Journal of Food Technology Research
2023 Vol. 10, No. 2, pp. 37-46
ISSN(e): 2312-3796
ISSN(p): 2312-6426
DOI: 10.18488/jftr.v10i2.3410
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Effect of simultaneous intake of rare sugars allitol and D-allulose on intra-abdominal fat accumulation in rats

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Article History
Received: 28 March 2023
Revised: 13 July 2023
Accepted: 24 July 2023
Published: 2 August 2023

Keywords
Allitol
Body fat
D-allulose
Rare sugar
Rats
Synergistic effect.

ABSTRACT

Allitol, one of the sugar alcohols, is a rare sugar produced by reducing D-allulose contained in Itea, a deciduous shrub belonging to the Saxifrageaceae family. We previously found that the long-term feeding of rats with a diet supplemented with Itea powder suppressed obesity. The present study aimed to further investigate whether this effect on body fat accumulation may be attributed to the simultaneous intake of allitol and D-allulose in Itea. Thirty-two male 3-week-old Wistar rats were randomly divided into four groups of eight. The rats had ad libitum access to the control (C), 3% allitol (A), 3% D-allulose (P), or 3% allitol + 3% D-allulose (AP) diet for 8 weeks, after which the rats were euthanized. D-Allulose significantly lowered the final body weight, weight gain, and quantities of perirenal, mesenteric, and total abdominal adipose tissue. Allitol significantly decreased the mesenteric adipose tissue weight. However, we found no combined effect between D-allulose and allitol on all abdominal fat. The liver glucose-6-phosphate dehydrogenase activity was significantly higher in the D-allulose-supplemented groups. Cecal weight, surface area, and content weight were found to be significantly higher in Groups A and AP than those in Groups C and P, suggesting a higher intestinal fermentability of allitol. Our results suggest that allitol and D-allulose have anti-obesity properties that are affected through different mechanisms but are non-synergistic.

Contribution/Originality: Allitol is a rare sugar produced by reducing D-allulose contained in Itea, a deciduous shrub belonging to the Saxifrageaceae family. In this study, we investigated for the first time the effect of the simultaneous intake of allitol and D-allulose on body fat accumulation under ad libitum feeding conditions in rats.

1. INTRODUCTION

Obesity is medically defined as conditions of increased body weight, and more specifically, the increase of adipose tissue to an extent sufficient to adversely affect health. The prevalence of this heterogeneous group of disorders has seen an alarming increase in developed countries (Listenberger et al., 2003; Rakhra, Galappaththy, Bulchandani, & Cabandugama, 2020). The causes of obesity are complex, but lifestyle factors, especially high-energy diets and inactive lifestyles, are risk factors for the development of obesity (Kim et al., 2000). Obesity rates among adults, children, and

adolescents are increasing rapidly in countries where high dietary fat intake is a major contributor to obesity (Canbakan et al., 2008). Obesity is associated with various comorbidities, collectively referred to as metabolic syndromes, among which the most relevant are hyperlipidemia (Fried et al., 2008), type Ⅱ diabetes (Montan et al., 2019), non-alcoholic fatty liver (Fan, Wang, & Du, 2018), and cardiovascular diseases, including heart failure or coronary heart disease (Lavie, Morshedi-Meibodi, & Milani, 2008). Hence, the prevention of obesity is essential to avoid metabolic syndrome, especially after middle age.

Some rare sugars have attracted attention in recent years as functional foods with anti-obesity effects. Rare sugars are monosaccharides and their derivatives that are inherently rarer than common sugars such as D-glucose and D - fructose (The International Society of Rare Sugars [ISRS]). They are used in supplements, functional food additives, and pharmaceuticals. Recently, several studies suggest that rare sugars exert benefits on health as low-energy carbohydrate sweeteners and bulking additives (Chung, Oh, & Lee, 2012; Hayashi et al., 2014; Matsuo et al., 2001a; Matsuo et al., 2001b; Ochiai et al., 2017). For the last three decades, some rare sugars, such as D-allulose (D-psicose), D-sorbose, D-tagatose, and L-sugars, have been developed as alternative sweeteners (Vastenavond et al., 2011; Yamada et al., 2014). Allitol is a sugar alcohol in which D- and L-hexoses are cross-linked in a central strategy called Izumoring (Granström, Takata, Tokuda, & Izumori, 2004) and is produced by reducing D-allulose (Figure 1) (Takeshita, Ishida, Takada, & Izumori, 2000). ISRS defines allitol as a rare sugar. This is because allitol is present in trace amounts in commercially available sugars and is difficult to synthesize using chemical methods (Hassanin et al., 2017). Previously, we demonstrated the anti-obesity effects of allitol in rats, which may be equal to or greater than that of D-allulose. Allitol has also been deemed partially safe for consumption (Shunsuke Higaki, Inai, & Matsuo, 2022a).

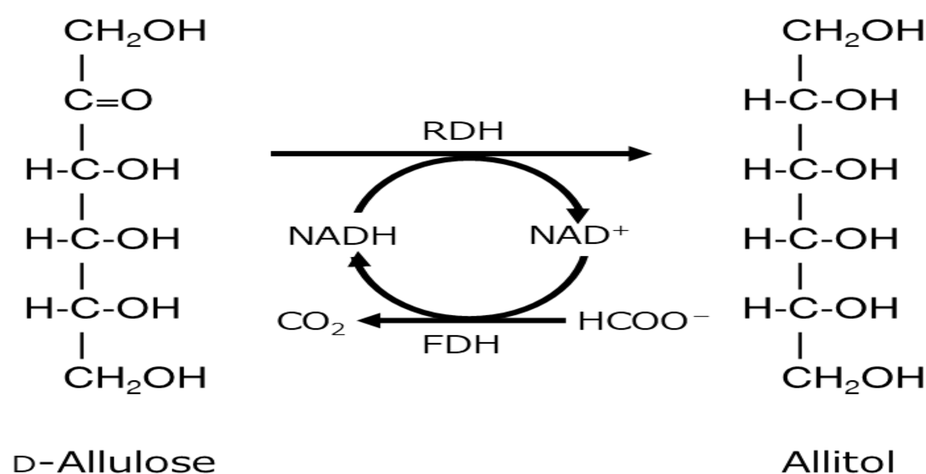

Figure 1. Diagram demonstrating the reduction of D-allulose to allitol. RDH, ribitol dehydrogenase. FDH, formate dehydrogenase.

In addition, allitol occurs naturally in *Itea*, a deciduous shrub belonging to the Saxifrageaceae family, along with D-allulose (Hough & Stacey, 1966). Our previous study revealed that the long-term feeding of rats with a dietary supplement of dried *Itea* powder suppresses body fat accumulation, even though contains very small amounts of rare sugars (Higaki, Inai, & Matsuo, 2022b, 2022c). We hypothesized that this effect is due to the simultaneous intake of D-allulose and allitol in *Itea*. Thus, we investigated the effect of the simultaneous intake of allitol and D-allulose on body fat accumulation, especially intra-abdominal fat accumulation to accurately assess the contribution of rare sugars to body fat in rats.

2. MATERIALS AND METHODS

All animal procedures were approved by the Animal Care and Use Committee of Kagawa University (approval number: 22621).

2.1. Materials

Rare sugars (allitol and D-allulose [D-psicose]) were received from the International Institute of Rare Sugar Research and Education (Kagawa, Japan). Soybean oil was purchased from Yamakei Industry Co. Ltd. (Osaka, Japan) and had the following composition: 10.3% palmitic acid, 3.8% stearic acid, 24.3% oleic acid, 52.7% linoleic acid, and 7.9% α-linolenic acid. Mineral and vitamin mixtures (AIN-76A) were obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan). The other ingredients in the diet were food-grade and obtained from Fonterra (Auckland, New Zealand), Mitsui DM Sugar Holdings (Tokyo), Nippon Paper Industries (Tokyo, Japan), and Oji Cornstarch (Tokyo, Japan), respectively. Other reagents were obtained from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan).

2.2. Animals, Diets, and Experimental Design

Thirty-two male 3-week-old Wistar rats were purchased from Japan SLC (Shizuoka, Japan) and were individually caged at 22 ± 1°C, with light from 08:00 to 20:00. For 3 days, the rats were fed MF, a commercial rodent diet (Oriental Yeast Co., Ltd., Tokyo, Japan), and had free access to water. High doses of allitol or D-allulose can induce diarrhea in rats (data not shown); hence, all rats were acclimated with 1–3% allitol and D-allulose incrementally for 9 days. After the acclimatization period of 12 days, rats were randomly divided into the following four groups: C (control), allitol (A), D-allulose (P), and allitol + D-allulose (AP) groups. The rats had the experimental diets and water *ad libitum* for 8 weeks. Table 1 shows the dietary composition of each group.

Table 1. Composition of experimental diets.

Ingredients	C	A	P	AP
Casein	200.0	200.0	200.0	200.0
DL-Methionine	3.0	3.0	3.0	3.0
Corn starch	349.9	349.9	349.9	349.9
Sucrose	300.0	270.0	270.0	240.0
D-Allulose (D-Psicose)	0.0	0.0	30.0	30.0
Allitol	0.0	30.0	0.0	30.0
Soybean oil	50.0	50.0	50.0	50.0
Mineral mixture[1]	35.0	35.0	35.0	35.0
Vitamin mixture[1]	10.0	10.0	10.0	10.0
Cellulose	50.0	50.0	50.0	50.0
Chorine chloride	2.0	2.0	2.0	2.0
Butylhydroxytoluene	0.1	0.1	0.1	0.1
	1000.0	1000.0	1000.0	1000.0

Note: [1]Based on the AIN76 mixture. C, A, P, and AP are abbreviations for the control, allitol, D-allulose, and allitol + D-allulose diets, respectively.

Body weight and food intake were recorded daily. After the feeding period, all rats were euthanized by beheading at 09:00 h without fasting. Blood was collected to obtain the serum. The abdominal adipose tissues (epididymal, perirenal, and mesenteric), heart, kidney, spleen, and liver were quickly removed and stored at −80°C until analysis. Carcass samples were procured by removing the head and remains from the intra-abdominal and intra-thoracic tissues. They were stored at −20°C until carcass fat analysis was conducted.

2.3. Biochemical Analysis

Serum insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, free fatty acid, triglyceride, glucose, and acetic acid concentrations were determined using the appropriate kits (Glucose CⅡ-test, LBIS Rat Insulin enzyme-linked immunosorbent assay (ELISA) Kit, Cholesterol E-Test, HDL-Cholesterol E-Test, non-esterified fatty acids (NEFA) C-Test, Triglyceride E-Test [FUJIFILM Wako Chemicals, Osaka, Japan], and EnzyChrom Acetate Assay Kit [Funakoshi Co., Ltd., Tokyo, Japan], respectively).

Liver lipids were extracted using the method described by Matyash, Liebisch, Kurzchalia, Shevchenko, and Schwudke (2008), and the liver triglyceride and cholesterol contents were determined using the appropriate kits (Triglyceride E-Test and Cholesterol E-Test, [FUJIFILM Wako Chemicals], respectively). Liver glucose-6-phosphate dehydrogenase (G6PD) and malate dehydrogenase (MDH) activities were determined using the appropriate kits (QuantiChrom™ Glucose-6-Phosphate Dehydrogenase Kit and EnzyChrom™ Malate Dehydrogenase Assay Kit, [BioAssay Systems, CA, USA], respectively). Carcass fat mass was determined using the method described by Mickelsen and Anderson (1959).

2.4. Data Analysis

All data were analyzed using a two-way Analysis of Variance (ANOVA) and the Tukey–Kramer test (Bell Curve for Excel, SSRI, Tokyo, Japan). Statistical significance was set at $p < 0.05$.

3. RESULTS

3.1. Body Weight, Food Intake, Tissue Weights, and Carcass Fat (Table 2)

Dietary D-allulose significantly reduced the final body weight and weight gain, whereas allitol did not. Energy intake was suppressed by D-allulose supplementation, but no differences were observed in food intake, food efficiency, and energy efficiency among all groups. D-Allulose significantly increased liver and kidney weights, which were greater in Groups P and AP than those in Groups C and A. The weights of the heart and spleen did not differ among all groups. Dietary allitol significantly increased the cecal weight, surface area, and content weight, which were significantly greater in Groups A and AP than in Groups C and P. The weights of mesenteric, perirenal, and total intra-abdominal adipose tissues were significantly reduced by dietary D-allulose, whereas dietary allitol reduced only mesenteric adipose tissue. The perirenal adipose tissue weight was significantly higher in Group C than in Group AP, while mesenteric adipose tissue weight was significantly higher in Group C than in Groups P and AP. Intra-abdominal fat accumulation was also suppressed by both allitol and D-allulose, but no synergistic interaction was observed between the two. No differences were observed in carcass fat among the four groups. We found no interaction between allitol and D-allulose addition for all indicators of body weight gain, food intake, tissue weights, and carcass fat.

Table 2. Body weight, food intake, tissue weights, and carcass fat weight.

	Groups	C	A	P	AP	2x2 ANOVA		
						A	P	AxP
Body weight								
Initial	(g)	114.1±2.1	114.2±1.8	113.9±1.9	114.1±1.8	n.s.	n.s.	n.s.
Final	(g)	331.3±2.2	330.8±4.9	321.9±4.1	319.1±6.8	n.s.	*	n.s.
Gain	(g)	217.2±1.3	216.6±4.2	208.0±3.4	205.0±6.1	n.s.	*	n.s.
Food intake	(g/day)	17.1±0.1	17.3±0.2	16.9±0.2	16.8±0.4	n.s.	n.s.	n.s.
Food efficiency	(g/g)	0.220±0.002	0.218±0.003	0.213±0.003	0.213±0.004	n.s.	n.s.	n.s.
Energy intake	(kcal/day)	62.9±0.5	62.0±0.8	59.4±0.7	57.5±1.5	n.s.	*	n.s.
Energy efficiency	(mg/kcal)	60.1±0.6	60.7±0.9	60.8±0.8	62.1±1.0	n.s.	n.s.	n.s.
Heart	(g)	0.81±0.02	0.83±0.02	0.79±0.01	0.79±0.01	n.s.	n.s.	n.s.
Liver	(g)	12.3±0.1	12.4±0.3	13.3±0.3	13.2±0.3	n.s.	**	n.s.
Kidneys	(g)	$2.07±0.03^{b}$	$2.12±0.04^{b}$	$2.43±0.05^{a}$	$2.32±0.05^{a}$	n.s.	**	n.s.
Spleen	(g)	0.72±0.01	0.70±0.02	0.72±0.02	0.67±0.01	n.s.	n.s.	n.s.
Cecum								
Weight	(g)	$4.91±0.21^{b}$	$6.80±0.25^{a}$	$4.90±0.3^{b}$	$6.74±0.22^{a}$	**	n.s.	n.s.
Surface area	(cm^2)	$16.8±0.8^{b}$	$23.2±2.1^{a}$	$17.6±0.1^{b}$	$27.3±1.0^{a}$	**	n.s.	n.s.
Conent	(g/cecum)	$3.18±0.17^{b}$	$4.83±0.15^{a}$	$3.46±0.13^{b}$	$4.98±0.22^{a}$	**	n.s.	n.s.
Intra-abdominal adipose tissues								
Epididymal	(g)	9.15±0.65	9.37±0.69	8.54±0.40	8.12±0.38	n.s.	n.s.	n.s.
Perirenal	(g)	$8.65±0.33^{a}$	$8.08±0.48^{ab}$	$7.67±0.19^{ab}$	$7.22±0.32^{b}$	n.s.	*	n.s.
Mesenteric	(g)	$7.34±0.29^{a}$	$6.41±0.23^{ab}$	$5.95±0.03^{b}$	$5.53±0.27^{b}$	*	**	n.s.
Total	(g)	25.1±1.2	23.9±1.3	22.2±0.8	20.9±0.8	n.s.	*	n.s.
Carcass fat	(g)	30.5±0.8	28.3±1.4	28.7±1.3	29.7±2.8	n.s.	n.s.	n.s.

Note: Values are the mean ± SE for 8 rats. $^{*}p < 0.05$; $^{**}p < 0.01$; n.s., not significant (two-way ANOVA).
^{a,b,ab}Within a row, values with different superscripts are significantly different ($p < 0.05$).
C, A, P, and AP are abbreviations for the control, allitol, D-allulose, and allitol + D-allulose groups, respectively.

3.2. Liver and Serum Biochemical Test Results (Table 3)

Dietary allitol significantly decreased liver cholesterol level, which was significantly lower in Group PA than in Group C. D-Allulose supplementation significantly decreased liver G6PD activity, which was significantly lower in Groups P and PA than in Group C. No differences were found in liver triglyceride content and MDH activity among all groups.

Dietary D-allulose significantly increased serum glucose, total cholesterol, non-HDL cholesterol, and triglyceride concentrations and significantly decreased serum HDL cholesterol levels. Serum total cholesterol was significantly higher in Group P than in Group C, whereas serum triglyceride was significantly higher in Group AP than in Group C. No differences were found in serum insulin, free fatty acids, and acetic acid concentrations among the four groups. No interactions were detected between dietary allitol and D-allulose supplementation for almost all indicators of liver and serum biochemical tests, except for serum free fatty acids and acetic acid.

Table 3. Liver and serum biochemical test results.

	Group	C	A	P	AP	2x2 ANOVA		
						A	P	AxP
Liver								
Triglyceride	(mg/g)	8.61±2.72	11.1±1.26	8.60±0.81	9.52±0.79	n.s.	n.s.	n.s.
Cholesterol	(mg/g)	5.89±0.52^{a}	4.74±0.18^{ab}	5.04±0.14^{ab}	4.69±0.15^{b}	*	n.s.	n.s.
G6PD activity	(U/g tissue)	6.45±0.45^{a}	6.47±0.52^{a}	4.82±0.31^{ab}	4.31±0.40^{b}	n.s.	**	n.s.
MDH activity	(U/g tissue)	14.9±0.4	15.8±0.5	15.9±0.4	16.4±0.4	n.s.	n.s.	n.s.
Serum								
Glucose	(mg/dL)	152.3±4.2	153.7±6.6	173.8±6.7	161.8±5.7	n.s.	*	n.s.
Insulin	(ng/mL)	4.07±0.60	3.44±0.46	3.13±0.45	2.74±0.37	n.s.	n.s.	n.s.
Total cholesterol	(mg/dL)	118.4±7.9^{b}	134.1±6.8^{ab}	152.3±5.9^{a}	139.8±7.3^{ab}	n.s.	*	n.s.
HDL-cholesterol	(mg/dL)	28.7±1.5	29.4±2.1	26.6±2.0	21.8±2.3	n.s.	*	n.s.
non-HDL-cholesterol	(mg/dL)	89.9±7.6	104.6±5.0	125.8±6.9	118.0±7.2	n.s.	*	n.s.
Free fatty acids	(mEq/L)	533±17	668±24	555±33	566±21	n.s.	n.s.	*
Triglyceride	(mg/dL)	83.8±11.4^{b}	118.7±6.3^{ab}	121.8±12.2^{ab}	143.2±20.2^{a}	n.s.	*	n.s.
Acetic acid	(mg/dL)	10.2±1.7	15.8±1.9	13.6±2.4	9.94±1.6	n.s.	n.s.	*

Note: Values are the mean ± SE for 8 rats. *$p < 0.05$, n.s., not significant (two way ANOVA).
^{a,b,ab}Within a row, values with different superscripts are significantly different ($p < 0.05$).
C, A, P, and AP are abbreviations for the control, allitol, D-allulose, and allitol + D-allulose groups, respectively.
G6PD, glucose-6-phosphate dehydrogenase; MDH, malate dehydrogenase.

4. DISCUSSION

Many studies have reported the anti-obesity effects of D-allulose (Chung et al., 2012; Han et al., 2016; Hayashi et al., 2014; Hossain et al., 2015; Matsuo et al., 2001a; Matsuo et al., 2001b; Nagata, Kanasaki, Tamaru, & Tanaka, 2015; Ochiai et al., 2017; Ochiai, Onishi, Yamada, Iida, & Matsuo, 2014). These effects have been attributed to a combination of factors, such as suppression of fatty acid synthesis in the liver, promoting of lipid metabolism, enhancing energy expenditure, and reducing chylomicron synthesis in small intestinal mucosal epithelial cells. In this study, we confirmed that dietary D-allulose inhibits body fat accumulation occurred with and without dietary allitol supplementation (Table 2). We had previously demonstrated the anti-obesity effect of allitol in rats may be equal to or greater than D-allulose (Higaki et al., 2022a). However, in the present study, body fat reduction by allitol alone was confirmed only by quantifying mesenteric adipose tissue, despite the confirmed strong anti-obesity influence of D-allulose. In addition, no synergy was observed between dietary D-allulose and allitol in reducing intra-abdominal adipose tissue. The different results between our previous and current studies can be due to differences in the experimental conditions, specifically in the diet fed to the experimental rats. Previously, 5% allitol and D-allulose

supplementation under the fixed feeding was applied, whereas only 3% supplementation under *ad libitum* feeding was used in the present study.

In our previous studies, supplementation with dietary allitol led to cecal hypertrophy and significantly increased the serum and cecal short-chain fatty acids (SCFAs) (Matsuo et al., 2022b; Matsuo, Ono, Mochizuki, Yoshihara, & Akimitsu, 2022a). These findings indicate that allitol has high intestinal fermentability. We had also suggested that allitol is more fermentable than maltitol (Matsuo et al., 2022a) and fructooligosaccharides (Matsuo, Ono, Mochizuki, Yoshihara, & Akimitsu, 2023), implying that SCFAs are obtained by gut microbiome-induced fermentation of dietary allitol. SCFAs, also known as volatile fatty acids, are organic carboxylic acids with less than 6 carbon atoms, such as acetic acid, propionic acid, butyric acid, and valeric acid. Of these, acetic acid, propionic acid, and butyric acid are abundantly very high (≥95%) (Cook & Sellin, 1998), with approximate molar ratios of 60:20:20, respectively (Cummings, Pomare, Branch, Naylor, & MacFarlane, 1987; Louis & Flint, 2017). Except for a small portion of SCFAs that come directly from food, most SCFAs are produced via intestinal microbial anaerobic fermentation. Approximately 500–600 mmol of SCFAs per day are produced in the intestinal tract, depending on diet, microbiome abundance and quality, and residence time in the intestinal tract (Macfarlane & Macfarlane, 2003). Many SCFAs produced in the intestines are used as energy sources (oxidation), glucose (gluconeogenesis), or fat (lipogenesis) substrates. In humans, SCFAs provide about 10% of the daily energy requirements (Vadder et al., 2016). Whether SCFAs are consumed as an energy substrate or used for fat synthesis or other physiological materials depends on the state of energy balance throughout the body (Vadder et al., 2016). SCFAs are involved in the regulation of almost every cell and physiological function in the body by acting as ligands for G protein-coupled receptors (GPCRs) (He et al., 2020). G protein-coupled receptor (GPR)41 (free fatty acid receptor 3, FFAR3) and GPR43 (FFAR2) are the most important receptors of SCFAs in the GPCR family (He et al., 2020). Shimizu, Ohue-Kitano, and Kimura (2019) and Kimura (2014) reported that FFAR3 and FFAR2 are abundantly expressed in the sympathetic ganglion and adipose tissues, respectively. They found that SCFAs increase energy consumption and lipolysis through these receptors. Furthermore, He et al. (2020) reviewed many experimental studies on SCFAs and their association with signaling pathways via FFAR3 and FFAR2 in glucose and lipid metabolism and concluded that SCFAs can increase fatty acid oxidation, inhibit lipogenesis, increase thermogenesis, and decrease fat accumulation. From these viewpoints, we speculated that simultaneous intake of allitol with D-allulose, which inhibits lipogenesis, would suppress its utilization as a lipogenesis substrate, thereby enhancing the anti-obesity effect (Figure 2).

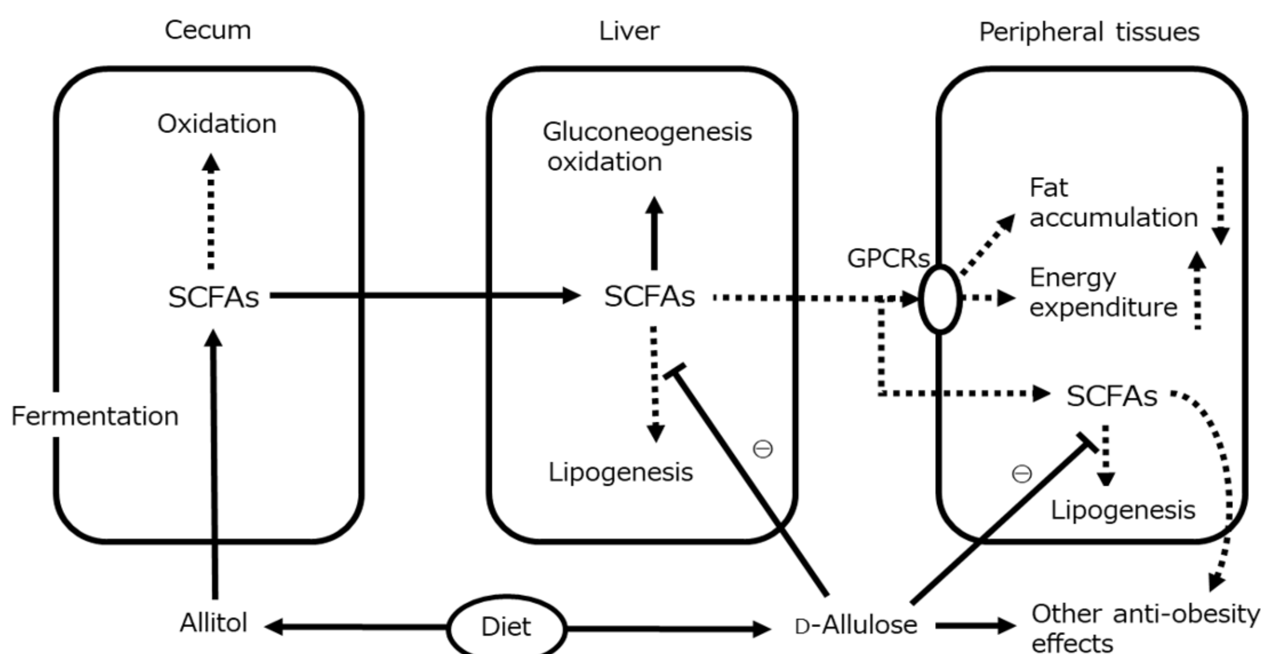

Figure 2. Illustration of the hypothesis on the synergistic effect of D-allulose and allitol against obesity. SCFAs, Short-chain fatty acids. GPCRs, G protein-coupled receptors.

In the present study, hepatic G6PD activity was suppressed by D-allulose (Table 3), confirming the inhibitory effect of D-allulose on lipogenesis. However, a synergy between allulose and allitol was not confirmed. SCFAs produced by the fermentation of allitol may have been used to increase serum glucose for gluconeogenesis or oxidation, resulting in decreased amounts of SCFAs in the systemic circulation. This can be inferred from the fact that the serum glucose level increased with D-allulose supplementation (Table 3). Notably, in this context, the simultaneous intake of allitol and D-allulose synergistically reduced serum acetic acid concentration (Table 3).

Conversely, dietary D-allulose significantly increased the serum total and non-HDL-cholesterol, and triglyceride concentrations, and significantly decreased HDL-cholesterol concentration. In this regard, Ochiai et al. (2014) suggested that serum triglyceride concentration was significantly higher in 5% D-allulose diet-fed rats than in control rats. Previously, it was reported that serum HDL-cholesterol was significantly lower in Golden Syrian hamsters fed a 3% D-allulose diet than those fed a normal diet (Kanasaki et al., 2019). There are several studies on the effects of D-allulose on lipid metabolism (Smith et al., 2022). They have suggested that D-allulose enhances HDL-cholesterol uptake into rat's primary hepatocytes via scavenger receptor class B type 1 (SR-B1). Kim, Kim, Kim, and Sung (2017) reported that dietary D-allulose supplementation decreased the expression of lipoprotein lipase (LPL), an enzyme that hydrolyzes triglycerides in lipoproteins. The increase in serum triglyceride concentration with D-allulose supplementation may decrease fatty acid uptake from serum lipoproteins into adipose tissues by suppressing LPL activity. Our present results partially support these findings.

Finally, safety as a supplement and food additive, D-allulose was generally recognized as safe (GRAS Notice No. 498, Food and Drug Administration in the USA, 2017), and can be used in various food and dietary supplements. As shown in Table 2, the kidney weight was significantly higher in the D-allulose-supplemented groups. Similar results have been suggested in previous studies (Matsuo et al., 2001a; Matsuo et al., 2001b; Ochiai et al., 2017; Ochiai et al., 2014). In our previous study of chronic toxicity using rats, the administration of 3% D-allulose resulted in kidney hypertrophy without any adverse health effects (Yagi & Matsuo, 2009). In addition, we suggested that feeding rats a diet containing 3% D-allulose for 4 weeks could increase the weights of the liver and kidney without any pathological damage, while 10 weeks of D-allulose interruption reversed these effects (Ochiai et al., 2019). In a clinical study evaluating 12-week ingesting of an isomerized carbohydrate containing 6% D-allulose, Hayashi et al. (2014) reported the absence of adverse effects on studies of lipid and carbohydrate metabolism or abnormal blood parameters, and renal and hepatic functions. Thus, these findings suggested that D-allulose is safe in humans and animals.

5. CONCLUSIONS

The present study confirmed that D-allulose supplementation mediated the suppression of body fat accumulation with or without dietary allitol supplementation. However, a synergistic interaction between allitol and D-allulose supplementation was not observed in the reduction of intra-abdominal adipose tissue weight. Because the physiological functions of allitol are unknown, further studies are needed to clarify the anti-obesity effects and metabolic pathways of allitol.

Funding: This study received no specific financial support.
Ethical Statement: The Ethical Committee of the Kagawa University, Japan has granted approval for this study (Ref. No. 22621).
Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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