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Anti-Obesity Effects of Dietary D-Allulose and Medium-Chain Triglycerides in High-Fat Diet-Fed Rats

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Abstract

D-Allulose, a rare sugar, exerts anti-obesity effects by inhibiting hepatic lipogenesis and promoting energy expenditure. Medium-chain triglycerides (MCTs) consist of three medium-chain fatty acids connected by glycerol. MCTs have been extensively investigated for their ability to reduce body fat accumulation. We previously investigated the anti-obesity effects of a combination of dietary D-allulose and MCT (5% - 13%) in rats; however, we could not confirm the anti-obesity effects of MCT or observed synergetic effects between D-allulose and MCT on body fat loss. We speculated that our previous studies were influenced by the excessive amount of MCT in the diets. Therefore, in this study, we aimed to investigate the anti-obesity effects of the simultaneous intake of D-allulose and MCT in rats fed an obesity-inducing high-fat diet with a low amount of MCTs (2%). Thirty-two male Wistar rats (3-week-old) were randomly divided into four groups: control, D-allulose, MCT, and D-allulose + MCT groups. Rats in each group were fed *ad libitum* on a control (no D-Allulose or MCT), 5% D-allulose, 2% MCT, or 5% D-allulose + 2% MCT diets for 16 weeks. Abdominal adipose tissue weights were significantly lower in the D-allulose diet group than in the control group, whereas no differences were observed between results of the MCT-supplemented groups. The total body fat mass was significantly lower in the D-allulose and MCT diet groups than in the control group, but no differences were observed between the MCT-supplemented groups. These results suggested that anti-obesity effects of dietary D-allulose were observed, and the effects of dietary MCTs were weaker than those of D-allulose. Moreover, we confirmed the interaction between dietary D-allulose and MCT on indicators of obesity. Interestingly, their effects were not synergistic, as MCT supplementation offset the anti-obesity effects of dietary D-allulose. However, the specific

mechanisms underlying those effects remain unknown, warranting further investigation.

Keywords

Rare Sugar, D-Allulose, Medium-Chain Triglycerides, Body Fat, Rat

1. Introduction

Recently, rare sugars have been widely recognized for their distinctive nutritional properties. Rare sugars are rarely found in nature compared to common sugars and are used in functional foods. These are beneficial to human health when used as low-calorie carbohydrate sweeteners and bulking agents [1]-[3]. Specifically, D-allulose is calorie-free and has a variety of food nutritional functions. Multiple studies have demonstrated the anti-obesity effect of D-allulose via the inhibition of hepatic lipogenesis [4] [5] and promotion of energy expenditure [6] [7].

Medium-chain triglycerides (MCTs) consist of three medium-chain fatty acids (MCFAs) with six to twelve carbon atoms connected to a glycerol [8]. MCTs are broken down in the small intestine and mostly absorbed as free fatty acids, which are transported to the liver via the portal vein and subsequently taken up by the mitochondria, without the need for carnitine palmitoyltransferase (CPT), facilitating rapid β-oxidation [9]. This physiological process favors the hepatic oxidation of MCFAs over that of long-chain fatty acids (LCFAs) and consumes more energy in the liver, preventing obesity. MCTs have been extensively investigated for their anti-obesity effects in rats and humans [8] [10] [11]. However, excessive intake of MCTs may stimulate hepatic lipogenesis [12] [13] and an anti-obesity effect may not be observed.

Because D-allulose inhibits hepatic lipogenesis, simultaneous intake of MCT and D-allulose may reduce the de novo lipogenesis induced by MCFAs and enhance their efficiency in preventing obesity. We previously investigated the anti-obesity effects of a combination of dietary D-allulose and MCT (5% - 13%) in rats fed a high-carbohydrate [14] or a high-fat diet [15] for eight weeks. However, we could not confirm the anti-obesity effects of MCT or observed synergetic effects between D-allulose and MCT on body fat loss. We speculated that our previous studies were influenced by the excessive amount of MCT in the diets. Therefore, in this study, we aimed to investigate the anti-obesity effects of the simultaneous intake of D-allulose and MCT in rats fed an obesity-inducing high-fat diet with a low amount of MCTs (2%). Furthermore, the feeding period was extended to 16 weeks to make it easier to detect anti-obesity effects.

2. Materials and Methods

All animal procedures were approved by the Animal Care and Use Committee

for Kagawa University (Approval number: 23617).

2.1. Materials

MCT oil was purchased from Nisshin Oillio Group Ltd. (Tokyo, Japan). The fatty acid composition of MCT oil was 75.0% octanoic acid and 25.0% decanoic acid. Soybean oil and beef tallow, long-chain triglycerides (LCTs), were purchased from Yamakei Industry Co. Ltd. (Osaka, Japan). The soybean oil was composed of 10.3% palmitic acid, 3.8% stearic acid, 24.3% oleic acid, 52.7% linoleic acid, and 7.9% *α*-linolenic acid. The beef tallow consisted of 24.0% palmitic acid, 16.6% stearic acid, 46.5% oleic acid, 2.8% linoleic acid, and 0.3% *α*-linolenic acid. D-allulose was obtained from the International Institute of Rare Sugar Research and Education (Kagawa, Japan). Vitamin and mineral mixtures (AIN-76A) were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan).

2.2. Animals, Diets, and Experimental Design

Thirty-two male Wistar rats (3-week-old) were obtained from Japan SLC (Shizuoka, Japan), adapted to the experimental environment for three days, and then randomly divided into four groups: control (C), D-allulose (A), MCT (M), and D-allulose + MCT (AM) groups. Table 1 shows the dietary composition of each group. The A and AM diets contained 5% D-allulose, whereas the M and AM diets contained 2% MCT. All experimental diets were obesity-inducing high-fat diets with high concentrations of sucrose added to increase fat synthesis in the

Table 1. Composition of experimental diets.

Ingredients (g/kg diet)	C	A	M	AM
Casein	200.0	200.0	200.0	200.0
DL-Methionine	3.0	3.0	3.0	3.0
Corn starch	149.9	149.9	149.9	149.9
Sucrose	300.0	250.0	300.0	250.0
D-allulose (D-Psicose)	0.0	50.0	0.0	50.0
Soybean oil	20.0	20.0	20.0	20.0
Beef tallow	230.0	230.0	210.0	210.0
MCT	0.0	0.0	20.0	20.0
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

[1]Based on the AIN-76 mixture. C, A, M, and AM are the control, D-allulose, MCT, and D-allulose + MCT diets, respectively.

liver. All animals were individually caged at 22˚C ± 1˚C, under light from 08:00 to 20:00, and had free access to the experimental diets and water for 16 weeks. The body weight and food intake were recorded daily. After the experimental period, all rats were euthanized via decapitation at 09:00 after 5 h of fasting. Blood was collected to obtain the serum. The heart, liver, kidneys, spleen, and abdominal adipose tissues (epididymal, perirenal, and mesenteric) were quickly removed and stored at −80˚C until analysis. Carcass samples were obtained by removing the head and remaining intra-abdominal and intra-thoracic tissues, and stored at −20˚C until analysis of carcass composition.

2.3. Biochemical Analysis

Serum concentrations of glucose, insulin, total cholesterol, free fatty acids, triglycerides, and β-hydroxybutyric acid were determined using kits (Glucose CII-test, LBIS Rat Insulin ELISA Kit, Cholesterol E-Test, NEFA C-Test, Triglyceride E-Test [FUJIFILM Wako Chemicals, Osaka, Japan], and F-kit d-3-hydroxybutyric acid [J.K. International, Tokyo, Japan], respectively). Liver lipids were extracted using the method described by Matyash *et al.* [16], and liver triglyceride and cholesterol levels were determined using kits (Triglyceride E-Test and Cholesterol E-Test [FUJIFILM Wako Chemicals, Osaka, Japan]). Liver glucose-6-phosphate dehydrogenase (G6PD) and malate dehydrogenase (MDH) activities were determined using the QuantiChromTM Glucose-6-Phosphate Dehydrogenase Kit and EnzyChromTM Malate Dehydrogenase Assay Kit, respectively (BioAssay Systems, CA, USA). The carcass fat content was determined using the method described by Mickelsen and Anderson [17]. The total body fat was calculated as described by Paik and Yearick [18].

2.4. Data Analysis

Data from Groups C, A, M, and AM were analyzed using a two-way analysis of variance (ANOVA). If the ANOVA results revealed significant interactions, multiple comparisons of the results of all groups were performed using the Tukey–Kramer test. Statistical significance was set at $p < 0.05$. Excel Statistics (Social Survey Research Information Co., Ltd., Tokyo, Japan) was used for data analyses.

3. Results and Discussion

3.1. Body Weight, Food Intake, Tissue Weight, and Body Fat

The mean body weight of rats in each group increased throughout the experimental period and was highest in Group C and lowest in Group A (**Figure 1**). Dietary D-allulose significantly reduced the final body weight, weight gain, and food efficiency (**Table 2**). Two-way ANOVA confirmed the interaction between D-allulose and MCT and their effects on final body weight, weight gain, and food intake, leading to multiple comparisons (**Table 2**). The final body weight and weight gain were significantly lower in Group A than that in Group C, but no

differences were observed between results of MCT-supplemented groups, Groups M and AM (Table 2). Food intake did not differ among the groups. Heart and spleen weights were not affected by either dietary D-allulose or MCT; however, dietary D-allulose significantly increased the liver and kidney weights (Table 2). Liver weight was significantly higher in Groups C and AM than that in Group M (Table 2). Dietary D-allulose significantly reduced the epididymal, mesenteric, and total adipose tissue weights (Table 2). Two-way ANOVA confirmed the interaction between two factors affecting all intra-abdominal adipose tissue weights, leading to multiple comparisons. The epididymal, mesenteric, and total adipose tissue weights were significantly lower in Group A than that in Group C, whereas no differences were observed between results of MCT-supplemented groups, Groups M and AM (Table 2). Dietary D-allulose reduced the carcass fat and total body fat weights; however, these differences were not statistically significant. Two-way ANOVA confirmed the interaction between two factors affecting the weights and percentages of carcass and total body fat leading to multiple comparisons. The carcass and total body fat weights were significantly lower in Groups A and M than those in Group C, but no differences were observed between the MCT-supplemented groups, Groups M and AM (Table 2). We confirmed that dietary D-allulose inhibited body fat accumulation. D-allulose reduced almost all indicators of body fat accumulation, with and without MCT supplementation (Table 2). This result is consistent with our previous reports [14] [15]. Although weaker than those of D-allulose, the anti-obesity effects of MCT were observed on carcass and total body fat mass (Table 2). In our previous studies [14] [15], we could not confirm the anti-obesity effects of MCT (5% - 13% in diets). Therefore, the observed anti-obesity effects observed in this study may be due to the reduction in MCT content to 2%. Previously, we also showed that the dietary intake of 15% - 20% structured medium- and long-chain triglycerides (MLCTs; equivalent to 1.7% - 2.3% MCFAs) for eight weeks suppresses the intra-abdominal and carcass fat weights of rats [19]. The results of this study support our previous findings.

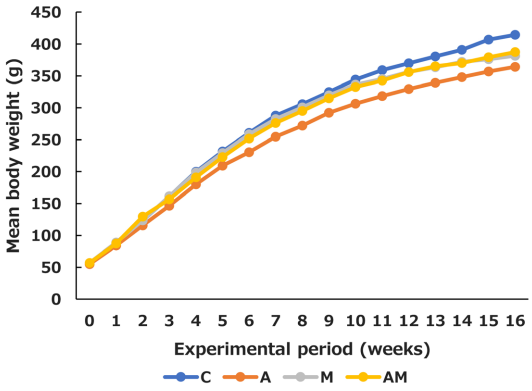

Figure 1. Weekly mean body weight of rats in each group. C, A, M, and AM are the control, D-allulose, MCT and D-allulose + MCT groups, respectively.

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

		C	A	M	AM	2 × 2 ANOVA		
						A	M	AxM
Body weight								
Initial	(g)	56.0 ± 2.9	55.0 ± 3.1	56.8 ± 2.2	57.2 ± 2.1	n.s.	n.s.	n.s.
Final	(g)	414.5 ± 11.1^{a}	364.2 ± 5.6^{b}	381.6 ± 4.7^{ab}	387.3 ± 9.9^{ab}	$p < 0.05$	n.s.	$p < 0.01$
Gain	(g)	385.5 ± 11.8^{a}	309.2 ± 5.6^{b}	324.8 ± 5.7^{ab}	330.1 ± 10.2^{ab}	$p < 0.05$	n.s.	$p < 0.01$
Food intake	(g/day)	15.0 ± 0.3	13.8 ± 0.3	14.2 ± 0.2	14.6 ± 0.4	n.s.	n.s.	$p < 0.05$
Food efficiency	(g/g)	0.215 ± 0.003	0.200 ± 0.000	0.207 ± 0.004	0.203 ± 0.003	$p < 0.05$	n.s.	n.s.
Visceral tissue weights								
Heart	(g)	0.913 ± 0.073	0.862 ± 0.060	0.878 ± 0.027	0.919 ± 0.095	n.s.	n.s.	n.s.
Liver	(g)	11.2 ± 1.1^{a}	10.9 ± 1.0^{ab}	9.6 ± 0.4^{b}	11.7 ± 0.9^{a}	$p < 0.05$	n.s.	$p < 0.01$
Kidneys	(g)	2.32 ± 0.33	2.64 ± 0.42	2.29 ± 0.37	2.73 ± 0.41	$p < 0.05$	n.s.	n.s.
Spleen	(g)	0.752 ± 0.073	0.683 ± 0.038	0.681 ± 0.049	0.675 ± 0.098	n.s.	n.s.	n.s.
Intra-abdominal adipose tissue weights								
Epididymal	(g)	15.9 ± 2.1^{a}	12.3 ± 2.1^{b}	13.8 ± 1.3^{ab}	13.5 ± 2.0^{ab}	$p < 0.01$	n.s.	$p < 0.05$
Perirenal	(g)	15.2 ± 2.7	12.5 ± 0.8	13.2 ± 1.7	15.2 ± 3.0	n.s.	n.s.	$p < 0.05$
Mesenteric	(g)	11.7 ± 2.9^{a}	7.0 ± 0.9^{b}	8.4 ± 1.1^{b}	8.8 ± 1.8^{b}	$p < 0.01$	n.s.	$p < 0.01$
Total	(g)	42.8 ± 6.6^{a}	27.8 ± 11.0^{b}	35.4 ± 4.0^{ab}	37.5 ± 6.0^{ab}	$p < 0.05$	n.s.	$p < 0.01$
Carcass fat	(g)	46.6 ± 2.7^{a}	32.0 ± 2.6^{b}	35.6 ± 2.5^{b}	43.0 ± 2.9^{ab}	$p = 0.20$	n.s.	$p < 0.01$
	(%)	18.0 ± 0.9	14.3 ± 1.0	15.3 ± 0.9	18.2 ± 1.1	n.s.	n.s.	$p < 0.01$
Total body fat	(g)	83.0 ± 4.4^{a}	59.0 ± 3.4^{b}	64.2 ± 4.1^{b}	74.8 ± 4.4^{ab}	$p = 0.08$	n.s.	$p < 0.01$
	(%)	19.9 ± 0.7	16.2 ± 0.9	17.2 ± 0.8	19.3 ± 0.8	n.s.	n.s.	$p < 0.01$

Values are the mean ± SE for eight rats. n.s., not significant. Within a row, values with different superscripts are significantly different ($p < 0.05$). C, A, M, and AM are the control, D-allulose, MCT and D-allulose + MCT groups, respectively.

In this study, we demonstrated the interaction between dietary D-allulose and MCT, which are indicators of obesity, body weight gain, intra-abdominal adipose tissue weights, weights and percentages of carcass and total body fat. However, their effects were not synergistic, as MCT supplementation offset the anti-obesity effects of dietary D-allulose. The mechanisms responsible for these effects remain unknown. However, the following points may provide some insights. In hepatocytes, MCFAs are more prone to oxidation and behave more like glucose than fat [20]. In contrast to LCFAs, MCFAs do not require CPT for mitochondrial transport and readily cross the mitochondrial membrane [21]. Consequently, the oxidation of MCFA is higher than that of LCFA [9], which increases the influx of acetyl-CoA into the tricarboxylic acid cycle. Hence, MCFA oxidations result in increased levels of acetyl-CoA, leading to increased ketone body production or lipogenesis [22]. In contrast, D-allulose increases CPT activity and β-oxidation [23] [24], increasing the transport of LCFAs to mitochondria. The combination of D-allulose and MCT greatly increases the in-

flux of fatty acids into the liver mitochondria. This may be supported by the results on D-allulose-induced increase in the serum β-hydroxybutyrate concentration and hepatic activity of MDH, a tricarboxylic acid cycle enzyme. However, because of their limited capacity for ATP production and ketone body synthesis [25], excess fatty acids are used for lipogenesis via acetyl-CoA and citric acid, thus contributing to fat accumulation in peripheral adipose tissues. Notably, liver G6PD, an enzyme involved in lipogenesis, was not suppressed by dietary D-allulose. In addition to our previous reports [14] [15], this study confirmed that the anti-obesity effect of dietary D-allulose is weakened by MCT.

3.2. Liver and Serum Biochemical Test Results

Dietary MCT significantly reduced the liver triglyceride content, whereas dietary D-allulose significantly increased the liver cholesterol content (Table 3). Dietary D-allulose significantly increased liver MDH activity, whereas the liver G6PD activity was not affected by both dietary dietary D-allulose and MCT (Table 3). Dietary D-allulose significantly increased the serum concentrations of free fatty acids, triglycerides, and β-hydroxybutyric acid; however, serum glucose, insulin, and total cholesterol levels were not affected by both dietary dietary D-allulose and MCT (Table 3). Furthermore, no interaction was observed between D-allulose and MCT in the liver and serum indicators (Table 3).

Table 3. Liver and serum biochemical test results.

		C	A	M	AM	2 × 2 ANOVA		
						A	M	AxM
Liver								
Triglyceride	(mg/g)	38.0 ± 5.0	36.8 ± 6.5	17.7 ± 2.5	27.2 ± 5.8	n.s.	$p < 0.01$	n.s.
Cholesterol	(mg/g)	5.26 ± 0.93	6.23 ± 1.06	3.36 ± 0.33	5.97 ± 0.67	$p < 0.01$	n.s.	n.s.
G6PD activity	(U/g tissue)	3.87 ± 0.18	3.58 ± 0.27	3.61 ± 0.33	3.82 ± 0.33	n.s.	n.s.	n.s.
MDH activity	(U/g tissue)	8.31 ± 0.22	9.22 ± 0.26	9.06 ± 0.27	9.27 ± 0.11	$p < 0.05$	n.s.	n.s.
Serum								
Glucose	(mg/dL)	150.0 ± 6.0	161.7 ± 7.7	141.3 ± 5.3	152.4 ± 3.7	n.s.	n.s.	n.s.
Insulin	(ng/mL)	8.25 ± 1.97	5.73 ± 0.83	5.80 ± 0.83	4.74 ± 0.44	n.s.	n.s.	n.s.
Total cholesterol	(mg/dL)	134.0 ± 8.3	126.0 ± 17.4	100.0 ± 9.0	133.2 ± 10.3	n.s.	n.s.	n.s.
Free fatty acids	(mEq/L)	0.86 ± 0.06	1.11 ± 0.12	0.89 ± 0.07	1.05 ± 0.07	$p < 0.05$	n.s.	n.s.
Triglycerides	(mg/dL)	132.3 ± 13.9	170.2 ± 30.5	103.0 ± 16.2	165.5 ± 14.7	$p < 0.05$	n.s.	n.s.
β-hydroxybutyric acid	(μmol/L)	297.5 ± 73.3	882.1 ± 481.8	348.3 ± 54.0	1913.6 ± 563.0	$p < 0.01$	n.s.	n.s.

Values are the mean ± SE for eight rats. n.s., not significant. Within a row, values with different superscripts are significantly different ($p < 0.05$). C, A, M, and AM are the control, D-allulose, MCT and D-allulose + MCT groups, respectively. G6PD, glucose-6-phosphate dehydrogenase; MDH, malate dehydrogenase.

Here, dietary D-allulose significantly increased the serum triglyceride and free fatty acid levels. However, these results may have been temporary due to in-

creased lipid metabolism. We previously found that serum triglyceride levels are significantly higher in rats fed a 5% D-allulose diet than in the control rats [7]. Several studies have investigated the effects of D-allulose on lipid metabolism. Kim *et al.* [26] reported that dietary D-allulose supplementation decreases the expression of lipoprotein lipase, triglyceride-hydrolyzing enzyme. Increase in serum triglyceride levels with D-allulose supplementation may decrease the fatty acid uptake from serum lipoproteins into adipose tissues by suppressing the lipoprotein lipase activity.

In this study, dietary MCT significantly reduced the liver triglyceride level. Several studies have reported the inhibitory effects of dietary MCT on liver triglyceride accumulation. Ronis *et al.* [27] administered rats isocaloric diets containing 10% - 70% total energy of corn oil or diets in which corn oil was replaced with saturated fats (beef tallow and MCT) for 21 days via total enteral nutrition. They reported that increasing MCT in the diet reduced hepatic steatosis. In addition, Mourad *et al.* [28] explored the potential MCT supplementation alleviate high-fat diet-induced hepatic steatosis in mice. In contrast to obese mice that remained on a high-fat diet, those that were transitioned to MCT diet showed a remarkable decline in hepatic lipid accumulation to levels similar to those observed in obese mice transitioned to a low-fat diet. The findings of this study partially support these reports.

4. Conclusion

Here, we demonstrated the anti-obesity effects of dietary D-allulose. We found that the anti-obesity effects of dietary MCT were weaker than those of D-allulose. We also confirmed the interaction between dietary D-allulose and MCT on indicators of obesity, body weight gain, intra-abdominal adipose tissue weight, weight, percentage of carcass, and total body fat. However, these effects were not synergistic, as MCT supplementation offset the anti-obesity effects of dietary D-allulose. Further studies are necessary to elucidate the exact mechanisms underlying these effects.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper. This study was supported by a grant from the Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan). T.Y. and T.I. are employees of this company.

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