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Effects of dietary allitol on body fat accumulation and cecal morphology in rats

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Abstract

Allitol, a sugar alcohol obtained by reducing D-allulose, is a rare sugar because it is present in trace amounts in commercially available sugars. Allitol, along with D-allulose, is also found in *Itea*, a deciduous shrub of the family Saxifragaceae. Our previous study reported that long-term feeding of rats with a dietary supplement of dried *Itea* powder suppressed body fat accumulation. This effect may be due to D-allulose and allitol, suggesting that allitol might have an anti-obesity effect similar to D-allulose. Moreover, we demonstrated that allitol provides approximately 2 kcal/g of energy, similar to that provided by maltitol, revealing that allitol is fermentable in the intestine. Herein, we investigated the effects of dietary allitol on body fat accumulation and cecal morphology in rats by comparing it with fructooligosaccharide, a highly fermentable carbohydrate. Male Wistar rats were fed a control diet or an allitol-supplemented diet for 3 weeks (Experiment 1) and an experimental diet containing fixed amounts of sucrose, fructooligosaccharide, or allitol (0.4–1.2 g) for 20 days (Experiment 2). This study demonstrated that allitol has an anti-obesity effect in rats and more fermentable in the intestine of rats than fructooligosaccharide. These findings suggest that allitol may be useful as a functional sweetener. However, further studies must elucidate its metabolic pathways and physiological functions.

Key words : rare sugar, allitol, body fat, cecum, rat

Introduction

There is increasing concern over the excessive intake of purified sugars, which is associated with obesity and an increased risk of metabolic syndrome. Many non- or low-energy sweeteners are marketed as alternatives to sucrose and high-fructose corn syrup. Recently, rare sugars have also been identified as an alternative. Rare sugars are monosaccharides and their derivatives that are not commonly found in nature, as opposed to common sugars, such as D-glucose and D-fructose (The International Society of Rare Sugars [ISRS], 2001). These rare sugars are used in functional foods, supplements, and agricultural fertilizers. Recent studies have reported their beneficial effects on human health as low-calorie carbohydrate sweeteners and bulking agents [1–3].

For nearly two decades, some rare sugars, such as D-allulose, D-sorbose, D-tagatose, and L-sugars, have been developed as alternative carbohydrate sweeteners [4–6]. However, allitol as a sugar substitute has not been studied. Allitol is a sugar alcohol obtained by reducing D-allulose (Fig. 1)[7] which cross-links D-and L-hexoses using a central strategy called Izumoring [8]. Allitol is referred to as a rare sugar by ISRS because it is present in trace amounts in commercially available sugars and is challenging to synthesize using chemi-

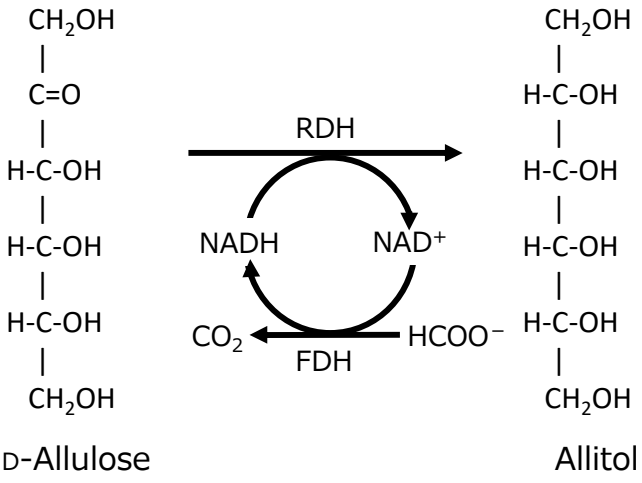

Figure 1　Molecular structures of D-allulose and allitol, and allitol production from D-allulose. RDH, ribitol dehydrogenase; FDH, formate dehydrogenase; NADH, dihydronicotinamide adenine dinucleotide; NAD^+, nicotinamide adenine dinucleotide.

cal methods [9]. Recently, allitol has been mass-produced by hydrogenating D-allulose and is now available for experimental purposes.

Allitol, along with D-allulose, is also found in *Itea*, a deciduous shrub of the family Saxifragaceae [10]. Our previous study reported that long-term feeding of rats with a dietary supplement of dried *Itea* powder suppressed body fat accumulation. This effect may be due to D-allulose and allitol in *Itea* [11], suggesting that allitol might have an anti-obesity effect similar to D-allulose. Moreover, we demonstrated that allitol provides approximately 2 kcal/g of energy, similar to that provided by maltitol, revealing that allitol is fermentable in the intestine [12]. Herein, we investigated the effects of dietary allitol on body fat accumulation and cecal morphology in rats by comparing it with fructooligosaccharide, a highly fermentable carbohydrate.

Materials and Methods

All animal procedures were approved by the Animal Care and Use Committee for Kagawa University (approval number: 20624).

Materials

Allitol was provided by the International Institute of Rare Sugar Research and Education (Kagawa, Japan). Fructooligosaccharide was procured from Meiji Food Materia Co., Ltd. (Tokyo, Japan). Vitamin and mineral mixtures (AIN-76A) were procured from Oriental Yeast Co. Ltd. (Tokyo, Japan). Soybean oil was procured from Yamakei Industry Co. Ltd. (Osaka, Japan); its composition was as follows: 52.7% linoleic acid, 24.3% oleic acid, 7.9% α-linolenic acid, 10.3% palmitic acid, and 3.8% stearic acid.

Experiment 1: Effects of dietary allitol on body fat accumulation and cecal surface area in rats fed a fixed diet.

Animals and experimental design. Fourteen male Wistar rats (3-weeks-old) were procured from Japan SLC (Shizuoka, Japan) and randomized into two groups of seven rats. They were individually caged at 22 ± 1 °C, under light from 08:00 h to 20:00 h. They were fed MF, a commercial rodent diet (Oriental Yeast Co., Ltd., Tokyo, Japan), and had access to water *ad libitum* for 3 days. The initial body weight of the rats was 47.3 ± 1.0 g (range: 44.1–52.3 g). The rats were then fed 10 g of synthetic (control, C) diet or the C diet supplemented with 1.0 g of allitol (A) (Table 1). The two groups of rats were fed the prescribed amount of diet (Table 1) at 09:00 h with free access to water for 3 weeks. The body weight was recorded daily. After the experimental period, all rats were euthanized by beheading at 09:00 h. The intra-abdominal adipose tissues (epididymal, perirenal, and mesenteric tissues) and the cecum were quickly removed.

Table 1 Composition of experimental diets (x10^{-2} g/daily meal).

Ingredients	C	A
Casein	200.0	200.0
DL-Methionine	3.0	3.0
Corn starch	649.9	649.9
Allitol	0.0	100.0
Soybean oil	50.0	50.0
Mineral mixture[1]	35.0	35.0
Vitamin mixture[1]	10.0	10.0
Cellulose	50.0	50.0
Chorine chloride	2.0	2.0
Butylhydroxytoluene	0.1	0.1
Total	1000.0	1100.0

[1]Based on the AIN-76 mixture.
C and A are abbreviations for the control and allitol diets, respectively.

Experiment 2: Comparison of dietary allitol and fructooligosaccharide for cecal morphology in rats.

Animals and experimental design. Fifty-five male Wistar rats (3-weeks-old) were procured from Japan SLC (Shizuoka, Japan). They were individually caged at 22 ± 1 °C, under light from 08:00 h to 20:00 h. They were fed MF, a commercial rodent diet (Oriental Yeast Co., Ltd., Tokyo, Japan), and had access to water *ad libitum* for 3 days. The basal diet comprised the following ingredients: casein, 600 g/kg; cornstarch, 195 g/kg; soybean oil, 50 g/kg; cellulose, 50 g/kg; mineral mixture, 70 g/kg; vitamin mixture, 20 g/kg (both mixtures are based on AIN-76); choline chloride, 5 g/kg; and DL-methionine, 10 g/kg. After the acclimation period, the rats (mean weight 49.3 ± 1.2 g [range: 46.7–54.2 g]) were randomly divided into 11 groups. One group of rats (day 0 control) was euthanized at the beginning of the study for body composition analysis. The remaining group of rats received 7 g of the basal diet to which a fixed amount of sucrose, fructooligosaccharide, or allitol (0.4–1.2 g of appropriate sweetener, see Table 2) was added for 20 days. On the final day of the experiment, the rats were fasted overnight (12 h) and euthanized by cervical dislocation. Residual food in their digestive

tract was discarded. The cecum was removed, and the weight of the cecal content and its surface area were measured.

Data analyses

In Experiment 1, all data were analyzed using an unpaired Student's t-test. In Experiment 2, data except for the day 0 control and the no supplement groups were analyzed using two-way ANOVA and Tukey–Kramer tests. Dunnett test was used to compare the non-supplement group with the other groups. Statistical significance was set at $p < 0.05$.

Results and Discussion

The results of Experiment 1 are shown in Fig. 2. Body weight gain did not differ between groups C and A; however, the intra-adipose tissue weight was significantly lower in group A than in group C. This suggests that dietary allitol has an anti-obesity effect, consistent with our previous findings [11]. The cecal surface area was significantly higher in group A than in group C. This is believed to be due to an increase in gut microbiota in allitol-fed rats. Allitol is considered to have high intestinal fermentability. In Experiment 2, we investigated the effects of dietary allitol on cecal morphology in rats by comparing it with fructooligosaccharide, a highly fermentable carbohydrate. Body weight gain increased with the incorporation of test carbohydrates in the diet, whereas it was higher in the rats fed a sucrose-supplemented diet than in the other rats (Table 2). Cecal content and surface area increased with increasing amounts of fructooligosaccharide and allitol, and the rate of increase in the cecal surface area was greater with allitol than with fructooligosaccharide (Table 2). The total amounts of fructooligosaccharide and allitol fed to the rats during the experimental period of 20 days were positively correlated with the weight of the cecal content and surface area (Fig. 3). The regression coefficients of fructooligosaccharide and allitol to total supplements were 0.116, r^2=0.973 and 0.113, r^2=0.950 for the cecal content, and 0.434, r^2=0.900 and 0.883, r^2=0.965 for the cecal surface area, respectively (Fig. 3). The cecal surface area was significantly greater in rats that consumed 24 g of allitol during the experimental period than in those that consumed 24 g of fructooligosaccharide (Table 2), thus revealing that allitol is fermentable in the intestine and its fermentability is stronger than that of fructooligosaccharide.

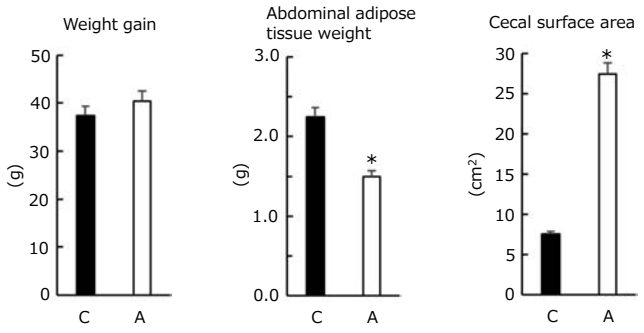

Figure 2 Weight gain, intra-abdominal adipose tissue weight, and cecal surface area in rats fed the control (C) diet and the allitol (A) diet. Values are means ± SE for 7 rats. *$p < 0.05$ vs. group C (Student's t-test).

Table 2 Final body weight and cecal content and surface area of rats with various levels of sucrose, fructooligosaccharide, and allitol supplementation.

Group	Supplement	Amount (g)[1]		Body weight[2]	Cecum[2]	
		Daily (g)	Total (g)	(g)	Content (g)	Surface area (cm^2)
1	None	0.0	0	77.4 ± 1.4	0.63 ± 0.21	4.1 ± 0.5
2	Sucrose	0.4	8	85.8 ± 1.0^{*bc}	0.59 ± 0.22^{d}	7.3 ± 0.9^{d}
3	Sucrose	0.8	16	89.6 ± 1.1^{*ab}	0.84 ± 0.08^{d}	6.3 ± 0.7^{d}
4	Sucrose	1.2	24	93.0 ± 1.7^{*a}	0.85 ± 0.11^{d}	7.6 ± 0.5^{d}
5	Fructooligosaccharide	0.4	8	84.4 ± 0.8^{bc}	1.58 ± 0.32^{c}	10.9 ± 1.3^{*bc}
6	Fructooligosaccharide	0.8	16	88.2 ± 2.2^{*b}	2.15 ± 0.38^{*c}	13.0 ± 2.1^{*bc}
7	Fructooligosaccharide	1.2	24	88.7 ± 1.8^{*b}	3.54 ± 0.53^{*a}	15.0 ± 1.8^{*b}
8	Allitol	0.4	8	81.2 ± 1.4^{c}	1.40 ± 0.13^{c}	10.0 ± 1.5^{c}
9	Allitol	0.8	16	84.0 ± 2.4^{bc}	2.85 ± 0.93^{*b}	15.0 ± 1.9^{*ab}
10	Allitol	1.2	24	90.4 ± 3.5^{*ab}	3.16 ± 0.64^{*ab}	26.0 ± 5.8^{*a}

[1]Over 20-day period.
[2]Values are means ± SE for 5 rats. Within a column except for the None group, the values with the different superscripts are significantly different ($p < 0.05$, ANOVA and Tukey-Kramer test).
*$p < 0.05$ vs. The none supplement group (Dunnett's test).

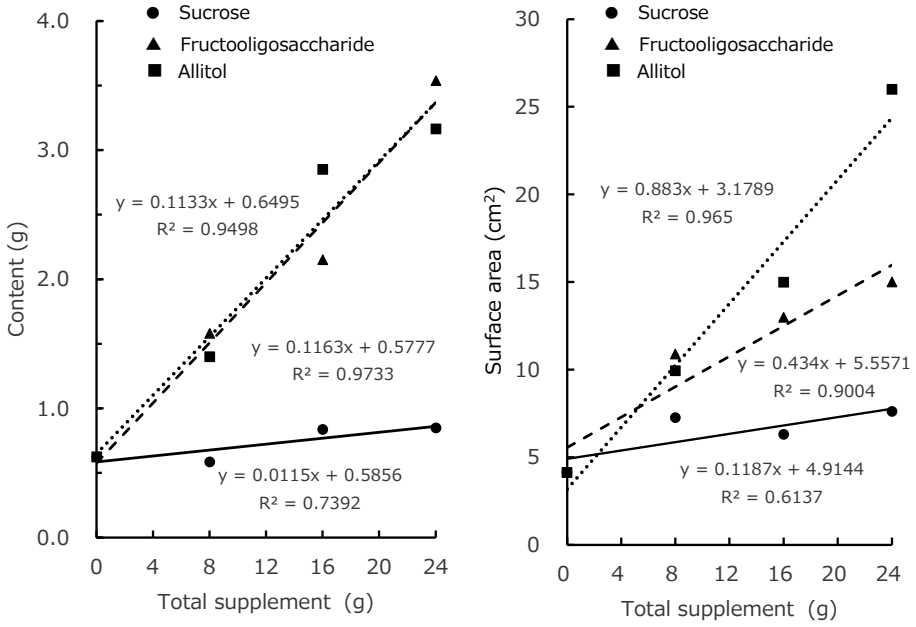

Figure 3 Cecal content (left) and surface area (right) of rats fed diets with various levels of sucrose, fructooligosaccharide, and allitol. Values are means for 5 rats. Regression lines were calculated from the values given in Table 2.

Sugar alcohols and oligosaccharides are converted to short-chain fatty acids (SCFAs) via intestinal fermentation. Our results suggest that ingestion of allitol may produce more SCFAs than ingestion of fructooligosaccharide. Recent studies have identified gut bacterial strains, structural components, and various metabolites that significantly affect the host energy metabolism [13]. SCFAs produced by intestinal bacterial fermentation have are essential nutrients that contribute to the maintenance of host energy homeostasis via immune and epigenetic systems. Shimizu et al. [13] and Kimura [14] reported that G-protein-coupled receptors (GPR41 and GPR43) are activated by SCFAs, and the intake of non-digestible carbohydrates plays an important role in determining the diversity and formation of the gut microbiota. Additionally, Yamashita [15] demonstrated that acetate, an SCFA, results in a high rate of oxygen consumption and small sized lipid droplets in white and brown adipose tissues. Taken together, these findings suggest that dietary allitol may be an effective precursor of SCFAs and thus act as a functional sweetener.

Rare sugar alcohols are also known to increase the water content in the small intestine, thereby reducing transit times. Consequently, rare sugar alcohols can be used to treat constipation or obesity [9]. Allitol acts as a laxative, suggesting its potential for therapeutic use. Osaka [16] previously studied the laxative effect of allitol on the small intestinal transit time and luminal water content after administration in mice. Diarrhea was induced at an allitol dose of 4.96 g/kg, which significantly increased water content in the lumen of the small intestine and cecum. In the current study, diarrhea was not observed in the rats after several days of acclimation, and they were adapted to allitol intake. However, the physiological effects of allitol have not been extensively investigated; therefore, the use of allitol as a food supplement should be considered cautiously.

This study demonstrated that allitol, a rare sugar, has an anti-obesity effect in rats, and it is more fermentable in the intestine of rats than fructooligosaccharide. These findings suggest that allitol may be useful as a functional sweetener. However, further studies must elucidate its metabolic pathways and physiological functions.

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アリトールがラットの体脂肪蓄積と盲腸形態に及ぼす影響

松尾達博・小野香音・望月　進・吉原明秀・秋光和也

要　約

希少糖アリトールはD-アルロースを還元して得られる糖アルコールであり，落葉性灌木の一つであるズイナにD-アルロースと共に高濃度に含有している．我々はこれまでに乾燥ズイナ粉末添加食をラットに長期間摂取させると，体脂肪蓄積が抑制されることを報告した．この効果はズイナに含有するD-アルロースとアリトールによるものであり，アリトールがD-アルロースと同様の抗肥満作用を有することが推察された．さらに，アリトールはマルチトールなどの糖アルコールと同様，腸内発酵性であり，約2 kcal/gのエネルギー価を持つことを明らかにした．本研究では，アリトールがラットの体脂肪蓄積および盲腸形態に及ぼす影響について，高発酵性糖類であるフラクトオリゴ糖と比較した．Wistar系雄ラットに対照食あるいはアリトール添加食を与え3週間飼育した（実験1）．また，高タンパク質の基礎食にショ糖，アリトールおよびフラクトオリゴ糖を規定量（0.4～1.2 g）添加した餌を20日間与えた（実験2）．その結果，アリトールは抗肥満作用を有すること，およびフラクトオリゴ糖よりも高い腸内発酵性を持つことが明らかになった．これらのことから，アリトールは機能性甘味料として有益である可能性があるが，アリトールの代謝経路や生理学的特性については，今後，詳細な検討が必要である．