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# Effects of D-allulose on body fat accumulation in rats fed severely carbohydrate-restricted diets containing beef tallow or soybean oil

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## ABSTRACT

**Purpose:** The carbohydrate-restricted diet has been recognized to be effective into preventing and alleviating lifestyle-related diseases, such as obesity and type 2 diabetes. The rare sugar D-allulose is a functional monosaccharide with anti-obesity effects. In the present study, we examined the effects of dietary D-allulose on body fat accumulation in rats fed severely carbohydrate-restricted diets containing high concentrations of different fats, beef tallow, or soybean oil.
**Methods:** Male Wistar rats (n = 35, 3-week-old) were divided into 5 groups: One chow-fed control (C) group, and four carbohydrate-restricted groups, namely, beef tallow (B), beef tallow + D-allulose (BA), soybean oil (S), and soybean oil + D-allulose (SA), with free access to the diet and water for 8 weeks. The B and BA diets contained 23% beef tallow and 2% soybean oil, whereas the S and SA diets contained 25% soybean oil. Furthermore, the BA and SA diets contained 5% D-allulose.
**Results:** The final body weight, weight gain, and food intake were significantly higher, and food efficiency was significantly lower in the control group compared to the other carbohydrate-restricted groups. Intra-abdominal adipose tissue, carcass fat, and total body fat weights were not influenced by dietary fat type or D-allulose supplementation, except for the epididymal adipose tissue weight. In contrast, carbohydrate restriction suppressed body weight gain in rats, but remarkably increased body fat accumulation.
**Conclusion:** Under carbohydrate-restricted conditions, no anti-obesity effects of dietary D-allulose were observed, regardless of the dietary fat type. The causes of these effects are unknown. However, they may be influenced by a very low carbohydrate and high protein diet. Further research is required to elucidate the effects of D-allulose under various nutrient compositions with different fat, carbohydrate, and protein energy ratios.

**Keywords:** carbohydrate; D-allulose; beef tallow; soybean oil; body fat; rat

## INTRODUCTION

Obesity is defined as a state in which body fat, particularly visceral fat, increases excessively to the extent that it causes pathological abnormalities. The prevalence of various obesity-

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**Conflict of Interest**

There are no financial or other issues that might lead to conflict of interest.

**Author Contributions**

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associated diseases continues to increase worldwide [1,2]. Obesity is a chronic disease that develops via complex mechanisms. Its onset is thought to be primarily due to an energy-dense diet and inactive lifestyle [3]. Globally, the prevalence of obesity is rapidly increasing among children, adolescents, and adults in countries with high dietary fat and sucrose intakes [4]. Obesity is associated with various chronic diseases collectively known as metabolic syndrome, among which the following are highly correlated: dyslipidemia [5], type 2 diabetes mellitus [6], and non-alcoholic fatty liver [7], and cardiovascular diseases such as heart failure and coronary artery disease [8]. For these reasons, it is important to prevent and eliminate obesity, especially to suppress the onset of metabolic syndrome in middle-aged individuals.

In recent years, carbohydrate-restricted diets (very low-carbohydrate diets) have attracted attention as an effective dietary therapy to prevent and improve lifestyle-related diseases, such as obesity and type 2 diabetes [9]. This diet restricts carbohydrate consumption compared to the average diet. Therefore, carbohydrate-rich foods (including sugar, bread, and pasta) are restricted and replaced with foods high in fats and proteins (beef, pork, chicken, fish and shellfish, eggs, dairy products, soy products, seeds, etc.) [10]. The anti-obesity and anti-diabetic effects of a carbohydrate-restricted diet are based on the suppression of postprandial hyperglycemia and hyperinsulinemia, resulting in abdominal fat deposition, glycation, and oxidative stress, which are risk factors for the development of hypertension and cardiovascular disease [11,12]. Carbohydrates are the only nutrients that strongly increase postprandial blood glucose concentration in diabetes [12,13]. In 2013, the American Diabetes Association recommended a carbohydrate-restricted diet as the first option for diabetes treatment [14].

Rare sugars are defined as monosaccharides and their derivatives that are rarely found in nature compared to common sugars, such as D-glucose and D-fructose (International Society of Rare Sugars), and they are used in functional foods, supplements, and agricultural fertilizers. Recent research has suggested that rare sugars may have beneficial health effects when used as low-calorie sweeteners and bulking agents to replace sucrose and energy-containing polysaccharides [15-17]. For a quarter of a century, rare sugars such as D-allulose (D-psicose), D-sorbose, D-tagatose, and various L-sugars have been developed as alternative sweeteners [18-20]. D-Allulose, in particular, is the C-3 epimer of D-fructose, has zero calories, and is mildly sweetened (approximately 70%) relative to sucrose. The anti-obesity effects of D-allulose are due to multiple mechanisms, including the suppression of lipogenesis in the liver and adipose tissue [18,21] and increased energy expenditure [22].

In the present study, we examined the effects of dietary D-allulose on body fat accumulation in rats fed severely carbohydrate-restricted diets containing high concentrations of different fats, beef tallow, or soybean oil. Soybean oil represents fat derived from plant-based diets, and beef tallow represents fat derived from animal-based diets. Different types of dietary fats were used because plant-based carbohydrate-restricted diets have lower coronary heart disease mortality rates than animal-based diets [23-25].

## METHODS

All animal procedures were approved by the Animal Care and Use Committee for Kagawa University (approval number: 17629).


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## Materials

Beef tallow and soybean oil were purchased from Yamakei Industry Co. Ltd. (Osaka, Japan), with the following composition: beef tallow, 29.0% palmitic acid, 9.2% stearic acid, 26.7% oleic acid, 3.6% linoleic acid; soybean oil, 10.3% palmitic acid, 3.8% stearic acid, 24.3%, oleic acid, 52.7% linoleic acid, 7.9% linoleic acid, and α-linolenic acid. D-allulose was obtained from the International Institute of Rare Sugar Research and Education (Kagawa, Japan). The vitamin and mineral mixtures (AIN-76A) were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). Other chemical reagents were purchased from FUJIFILM Wako Pure Chemicals (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan).

## Animals and diets

Male Wistar rats (n = 35, 3-week-old) were purchased from Japan SLC (Shizuoka, Japan), adapted to the laboratory environment for 3 days, and randomly divided into 5 groups. Rats fed a commercial rodent diet (MF, Oriental Yeast Co. Ltd.) were established as the control (C) group. The remaining animals were divided into the following 4 groups: beef tallow (B), beef tallow + D-allulose (BA), soybean oil (S), and soybean oil + D-allulose (SA). The dietary composition of each group is presented in **Table 1**. The B and BA diets contained 23% beef tallow and 2% soybean oil, whereas the S and SA diets contained 25% soybean oil. Furthermore, the BA and SA diets contained 5% D-allulose.

## Experimental design

All animals were housed in individual cages at a room temperature of 22 ± 1°C, approximately 60% humidity, and a light/dark cycle with a light period of 8:00 to 20:00, with free access

**Table 1.** Composition of experimental diets

| Diets | C | B | BA | S | SA |
|---|---|---|---|---|---|
| Ingredients (g/kg diet) | 0.0 | 500.0 | 500.0 | 500.0 | 500.0 |
| Casein | 0.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| DL-Methionine | 0.0 | 149.0 | 99.9 | 149.9 | 99.9 |
| Corn starch | 0.0 | 0.0 | 50.0 | 0.0 | 50.0 |
| D-allulose | 0.0 | 230.0 | 230.0 | 0.0 | 0.0 |
| Beef tallow | 0.0 | 20.0 | 20.0 | 250.0 | 250.0 |
| Soybean oil | 0.0 | 35.0 | 35.0 | 35.0 | 35.0 |
| Mineral mixture[1)] | 0.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Vitamin mixture[1)] | 0.0 | 50.0 | 50.0 | 50.0 | 50.0 |
| Cellulose | 0.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Chorine chloride | 0.0 | 0.1 | 0.1 | 0.1 | 0.1 |
| Butylhydroxytoluene | 1,000.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| MF[2)] | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 |
| Total weight | | | | | |
| Nutritional composition (g/kg diet) | | | | | |
| Carbohydrate[3)] | 553.0 | 143.3 | 150.2 | 143.3 | 150.2 |
| Fat | 51.0 | 250.6 | 250.3 | 251.1 | 250.7 |
| Protein | 231.0 | 434.1 | 434.1 | 434.1 | 434.1 |
| Energy (kcal/g)[4)] | 3.6 | 4.6 | 4.4 | 4.6 | 4.4 |
| Energy ratio (%) | | | | | |
| Carbohydrate | 61.5 | 12.6 | 13.1 | 12.5 | 13.1 |
| Fat | 12.8 | 49.4 | 49.1 | 49.5 | 49.1 |
| Protein | 25.7 | 38.0 | 37.8 | 38.0 | 37.8 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

C, control; B, beef tallow; S, soybean oil; A, D-allulose.
[1)]Based on the AIN-76 mixture (Oriental Yeast Co., Ltd., Tokyo, Japan).
[2)]Commercial rodent diet (Oriental Yeast Co., Ltd.).
[3)]Carbohydrate present D-allulose, 86.3% of corn starch, 11.8% of mineral mixture, and 98.1% of vitamin mixture.
[4)]Carbohydrate (excluding D-allulose), fat, and protein provide energy at 4, 9, and 4 kcal/g, respectively.


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to experimental food and water for 8 weeks. Body weight and food intake were recorded daily. After the experimental period, all rats were euthanized by decapitation with guillotine at 09:00 a.m. without fasting. Blood was collected to obtain the serum. The heart, liver, kidney, spleen, and intra-abdominal adipose tissues (epididymal, perirenal, and mesenteric) were immediately removed and stored at −80°C until analysis. The head and remaining intraperitoneal and intrathoracic tissues were then removed to serve as carcass samples and stored at −20°C until carcass fat analysis.

### Biochemical analyses

Serum concentrations of glucose, insulin, triglyceride, total cholesterol, high density lipoprotein (HDL)-cholesterol, free fatty acids, phospholipids, total protein, albumin, albumin/globulin ratio, leptin, and adiponectin were determined using kits (Glucose CII-test, LBIS Rat Insulin ELISA Kit, Triglyceride E-Test, Cholesterol E-Test, HDL-Cholesterol E-Test, NEFA C-Test, Phospholipids C-Test, and A/G B-Test, Mouse/Rat Leptin ELISA Kit [Morinaga Institute of Biological Science, Inc., Kanagawa, Japan], and LBIS Adiponectin Mouse/Rat ELISA Kit [FUJIFILM Wako Pure Chemicals], respectively). Other serum biochemical tests, including aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bile acid, urea nitrogen, creatinine, sodium, potassium, chlorine, calcium, phosphorus, acetoacetic acid, β-hydroxybutyric acid, and total ketone bodies, were performed by FUJIFILM Corporation (Tokyo, Japan). The liver glycogen content was determined as described by Lo et al. [26]. The liver lipids were extracted using the method described by Folch et al. [27], and liver triglyceride and cholesterol contents were determined using Triglyceride E-Test, and Cholesterol E-Test, respectively (FUJIFILM Wako Pure Chemicals). Carcass fat was analyzed using the method described by Mickelsen and Anderson [28]. Total body fat was calculated as described by Paik and Yearick [29].

### Data analyses

Dunnett's test was used to compare Group C with the other 4 groups. Data from groups B, BA, S, and SA were tested for the effects of D-allulose and dietary fats using a 2-way analysis of variance (ANOVA). If significant differences were found in each element, the Tukey-Kramer test for multiple comparisons was performed. Statistical significance was set at $p < 0.05$. Excel Statistics (Social Survey Research Information Co. Ltd., Tokyo, Japan) was used for the data analysis.

## RESULTS

### Body weight, food intake, tissue weight, and body fat

Dunnett's test results showed that the final body weight, weight gain, and food intake were significantly higher and food efficiency was significantly lower in Group C than in the other groups (**Table 2**), indicating that carbohydrate restriction reduces body weight gain in rats. In addition, except for Group C, these values did not differ among the other 4 groups. Based on the 2-way ANOVA results, food efficiency was significantly influenced by dietary fat type; however, the values did not differ significantly among Groups B, BA, S, and SA in the Tukey-Kramer test. Kidney weight was significantly higher in Group BA than in Group C, whereas heart, liver, and spleen weights did not differ among the 5 groups. The animal-based diet (Group B) increased intra-abdominal adipose tissue and total body fat weight, while D-allulose tended to suppress these increases. However, intra-abdominal adipose


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**Table 2.** Body and tissue weights, food intake and body fat

| Groups | C | B | BA | S | SA | 2 × 2 ANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | F | A | F × A |
| Body wight | | | | | | | | |
| Initial (g) | 98.4 ± 1.0 | 98.4 ± 1.0 | 98.3 ± 0.9 | 98.4 ± 0.8 | 98.1 ± 1.0 | 0.63 | 0.40 | 0.23 |
| Final (g) | 306.5 ± 5.7 | 282.4 ± 4.1* | 271.4 ± 4.7* | 278.6 ± 5.4* | 280.2 ± 5.4* | 0.67 | 0.42 | 0.28 |
| Gain (g) | 208.1 ± 6.6 | 184.0 ± 4.3* | 173.1 ± 5.1* | 180.2 ± 6.0* | 182.1 ± 5.3* | 0.63 | 0.40 | 0.23 |
| Food intake (g/d) | 15.9 ± 0.2 | 11.2 ± 0.2* | 11.0 ± 0.2* | 10.7 ± 0.2* | 10.9 ± 0.3* | 0.22 | 0.85 | 0.36 |
| Food efficiency (g/g) | 0.228 ± 0.005 | 0.286 ± 0.003* | 0.275 ± 0.004* | 0.292 ± 0.006* | 0.291 ± 0.003* | **0.02** | 0.16 | 0.25 |
| Tissue weights | | | | | | | | |
| Heart (mg) | 771 ± 21 | 652 ± 80 | 713 ± 19 | 752 ± 33 | 778 ± 24 | 0.09 | 0.35 | 0.71 |
| Liver (g) | 10.52 ± 0.39 | 9.99 ± 0.53 | 9.74 ± 0.36 | 9.64 ± 0.27 | 10.43 ± 0.57 | 0.71 | 0.55 | 0.26 |
| Kidneys (g) | 1.96 ± 0.05 | 2.01 ± 0.05 | 2.19 ± 0.06* | 1.99 ± 0.07 | 2.17 ± 0.08 | 0.32 | 0.05 | 0.53 |
| Spleen (mg) | 674 ± 21 | 630 ± 24 | 631 ± 17 | 669 ± 19 | 660 ± 17 | 0.71 | 0.55 | 0.26 |
| Intra-abdominal adipose tissues | | | | | | | | |
| Epididymal (g) | 4.79 ± 0.19 | 6.46 ± 0.38* | 5.28 ± 0.29 | 5.32 ± 0.28 | 5.54 ± 0.32 | **0.04** | 0.19 | 0.15 |
| Perirenal (g) | 4.15 ± 0.25 | 6.52 ± 0.32* | 5.76 ± 0.47* | 5.82 ± 0.33* | 6.29 ± 0.40* | 0.93 | 0.81 | 0.15 |
| Mesenteric (g) | 3.79 ± 0.18 | 4.43 ± 0.24 | 3.92 ± 0.22 | 4.32 ± 0.25 | 4.32 ± 0.41 | 0.61 | 0.39 | 0.39 |
| Total (g) | 12.73 ± 0.56 | 17.31 ± 0.89* | 14.96 ± 0.94 | 15.46 ± 0.81 | 16.16 ± 1.10* | 0.73 | 0.39 | 0.12 |
| Carcass fat | | | | | | | | |
| (g) | 14.3 ± 0.8 | 17.1 ± 0.8 | 16.0 ± 1.2 | 16.2 ± 0.6 | 15.7 ± 1.0 | 0.54 | 0.37 | 0.69 |
| (%) | 9.8 ± 0.6 | 12.7 ± 0.5* | 12.1 ± 0.6* | 12.1 ± 0.4* | 11.7 ± 0.6* | 0.32 | 0.32 | 0.79 |
| Total body fat | | | | | | | | |
| (g) | 25.2 ± 1.2 | 31.9 ± 1.5* | 28.6 ± 2.0 | 29.3 ± 1.1 | 29.4 ± 1.8 | 0.61 | 0.35 | 0.32 |
| (%) | 8.2 ± 0.4 | 11.3 ± 0.5* | 10.5 ± 0.5* | 10.5 ± 0.3* | 10.5 ± 0.5* | 0.43 | 0.38 | 0.45 |

Values are means ± standard error for 7 rats. In the 2 × 2 ANOVA, words in bold are significant at $p < 0.05$.
C, control; B, beef tallow; S, soybean oil, A, D-allulose; F, dietary fat type; ANOVA, analysis of variance.
*$p < 0.05$ vs. Group C (Dunnett's test).

tissue, carcass fat, and total body fat weights were not influenced by dietary fat type or D-allulose supplementation in the 2-way ANOVA, except for the epididymal adipose tissue weight. In addition, there was no significant difference in epididymal adipose tissue weight between the B, BA, S, and SA groups in the Tukey-Kramer test. By contrast, carbohydrate restriction increased the body fat indicators. The total intra-adipose tissue and total body fat weights were significantly higher in Group B than in Group C. Carcass fat and total body fat percentages were significantly lower in Group C than in the other carbohydrate-restricted groups, and these values did not differ among the 4 groups. We detected no interaction between dietary fat type and D-allulose supplementation for any of the body or tissue weights, food intake, or body fat indicators.

## Concentrations of serum components

Dunnett's test results showed that the serum insulin concentration was significantly higher in Groups B, S, and SA than in Group C (**Table 3**). Serum leptin was significantly higher in Group S, and adiponectin was significantly higher in Groups BA, S, and SA than in Group C. The serum urea nitrogen concentration was significantly lower in Group C than in the other carbohydrate-restricted groups. Serum β-hydroxybutyric acid and total ketone bodies were significantly higher in Group SA than in Group C. The serum triglyceride concentration was significantly lower in Groups S and SA, and the AST level was significantly lower in Group S than in Group C. In contrast, D-allulose decreased serum insulin concentration and increased serum adiponectin concentration in the B and BA diet groups, whereas these parameters did not differ significantly in the 2-way ANOVA. Based on the 2-way ANOVA results, dietary fat type significantly influenced serum triglyceride, urea nitrogen, and leptin concentrations; however, the values did not differ significantly between Groups B, BA, S, and SA in the Tukey-Kramer test. The concentrations of other serum components were not influenced by dietary fat type or D-allulose supplementation. In addition, we detected


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**Table 3.** Concentrations of serum components

| Groups | C | B | BA | S | SA | 2 × 2 ANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | F | A | F × A |
| Glucose (mg/dL) | 135.8 ± 3.6 | 134.0 ± 7.3 | 127.7 ± 8.2 | 141.1 ± 11.3 | 138.9 ± 5.7 | 0.29 | 0.62 | 0.82 |
| Glycoalbumin (%) | 4.4 ± 0.2 | 4.1 ± 0.3 | 3.5 ± 0.5 | 4.4 ± 0.2 | 4.4 ± 0.2 | 0.06 | 0.27 | 0.36 |
| Insulin (ng/mL) | 2.8 ± 0.5 | 3.8 ± 0.2* | 3.4 ± 0.2 | 3.8 ± 0.2* | 3.7 ± 0.3* | 0.54 | 0.33 | 0.47 |
| Triglyceride (mg/dL) | 121.5 ± 19.7 | 81.3 ± 18.3 | 82.4 ± 18.9 | 49.1 ± 12.2* | 47.4 ± 9.7* | **0.04** | 0.98 | 0.93 |
| Free fatty acids (mEq/L) | 0.86 ± 0.05 | 0.66 ± 0.08 | 0.81 ± 0.10 | 0.74 ± 0.13 | 0.67 ± 0.12 | 0.77 | 0.73 | 0.35 |
| Total cholesterol (mg/dL) | 66.7 ± 3.4 | 74.2 ± 4.1 | 71.1 ± 6.0 | 62.7 ± 5.0 | 74.4 ± 6.1 | 0.46 | 0.43 | 0.19 |
| HDL-cholesterol (mg/dL) | 27.8 ± 2.9 | 32.1 ± 2.5 | 39.9 ± 4.9 | 33.5 ± 5.8 | 29.2 ± 5.2 | 0.33 | 0.71 | 0.21 |
| Phospholipid (mg/dL) | 144.5 ± 11.3 | 121.8 ± 5.3 | 139.7 ± 12.2 | 107.8 ± 13.2 | 121.7 ± 27.9 | 0.35 | 0.35 | 0.91 |
| Leptin (ng/mL) | 1.53 ± 0.67 | 2.48 ± 0.53 | 2.24 ± 0.47 | 5.23 ± 1.15* | 4.06 ± 1.26 | **0.02** | 0.45 | 0.62 |
| Adiponectin (pg/mL) | 21.2 ± 2.3 | 26.7 ± 2.1 | 32.2 ± 2.8* | 31.6 ± 3.2 | 31.3 ± 1.9* | 0.44 | 0.32 | 0.27 |
| Total protein (g/dL) | 5.9 ± 0.2 | 5.8 ± 0.2 | 5.7 ± 0.3 | 5.5 ± 0.4 | 5.8 ± 0.3 | 0.76 | 0.72 | 0.57 |
| Albumin (g/dL) | 3.5 ± 0.1 | 3.5 ± 0.1 | 3.4 ± 0.2 | 3.4 ± 0.2 | 3.6 ± 0.2 | 0.88 | 0.77 | 0.50 |
| Albumin/Globulin | 2.4 ± 0.2 | 2.3 ± 0.1 | 2.3 ± 0.1 | 2.1 ± 0.2 | 2.2 ± 0.1 | 0.75 | 0.72 | 0.51 |
| AST (IU/L) | 195.5 ± 13.4 | 169.3 ± 12.2 | 172.0 ± 15.9 | 150.9 ± 11.5* | 164.1 ± 8.3 | 0.30 | 0.52 | 0.67 |
| ALT (IU/L) | 58.7 ± 4.6 | 53.2 ± 3.5 | 66.0 ± 9.5 | 50.1 ± 4.6 | 55.3 ± 3.2 | 0.26 | 0.14 | 0.52 |
| γ-GTP (IU/L) | 0.23 ± 0.05 | 0.25 ± 0.10 | 0.19 ± 0.06 | 0.44 ± 0.16 | 0.23 ± 0.08 | 0.29 | 0.21 | 0.50 |
| LDH (IU/L) | 1,221 ± 92 | 1,128 ± 99 | 1,392 ± 341 | 946 ± 61 | 995 ± 60 | 0.14 | 0.42 | 0.57 |
| Total bile acid (μmol/L) | 4.2 ± 0.3 | 12.3 ± 6.7 | 8.7 ± 2.3 | 5.6 ± 0.5 | 4.9 ± 0.7 | 0.11 | 0.50 | 0.65 |
| Urea nitrogen (mg/dL) | 19.7 ± 0.9 | 28.0 ± 1.2* | 26.7 ± 1.3* | 25.4 ± 1.3* | 22.6 ± 1.9 | **0.04** | 0.18 | 0.60 |
| Creatinine (mg/dL) | 0.38 ± 0.02 | 0.38 ± 0.02 | 0.30 ± 0.02 | 0.33 ± 0.02 | 0.38 ± 0.02 | 0.30 | 0.67 | 0.67 |
| Sodium (μEq/L) | 132.0 ± 5.4 | 132.5 ± 5.3 | 128.7 ± 5.4 | 137.3 ± 4.4 | 144.7 ± 1.3 | 0.06 | 0.68 | 0.21 |
| Potassium (μEq/L) | 8.7 ± 0.7 | 10.4 ± 1.5 | 9.2 ± 1.4 | 7.2 ± 0.5 | 7.6 ± 0.4 | 0.06 | 0.69 | 0.45 |
| Chlorine (μEq/L) | 96.0 ± 3.3 | 97.8 ± 2.8 | 94.7 ± 3.4 | 98.3 ± 3.1 | 102.0 ± 1.2 | 0.17 | 0.92 | 0.23 |
| Calcium (mg/dL) | 9.5 ± 0.4 | 9.0 ± 0.4 | 8.5 ± 0.6 | 8.8 ± 0.6 | 9.1 ± 0.5 | 0.67 | 0.88 | 0.51 |
| Phosphorus (mg/dL) | 7.6 ± 0.2 | 7.5 ± 0.5 | 7.3 ± 0.6 | 6.6 ± 0.3 | 7.0 ± 0.3 | 0.23 | 0.91 | 0.51 |
| Acetoacetic acid (μmol/L) | 24.5 ± 3.5 | 18.0 ± 4.4 | 20.0 ± 3.3 | 22.1 ± 2.7 | 34.1 ± 9.6 | 0.13 | 0.24 | 0.40 |
| β-Hydroxybutyric acid (μmol/L) | 208.8 ± 24.4 | 290.0 ± 83.2 | 349.6 ± 31.9 | 317.1 ± 64.9 | 506.3 ± 98.7* | 0.22 | 0.10 | 0.39 |
| Total Ketone bodies (μmol/L) | 233.3 ± 27.2 | 307.7 ± 86.3 | 369.6 ± 32.8 | 339.3 ± 66.4 | 540.4 ± 107.4* | 0.21 | 0.11 | 0.38 |

Values are means ± standard error for 7 rats. In the 2 × 2 ANOVA, words in bold are significant at $p < 0.05$.
C, control; B, beef tallow; S, soybean oil, A, D-allulose; F, dietary fat type; ANOVA, analysis of variance; HDL, high density lipoprotein, AST, aspartate transaminase; ALT, alanine transaminase; γ-GTP, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase.
*$p < 0.05$ vs. Group C (Dunnett's test).

no interaction between dietary fat type and D-allulose supplementation at any serum component concentrations.

## Contents of liver glycogen and lipids

Dunnett's test results showed that liver glycogen was significantly higher in Group B than in Group C (**Table 4**). Liver total lipid and cholesterol contents were significantly higher in Groups BA and SA, and liver triglyceride content was significantly higher in Groups BA, S, and SA than in Group C (**Table 4**). Based on the 2-way ANOVA results, dietary fat type significantly influenced liver glycogen. Based on the Tukey-Kramer test, liver glycogen content was significantly lower in Group S than in Group B and was significantly lower in

**Table 4.** Contents of liver glycogen and lipids

| Groups (mg/g) | C | B | BA | S | SA | 2 × 2 ANOVA (p-value) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | F | A | F × A |
| Glycogen | 10.3 ± 0.9 | 16.6 ± 1.8*[a] | 13.2 ± 2.1[ab] | 9.1 ± 1.4[bc] | 5.9 ± 1.4[c] | **$2.4 \times 10^{-4}$** | 0.07 | 0.95 |
| Total lipid | 101.0 ± 7.5 | 113.6 ± 6.3 | 127.7 ± 4.3* | 113.2 ± 6.5 | 133.2 ± 4.5* | 0.65 | **$4.9 \times 10^{-3}$** | 0.59 |
| Triglyceride | 27.8 ± 1.4 | 56.3 ± 5.0 | 80.9 ± 6.9* | 69.9 ± 11.2* | 70.0 ± 14.6* | 0.89 | 0.23 | 0.24 |
| Cholesterol | 11.9 ± 1.0 | 16.5 ± 1.0 | 18.8 ± 1.2* | 15.6 ± 2.2 | 18.1 ± 1.9* | 0.65 | 0.16 | 0.93 |

Values are means ± standard error for 7 rats. In the 2 × 2 ANOVA, words in bold are significant at $p < 0.05$.
C, control; B, beef tallow; S, soybean oil, A, D-allulose; F, dietary fat type; ANOVA, analysis of variance.
*$p < 0.05$ vs. Group C (Dunnett's test).
Values with different superscripts are significantly different (excluding Group C; $p < 0.05$).


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Group SA than in Group BA. Liver triglyceride and cholesterol levels were not influenced by dietary fat type or D-allulose supplementation. In addition, we detected no interaction between dietary fat type and D-allulose supplementation for liver glycogen or lipid contents.

## DISCUSSION

This study yielded 3 important results regarding the anti-obesity effects. First, carbohydrate restriction suppressed body weight gain in rats but remarkably increased body fat accumulation. Several clinical studies have demonstrated the anti-obesity effects of carbohydrate-restricted diets [30-32]. Therefore, we expected that the ingestion of carbohydrate-restricted diets could decrease body fat accumulation in rats as well as serum glucose, glycoalbumin, and insulin levels. However, the results of this study suggest that serum glucose and glucoalbumin concentrations were unchanged by the carbohydrate-restricted diet, that is, very high-fat diets, whereas insulin concentration was increased, which may have induced body fat accumulation associated with insulin resistance. In this context, it is worth noting that the adipocytokines leptin and adiponectin increased with body fat accumulation, particularly in the beef tallow diet groups. We previously reported that a carbohydrate-restricted diet increased intra-abdominal fat mass in normal rats [33], and similar results have been reported in other studies [34,35]. Previously, Drabińska et al. [34] demonstrated that following a high-fat diet during adolescence to induce obesity in Wistar rats, carbohydrate restriction for 4 weeks in maturity resulted in slowing down weight gain, but higher adiposity compared to a standard diet, the latter resulting in a deterioration of liver parameters. Mel et al. [35] suggested that a minute amount of dietary carbohydrates in a carbohydrate-restricted diet can dramatically worsen insulin resistance in mice, and 10–25% carbohydrate in a carbohydrate-restricted diet can cause maximal insulin resistance in obese mice. This study partially supports these findings. In view of the above, the anti-obesity effects of carbohydrate-restricted diets may differ between rats and humans.

Secondly, surprisingly, no anti-obesity effects of dietary D-allulose were observed under carbohydrate-restricted conditions. Other studies have demonstrated that the mechanism underlying the anti-obesity effect of D-allulose is complex [36], through the suppression of hepatic lipogenesis [18,21] and increased energy expenditure [22]. Although the underlying molecular mechanisms remain largely unclear, we expect that D-allulose supplementation will be effective under high-carbohydrate (low-fat) conditions because D-allulose is metabolized as a monosaccharide with zero to extremely low calories and suppresses lipogenesis induced by carbohydrate intake. However, the anti-obesity effect of D-allulose in rats fed high-fat diets has not clearly been established; in fact, there have been adverse results [37-39]. Gou et al. [37] demonstrated that 5% supplementation of D-allulose with a high-fat diet (an energy ratio of 61.6% fat) for 4 weeks improved insulin resistance in male Wistar rats but did not suppress abdominal fat accumulation. Pongkan et al. [38] also suggested that oral administration of D-allulose (0.19 g/kg/day) for 12 weeks improved insulin resistance but did not suppress body weight gain and abdominal fat accumulation in rats fed a high-fat diet (an energy ratio of 60% fat). In addition, Chung et al. [39] reported that 2.5 and 5.0% addition of D-allulose in a high-fat diet (an energy ratio of 38.8% fat) for 8 weeks suppressed body weight gain but did not suppress white abdominal fat accumulation in male Sprague-Dawley rats. Our present study, using a 50–52% fat-to-energy diet, supports these previous studies.


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Thirdly, under carbohydrate restriction, the dietary fat type did not affect carcass fat, intra-abdominal adipose tissue, or total body fat weight. In addition, dietary fat type did not influence serum constituents and liver fat. We previously reported that compared with a diet high in unsaturated fat, a diet high in saturated fat promotes body fat accumulation and exacerbates insulin resistance in rats [40,41]. The possible mechanism by which insulin resistance increases due to increased dietary saturated fat intake is a decrease in cell membrane responsiveness to insulin action due to a decrease in binding affinity [42]. Other researchers have reported oversynthesis of ceramides due to carbohydrate-restricted diets rich in saturated fatty acids (such as palmitic acid), which may also induce insulin resistance [43]. The results of this study were inconsistent with those of previous studies. The causes of these effects are unknown; however, they may be influenced by a very low-carbohydrate and high-protein diet.

Regarding food safety, D-allulose has been approved and is generally accepted as safe for use in various foods and dietary supplements (GRAS Notice No. GRN 498; Food and Drug Administration, USA, 2017). In our previous chronic toxicity study using rats, dietary administration of 3% D-allulose resulted in normal weight gain without adverse health effects [44]. In a human study evaluating the consumption of rare sugar syrup containing 6% D-allulose for 12 weeks, Hayashi et al. [15] found that there were no adverse effects on lipid and carbohydrate metabolism and liver and kidney function in blood parameters. Therefore, the results of these studies demonstrate that D-allulose is a safe monosaccharide for animals and humans.

In the present study, dietary D-allulose significantly increased liver total lipids and tended to increase liver triglyceride and cholesterol contents, but the increase was not significant. In this regard, Kanasaki et al. [45] found that although serum triglyceride levels were not significantly different, hepatic triglyceride content was significantly higher in hamsters fed a 3% D-allulose diet than in control hamsters fed a normal diet. However, the reasons underlying these observations remain unclear.

Finally, regarding the composition of the experimental diets, the nutrient composition of the B and BA diets, or the S and SA diets, is slightly different because D-allulose (which has zero energy value) is replaced with cornstarch (the main carbohydrate source) when preparing the BA and SA diets (**Table 1**). To make the nutrient composition of diets with and without D-allulose the same, another zero-calorie sweetener, such as erythritol or cellulose, must be used. However, as these alternative carbohydrates (erythritol, cellulose, etc.) have different functionalities from D-allulose, there may be problems in using them as a control substance. Previously, we have tested various sugars (cornstarch, sucrose, fructose, cellulose, erythritol, etc.) as controls for D-allulose, but the results did not seem to differ significantly. Regarding these issues, we would like to consider using D-allulose and other zero-calorie sugars to completely match the nutritional composition in future studies.

## SUMMARY

In the present study, we examined the effects of dietary D-allulose on body fat accumulation in rats fed severe carbohydrate-restricted diets containing high concentrations of different fats, beef tallow, or soybean oil. However, carbohydrate restriction suppressed body weight gain in rats but remarkably increased body fat accumulation. Under carbohydrate-restricted


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conditions, no anti-obesity effects of dietary D-allulose were observed, regardless of the dietary fat type. The causes of these effects are unknown; however, they may be influenced by a very low-carbohydrate and high-protein diet. Further research is required to elucidate the effects of D-allulose under various compositions of nutrients.

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