Effects of Oral Acute Administration and Subchronic Feeding of Several Levels of D-Psicose in Rats

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Summary The effects of oral acute administration and subchronic (34d) feeding of several levels of D-psicose, a C3-epimer of D-fructose, were studied in rats. In the acute administration test, five groups of eight male Wistar rats (3 wk old) were orally given D-psicose in doses of 8, 11, 14, 17, and 20 g/kg. Three rats receiving 14 g/kg, three rats receiving 17 g/kg and eight rats receiving 20 g/kg of D-psicose died within 2 d after administration. The calculated LD50 values were 16.3 g/kg by the Behrens-Karber method and 15.8 g/kg by the Litchfield-Wilcoxon method. In the subchronic feeding test, eight groups of seven male Wistar rats (3 wk old) were fed diets containing 0 (control), 10, 20, 30, and 40% for 34 d. One rat fed 30% D-psicose diet and five rats fed 40% D-psicose diet died during the experimental period. Body weight gain, food intake and food efficiency were more extensively suppressed by the higher D-psicose diets. The weights of heart, spleen and abdominal adipose tissue were smaller in the order of dietary D-psicose concentration. Cecal weight increased with increasing D-psicose concentration in the diets. Cecal hypertrophy was observed in rats fed 10–40% D-psicose diets. These results suggest that D-psicose differs in nutritional characteristics from D-glucose or D-fructose. The feeding of diets extremely high in D-psicose seems to be harmful to the intestinal tract.

Key Words D-psicose, acute administration, LD50 value, subchronic feeding, rat

D-Psicose (D-ribo-2-hexulose), a C-3 epimer of D-fructose, is a "rare sugar" present in small quantities in commercial mixtures of D-glucose and D-fructose obtained from the hydrolysis of sucrose or isomerization of D-glucose (1). D-Psicose is also present in processed cane and beet molasses (2), and is found in wheat (3), Itea plants (4), and in the antibiotic psicofranine (5). Because of the very small amounts of D-psicose in natural products, few studies of D-psicose metabolism in animals have been conducted.

Recently, we developed a new method to produce D-psicose enzymatically on a large scale (6), making it possible to conduct such studies. We have suggested that D-psicose supplements suppress hepatic lipogenic enzyme activity and reduce intra-abdominal fat accumulation compared to D-glucose or D-fructose in rats (7). Moreover, we demonstrated that D-psicose is a sweet monosaccharide that provides no energy to growing rats (8). Thus, D-psicose may be useful as a sweetener for obese people as an aid for weight reduction.

In this study, we examined the effects of oral acute administration and subchronic (34 d) feeding of several levels of D-psicose to rats to gather basic data regarding the safety of using D-psicose as a sugar substitute.

MATERIALS AND METHODS

All procedures involving animals were approved by the Experimental Animal Care Committee of Kagawa University.

Animals. Male Wistar rats (3 wk old) were obtained from Japan SLC (Shizuoka, Japan). They were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) and water ad libitum until 4 wk old. They were individually caged at 24 ± 1°C, with light from 08:00 to 20:00 h.

Experiment 1. Oral acute administration of several levels of D-psicose

Experimental design. D-Psicose was prepared from D-fructose by immobilized D-tagatose 3-epimerase (6) and diluted to a 50% water solution. Five groups of eight rats (76 ± 2 g) were fasted 12 h from 22:00 h and orally given D-psicose in single doses of 8, 11, 14, 17, and 20 g/kg body weight using a stainless sonde attached to a 20 mL syringe. The rats were fasted 4h after D-psicose administration and then given free access to CE-2 and water.

Experiment 2. Subchronic feeding of several levels of D-psicose

Experimental design. Five groups of seven rats were fed on diets containing 0, 10, 20, 30, and 40% D-psico-
Table 1. Composition of experimental diets (Experiment 2).1

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0% d-psicose</th>
<th>10% d-psicose</th>
<th>(g/kg diets)</th>
<th>20% d-psicose</th>
<th>30% d-psicose</th>
<th>40% d-psicose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>α-Methionine</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>650.0</td>
<td>550.0</td>
<td>450.0</td>
<td>350.0</td>
<td>250.0</td>
<td>400.0</td>
</tr>
<tr>
<td>d-Psicose</td>
<td>0.0</td>
<td>100.0</td>
<td>200.0</td>
<td>300.0</td>
<td>400.0</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
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<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
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<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mixture3</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>1,000.0</td>
<td>1,000.0</td>
<td>1,000.0</td>
<td>1,000.0</td>
<td>1,000.0</td>
<td>1,000.0</td>
</tr>
</tbody>
</table>

1 Butylated hydroxytoluene (0.01 g/kg diet) was added to all the diets as an antioxidant.
2 Based on AIN-76A.

cose and were given free access to water for 34 d. The compositions of experimental diets are shown in Table 1. The vitamin and mineral mixtures were based on AIN-76A (9, 10). Butylated hydroxytoluene (0.01 g/kg diet) was added to all the diets as an antioxidant (10). Forty percent dietary d-psicose was chosen as the maximum amount after referencing a previous report by Suzuki and Sato (11). Body weight gain and food intake were recorded everyday. The number of rats surviving and displaying diarrhea were observed. After 34 d, the rats were fasted 3 h from 07:00 h and then sacrificed by decapitation. Blood was collected to obtain serum, and liver, heart, spleen, kidneys, cecum and intra-abdominal adipose tissues were quickly removed and weighed. Carcass samples were obtained by removing the remains of intra-abdominal and intra-thorax tissues and stored at −20°C until analysis of carcass composition.

Analyses. Serum glucose and triacylglycerol concentrations were determined by a method reported previously (12, 13). Liver total lipid was extracted by the method of Folch et al. (14), and liver triacylglycerol was measured by the method of Fletcher (13). Carcass fat and protein were analyzed using the method reported by Mickelsen and Anderson (15).

Data analyses. All data were analyzed by a factorial analysis of variance (ANOVA) and post hoc Scheffe’s tests. Differences were considered statistically significant at p<0.05.

RESULTS

Experiment 1. Oral acute administration of several levels of d-psicose

All rats displayed diarrhea 1–24 h after d-psicose administration. The high-dosage groups (17 and 20 g/kg body weight) became quite weak. Three rats receiving 14 g d-psicose/kg body weight, three rats receiving 17 g/kg and eight rats receiving 20 g/kg died within 48 h after administration (Fig. 1). No abnormalities were observed in the surviving rats after 3 d. The calculated LD50 values were 16.3 g/kg body weight by the Behrens-Karber method and 15.8 g/kg by the Litchfield-Wilcoxon method. All rats that died during the experimental period were dissected. In rats that died after receiving 17 or 20 g d-psicose/kg body weight, bleeding was observed in the mucous layers of the stom-
Experiment 2. Subchronic feeding of several levels of D-psicose

Number surviving, body weight gain, food intake and carcass composition. One rat fed the 30% D-psicose diet and five rats fed 40% D-psicose died during the experimental period (Table 2). The rats fed the 20, 30, and 40% diets displayed diarrhea for the first 8 d (Table 2). Body weight gain was more suppressed by the feeding of a higher D-psicose diet (Table 3). A significant difference in weight gain was observed between the 0, 10, 20, and 30% D-psicose groups (p<0.05) (Table 3). Food intake and food efficiency were lower (p<0.05) in rats fed a higher D-psicose diet (Table 3). Carcass fat content and percentage of carcass fat decreased significantly (p<0.05) with increasing D-psicose in the diet (Table 3). Carcass protein content decreased as the amount of D-psicose increased, whereas the percentage of carcass protein was significantly (p<0.05) higher in the 0 and 10% D-psicose groups than in the 20 and 30% D-psicose groups (Table 3).

Tissue weights and cecal enlargement. The weights of heart and spleen were smaller (p<0.05) in rats fed higher D-psicose concentration diets (Table 4). Liver and kidney weights were heavier (p<0.05) in rats fed the 10% D-psicose diet than in rats fed the 0 and 30% D-psico-
The subchronic test suggests that body weight gain and food intake were suppressed more by the feeding of higher D-psicose diets. Similar effects were seen in alterations in the weights of tissues including heart, spleen and intra-abdominal adipose tissues, and in the carcass fat content. Inversely, cecal weight was greater in rats fed a higher D-psicose diet, to the point of cecal enlargement. Many of the effects were assumed to be secondary to a decrease in food consumption or the consumption of large amounts of a non-nutritive, poorly absorbed, osmotically active substance. The World Health Organization has looked extensively at the relationship between the consumption of non-nutritive substances in the diet as a cause of decreased weight gain and also reported an association with cecal enlargement (20, 21). In addition to the documented effect of specific dietary components on cecal weight, certain organs have been shown to be more sensitive to nutritional impact if it occurs when the animals are young (18).

More interestingly, food efficiency was also suppressed by the feeding of higher concentrations of D-psicose in the diet. Goldsmith (18) reported no statistically significant differences in food efficiency at various levels of saccharose in treated animals as compared to control animals. These results suggest that D-psicose could have another unknown toxic effect, such as interaction with other macro- or micronutrients. From these points of view, it is also suggestive that liver and kidney weights and liver triacylglycerol contents were heavier in rats fed the 10% D-psicose diet, and that the relative weight per body weight of liver and kidney were greater in rats fed the 10–30% D-psicose diets than the 0% diet. Bar et al. (22) demonstrated that rats fed diets with 5–20% D-tagatose, an incompletely absorbed ketohexose (stereoisomer of D-fructose), exhibited increased liver weights, but no histopathological alterations. They also suggested that D-tagatose at dietary levels of 5–20% increases liver glycogen deposition and relative weight in rats (22, 23). It is not clear whether or not the cause of D-psicose-induced liver enlargement is due to liver glycogen deposition. A more detailed study is required to clarify the mechanism of D-psicose-induced liver enlargement.

In conclusion, the present results suggest that D-psicose displays nutritional characteristics unlike other monosaccharides such as D-glucose or D-fructose. The feeding of diets extremely high in D-psicose seems to be harmful to the intestinal tract, suggesting that D-psicose...
should be used carefully, if at all, as a dietary fiber-like substance or sweetener in food manufacturing.

REFERENCES