



## Applied nutritional investigation

## d-Allulose enhances postprandial fat oxidation in healthy humans



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## ABSTRACT

**Objective:** d-Allulose, a C-3 epimer of d-fructose, has been reported to decrease body weight and adipose tissue weight in animal studies and is expected to be a potent antiobese sweetener. Our animal study suggested that one of the mechanisms of d-allulose's antiobesity function is an increase in energy expenditure. However, a few studies have thus far explored the underlying mechanism in humans. The aim of this study was to examine the effects of a single ingestion of d-allulose on postprandial energy metabolism in healthy participants.

**Methods:** Thirteen healthy men and women (mean age of  $35.7 \pm 2.1$  y and body mass index  $20.9 \pm 0.7$  kg/m<sup>2</sup>) were studied. The study was a randomized, single-blind crossover design with a 1-wk washout period. At 30 min after taking 5 g of d-allulose or 10 mg of aspartame without any sugar as a control, overnight-fasted participants ingested a standardized meal, and energy metabolism was evaluated by a breath-by-breath method. During the experiment, blood was collected and biochemical parameters such as plasma glucose were analyzed.

**Results:** In the d-allulose-treated group, the area under the curve of fat oxidation was significantly higher than in the control group ( $10.5 \pm 0.4$  versus  $9.6 \pm 0.3$  kJ·4 h·kg<sup>-1</sup> body weight [BW];  $P < 0.05$ ), whereas that of carbohydrate oxidation was significantly lower ( $8.1 \pm 0.5$  versus  $9.2 \pm 0.5$  kJ·4 h·kg<sup>-1</sup> BW;  $P < 0.05$ ). Furthermore, plasma glucose levels were significantly lower, and free fatty acid levels were significantly higher in the d-allulose group than in the control group. No other parameters such as insulin, total cholesterol, or triacylglycerol were modified.

**Conclusion:** d-Allulose enhances postprandial fat oxidation in healthy humans, indicating that it could be a novel sweetener to control and maintain healthy body weight, probably through enhanced energy metabolism.

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## Introduction

Obesity is one of the factors affecting most of the prevalent lifestyle-related health complications such as coronary heart disease, diabetes, and certain types of cancer [1]. Obesity results from an imbalance between energy intake and energy expenditure [1]. However, it is difficult to decrease energy intake from

foods because advanced food technology has introduced energy-packed or energy-hidden foods to the market. Therefore, an increase in energy expenditure has emerged as an attractive strategy for treating or preventing obesity. To this end, food ingredients to burn energy have been explored.

d-Allulose (previously referred to as d-psicose), a C-3 epimer of d-fructose, has 70% sweetness of sucrose, is rarely found in nature, and therefore is referred to as a rare sugar [2]. d-Allulose is also formed from d-fructose during cooking and is present in a very small amount in various foods such as fruit juices and cola drinks [3]. The d-allulose content per 100 g ranges from 0.5 to 130.6 mg; fruit juice contains 21.5 mg and cola drink 38.3 mg. The daily intake of d-allulose is ~0.2 g [3]. About 70% of

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d-allulose is absorbed and then is excreted via urine. The remaining is not fermented in the large intestine and is excreted into feces [4]. Furthermore, d-allulose is a noncaloric sweetener [5] and has been approved as a Generally Recognized as Safe ingredient by the Food and Drug Administration in 2014.

Studies showed that d-allulose is a potential antidiabetic and antiobese sweetener; 0.2 g/kg of d-allulose decreases absorption of sugar by inhibiting intestinal  $\alpha$ -glucosidase in Wistar rats compared with nontreated animals [6]. Intake of 0.2 g/kg of d-allulose activates the translocation of glucokinase from the nucleus to the cytosol in the liver that facilitates glycogen biosynthesis in Wistar and Goto-Kakizaki (type 2 diabetes mellitus [T2DM] model) rats [7]. In healthy humans, a single ingestion of 5 g of d-allulose improves insulin sensitivity after administering maltodextrin [8]. Feeding the 5% d-allulose solution enhances insulin sensitivity in T2DM model Otsuka Long-Evans Tokushima Fatty rats compared with only water [9]. Furthermore, d-allulose decreases body weight and abdominal adipose tissue weight in animal studies [10–13]. The proposed underlying mechanism of d-allulose-induced antiobese action is that it suppresses the activity of hepatic lipogenic enzymes such as fatty acid synthase (FAS) and glucose-6-phosphate dehydrogenase [10,12], augments the hepatic carnitine palmitoyl-transferase activity essential for fatty acid oxidation [13], and increases energy expenditure [12,13].

However, no studies thus far have been done to see if and how d-allulose modifies energy metabolism in humans. Furthermore, to apply d-allulose to a broad range of diet regimens, clinical studies are imperative. We thus investigated the effects of a single ingestion of d-allulose on postprandial energy metabolism in 13 healthy individuals. We measured short-term (4-h) energy metabolism as d-allulose is metabolized and excreted from the body quickly after ingestion [14] and has an immediate effect on lipid metabolism in rats [13]. To our knowledge, the present study showed for the first time that d-allulose increased fat oxidation and decreased carbohydrate oxidation in healthy humans. This result suggests the possibility that d-allulose may help control healthy body weight, partially via enhanced energy metabolism in humans.

## Materials and methods

### Participants

Thirteen healthy volunteers (five men and eight women) were recruited. Participants were excluded if they were diagnosed with diabetes or metabolic disorders such as cardiovascular diseases. Individuals with a mean age of  $35.7 \pm 2.1$  y and body mass index (BMI) of  $20.9 \pm 0.7$  kg/m<sup>2</sup> participated in the study. This study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan). Written informed consent was obtained from all participants before the experiment.

### Test samples

The test sample used in this study was 5 g of d-allulose with >99% purity (Matsutani Chemical Industry Co., Ltd.). As a control, 10 mg of aspartame (Ajinomoto Co., Inc. Tokyo, Japan) was used without any sugar. Each sample was dissolved in 150 mL of water and their sweetness was equivalent.

### Experimental design

This was a randomized, single-blind study, and all participants received crossover treatment after a 1-wk washout period. They were asked to avoid overeating and excessive exercise, and consumed a specified evening meal (about 450 kcal containing ~15 g of fat [30% as calorie] and ~30 g of protein [27% as calorie]). The specified evening meal was given before 2100 so that participants fasted at least 12 h before the experiment.

Participants first took the beverage containing d-allulose or aspartame, and then 30 min later, ingested a standardized breakfast over 10 min. Breakfast consisted of 200 g of cooked rice, 166 g of hamburger steak, and 150 mL of water, which provided 571 kcal containing 61% of calories as carbohydrate, 25% as fat, and 14% as protein. After breakfast, energy metabolism was evaluated by a breath-by-breath method, blood was collected, and biochemical parameters such as plasma glucose were analyzed.

### Energy metabolism assessment

Energy metabolism was measured by a breath-by-breath method using a respiratory gas analyzer (AE-310 S; Minato Medical Science Co., Ltd. Osaka, Japan). After breakfast, participants sat quietly with a face mask on, and then energy metabolism was measured for 6 min intervals every 30 min throughout the experiment (4 h). Data obtained during the last 3 min of each measurement period were analyzed and averaged by calculating minute by minute. Assuming that protein oxidation accounts for 15% energy expenditure, resting energy expenditure (REE), carbohydrate energy expenditure (CEE), and fat energy expenditure (FEE) were calculated by the method of Elia and Livesey [15], and expressed as per body weight (BW). Respiratory quotient (RQ) also was analyzed. The area under the curve (AUC) was calculated as the area between the curve and the x axis ( $y = 0$ ) [16].

### Plasma metabolite analyses

During the measurement of energy metabolism, blood was collected, and the levels of plasma glucose, insulin, total cholesterol (TC), triacylglycerol (TG), and free fatty acids (FFA) were analyzed. Changes in parameters such as glucose and insulin were calculated by subtracting values at each time point from baseline values ( $\Delta$ ).

### Statistical analysis

All of the values are expressed as mean  $\pm$  SEM. Two-way analysis of variance was used to analyze time-course changes (treatment group  $\times$  time). When a significant effect of each group or interaction was found, a paired Student's *t* test was carried out at each time point.  $P < 0.05$  was considered statistically significant. Statistics were determined by using SPSS 13.0 J (IBM Co., Armonk, NY, USA).

## Results

### Energy metabolic parameters

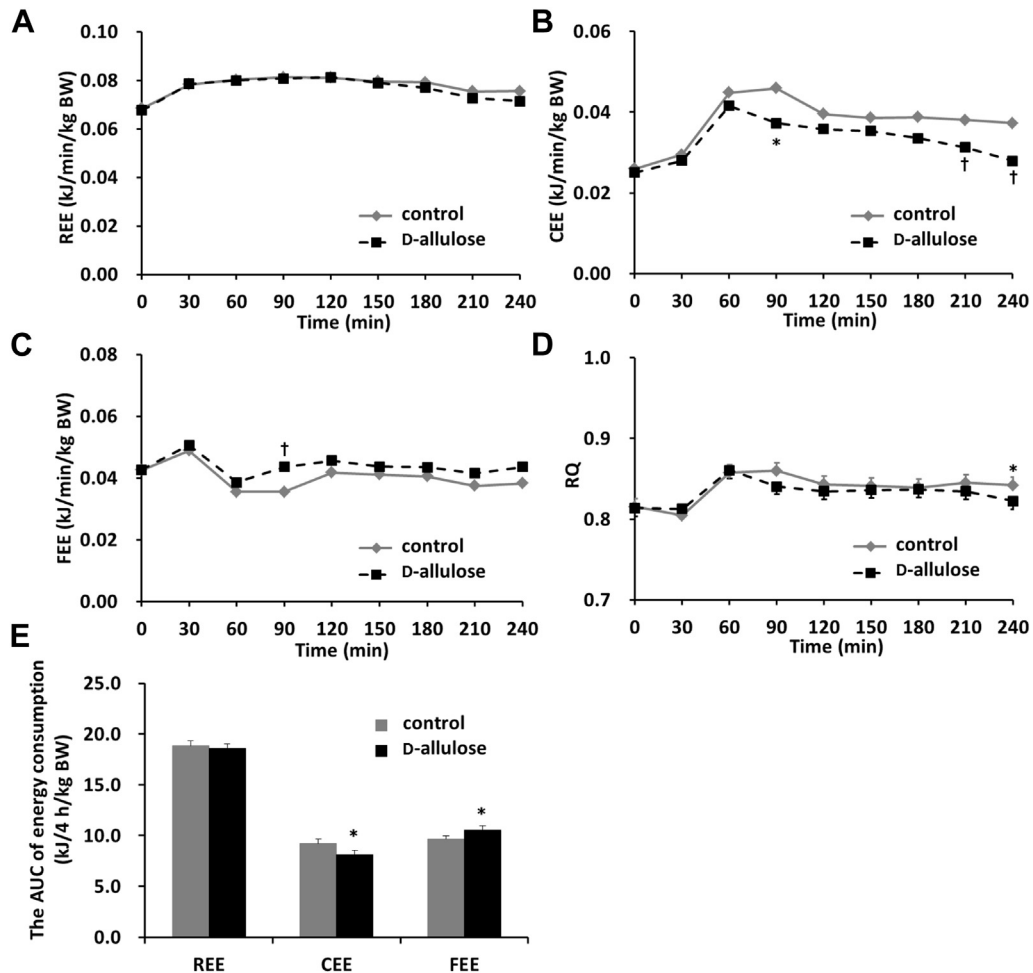
No significant difference in REE between the two groups was demonstrated (Fig. 1A). However, ingesting d-allulose caused a significant increase in FEE at 90 min compared with the control group (Fig. 1C). CEE and RQ were significantly lower in the d-allulose group than in the control group; CEE at 90, 210, and 240 min (Fig. 1B), and RQ at 240 min (Fig. 1D) were lower. The AUC of REE, CEE, and FEE for 4 h after a meal are summarized in Figure 1E. The AUC of FEE was significantly increased ( $10.5 \pm 0.4$  versus  $9.6 \pm 0.3$  kJ  $\cdot$  4 h  $\cdot$  kg<sup>-1</sup> BW;  $P < 0.05$ ), whereas that of CEE was significantly decreased ( $8.1 \pm 0.5$  versus  $9.2 \pm 0.5$  kJ  $\cdot$  4 h  $\cdot$  kg<sup>-1</sup> BW;  $P < 0.05$ ) in the d-allulose group compared with the control group (Fig. 1E).

### Plasma metabolite parameters

In the d-allulose-treated group,  $\Delta$ plasma glucose at 90 min (Fig. 2A) was significantly lower. After a single ingestion of d-allulose, FFA was kept at higher levels compared with the control with the difference at 180 min and thereafter being significant (Fig. 2E). There were no differences in the levels of insulin, TC, and TG between two groups (Fig. 2B–D).

## Discussion

In previous animal studies, d-allulose decreased body weight and abdominal adipose tissue weight [10–13], suggesting that the 3% to 5% d-allulose diets may have antiobese action. The



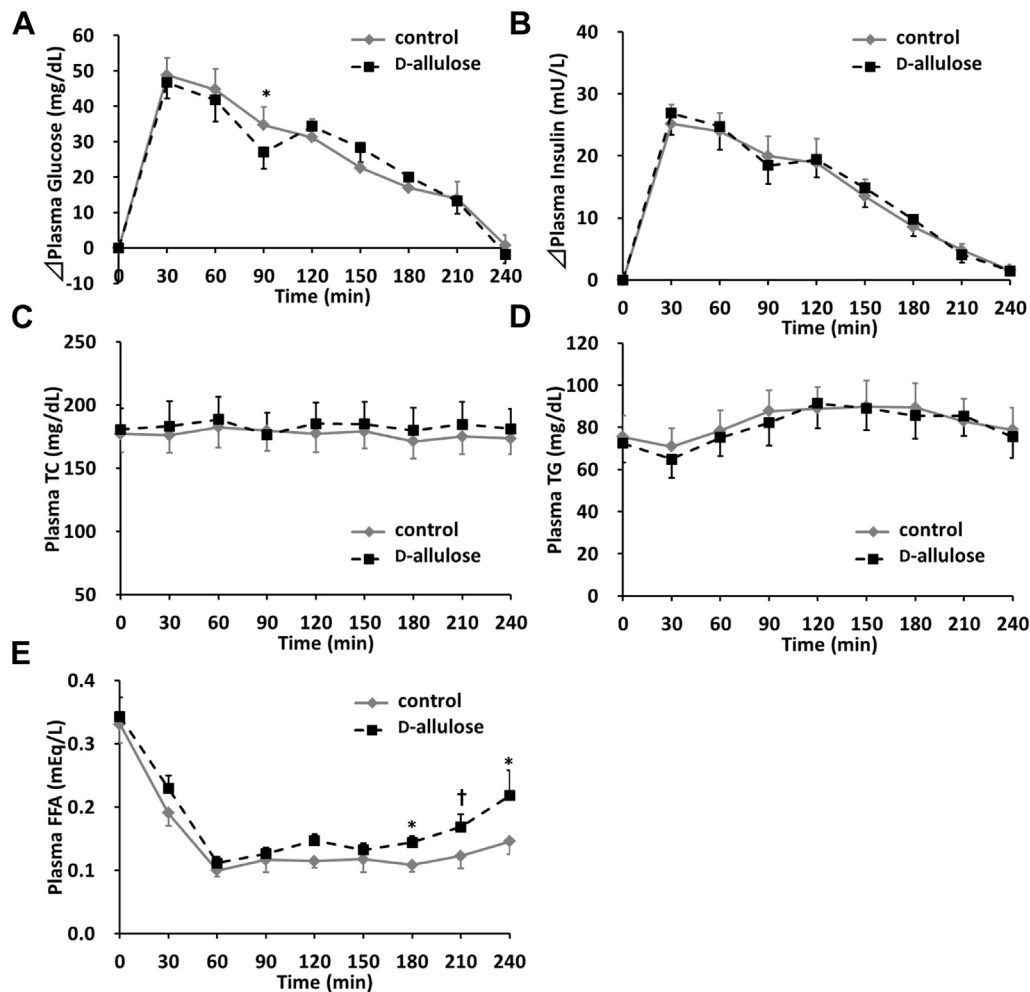
**Fig. 1.** Effects of d-allulose on various parameters in energy metabolism in humans: (A) REE, (B) CEE, (C) FEE, (D) RQ, and (E) the AUC of REE, CEE, and FEE. Data expressed as mean  $\pm$  SEM ( $n = 13$  in each group) by a paired Student's  $t$  test. AUC, area under the curve; CEE, carbohydrate energy expenditure; FEE, fat energy expenditure; REE, resting energy expenditure; RQ, respiratory quotient. \*Significant differences at  $P < 0.05$ . †Significant differences at  $P < 0.01$ .

proposed mechanism of d-allulose-induced antiobese action is that d-allulose suppresses hepatic lipogenesis [10,12] and augments hepatic fatty acid oxidation, resulting in enhanced energy expenditure [13]. In partial agreement with previous animal studies [13], the present study demonstrated that a single ingestion of 5 g d-allulose before a meal significantly enhanced postprandial fat oxidation and spared carbohydrate oxidation compared with the control group. To the best of our knowledge, this is the first study to demonstrate the potential of d-allulose in controlling and maintaining healthy body weight through energy metabolism.

With regard to the mechanisms of d-allulose's antiobese action, intestinal events may be suggested. d-Allulose is known to have an inhibitory action of  $\alpha$ -glucosidase, which breaks down sucrose into glucose in the small intestine [6]. It is reported that a single ingestion of coffee polyphenols (CPP), an  $\alpha$ -glucosidase inhibitor, also exhibited higher postprandial fat oxidation and energy expenditure, and lowered carbohydrate oxidation, resulting in decreased RQ [17–19]. Because glucose metabolism is known to regulate energy metabolism via glycolysis and fatty acid synthesis, decreased absorption of glucose may be in part responsible for an increase in fat oxidation and a decrease in carbohydrate oxidation.

Although the levels of insulin did not decrease in the present study, d-allulose is reported to improve glucose tolerance and to enhance insulin sensitivity in humans [8]. Increases in insulin have been known to reduce fatty acid oxidation accompanied with reduced levels of plasma FFA [20]. However, FFA levels were augmented by d-allulose concomitantly with upregulated fat oxidation and downregulated carbohydrate oxidation in the present study (Fig. 2E), thus indicating that insulin may be involved in d-allulose-induced fat oxidation. Increased insulin sensitivity could shift an energy source toward fat.

Ochiai et al. found that d-allulose increases glycogen contents in the liver, which may be due to hepatic glucokinase [12]. Glucokinase activity in the liver is a key step of glycolysis, glycogen synthesis, and pentose pathway [21]. Furthermore, translocation of glucokinase from the nucleus to the cytoplasm is reported to be impaired in T2DM [22], implying an important role for glucokinase in glucose metabolism. Our previous study showed that d-allulose promotes glucokinase translocation in the liver, activating glucokinase activity [7]. Thus, it is assumed that d-allulose activates glucokinase activity and stimulates glycogen synthesis, consequently sparing glucose. However, contradictory results on the role of glucokinase in energy metabolism have been reported. Overexpression of glucokinase



**Fig. 2.** Effects of d-allulose on biological parameters in humans: (A)  $\Delta$ plasma glucose, (B)  $\Delta$ plasma insulin, (C) plasma TC, (D) plasma TG, and (E) plasma FFAs. Data expressed as mean  $\pm$  SEM ( $n = 13$  in each group) by a paired Student  $t$  test. FFA, free fatty acid; TC, total cholesterol; TG, triacylglycerol. \*Significant differences at  $P < 0.05$ . †Significant differences at  $P < 0.01$ .

in the liver increases energy expenditure, probably through effects secondary to lowering plasma insulin and glucose [23], whereas hepatic glucokinase expression suppresses brown adipose tissue thermogenesis via neural signals, which is associated with downregulated energy metabolism [24]. Thus, further studies using animal model and humans with impaired glucose tolerance or diabetes are required to see whether and how glucose metabolism modulated by d-allulose plays a role in regulating lipid and energy metabolism.

In the present study, FFA was kept at higher levels after an ingestion of d-allulose throughout the experiment (Fig. 2E), indicating that d-allulose elevated a mobilization of fat from adipose tissue as a fuel. This result may support previous studies in which d-allulose decreases adipose tissue weight and upregulates fat oxidation in rats via proliferator-activated receptor  $\alpha$  [12,13]. However, it is unclear where in the body energy expenditure is enhanced or precisely what triggers an increase in energy metabolism.

There are some limitations in the present study. First, the measurement was carried out only for 4 h. d-Allulose is metabolized and excreted from the body quickly after ingestion [14], and it has an immediate effect on lipid metabolism in rats [13]. A metabolomics approach recently demonstrated that acute intake

of sugar-sweetened beverages alters dietary biomarkers within several hours [25], indicating that carbohydrate in beverages has the potential to modify metabolism in the short term. Thus, a 4-h measurement of some parameters may be appropriate to see differences. Second, in the present study, postprandial energy expenditure showed no significant difference, in contrast to the result showing that in rats fed d-allulose over a long-term period, postprandial energy expenditure was significantly increased during the latter half of the dark period [12] or during the late of the light period [13]. The contradictory results also were observed in CPP-fed mice and humans [17,18]. The similar discrepancy may be due to experimental conditions, a single ingestion versus chronic feeding of d-allulose and animal study versus human study. It is thus necessary to do long-term studies to investigate whether and, if so, how energy expenditure might be increased by d-allulose in humans. Finally, in the present study, participants were not stratified by sex because of a relatively small number of men. Sex difference in energy expenditure may exist because body fat size is generally greater in women than in men and the mobilization of fatty acid from adipose tissue may be influenced by plasma hormones such as estrogen [26]. Previous studies, however, have revealed that feeding d-allulose causes an increase in energy expenditure and fat oxidation in male rats [12,13]. BMI

of the study participants was within a low range ( $21.7 \pm 1.4$  in male versus  $20.4 \pm 0.9$  kg/m<sup>2</sup> in female). Therefore, sex difference in fat size between male and female participants may not mislead to our conclusion. However, further studies using a large number of male and female participants with or without are needed to confirm the antiobese action of d-allulose.

We investigated the effect of d-allulose on energy metabolism only in healthy individuals, which could not be extrapolated to those with various diseases such as diabetes and obesity. Given that high-fructose corn syrup-enriched beverages have been used in the food industry for many years and the noncaloric value of d-allulose at a low dose has been reported, d-allulose may be applied to a broad range of diet regimens. Further studies would be imperative to see the effects of d-allulose on energy and lipid metabolism in humans under different conditions.

## Conclusion

At a low dose, d-allulose enhanced postprandial fat oxidation and decreased carbohydrate oxidation in healthy humans. This indicates that d-allulose has the potential to be an antiobese sweetener in humans. Further studies in humans are needed to confirm these effects using a large number of individuals with various diseases such as diabetes and obesity and feeding d-allulose over a long-term period.

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