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# Protective Effects of Dietary 1,5-Anhydro-D-glucitol as a Blood Glucose Regulator in Diabetes and Metabolic Syndrome

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**ABSTRACT:** 1,5-Anhydro-D-glucitol (1,5-AG) is fairly widespread in food products. It is also one of the major polyols in the human body, and its concentration is homeostatically regulated. We report here on the beneficial effects of 1,5-AG in preventing hyperglycemia and its role in improving metabolic syndrome. The findings revealed that it does not affect blood glucose levels itself under normal conditions but clearly has a suppressive effect on the levels of dietary sugars, such as glucose, maltose, and sucrose. A long-term administration study revealed that feeding *db/db* diabetic mice 3% 1,5-AG for 8 weeks significantly decreased blood glucose levels compared to untreated mice (339 ± 30 versus 438 ± 34 mg/dL; $p < 0.05$). Furthermore, this treatment also significantly suppressed serum cholesterol levels (110.2 ± 18.0 versus 168.4 ± 9.8 mg/dL; $p < 0.01$). 1,5-AG did not inhibit intestinal $\alpha$-glucosidase activities but regulated liver glucose levels via affecting both the glycogenolysis and gluconeogenesis pathways. Furthermore, the oral administration of 1,5-AG significantly increased urinary glucose excretion in hyperglycemic conditions. These results clearly suggest that dietary 1,5-AG acts as a modulator of glucose levels in hyperglycemia. 1,5-AG therefore represents a new class of promising functional sweeteners, where the daily consumption of 1,5-AG with meals could inhibit the progress of hyperglycemia and metabolic syndrome.

**KEYWORDS:** *1,5-Anhydro-D-glucitol, 1,5-AG, sugar alcohol, sweeteners, hyperglycemia, diabetes, metabolic syndrome, gluconeogenesis, glycogen-degrading enzymes, urinary glucose excretion*

## INTRODUCTION

Diabetes mellitus is one of the most common chronic metabolic diseases. Today, the worldwide prevalence of diabetes is assuming pandemic dimensions, with changing lifestyles leading to reduced physical activity and increased obesity. In 2010, 285 million people were reported to be suffering from diabetes, and this number is estimated to increase to 439 million by 2030.[1] Prolonged hyperglycemia is a primary causal factor of not only diabetic complications but also several related diseases, such as hypertension, dyslipidemia, arteriosclerosis, microangiopathy, and macroangiopathy.[2−5] Therefore, the careful control of blood glucose levels is effective in reducing diabetic complications. Various strategies have been proposed to prevent the development of hyperglycemia and diabetes mellitus. Recently, high-intensity artificial sweeteners (e.g., aspartame, acesulfame potassium, and sucralose) and sugar alcohols (e.g., sorbitol, erythritol, xylitol, maltitol, and lactitol) have been developed for nutritional and therapeutic use to manage problems associated with hyperglycemia-, excessive-weight-, and diabetes-related complications. They are considered to be low-calorie sweeteners and do not promote dental cavities. Despite these beneficial effects, several studies have reported that there are risks associated with the consumption of high levels of artificial sweeteners.[6] Furthermore, some reports suggest that the excessive intake of high-intensity artificial sweeteners can cause a loss of sensitivity to sweetness and might constitute a new trigger for the development of obesity and overeating.[7−9] Similarly, there are several aspects associated with the use of sugar alcohols that could be improved. For example, almost all sugar alcohols, except for erythritol, are not absorbed from the small intestine. They remain in the intestine and colon for a long period of time and are associated with a high frequency of flatulence, diarrhea, and laxative effects. Furthermore, while sugar alcohols have lower calories than glucose and sucrose, they still can result in moderate increases in blood glucose levels. However, none of these conventional sugar alcohols are able to directly suppress hyperglycemia.

Thus, the objective of this study was to identify effective natural sweeteners that directly affect hyperglycemia-, excessive-weight-, and diabetes-related complications. Our initial focus was on 1,5-anhydro-D-glucitol (1,5-AG). 1,5-AG is a cyclic sugar alcohol that adopts a C1-chair conformation in solution. It is structurally similar to D-glucose, except that it contains a methylene group at the anomeric position (Figure 1). 1,5-AG is

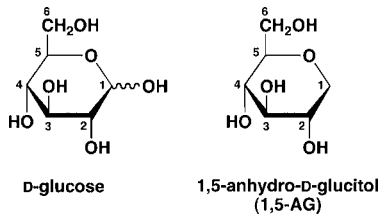


**Figure 1.** Structural similarity of D-glucose and 1,5-AG.

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60−65% as sweet as sucrose. Over 40 years have passed since 1,5-AG was discovered as the first natural glucose mimic in human cerebrospinal fluid.[10] 1,5-AG was later isolated from the plant families Proteaceae[11] and Polygalaceae[12] and was further found to be produced by *Escherichia coli*.[13] It is noteworthy that 1,5-AG is also fairly widespread in our daily food. The largest food source is soybeans, which contain approximately 22.4 ± 8.4 μg/g, while bread, rice, and buckwheat noodles contain 3.3 ± 1.1, 3.8 ± 1.4, and 3.8 ± 1.6 μg/g, respectively. 1,5-AG is also present in low levels in meats, fish, fruits, vegetables, tea, milk, and cheese.[14] In previous reports, the mean 1,5-AG intake through daily food was estimated to be ∼4.38 mg/day.[14] An additional point is that 1,5-AG is one of the major polyols present in human serum. The pool of 1,5-AG in the whole body amounts to a constant 500−1000 mg, and this concentration is homeostatically regulated. Plasma 1,5-AG levels are normally stable but are specifically reduced in diabetic subjects.[15,16] 1,5-AG intake is balanced by urinary excretion, and nearly 99.9% is reabsorbed by the kidney.[14] 1,5-AG is actively reabsorbed by a 1,5-AG/D-fructose/D-mannose selective transporter, which is referred to as the sodium glucose transporter (SGLT)-4. Because the reabsorbtion of 1,5-AG is competitively inhibited by urinary D-glucose, serum 1,5-AG levels are lower in diabetic patients.[17] On the basis of this property, serum 1,5-AG is used as a new clinical marker of glycemic control in diabetic patients.[18] The reference range for Japanese males (14.9−44.7 μg/mL) is slightly higher than for females (12.4−28.8 μg/mL). In comparison to glycated hemoglobin (HbA1c), which reflects mean glucose levels over a 1−3 month period, serum 1,5-AG levels reflect more recent glycemic control (1−3 days) and are a more sensitive indicator of changes in plasma glucose levels.

1,5-AG is pooled, and its level is regulated homeostatically, whereas the physiological role, biosynthesis, and metabolism of this molecule remain obscure at present. Considering this remarkable evidence, we hypothesized that 1,5-AG acts as a modulator of glucose levels and the positive effects on hyperglycemia might be gained in the case of 1.5-AG loading. To test this hypothesis, we examined the beneficial effects of 1,5-AG in preventing hyperglycemia and protecting or improving metabolic syndrome. We investigated the suppressive effects of 1,5-AG on blood glucose levels using a carbohydrate loading test *in vivo* and measured its effect on body weight, blood glucose, and serum cholesterol levels using *db*/*db* diabetic model mice.

## MATERIALS AND METHODS

**Materials.** Phosphoglucomutase, glucose-6-phosphate dehydrogenase, glucose-1,6-bisphosphate, glycogen phosphorylase b, and α-D-glucose 1-phosphate dipotassium salt hydrate were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO). Glycogen was purchased from Nacalai Tesque (Kyoto, Japan). Erythritol and other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). 1,5-AG was prepared according to a previously reported method.[19] Purity for 1,5-AG was established to be ≥98% by high-performance liquid chromatography (HPLC) analysis. HPLC conditions: a Shodex NH2P-40 3E column (3.0 × 250 mm) used 90:10 $CH_3CN/H_2O$ (flow rate of 0.3 mL/min) as the eluent and a Shimadzu RID-10 refractive index detector (retention time of 22.8 min for 1,5-AG).

**Animals.** Male ddy, C57BLKS/J Iar-+*Lepr*$^{db}$/+*Lepr*$^{db}$ (homo) and C57BLKS/J Iar-m+/m+ (misty) mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The mice were allowed *ad libitum* access to food (basal 18% casein diet, Labo MR stock, Nosan Corporation, Ltd., Kanagawa, Japan), unless stated otherwise. Animal rooms were maintained at 24 ± 1 °C, 60 ± 5% relative humidity, and a 12 h light/dark cycle, with lights on at 8:30 a.m. The animal experimental protocols in this study were approved by the Animal Experiments Committee of the University of Toyama.

**Single-Dose and Combined Administration Study.** Male ddy mice (7 weeks of age) were fasted overnight for 17 h. 1,5-AG and D-glucose were dissolved in a 0.9% NaCl solution. The mice were divided into two groups (groups A and B). (A) In this group, 1,5-AG (2.5 g/kg of body weight) and D-glucose (2.5 g/kg of body weight) were independently administered to the mice via a stomach tube. (B) In this group, both 1,5-AG (100 or 500 mg/kg of body weight) and D-glucose (2.5 g/kg of body weight) were mixed and administered to mice via a stomach tube. A control group was loaded with D-glucose (2.5 g/kg of body weight) only. Blood samples for glucose measurements were obtained from the tail vein at 0, 15, 30, 60, and 120 min after D-glucose loading. The blood glucose levels were measured using a portable kit, StatStrip Xpress (Nova Biochemical Co., Ltd., Waltham, MA).

**Disaccharide Loading Test.** Male ddy mice (7 weeks of age) were fasted overnight for 17 h. Maltose (A, 2.5 g/kg of body weight) or sucrose (B, 2.5 g/kg of body weight) as well as the test samples, 500 mg/kg of body weight for 1,5-AG and erythritol, were dissolved in a 0.9% NaCl solution and administered to the mice via a stomach tube. A control group was loaded with disaccharide only. Each experiment was performed with 5 mice per group. Glucose measurements were the same as those used in the single-dose administration study.

**Long-Term Administration Study of *db*/*db* Mice.** Male C57BLKS/J Iar-+*Lepr*$^{db}$/+*Lepr*$^{db}$ (homo) and C57BLKS/J Iar-m+/m+ (misty) mice (5 weeks of age) were housed in wire-bottomed cages under a conditional lighting regimen with dark light. The room temperature (about 24 ± 1 °C) and humidity (about 60 ± 5%) were controlled automatically. Each experiment was performed using 10 mice per group. The mice were divided into three groups (groups A, B, and C), and blood glucose levels were measured by the portable StatStrip Xpress (Nova Biochemical Co., Ltd., Waltham, MA) at the end of 1, 2, 4, 6, and 8 weeks. C57BLKS/J Iar-m+/m+ (misty) mice were used as controls (group C). Groups A (nontreated diabetic group) and C (misty) were fed a basal 18% casein diet (Labo MR stock, Nosan Corporation, Ltd., Kanagawa, Japan). Group B (1,5-AG feeding group) was fed the same basal 18% casein diet but with 3% 1,5-AG added. Food intake (g $day^{-1}$ $mouse^{-1}$) was calculated as the average for each mouse. After 8 weeks, serum cholesterol levels were measured by means of the cholesterol E-test Wako (Wako Pure Chemical Industries, Osaka, Japan).

**Assay of the Enzyme Activity.** Brush border membranes were prepared from rat small intestines according to the method described by Kessler et al.[20] and were used as the source of rat intestinal glucosidases. The activities of the rat intestinal α-glucosidases were determined using the appropriate disaccharides as substrates. The released D-glucose was determined colorimetrically using the Glucose CII kit from Wako (Wako Pure Chemical Industries, Osaka, Japan). Glycogen phosphorylase b activity was measured in the direction of glycogenolysis using 2 mg/mL glycogen as the substrate in 45 mM phosphate buffer at pH 6.8, containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.34 mM $NADP^+$, 4 mM glucose-1,6-bisphosphate, 15 mM magnesium chloride, 1 mM adenosine 5′-monophosphate (AMP), phosphoglucomutase (0.8 units/mL), glucose 6-phosphate (glucose-6-P) dehydrogenase (3 units/mL), and glycogen phosphorylase b. The rate of enzyme-catalyzed reaction in the medium can be followed by the increase in absorbance at 340 nm and 25 °C because of the formation of NADPH.[21]

**Pyruvate Tolerance Test.**[22] Male ddy mice (7 weeks of age) were fasted overnight for 17 h. Each experiment was performed using 5 mice per group. The mice were injected intraperitoneally with 2.0 g/kg of pyruvate dissolved in saline. 1,5-AG (500 mg/kg of body weight) was administered to mice via a stomach tube 30 min before the pyruvate injection. A control group was loaded with saline only. Blood samples for glucose measurements were obtained from the tail vein at 0, 15, 30, 60, and 120 min after pyruvate loading. The blood glucose levels were measured by a portable kit, StatStrip Xpress (Nova Biochemical Co., Ltd., Waltham, MA).

**Urinary Glucose Excretion in Mice.** Male ddy mice (8 weeks of age) were fasted for about 17 h. Each experiment was performed using 5 mice per group. The mice were then given an oral dose of saline or 1,5-AG at a dose of 1.0 g/kg of body weight and placed in metabolic cages. At 1 h after the treatment, the mice were orally dosed with D-glucose (2.5 g/kg of body weight). Urine was collected for 20 h after the dosing of 1,5-AG. The urinary glucose concentration was measured using a portable digital urinary analyzer, model UG-201 (Tanita, Ltd., Tokyo, Japan).

**Maillard Reaction Test.** The test solution was divided into four groups (groups A, B, C, and D). Groups A (D-glucose) and B (1,5-AG) were dissolved in 50 mM acetate buffer (pH 4.5) and adjusted to a 5% solution. Groups C (D-glucose) and D (1,5-AG) were dissolved in 50 mM phosphate buffer (pH 7.5) and adjusted to a 5% solution. Each reaction mixture containing 1.0% glycine was filtered through a membrane filter (0.44 $\mu$m). After the filtrate was allowed to settle in a propylene tube, it was sealed and heated for 20 min at 121 °C in an autoclave.

## RESULTS AND DISUSSION

**Effects of Single-Dose and Combined Administrations of 1,5-AG and D-Glucose.** In the first experiment, we compared the single-dose administration of 1,5-AG and D-glucose against blood glucose levels (Figure 2A). Blood glucose levels were measured using a portable StatStrip Xpress kit. The administration of D-glucose (2.5 g/kg of body weight p.o.) to fasted mice resulted in a rapid increase in blood glucose levels from 103 ± 7 mg/dL to a maximum of 285 ± 19 mg/dL after 30 min. Thereafter, the blood glucose levels recovered to the pretreatment level at 120 min. When 1,5-AG was compared to D-glucose, 1,5-AG (2.5 g/kg of body weight p.o.) had no effect on blood glucose levels at any of the time points measured (Figure 2A). Thus, we next focused on the combined effects of 1,5-AG with D-glucose. In the case of the oral administration of D-glucose (2.5 g/kg of body weight) and 1,5-AG (100 or 500 mg/kg of body weight), 1,5-AG clearly suppressed postprandial hyperglycemia in a dose-dependent manner (Figure 2B). These results suggest that 1,5-AG itself does not increase blood glucose levels but clearly exerts a suppressive effect in the presence of D-glucose.

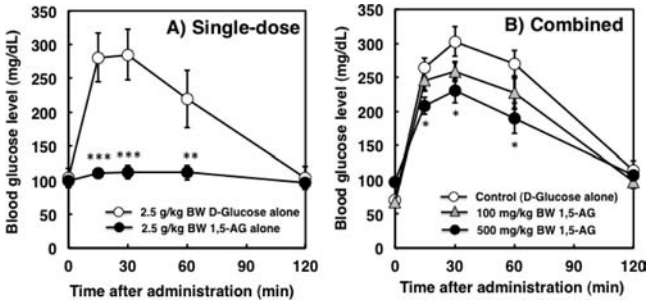


**Figure 2.** Effects of single-dose and combined administrations of D-glucose and 1,5-AG on blood glucose levels. (A) Blood glucose concentrations of male ddy mice ($n$ = 5) after an oral load with (○) 2.5 g/kg of body weight D-glucose or (●) 2.5 g/kg of body weight 1,5-AG, independently. (B) Blood glucose concentrations of male ddy mice ($n$ = 5) after an oral load with 2.5 g/kg of body weight D-glucose with (△) 100 mg/kg of body weight 1,5-AG and (●) 500 mg/kg of body weight 1,5-AG by oral administration (p.o.). (○) Control group ($n$ = 5) was loaded with 2.5 g/kg of body weight D-glucose alone. Each value represents the mean ± standard error of the mean (SEM) ($n$ = 5). Statistical significance compared to the D-glucose-loading group is as follows: (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.

**Disaccharide Loading Test.** To understand the suppression basis for 1,5-AG with carbohydrate, we furthermore investigated the influence of 1,5-AG and erythritol, which was used as a reference compound, on blood glucose levels after an *in vivo* disaccharide loading (Figure 3). Erythritol is a C4 polyol that is fairly widespread in fruits and daily food, similar to 1,5-AG. The control group was loaded with saline only. In comparison to the controls, 1,5-AG caused a significant lowering in blood glucose levels at 15 and 30 min compared to a placebo administration after maltose (2.5 g/kg of body weight) loading (Figure 3A). For comparison, we next investigated the influence of 1,5-AG using a sucrose-loading test (Figure 3B). The administration of sucrose (2.5 g/kg of body weight p.o.) to fasted mice resulted in a rapid increase in blood glucose levels from 92 ± 8 mg/dL to a maximum of 181 ± 14 mg/dL after 30 min. Thereafter, blood glucose levels recovered to the pretreatment level by 120 min. 1,5-AG resulted in significantly lower blood glucose levels at 15 and 30 min compared to the administration of the placebo. In sharp contrast, erythritol showed no suppressing effects in both loading tests. These results clearly suggest that the suppression effects of 1,5-AG are not dependent upon the dietary sugar type, which include blood-glucose-increasing sugars, such as glucose, maltose, and sucrose. This is one of the advantages of 1,5-AG over other functional sugars and sugar alcohols. For example, L-arabinose is able to inhibit intestinal sucrase activity and, thereby, delay sucrose digestion, but its effects are limited to sucrose loading only.[23,24]

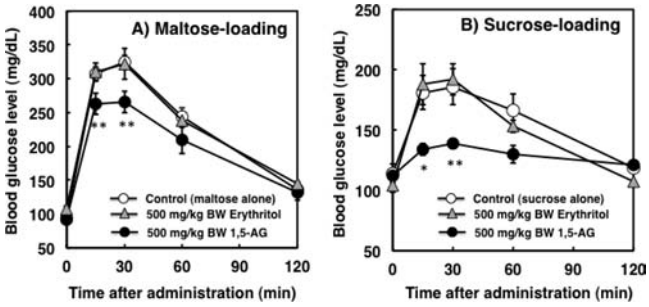


**Figure 3.** Effects of erythritol and 1,5-AG on blood glucose levels. Blood glucose concentrations of male ddy mice ($n$ = 5) after an oral load of 2.5 g/kg of body weight (A) maltose or (B) sucrose, with 500 mg/kg of body weight (△) erythritol and (●) 1,5-AG. (○) Control group was loaded with maltose or sucrose alone. Each value represents the mean ± SEM ($n$ = 5). Statistical significance compared to the control group is as follows: (*) $p < 0.05$ and (**) $p < 0.01$.

**Long-Term Administration Study against *db*/*db* Mice.** On the basis of these findings, it appears that the short-term administration of 1,5-AG might result in the suppression of postprandial hyperglycemia. Thus, we next focused on a long-term administration study using *db*/*db* diabetic mice and whether 1,5-AG is able to suppress hyperglycemia. Moreover, we also investigated the effect of 1,5-AG on body weight and serum cholesterol levels. The mice were divided into three groups (groups A, B, and C): nontreated C57BLKS/J Iar-+*Lepr*$^{db}$/+*Lepr*$^{db}$ (*db*/*db*) mice ($n$ = 10) with a mean body weight of 22.3 ± 1.0 g at 7 weeks of age (group A), 1,5-AG-treated *db*/*db* mice ($n$ = 10) with a mean body weight of 21.4 ± 0.9 g at 7 weeks of age (group B), and nondiabetic C57BLKS/J Iar-m+/m+ (misty) mice ($n$ = 10) with a mean body weight of 17.0 ± 0.3 g at 7 weeks of age (group C). Groups A and C were


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fed a basal 18% casein diet. Group B was fed the same basal 18% casein diet but with 3% 1,5-AG added. In comparison to the *m*/*m* misty mice (group C), the *db*/*db* diabetic mice (groups A and B) obviously showed overeating characteristics during this experimental period (Figure 4A). However, no

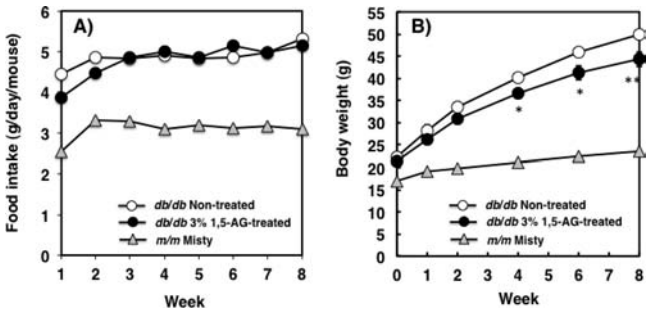


**Figure 4.** (A) Average daily food intake and (B) changes in body weight with or without 3% 1,5-AG treatment over 8 weeks. (○) Nontreated *db*/*db* mouse ($n$ = 10) and (△) misty mice ($n$ = 10) were fed a basal 18% casein diet. (●) 1,5-AG-treated *db*/*db* mice ($n$ = 10) were fed a 3% 1,5-AG-containing basal 18% casein diet. Food intake (g day$^{-1}$ mouse$^{-1}$) was calculated as the average of each mouse.

significant differences were found between groups A and B. Total feeding weights of each group were 273.1, 268.1, and 173.4 g, respectively. These results suggest that 1,5-AG itself does not induce overeating or refusal to feed in diabetic mice. It is noteworthy that the 1,5-AG treatment (group B) significantly reduced body weight compared to nontreated diabetic mice (group A) after 8 weeks (50.0 ± 0.7 versus 44.3 ± 1.6 g; $p$ < 0.01), even though there was no significant difference in food intake (Figure 4B). Furthermore, we monitored the blood glucose levels of each group at 1, 2, 4, 6, and 8 weeks after the experiments (Figure 5A). The nontreated *db*/*db* diabetic mice

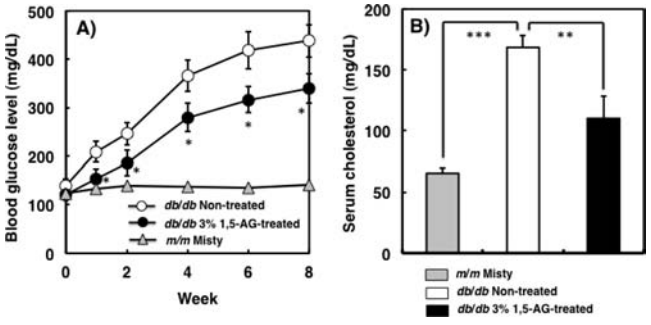


**Figure 5.** Effects of the long-term administration of 1,5-AG on (A) blood glucose and (B) serum cholesterol levels. Nontreated *db*/*db* mice, 3% 1,5-AG-fed *db*/*db* mice, and misty mice were kept in wire-bottomed cages for 8 weeks. Each value represents the mean ± SEM ($n$ = 10). (A) Statistical significance compared to nontreated *db*/*db* mice is as follows: (∗) $p$ < 0.05. (B) Statistical significance compared to nontreated *db*/*db* mice versus misty mice and nontreated *db*/*db* mice versus 3% 1,5-AG-fed *db*/*db* mice is as follows: (∗∗) $p$ < 0.01 and (∗∗∗) $p$ < 0.001.

(group A) showed a significant elevation in blood glucose levels compared to the misty mice (209 ± 21 versus 133 ± 6 mg/dL) at week 2. Thereafter, blood glucose levels continued to rise gradually and reached 438 ± 34 mg/dL at week 8. The next notable point is that treatment of 3% 1,5-AG (group B) for 8 weeks resulted in a significant decrease in blood glucose levels compared to the untreated *db*/*db* diabetic mice (339 ± 30 mg/dL). To check whether 1,5-AG treatment had any influence on lipid metabolism, we measured the serum cholesterol levels after 8 weeks (Figure 5B). In comparison to the *m*/*m* misty mice (group C), the *db*/*db* diabetic mice (group A) had significantly increased serum cholesterol levels (65.5 ± 4.2 versus 168.4 ± 9.8 mg/dL; $p$ < 0.001). This study revealed that the 3% 1,5-AG-treated group significantly suppressed serum cholesterol levels compared to untreated *db*/*db* diabetic mice (110.2 ± 18.0 versus 168.4 ± 9.8 mg/dL; $p$ < 0.01). These results suggest that the long-term administration of 1,5-AG suppressed not only hyperglycemia but also serum cholesterol levels. In addition, the 3% 1,5-AG-treated group did not appear to induce flatulence or diarrhea during this period. 1,5-AG differs from straight-chain sugar alcohols, such as xylitol, sorbitol, and mannitol, because it is rapidly absorbed in the small intestine. This pharmacokinetic property consequently enables postprandial hyperglycemia to be quickly suppressed, with no obvious gastrointestinal complications.

**Effects of 1,5-AG on Glycogen-Degrading Enzymes and Gluconeogenesis.** Type II diabetes is a heterogeneous disease characterized by hyperglycemia, which is caused by a disorder involving insulin secretion, insulin resistance in target tissues, and activation of the hepatic glucose production pathway in the liver.[25,26] Hepatic glucose levels are affected by the glycogenolysis (the breakdown of glycogen) and gluconeogenesis (the synthesis of hexoses from 3-carbon precursors) pathways. A number of studies have suggested that more than 70% of the total hepatic glucose production is due to the breakdown of glycogen in type II diabetes patients.[27] Furthermore, hepatic insulin resistance is also affected by a lack of suppression of glycogenolysis.[28] A possible strategy for suppressing hepatic glucose production and lowering blood glucose levels in type II diabetes may be through the inhibition of glycogenolysis and gluconeogenesis.[29] We first evaluated the ability of 1,5-AG to inhibit rat intestinal maltase, isomaltase, and sucrase activities. 1-Deoxynojirimycin (DNJ) was also tested to extend our understanding of the inhibition mechanisms of 1,5-AG. DNJ is an analogue of 1,5-AG, in which the ring oxygen has been replaced by nitrogen. We previously reported that the $IC_{50}$ values of DNJ toward rat intestinal maltase, sucrase, and isomaltase are 0.36, 0.21, and 0.30 $\mu$M, respectively.[30] In sharp contrast, 1,5-AG did not inhibit intestinal $\alpha$-glucosidases, even at concentrations as high as 1000 $\mu$M. This result indicates that the suppression effect of 1,5-AG against hyperglycemia is not due to the inhibition of intestinal $\alpha$-glucosidase enzymes. Thus, we therefore focused on its effect on glycogen-degrading enzymes. Glycogen phosphorylase (EC 2.4.1.1) is a key enzyme in the regulation of glycogen metabolism and catalyzes the degradative phosphorolysis of glycogen to produce glucose 1-phosphate. Glycogen phosphorylase exists in two interconvertible forms, namely, the dephosphorylated low-activity form, glycogen phosphorylase b, and the Ser14-phosphorylated high-activity form, glycogen phosphorylase a. Glycogen phosphorylase b can be converted into glycogen phosphorylase a through phosphorylation by a phosphorylase kinase. Glycogen phosphorylase is an allosteric enzyme, and allosteric effectors in both forms can promote equilibrium between a less active T state and a more active R state. Under normal conditions, glycogen phosphorylase b is predominantly in the T state and glycogen phosphorylase a is in the R state. AMP, an allosteric effector, activates glycogen phosphorylase b by changing its

conformation from the T state to the R state. This study revealed that 1,5-AG showed inhibitory activity against glycogen phosphorylase b, with an $IC_{50}$ value of 671 $\mu$M. In contrast, DNJ did not inhibit this enzyme. Considering this interesting activity, we envisaged that 1,5-AG might be used to alter liver glycogenolysis and gluconeogenesis. To test this hypothesis, we investigated the influence of 1,5-AG on blood glucose levels after an *in vivo* pyruvate loading (Figure 6). The

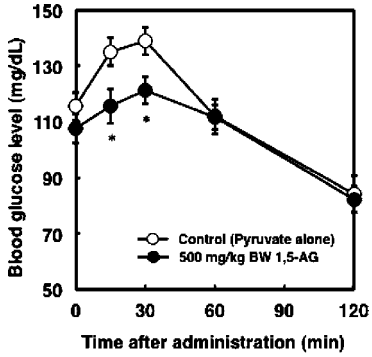


**Figure 6.** Effects of 1,5-AG on liver gluconeogenesis. Blood glucose concentrations of a male ddy mouse ($n$ = 5) after an oral load with 2.0 g/kg of pyruvate with (●) 500 mg/kg of body weight 1,5-AG. (○) Control group was loaded with pyruvate alone. Each value represents the mean ± SEM ($n$ = 5). Statistical significance compared to the control group is as follows: (*) $p < 0.05$.

administration of pyruvate (2.0 g/kg of body weight intraperitoneally) to fasted mice resulted in a rapid increase in blood glucose levels, from mg/dL 108 ± 5 to a maximum of 121 ± 5 mg/dL after 30 min. Thereafter, blood glucose levels recovered to the pretreatment level at 120 min. 1,5-AG resulted in significantly lower blood glucose levels at 15 and 30 min compared to a placebo administration (Figure 6). These results strongly suggest that 1,5-AG regulates liver glucose levels by virtue of its ability to affect both the glycogenolysis and gluconeogenesis pathways.

**Effects of 1,5-AG on Urinary Glucose Excretion *in Vivo*.** The kidney has a key role in the regulation of plasma glucose levels by mediating the reabsorption of glucose back into the plasma.[31] The reuptake of glucose in the proximal tubules is mediated by sodium-glucose cotransporter-2 (SGLT2), which is a high-capacity, low-affinity transporter that is exclusively found in the S1 segment of the proximal renal tubules.[32,33] It is thought that more than 90% of renal glucose is mediated by SGLT2. This process contributes to the maintenance of serum glucose levels and to retaining calories. Inhibiting this glucose reabsorption process is therefore emerging as a potential new approach to the treatment of diabetes because it should permit excessive glucose to be excreted and, therefore, reduce calories.[34,35] We therefore evaluated the ability of 1,5-AG to cause the excretion of urinary glucose (Figure 7). Urinary glucose excretion was 1.9-fold greater after an oral glucose (2.5 g/kg of body weight) loading in mice with vehicle compared to that in nontreated mice. The administration of 1,5-AG (1.0 g/kg of body weight) along with glucose (2.5 g/kg of body weight) significantly increased urinary glucose excretion compared to glucose alone (77.5 ± 4.8 versus 60.0 ± 4.5 mg/dL; $p < 0.05$). It is noteworthy that a single administration of 1,5-AG (1.0 g/kg of body weight p.o.) had no effect on urinary glucose excretion compared to the nontreated group (38.0 ± 4.5 versus 32.0 ± 4.5 mg/dL). These

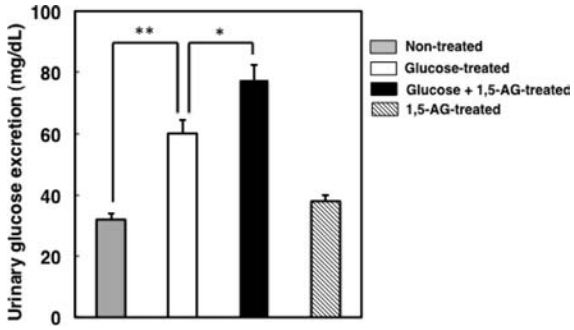


**Figure 7.** Effects of 1,5-AG on urinary glucose excretion. Urinary glucose excretion of male ddy mice ($n$ = 5) after an oral load of 2.5 g/kg of body weight D-glucose, with or without 1.0 g/kg of body weight 1,5-AG by oral administration (p.o.). The control group ($n$ = 5) was loaded with 2.5 g/kg of body weight D-glucose alone. Each value represents the mean ± SEM ($n$ = 4−5). Statistical significance compared to D-glucose alone is as follows: (*) $p < 0.05$ and (**) $p < 0.01$.

results suggest that the therapeutic use of 1,5-AG may be useful in treating diabetes mellitus by increasing the loss of filtered glucose and could also potentially improve hyperglycemia and excessive weight through the loss of excess calories.

**1,5-AG Does Not Function as a Precursor of Advanced Glycation Endoproducts.** Our present study revealed that the short- and long-term administration of 1,5-AG might be of benefit in the treatment of diabetes or metabolic syndrome. Thus, we next focused on its physical properties. Advanced glycation end-products (AGEs) are closely related to hyperglycaemia. AGEs are a complex and heterogeneous group of molecules formed as the result of the non-enzymatic reaction of reducing sugars with free amino groups of proteins, lipids, and nucleic acids.[36] The reaction involves the initial, reversible formation of a Schiff base between a reducing sugar and the amino group of a protein, such as lysine and arginine. These early stages are dependent upon the concentration of the reactants. Under hyperglycaemic conditions, the relatively unstable Schiff base undergoes a rearrangement to form more stable Amadori products. Complex oxidation, dehydration, and condensation reactions subsequently lead to the intra- and intermolecular cross-linking of the proteins to produce AGEs. For example, fructose can become phosphorylated to fructose-3-phosphate, which is broken down to 3-deoxyglucosone, ultimately forming AGEs that are capable of cellular damage.[37] These abnormal metabolic results have been reported to be factors responsible for diabetic complications, vascular damage, and Alzheimer's disease.[38,39] We examined the issue of whether 1,5-AG can serve as a precursor in the production of Amadori products. D-Glucose, which structurally resembles 1,5-AG, was used as a positive control. The reaction mixture contained 5% glucose or 1,5-AG in the presence of 1.0% glycine and was incubated for 20 min at 121 °C in an autoclave. With glucose, the resulting solution was colored brown. A color change was observed under both weak acidity (pH 4.5) and neutral (pH 7.5) conditions (A and C of Figure 8). In sharp contrast, when 1,5-AG was incubated with glycine under the same conditions, no change in color was found (B and D of Figure 8). These results suggest that dietary 1,5-AG, which differs from D-glucose, does not participate in the Amadori reaction and, therefore, poses minimal risk in terms of causing diabetic complications.

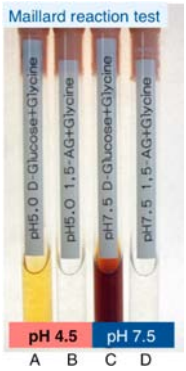


**Figure 8.** Comparison of 1,5-AG and D-glucose for participation in the Maillard reaction. The reaction mixture containing 5% glucose or 1,5-AG with the presence of 1.0% glycine was incubated for 20 min at 121 °C in an autoclave.

In this study, we report on the beneficial effects of 1,5-AG on the prevention of hyperglycemia and the protection or improvement of metabolic syndrome. The main features of 1,5-AG are as follows: (a) it does not affect blood glucose levels itself under normal conditions, while it obviously showed a suppressive effect on the levels of D-glucose; (b) disaccharide-loading tests revealed that it obviously differed from conventional functional sugars and sugar alcohols in that the suppressive effects did not depend upon the type of dietary sugar and included glucose, maltose, and sucrose; (c) the long-term administration of 1,5-AG significantly suppressed not only hyperglycemia but also serum cholesterol levels under diabetic conditions; furthermore, 1,5-AG treatment significantly reduced body weight after 8 weeks, whereas 1,5-AG itself did not induce overeating in diabetic mice; (d) 1,5-AG did not inhibit intestinal $\alpha$-glucosidase activities, while 1,5-AG regulates liver glucose levels via affecting both the glycogenolysis and gluconeogenesis pathways; and (e) the administration of 1,5-AG significantly increased urinary glucose excretion in hyperglycemic conditions. Further studies will clearly be needed to more completely understand the physiological significance of 1,5-AG, but our results clearly suggest that 1,5-AG acts as a modulator of glucose levels in hyperglycemic conditions. 1,5-AG is supplemented through daily foods. Furthermore, it is pooled in the body, and its level is regulated homeostatically. Considering this remarkable evidence, 1,5-AG represents a new class of promising functional sweeteners that has the potential for preventing postprandial hyperglycemia and metabolic syndrome.

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### Notes

The authors declare no competing financial interest.

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