

Original Article

Dietary D-psicose, a C-3 epimer of D-fructose, suppresses the activity of hepatic lipogenic enzymes in rats

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D-Psicose (D-ribo-2-hexulose), a C-3 epimer of D-fructose, is present in small quantities in commercial carbohydrate complexes or agricultural products. Wistar male rats were fed experimental diets which consisted of 5% D-psicose, cellulose, D-fructose or D-glucose for 28 days. Abdominal adipose tissue weight was significantly lower ($P < 0.05$) in rats fed the D-psicose diet than in rats fed a D-fructose and D-glucose diets, even though the four dietary groups were offered the same amount throughout the experimental period. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were significantly lower ($P < 0.05$) in rats fed the D-psicose diet than in rats fed the D-fructose and D-glucose diets. However, lipoprotein lipase activities in the heart, soleus muscle and perirenal adipose tissue were the same. These results suggest that a supplement of D-psicose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed a D-psicose diet might result from lower lipogenesis in the liver.

Key words: abdominal fat, D-psicose, Japan, lipogenesis, liver, rat.

Introduction

Sucrose is used in human nutrition worldwide. However, a number of sugar substitute sweeteners have been developed because of the disadvantages of sucrose intake, namely, lipogenic potency^{1,2} and high insulinogenicity.^{3,4} Low-lipogenic or low-insulinogenic sweeteners may play a useful role as one factor helping to make severely energy-restricted diets bearable. Such sweeteners typically have an energy content approximately one-half that of sucrose, 16.5 kJ/g.⁵ The major among these are 'polyols', sugar alcohols with a generally sweet taste, but have a lower intensity than sucrose. They also have an undesirable cooling effect induced by heat absorption by being melted in the mouth.^{5–7}

For nearly two decades, some isomers of monosaccharides, for example, L-sugars or D-tagatose, have been developed as alternative carbohydrate sweeteners and bulking agents.^{8–10} However, D-psicose (D-ribo-2-hexulose), a C-3 epimer of D-fructose, has not yet been studied as a sugar substitute. D-Psicose is present in small quantities in commercial mixtures of D-glucose and D-fructose obtained from hydrolysis of sucrose or isomerization of D-glucose.¹⁰ D-Psicose is also present in processed cane and beet molasses,¹¹ and is found in wheat¹², *Itea* plants,¹³ and in the antibiotic psicofranine.¹⁴ Whistler *et al.* reported that when D-[U-¹⁴C] psicose was orally injected into fasting rats, the radioactivities were analyzed by the urine (37%), faeces (13%), breath (15%) and carcass (39%).¹⁵ These findings suggested that D-psicose might have low available energy, even though a portion of D-psicose was absorbed into the animal's metabolic system. However, information on the effects of long-term dietary D-psicose intake is limited. In this study, we examined the effects of D-psicose supplement (5%) in diet as a sugar substitute on lipid metabolism and accumulation in

rats compared with cellulose (control as zero energy carbohydrate), D-fructose (control as ketohexose) or D-glucose (control as main monosaccharide).

Methods

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

Animals and diets

Twenty-four male Wistar rats (3-week-old) were obtained from Japan SLC (Shizuoka, Japan) and randomized into four groups. Rats were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) and water *ad libitum* until 4-weeks-old. Rats were fed a synthetic high carbohydrate diet including 60% α -starch and 5% cellulose, D-glucose, D-fructose or D-psicose (Table 1). As cellulose was used as a control for the zero energy carbohydrate, the other three diets contained little fibre. Cellulose, D-glucose and D-fructose were purchased from Wako Pure Chemical Industries (Osaka, Japan). D-Psicose (Fig. 1) was prepared from D-fructose by immobilized D-tagatose 3-epimerase.¹⁶ The vitamin and mineral mixtures based on Harper's mixture were used.¹⁷

Experimental design

Rats were individually caged at $24 \pm 1^\circ\text{C}$, with light from 08.00 to 20.00 h. Each group of rats ($n = 6$ per group) were

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Table 1. Composition of experimental diets

Ingredients	Amount g/kg diet
α -Starch	601.7
Casein	238.5
Carbohydrate*	50.0
Cornoil	50.0
Vitamin mixture [†]	8.5
Mineral mixture [†]	49.8
Choline chloride	1.5

*Cellulose, glucose, fructose and psicose were used as experimental carbohydrates. [†]Based on the Harper's mixtures.

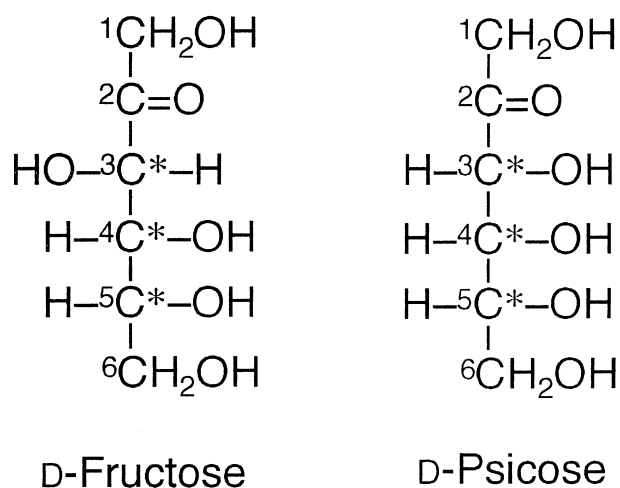


Figure 1. Molecular structures of D-fructose and D-psicose. Asterisk designates chiral carbon.

given free access to the cellulose, D-glucose, D-fructose or D-psicose diet and water for 28 days. After 28 days of the experimental diet, the rats were fasted overnight (12 h) and their heart punctured under anaesthesia conditions. Liver, heart, perirenal adipose tissue, soleus muscle and plantaris muscle were quickly removed and stored at -40°C until analysis. Carcass samples were obtained by removing the head, intrapectral and intra-abdominal tissues and were stored at -20°C until analysis of carcass composition.

Analyses

Plasma glucose, triacylglycerol, free fatty acids and total cholesterol concentrations were determined by the method reported previously.^{18–21} Plasma insulin concentration was determined using an enzyme immunoassay kit (Amersham Pharmacia Biotech UK, Buckinghamshire, England).

Lipoprotein lipase (LPL; EC3.1.1.34) activities of the heart, soleus muscle and perirenal adipose tissue were measured as described previously.²² The tissues were prepared by the method of Mori *et al.*²³ The substrate for LPL was prepared according to the method of Nilsson-Ehle and Schotz, but unlabeled triolein was used instead of [³H]triolein.²⁴ The LPL activity assay was performed by incubation of the extract with the substrate at 37°C for 30 min. The free fatty acids released during the incubation were measured by

the method reported previously.²⁰ One unit of LPL activity was defined as that catalyzing the release of 1 μmol of free fatty acid per hour.

Activities of lipogenic enzyme, fatty acid synthase (FAS; EC2.3.1.85) and glucose 6-phosphate dehydrogenase (G6PD; EC1.1.1.8) were measured spectrophotometrically using liver samples and perirenal adipose tissue cytosol according to previously described methods.^{25,26} The enzyme activities were measured at 37°C and expressed as units per gram of wet tissue weight. One unit of the enzymes oxidized 14 nmol NADPH per min for FAS, and converted 2 μmol NADP⁺ per min for G6PD.

The activities of citrate synthase in the liver, heart and plantaris muscle were determined by the method described by Srere.²⁷ The method established whether the dietary carbohydrate supplements had any effect on aerobic metabolism.

Data analysis

All data were analyzed by a factorial analysis of variance (ANOVA) and Fisher's PLSD tests. Differences were considered statistically significant at $P < 0.05$.

Results

Bodyweight, bodyfat and food conversion efficiency

Bodyweight gain, food intake and food conversion efficiency did not differ among the four groups (Table 2). Although each group of rats consumed the same amount of food, the type of dietary carbohydrate had a significant effect on abdominal adipose tissue weight (Table 2). Abdominal adipose tissue weight was significantly lower in the D-psicose diet group than in the D-glucose and D-fructose groups, whereas the carcass fat and protein contents were not affected by dietary carbohydrate (Table 2).

Plasma glucose, insulin, triacylglycerol, free fatty acids and total cholesterol concentrations were not significantly different among the four dietary groups (Table 3).

Enzyme activities in various tissues

The LPL activities in the heart, soleus muscle and perirenal adipose tissue were not affected by the dietary carbohydrate (Table 4). Hepatic FAS and G6PD activities were significantly lower in the D-psicose diet group than in the D-glucose and D-fructose groups, whereas these enzyme activities were not significantly different between the cellulose and D-psicose diet groups (Table 5). The FAS and G6PD activities in the perirenal adipose tissue were not significantly different between the D-psicose diet group and other diet groups (Table 5). The activities of citrate synthase (an index of aerobic metabolism) in the liver, heart and plantaris muscle did not differ among the four dietary groups (Table 6).

Discussion

We have shown here that D-psicose in the diet, compared with D-fructose or D-glucose, appeared to promote less abdominal fat during the 28-day experimental period and that D-psicose suppressed hepatic lipogenic enzyme activities. As all four dietary groups of rats were offered the same amount of food throughout the experimental period, the difference among the four dietary groups was ascribed to the different dietary carbohydrate supplements.

Table 2. Effects of dietary carbohydrates on rat body weight, food efficiency, tissue weights and carcass composition

	Cellulose	Glucose	Fructose	Psicose
Bodyweight				
Initial (g)	89 ± 2	89 ± 2	89 ± 2	88 ± 1
Final (g)	190 ± 7	204 ± 5	200 ± 8	193 ± 7
Gain (g)	101 ± 5	115 ± 5	111 ± 7	105 ± 6
Food intake (g)	369 ± 14	376 ± 11	370 ± 18	374 ± 20
Food conversion (g/g) efficiency	0.27 ± 0.01	0.31 ± 0.01	0.30 ± 0.02	0.28 ± 0.01
Tissue weights				
Liver (g)	7.0 ± 0.4*	8.0 ± 0.3	7.8 ± 0.5*†	8.8 ± 0.3*
Heart (mg)	611 ± 22	649 ± 27	630 ± 21	620 ± 20
Soleus (mg)	128 ± 5	144 ± 7	137 ± 3	134 ± 5
Abdominal (g) adipose tissue	8.3 ± 0.8*†	9.6 ± 0.7*	9.8 ± 0.6*	6.8 ± 0.6†
Carcass				
Weight (g)	126 ± 4	136 ± 3	131 ± 5	126 ± 5
Fat (g)	16.3 ± 1.2	17.2 ± 1.1	17.2 ± 1.2	15.9 ± 1.0
(%)	13.0 ± 0.8	12.8 ± 0.9	13.1 ± 0.9	12.7 ± 0.5
Protein (g)	25.3 ± 1.5	26.8 ± 1.6	27.9 ± 2.1	27.8 ± 2.0
(%)	20.1 ± 0.9	19.7 ± 0.8	21.3 ± 1.0	21.0 ± 1.1

Values are means ± SEM for six rats. Within a row, values with different superscripts are significantly different ($P < 0.05$).

Table 3. Effects of dietary carbohydrates on plasma glucose, insulin, triacylglycerol, free fatty acids and total cholesterol concentrations of rats

	Cellulose	Glucose	Fructose	Psicose
Glucose (mg/100mL)	181 ± 8	184 ± 12	175 ± 11	190 ± 12
Insulin (ng/mL)	27.3 ± 19.6	51.0 ± 24.4	30.3 ± 12.6	29.9 ± 14.3
Triacylglycerol (mg/100mL)	151 ± 24	152 ± 22	125 ± 19	117 ± 16
Free fatty acids (mmol/L)	1.51 ± 0.08	1.25 ± 0.07	1.22 ± 0.11	1.19 ± 0.10
Total cholesterol (mg/100mL)	153 ± 8	130 ± 17	123 ± 20	146 ± 12

Values are means ± SEM for six rats.

Table 4. Effects of dietary carbohydrates on lipoprotein lipase activity in the heart, soleus muscle and perirenal adipose tissue of rats

	Cellulose	Glucose	Fructose	Psicose
Heart (U/g tissue)	24.8 ± 1.1	23.5 ± 1.9	25.1 ± 1.4	25.6 ± 1.1
Soleus (U/g tissue)	6.8 ± 0.9	6.7 ± 0.9	6.4 ± 0.4	7.3 ± 0.6
Perirenal adipose tissue (U/g tissue)	19.8 ± 1.9	19.0 ± 2.0	17.5 ± 1.9	20.0 ± 1.3

Values are means ± SEM for six rats.

Table 5. Effects of dietary carbohydrates on lipogenic enzyme activities in the liver and perirenal adipose tissue of rats

	Cellulose	Glucose	Fructose	Psicose
Fatty acid synthase				
Liver (U/g tissue)	74.0 ± 2.6†‡	80.2 ± 29*†	87.2 ± 3.6*	66.7 ± 5.0‡
Perirenal adipose tissue (U/g tissue)	15.9 ± 0.5†‡	17.1 ± 0.6†	21.2 ± 0.8*	19.5 ± 1.4*†
Glucose-6 phosphate dehydrogenase				
Liver (mU/g tissue)	1854 ± 88†‡	2017 ± 96*‡	2211 ± 58*	1673 ± 9‡
Perirenal adipose tissue (mU/g tissue)	1093 ± 87	1097 ± 55	1053 ± 71	925 ± 87

Values are means ± SEM for six rats. Within rows, values with different superscripts are significantly different ($P < 0.05$).

Table 6. Effects of dietary carbohydrates on citrate synthase activity in the liver, heart and plantaris muscle of rats

	Cellulose	Glucose	Fructose	Psicose
Liver (U/g tissue)	11.5 ± 0.5	11.7 ± 0.5	11.9 ± 0.6	11.0 ± 0.7
Heart (U/g tissue)	19.4 ± 0.8	18.4 ± 0.6	19.3 ± 0.9	18.0 ± 1.2
Plantaris (U/g tissue)	14.7 ± 0.3	13.6 ± 0.6	12.8 ± 0.8	13.4 ± 0.6

Values are means ± SEM for six rats.

Higher lipogenesis in the liver and adipose tissue promote hyperlipidemia and fat accumulation. It is suggested that this effect of the D-psicose diet is at least in part ascribable to lower lipogenic enzyme activity.²⁸ As FAS²⁵ and G6PD²⁶ are the lipogenic enzymes, we analyzed the activities of these enzymes in the liver and perirenal adipose tissue. The livers of rats fed the D-psicose diet appeared to have lower FAS and G6PD activities than those of rats fed the D-fructose and D-glucose diets. This finding is consistent with the lower level of abdominal fat accumulation in rats fed the D-psicose diet.

The carcass samples in this study consisted of muscle, bone, skin and subcutaneous fat. The fat content of the carcass sample was not significantly different among the four dietary groups, but the abdominal fat weight was significantly less in the D-psicose group than in the D-fructose and D-glucose groups. These results may indicate that dietary carbohydrate supplements affect bodyfat accumulation more in the abdominal adipose tissues than in the subcutaneous adipose tissue.

Lipoprotein lipase has its physiological site of action at the luminal surface of capillary endothelial cells where the enzyme hydrolyzes the triacylglycerol component of circulating lipoprotein particles, chylomicrons and very low density lipoproteins, to provide free fatty acids for tissue utilization in the heart and skeletal muscles or for fat accumulation in the adipose tissues.^{29,30} Lipoprotein lipase plays a primary role in triacylglycerol metabolism as well as an important role in certain metabolic disorders, including obesity.^{31,32} Genetic and diet-induced obesity are each clearly associated with increases in LPL protein levels in the adipose tissue of human³³ and rodents.³⁴ As LPL activities in the heart, soleus muscle and perirenal adipose tissue of the four groups were the same, less abdominal fat accumulation in rats fed the D-psicose diet may not be associated with the capacity to remove triacylglycerol from the bloodstream into adipose tissue, but the level of blood triacylglycerol synthesized in the liver.

In spite of lower lipogenesis in the liver of rats fed the D-psicose diet, serum triacylglycerol concentration on D-psicose group did not differ from other dietary groups. Serum glucose, insulin, free fatty acids and total cholesterol concentrations were also approximately the same in all four dietary groups. We speculate that these findings may be caused by killing at overnight fasting or 5% supplementation of test carbohydrates.

Bodyweight gain, abdominal fat weight and hepatic lipogenic enzyme activities of rats fed the cellulose diet were not significantly different from rats fed D-psicose diet. As cellulose is a non-metabolizable polysaccharide, these results suggest that the energy value of D-psicose might be effectively

zero. However, it was suggested that a large portion of D-psicose was metabolized in the rat's body.¹⁵ If most of D-psicose is absorbed, it should therefore be expected to have a strong thermic effect to account for its putative lack of net energy. It is not possible that D-psicose supplement enhances mitochondrial oxidative capacity because the activities of citrate synthase in the liver, heart and plantaris muscle did not differ among four dietary groups. Another possibility is that D-psicose may impair the absorption of other macronutrients. This possibility has not as yet been examined.

In conclusion, the present study demonstrates that a supplement of D-psicose in the diet suppressed hepatic lipogenic enzyme activities in rats compared with supplements of D-fructose or D-glucose in diets. The lower abdominal fat accumulation in rats fed D-psicose might result from lower lipogenesis in the liver. However, a more detailed study is required to clarify the availability of D-psicose as a sugar-substitute sweetener.

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