02**
BUN (mg/dL)	8.0 ~ 20.0	15.2 ± 3.2	14.8 ± 3.9	14.7 ± 3.4	14.3 ± 3.2	15.2 ± 3.8	14.7 ± 4.1
Cre (mg/dL)	M0.65 ~ 1.09 W0.46 ~ 0.82	0.660 ± 0.170	0.670 ± 0.173	0.670 ± 0.170	0.650 ± 0.170	0.660 ± 0.170	0.640 ± 0.170
Na (mEq/L)	135 ~ 145	141.7 ± 1.5	140.3 ± 1.7**	141.2 ± 1.1	141.4 ± 1.3	140.9 ± 1.0	140.8 ± 1.2
K (mEq/L)	3.5 ~ 5.0	4.20 ± 0.26	4.28 ± 0.24	4.32 ± 0.32	4.23 ± 0.31	4.29 ± 0.25	4.36 ± 0.25*
Cl (mEq/L)	98 ~ 108	104.2 ± 2.1	103.3 ± 1.70	103.3 ± 1.7	103.8 ± 2.1	103.3 ± 1.80	102.5 ± 1.4**
Ca (mg/dL)	8.2 ~ 10.0	9.18 ± 0.35	9.12 ± 0.25	9.27 ± 0.27	9.19 ± 0.33	9.27 ± 0.20	9.27 ± 0.25
IP (mg/dL)	2.5 ~ 4.5	3.44 ± 0.51	3.41 ± 0.35	3.44 ± 0.49	3.48 ± 0.44	3.64 ± 0.45*	3.63 ± 0.41
Mg (mg/dL)	1.7 ~ 2.6	2.14 ± 0.18	2.19 ± 0.16	2.18 ± 0.12	2.19 ± 0.13	2.19 ± 0.15	2.26 ± 0.12**
AMY (U/L)	39 ~ 134	61.2 ± 15.5	64.7 ± 17.5	70.4 ± 30.7	66.8 ± 17.9	67.3 ± 12.8	65.6 ± 17.4
Glucose (mg/dL)	70 ~ 109	126 ± 24	127 ± 25	132 ± 31	125 ± 26	131 ± 32	128 ± 34
Insulin (μU/mL)	2.2 ~ 12.4	7.76 ± 5.24	8.23 ± 5.41	8.14 ± 5.36	6.72 ± 4.22	7.69 ± 4.21	6.45 ± 4.10
HbA1c (%)	4.3 ~ 5.8	6.06 ± 0.63	6.07 ± 0.69	6.11 ± 0.70	6.03 ± 0.68	6.17 ± 0.67*	6.23 ± 0.73**
GA (%)	11.6 ~ 16.4	17.0 ± 3.45	17.1 ± 3.41	17.1 ± 3.54	16.9 ± 3.35	17.1 ± 3.38	17.0 ± 4.08
Total ketone body (μmol/L)	131 ≥	64.8 ± 35.1	83.1 ± 53.0	117.8 ± 135.2	130.1 ± 110.9*	84.2 ± 71.7	74.8 ± 45.4
AcAc (μmol/L)	55 ≥	21.2 ± 9.7	25.7 ± 15.2	34.4 ± 33.1	39.6 ± 31.3*	26.3 ± 18.8	24.7 ± 12.5
BOH (μmol/L)	85 ≥	43.6 ± 26.5	57.4 ± 38.6	83.4 ± 102.5	90.4 ± 80.8*	57.9 ± 53.5	50.7 ± 33.1

TP: total protein, Alb: albumin, A/G: albumin globulin ratio, T-Bil: total bilirubin, D-Bil: direct bilirubin, I-Bil: indirect bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactase dehydrogenase, CHE: cholinesterase, γ-GTP: γ-glutamyl transpeptidase, CPK: creatinine phosphokinase, T-Cho: total cholesterol, HDL-C: high-density lipoprotein, LDL-C: low-density lipoprotein, TG: triglyceride, FFA: free fatty acid, RLP-C: remnant like particles cholesterol, PL: phospholipids, UA: uric acid, BUN: blood urea nitrogen, Cre: creatinine, Na: sodium, K: potassium, Cl: chlorine, Ca: calcium, IP: inorganic phosphate, Mg: magnesium, AMY: serum amylase, HbA1c: haemoglobinA1c (measured using the Japan Diabetes Society guidelines), GA: glycoalbumin, AcAc: acetoacetic acid, BOH: 3-hydroxybutyric acid, M: men, W: women

There were significant differences from the week 0 value, as determined by Dunnett's test (*$p < 0.05$, **$p < 0.01$)

Table 3. Changes in the hematological parameters after the long-term intake of D-allulose.

Item	Standard range	week 0	week 2	week 4	week 8	week 12	Follow-up
WBC (/μL)	3500~9700	5782 ± 1399	6050 ± 1594	6057 ± 1392	6092 ± 1465	5913 ± 1340	6279 ± 1436*
RBC ($\times 10^4$/μL)	M438~577 W376~516	460 ± 39	459 ± 36	462 ± 37	460 ± 35	473 ± 37**	469 ± 38
Hb (g/dL)	M13.6~18.3 W11.2~15.2	13.9 ± 1.2	13.9 ± 1.1	13.9 ± 1.2	13.9 ± 1.0	14.1 ± 1.1	14.1 ± 1.1
Ht (%)	M40.4~51.9 W34.3~45.2	43.4 ± 3.3	43.8 ± 2.8	43.2 ± 3.1	43.2 ± 2.7	43.5 ± 2.7	43.6 ± 3.1
MCV (fL)	M86~104 W82~101	94.5 ± 3.4	95.5 ± 3.5*	93.6 ± 3.4	94.0 ± 4.2	92.1 ± 3.5**	93.1 ± 3.6**
MCH (pg)	M28.0~34.6 W26.3~34.7	30.3 ± 1.2	30.3 ± 1.0	30.0 ± 1.2	30.2 ± 1.1	29.8 ± 1.2**	30.0 ± 1.2*
MCHC (%)	M31.0~36.6 W30.0~36.6	32.0 ± 0.7	31.7 ± 0.9	32.1 ± 0.8	32.1 ± 1.0	32.4 ± 0.74*	32.3 ± 0.8
PLT ($\times 10^4$/μL)	14.0~37.9	23.8 ± 6.0	23.6 ± 6.0	23.8 ± 6.0	23.8 ± 5.5	24.0 ± 5.6	25.0 ± 6.4

WBC: white blood cells, RBC: red blood cells, Ht: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelets, M: men, W: women

There were significant differences from the week 0 value, as determined by Dunnett's test (*$p < 0.05$, **$p < 0.01$)

Table 4. Changes in urinary parameters after the long-term intake of D-allulose.

Item	Standard range	Grade	week 0	week 2	week 4	week 8	week 12	Follow-up
Urine protein	(-) ~ (±)	-	15	18	17	17	15	16
		±	1	0	1	0	1	0
		1+	1	0	0	1	2	2
		2+	1	0	0	0	0	0
		3+	0	0	0	0	0	0
		4+	0	0	0	0	0	0
Urine sugar	(-) ~ (±)	-	14	14	17	17	14	13
		±	2	1	0	0	1	2
		1+	0	1	0	1	2	0
		2+	0	2	0	0	0	1
		3+	1	0	1	0	1	1
		4+	1	0	0	0	0	1
Urine urobilinogen	(±)	-	0	0	0	0	0	0
		±	17	18	18	18	18	18
		1+	1	0	0	0	0	0
		2+	0	0	0	0	0	0
		3+	0	0	0	0	0	0
		4+	0	0	0	0	0	0
Occult blood	(-)	-	18	18	16	18	18	17
		±	0	0	1	0	0	1
		1+	0	0	1	0	0	0
		2+	0	0	0	0	0	0
		3+	0	0	0	0	0	0
		4+	0	0	0	0	0	0
Urine specific gravity	1.008~1.034		1.021 ± 0.007	1.019 ± 0.008	1.019 ± 0.007	1.017 ± 0.007*	1.019 ± 0.010	1.018 ± 0.008
Urinary microalbumin (mg/g · Cr)	18.0 >		73.9 ± 243.2	28.6 ± 59.2	27.6 ± 60.9	35.0 ± 79.7	41.8 ± 99.6	54.7 ± 154.9

urine protein, urine sugar, urine urobilinogen and occult blood. Values show the numbers of people. [-: negative, ±: false-positive, 1+: positive (mild), 2+: positive]

There was no significant difference in urine protein, urine sugar, urine unbilinogen, and occult blood from week 0 value, as determined by the Wilcoxon signed-rank test

There was a significant difference in urine specific gravity between week 0 and week 8 by Dunnett's test (*$p < 0.05$)

There was no significant difference in urine microalbumin by Dunnett's test

Table 5. Changes in anthropometric indicators after the long-term intake of D-allulose.

Item	week 0	week 2	week 4	week 8	week 12	Follow-up
Body weight (kg)	71.1 ± 15.7	71.3 ± 15.7	71.0 ± 15.5	71.0 ± 15.4	71.2 ± 15.6	71.0 ± 15.3
Body fat percentage (%)	31.0 ± 10.3	31.5 ± 10.3	31.3 ± 10.8	31.4 ± 10.3	32.1 ± 10.7**	31.7 ± 10.4*
Waist circumference (cm)	93.4 ± 12.1	93.6 ± 11.9	93.4 ± 11.9	92.9 ± 12.1	92.6 ± 11.7	92.5 ± 11.7
Systolic blood pressure (mmHg)	119.3 ± 13.3	116.9 ± 16.7	118.0 ± 14.0	118.5 ± 14.5	121.6 ± 15.6	123.9 ± 14.2
Diastolic blood pressure (mmHg)	74.1 ± 9.5	73.6 ± 12.3	72.3 ± 10.1	73.9 ± 10.0	72.8 ± 12.2	75.0 ± 11.4
Pulse (beats/min)	71.4 ± 10.1	72.0 ± 8.6	69.7 ± 8.4	69.9 ± 9.8	69.9 ± 11.1	71.8 ± 10.3

There were significant differences from the week 0 value, as determined by Dunnett's test (*p < 0.05, **p < 0.01)

Table 6. Changes in other parameters in blood and urine after the long-term intake of D-allulose.

Item	Standard range	week 0	week 12
ApoA-I (mg/dL)	M119~ W126~	137 ± 21	134 ± 22
ApoA-II (mg/dL)	M25.9~ W24.6~	30.3 ± 4.2	28.7 ± 4.8
ApoB (mg/dL)	M73~109 W66~101	120 ± 23	137 ± 29**
ApoC-II (mg/dL)	M1.8~4.6 W1.5~3.8	5.00 ± 1.78	5.39 ± 2.03
ApoC-III (mg/dL)	M5.8~ W5.4~9.0	9.92 ± 2.19	10.16 ± 2.49
ApoE (mg/dL)	M2.7~4.3 W2.8~4.6	4.75 ± 0.94	4.90 ± 1.04
LDL-C/apoB	-	1.24 ± 0.10	1.25 ± 0.10
ApoC-II/ApoC-III	-	0.506 ± 0.133	0.530 ± 0.133*
β2-microgrobulin (mg/dL)	0.9~1.9	1.61 ± 0.32	1.48 ± 0.27**
Cystatin-C (mg/L)	0.40~0.91	0.951 ± 0.123	0.975 ± 0.139
NAG activity (U/L)	0.0~10.0	6.98 ± 4.99	5.82 ± 6.73

NAG activity: N-acetyl-β-D-glucosaminidase activity, M: men, W: women
There were significant differences between week 0 and week 12, as determined by a paired t-test (*p < 0.05, **p < 0.01)

Table 7. The results of adverse events.

Adverse events	Number of episodes	Incidence(%)
Gastrointestinal symptoms	11	44.4
Infections	8	38.9
Disorders of musculoskeletal system and connective tissue	2	11.1
Abnormal changes in blood biochemical parameters	2	11.1
Others	6	27.8

toms. No serious clinical problems occurred during the consumption period, indicating that D-allulose is safe for individuals with borderline diabetes and type 2 diabetes.

We also evaluated the effect of D-allulose on markers of hepatic and renal functions and found significant improvements in hepatic functions (γ-GTP and ALP). Nagata *et al.* (2015) reported that liver enzyme activities were significantly lowered by D-allulose diet in rats, whereas gene expression of a transcriptional modulator of fatty acid oxidation was enhanced. D-allulose was also showed to prevent abdominal and hepatic fat accumulation in *ob/ob* mice (Itoh *et al.*, 2015). These results suggest that D-allulose could improve hepatic functions by mitigating fatty liver. In this study, UA and β2-microgrobulin values were significantly improved, and other indicators of renal function were all within the standard ranges. No serious changes related to the test substance were observed. Urine analysis findings were also not signifi-

cantly changed. Urine protein and occult blood were positive in two subjects each; however, these were not related to the changes in the renal blood parameters and were consequently evaluated to be unrelated with the intake of D-allulose by the principal physician. Urine sugar was positive in 4 subjects, but changes in urine sugar were transient, and their blood glucose concentration remained high during the consumption period. Hence, those changes were judged as individual physiological variations by the principal physician. These results indicate that long-term ingestion of D-allulose does not cause serious problems in renal functions in borderline diabetes and type 2 diabetes.

With respect to the effect of D-allulose on indicators of glucose and fat metabolism, HbA1c was significantly increased after 12 weeks of consumption and during follow-up compared to the first day of consumption. While D-allulose was reported to improve postprandial glucose metabolism (Hayashi *et al.*, 2010; Hossain *et al.*, 2011; Iida *et al.*, 2008; Matsuo and Izumori, 2006), HbA1c reflects the mean blood glucose over 2-3 months, which is calculated from seven blood glucose extractions (drawn before and 90 min after breakfast, lunch, and dinner, and before bedtime; McCarter *et al.*, 2006). However, no variations were also found in other indicators of fasting glucose metabolism (i.e., glucose and insulin). These findings raise the possibility that 12-week continuous ingestion of D-allulose might not affect fasting glucose metabolism in borderline diabetes and type 2 diabetes subjects. Moreover, this HbA1c fluctuation appears to be a seasonal fluctuation. HbA1c was reported to be highest in winter-spring and lowest in summer-autumn in most diabetes patients (Sakura *et al.*, 2010; Tseng *et al.*, 2005). Because this study was conducted from summer to winter, the value for HbA1c is supposed to increase gradually. The slight increased values for the body fat percentage after 12 weeks of consumption and during follow-up support this seasonal variation as it is generally accepted that the body fat of humans tend to rise in the transition from summer to winter. It was also reported that cholesterol has a seasonal variation, with a peak in the winter and a trough in the summer (Ockene *et al.*, 2004). There were also significant increases in T-Cho and LDL-C during the consumption period compared with the values on the first day of consumption. The RLP-C value also elevated significantly after 12 weeks of consumption. However, the mean values of T-Cho, LDL-C, and RLP-C at week 0 of consumption were higher than the standard ranges, and the majority of subjects had hypercholesterolemia. Hence, the subjects in our study might be susceptible to the seasonal variation due to inadequate capacity for cholesterol control.

Furthermore, the indicators related with ketone body increased significantly after 8 weeks of consumption compared to the values on the first day of consumption. As indicated above, these changes were attributed to increase in fat oxidation by D-allulose, which was shown in some animal and human reports (Iwasaki *et al.*, 2018; Kimura *et al.*, 2017; Nagata *et al.*, 2015; Ochiai *et al.*, 2014). The enhanced fat oxidation might be associated with lipoprotein lipase (LPL) activity and the quantity of triglyceride, which indicated no significant variation in this experiment. LPL catalyzes the hydrolysis of the triacylglycerol component of circulating chylomicrons and very-low-density lipoproteins (VLDLs), thereby providing non-esterified fatty acids and 2-monoacylglycerol for tissue utilization (Mead *et al.*, 2002). In this process, LDL is converted from VLDL. Matsuo *et al.* (2001) reported that soleus LPL activity was significantly higher in D-allulose-fed rats than in cellulose- or fructose-fed rats. Moreover, LPL activity depends on the ratio of apoC-II/apoC-III expression. A high ratio of apoC-II/apoC-III promotes LPL activity, while a low apoC-II/apoC-III ratio inhibits LPL function (Gonzales and Orlando, 2007). ApoC-II and apoC-III did not differ between the first day of consumption and after 12 weeks of consumption in our study. However, apoC-II/apoC-III ratio was significantly increased, showing that D-allulose can promote LPL activity, and consequently, the catabolism from VLDL to LDL might increase. Some reports showed that LDL-C increases after the intake of food ingredients (Foster *et al.*, 2010; Harris, 1996); however, these fluctuations were temporal. For example, a continuous 3- and 6-month low-carbohydrate diet was reported to increase LDL-C. However, plasma LDL-C concentrations over 12 months were similar to the value on the first day of consumption, and the changes in LDL-C concentrations did not statistically differ between groups (Foster *et al.*, 2010). Ingestion of n-3 fatty acids also consistently increased LDL-C concentrations in short-term studies within 10 weeks (Morgan *et al.*, 1995; Mori *et al.*, 2000). By contrast, most of studies conducted within 6-12 months showed only a small and statistically insignificant 5% increase in LDL-C (Eritsland *et al.*, 1995; Harris, 1996). T-Cho and LDL-C were increased in rats fed with D-allulose for 52 days (Chung *et al.*, 2012). Meanwhile, T-Cho did not increase in rats fed the D-allulose diet for 90 days, 12 months, and 18 months (Matsuo *et al.*, 2012; Yagi and Matsuo, 2009). In previous studies, LDL-C did not vary with 12-week D-allulose ingestion in both healthy and obese subjects (Han *et al.*, 2018; Hayashi *et al.*, 2010). Thus, experiments with longer term ingestion of D-allulose (more than

12 weeks) in humans with hypercholesterolemia might be needed.

Elevated cholesterol concentrations is generally accepted to cause CVD, which was reported to originate from strongly atherogenic small dense LDL (sdLDL) (Frcpath *et al.*, 2000; Koba *et al.*, 2008). Hirano *et al.* (2005) demonstrated that the LDL-C/apoB ratio was significantly correlated with LDL size and that the sdLDL ratio for total LDL can increase when the LDL-C/apoB ratio is less than 1.20). The LDL-C/apoB ratios in our results were higher than 1.2, and they did not change from the first day of consumption until after 12 weeks of consumption. Other blood parameters varied before and after consumption, but their mean values were within the standard ranges, and no serious variations were observed.

Twenty-nine adverse events occurred during the experiment; of these, only constipation might be related to the test substance. Although the association between the constipation episode in one subject and the test substance was unclear, it was judged to be not serious by the principal physician due to symptomatic improvement at follow-up.

This study has some limitations. First, the small sample size limits the generalizability of the findings to the general population. Second, we could not completely exclude other influencing factors such as seasonal variations and individual subject characteristics because this was an open study without a control group.

In conclusion, long-term D-allulose ingestion is safe for borderline diabetes and type 2 diabetes; no serious clinical problems occurred after 12 weeks of continuous D-allulose ingestion. Additionally, some indicators of hepatic function improved. These findings indicate that D-allulose, an almost zero calorie sweetener with the potential to suppress postprandial hyperglycemia and fat mass accumulation, might be useful in diabetes.

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