Materials and Methods

Animals and experimental design. Fourteen male Sprague-Dawley rats (3 wk old) were obtained from CLEA Japan, Inc. (Tokyo, Japan). The rats were housed individually at a temperature of 22 ± 2°C, with lights on from 07:00 to 19:00 h. All rats were fed CE-2, a commercial rodent diet (CLEA Japan, Inc.), and given water ad libitum until they turned 4 wk old. The rats were then randomly divided into two groups of 7 animals each (i.e., a control and d-sorbose group). The composition of the experimental diets is shown in Table 1. d-Sorbose was supplied by Rare Sugar Production Technical Research Laboratories, Co., Ltd. (Kagawa, Japan). The diet consumption was monitored once every 3–4 d and body weight was measured once a week. After 28 d of feeding, the rats were euthanized under ether anesthesia. Blood was collected from the abdominal aorta under non-fasting conditions using heparinized syringes. The blood was then centrifuged to obtain serum, which was stored at −20°C until biochemical analysis. The liver, kidneys, cecum and intra-abdominal adipose tissues were quickly removed and weighed. All procedures in this study of the rat model were approved by the Animal Experiment Committee of Matsutani Chemical Industry Co., Ltd. (No. 080916).

Biochemical analyses. Serum total cholesterol, HDL-cholesterol, triglyceride, phospholipids, glucose, free fatty acid, urea nitrogen, creatinine, uric acid, aspartate aminotransferase, and alanine aminotransferase were measured using the Cholesterol E-test kit, HDL-cholesterol E-test kit, Triglyceride E-test kit, Phospholipids C-test kit, Glucose C II-test kit, NEFA C-test kit, Urea nitrogen B-Test kit, Creatinine test kit, Uric acid C-test kit, Cholesterol E-test kit, HDL-cholesterol E-test kit, Triglyceride E-test kit, Phospholipids C-test kit, Glucose C II-test kit, NEFA C-test kit, Urea nitrogen B-Test kit, Creatinine test kit, Uric acid C-test kit, and Transaminase C II-test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. Absorption measurements were done using a Hitachi U-3210 spec-

Note

Dietary d-Sorbose Decreases Serum Insulin Levels in Growing Sprague-Dawley Rats

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Summary d-Sorbose is naturally occurring rare sugar. In this study, we examined the effects of dietary d-sorbose in rats. Four-week-old male Sprague-Dawley rats were fed either an AIN-93G-based control diet or a 3% d-sorbose diet for 28 d. Body weight and body fat accumulation were not different between the two diet groups. Dietary supplementation of d-sorbose lowered the serum insulin level (*p<0.05) significantly compared to the control, although the glucose was not changed. In addition, the relative weight of the cecum increased significantly in the d-sorbose group (**p<0.01). These findings suggest that intake of d-sorbose may improve the glucose metabolism by reducing insulin secretion, and d-sorbose can be used as a food ingredient.

Key Words d-sorbose, rare sugar, 28-d feeding, serum insulin level

D-Sorbose (d-xylo-2-hexulose), a C-3 and C-4 diastereomer of d-fructose, is a rare sugar. Because of the very small amounts of d-sorbose in natural products, few animal studies of d-sorbose have been conducted. Recently, using a new technique, d-sorbose was obtained from d-tagatose by the action of d-tagatose 3-epimerase (1), which is produced from d-galactose by the action of L-arabinose isomerase (2).

D-Sorbose has been reported to suppress postprandial blood glucose by the inhibition of α-glucosidases (i.e., sucrase and maltase) (3). D-Sorbose falls into the same category of d-ketohexose as d-fructose, d-psicose and d-tagatose. D-Fructose is a constituent of sucrose and high-fructose corn syrup, which is widely used in many food applications around the world. D-Psicose is a zero calorie sweetener (4, 5), which can suppress the elevation of postprandial blood glucose (6, 7) and improve lipid metabolism (8, 9). D-Tagatose is used as a low calorie sweetener (10). Both d-psicose and d-tagatose are relatively new sweeteners and safety assessments of these ingredients have been conducted and verified by acute and sub-chronic toxicity studies in rats (11, 12). However, no equivalent studies on d-sorbose have been reported. The experiments on d-psicose or d-tagatose were performed at a dose of 3% (13–15) or more. In this study, we used a dose of 3% for d-sorbose as a starting point. The objective of this study was to determine whether d-sorbose can be used as a food ingredient similar to d-psicose and d-tagatose.

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trophotometer (Hitachi, Tokyo, Japan). Insulin levels in the serum samples were determined using a Morinaga rat insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan) and a microplate reader (Multiskan MS; Labsystems, Kanagawa, Japan) and an enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

**Statistical analysis.** Data are expressed as mean ± SD, and analyzed by Student’s t-test. All analyses were performed by using SPSS version 13.0J (SPSS Japan Inc., Tokyo, Japan), a statistical software package. A statistically significant difference was considered to exist at \( p < 0.05 \).

### Results

The results of body weight, tissue weights, and dietary intake of rats in the two diet groups are shown in Table 2. The final body weight, weight gain, dietary intake, and feed efficiency did not differ between the control and D-sorbose groups. However, the mean cecum weight (\( \ast p < 0.05 \), and relative weight of the cecum to body weight (\( \ast \ast p < 0.01 \)) were significantly higher in the D-sorbose group than those in the control group, while no differences were observed in any other tissues. The cecal pH of the D-sorbose group was significantly lower than that of the control group (\( \ast \ast p < 0.05 \)).

The results of serum biochemical analyses are shown in Table 3. The serum concentrations of total cholesterol, HDL-cholesterol, triglyceride, phospholipids, glucose, free fatty acid, urea nitrogen, creatinine, and alanine aminotransferase were the same between the two groups, while the serum concentrations of insulin (\( \ast p < 0.05 \)), uric acid (\( \ast p < 0.05 \)), and aspartate aminotransferase (\( \ast \ast p < 0.01 \)) in the D-sorbose group were significantly lower than those in the control group.

### Discussion

The results of the present study suggest that a 3% addition of D-sorbose to the diet of rats increases the cecal weight significantly without affecting body weight gain, feed efficiency or weight of intra-abdominal adipose tissues. An addition of either D-psicose or D-tagatose has been reported to reduce the weight of intra-abdominal adipose tissue in rats at a concentration of 3% in the diet (16), but this did not alter the cecal weight. This assumes that the absorption rate of D-sorbose in the small intestine is less than that of other common ketohexoses. D-Sorbose, as well as L-arabinose (17), D-tagatose (18) and D-psicose (6), is reported to inhibit rat-intestinal sucrose activity (3). Thus, D-sorbose may...
help improve insulin resistance. Hence D-sorbose could have a smaller metabolic effect on the internal organs by comparison to other ketohexoses. Biochemical analysis of the serum showed the insulin level was decreased significantly in the D-sorbose group, while there was a decreasing trend for glucose concentration \((p=0.09)\). The dietary intake in the present study was lower in the D-sorbose group than in the control group. There is a possibility that the decrease of serum insulin level by D-sorbose was due to a lower food intake. A further study using pair-feeding is required to confirm this conclusion. It was reported that D-fructose feeding led to impaired insulin-stimulated glucose metabolism in rats, which was effected by the triglyceride levels \((19, 20)\).

In this study, serum triglyceride in the D-sorbose group was lower than in the control group, but this level did not differ significantly. We have not compared D-sorbose to D-fructose. However, D-sorbose may show properties that are different from those of D-fructose.

In conclusion, this is the first study to report the effects of D-sorbose as a food ingredient. No abnormalities were detected in our biochemical analysis after a 28-d feeding with 3% D-sorbose. However, our results indicate D-sorbose may be beneficial in terms of its effect on glucose metabolism. Thus, D-sorbose could be a valuable low-calorie sweetener and food ingredient. However, the metabolism of D-sorbose remains unclear and further studies are needed to clarify it.

REFERENCES