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Less Body Fat Accumulation with D-Psicose Diet *versus* D-Fructose Diet

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Summary D-Psicose (D-*ribo*-2-hexulose), a C-3 epimer of D-fructose, is present in small quantities in commercial carbohydrates and agricultural products. To evaluate the possibility of D-psicose as low-energy sugar substitute, we studied the effects on body fat accumulation of D-psicose compared with cellulose or D-fructose in rats. Wistar male rats were fed experimental diets including 5% D-psicose, cellulose or D-fructose for 21 days. Abdominal adipose tissue weight was significantly lower ($p<0.05$) in rats fed D-psicose than in those fed D-fructose. Gene expression of uncoupling protein-1 and β_3-adrenergic receptor in the brown adipose tissue did not differ among the dietary groups. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were significantly lower ($p<0.05$) in rats fed D-psicose, whereas lipoprotein lipase activities in the heart, soleus muscle, perirenal adipose tissue, and subcutaneous adipose tissue did not differ. These results suggest that supplementation of D-psicose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed D-psicose might have resulted from lower lipogenesis in the liver. D-Psicose could prove to be a superior sugar substitute.

Key Words: D-psicose, low-energetic sweetener, body fat, lipogenesis, rat

Sucrose is used for human nutrition the world over. However, a number of sugar substitutes have been developed because of the disadvantages of sucrose intake, namely, cariogenic potency [1, 2] and high insulinogenicity [3, 4]. Zero- or low-energy sweeteners can play a useful role as one factor in helping to make

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severely energy-restricted diets bearable. Such sweeteners typically have an energy content approximately one-half that of sucrose, 16.5 kJ/g [5]. Chief among these are "polyols," sugar alcohols with a generally sweet taste but lower intensity than sucrose. However, they also have undesirable cooling effects [5–7].

For nearly 2 decades, some isomers of monosaccharides (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 the hydrolysis of sucrose or isomerization of D-glucose [11]. D-Psicose is also present in processed cane and beet molasses [12]. It is found in wheat [13], *Itea* plants [14], and in the antibiotic psicofranine [15]. Human urine contains some 15–30 mg/liter, where it is presumably derived from the diet, since it disappears from the urine of subjects who have fasted 48 h [16]. It has also been reported in washings from human skin [17]. However, there are few studies concerning D-psicose in the diet and its metabolism.

Whistler *et al.* [18] reported that when D-[U-^{14}C] psicose was orally injected into fasting rats, the radioactivity was retained by the urine (37%), feces (13%), breath (15%), and carcass (39%). 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 D-psicose supplementation (5%) in the diet as a sugar substitute for its effect on body fat accumulation in rats in comparison with cellulose (control as a zero-energy carbohydrate) or D-fructose (control as a ketohexose). Moreover, we determined the effects of these dietary carbohydrates on the gene expression of uncoupling protein (UCP)-1 and β_3-adrenergic receptor in the brown adipose tissue (BAT) and on lipogenic enzyme activities in various tissues.

MATERIALS AND METHODS

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

Animals and diets. Eighteen male Wistar rats (3 weeks old) were obtained from Japan SLC, Inc. (Shizuoka, Japan) and randomized into 3 groups. The rats were fed CE-2, a commercial rodent diet (CLEA Japan, Tokyo), and provided water *ad libitum* through 4 weeks of age. The animals were fed a synthetic high carbohydrate including 60% α-starch and 5% cellulose, D-fructose or D-psicose (Table 1). Cellulose and D-fructose were purchased commercially from Shikoku Yashima Pure Chemicals Co., Ltd. (Kagawa, Japan). D-Psicose (Fig. 1) was prepared from D-fructose by immobilized D-tagatose 3-epimerase [19]. The vitamin and mineral mixtures based on the Harper's mixtures [20] were used, except that the iron concentration was 17 μg/g diet.

Table 1. Composition of experimental diets.

	Amount
Ingredients	g/kg diet
α-Starch	601.7
Casein	238.5
Carbohydrate[1]	50.0
Corn oil	50.0
Vitamin mixture[2]	8.5
Mineral mixture[2]	49.8
Choline chloride	1.5

[1]Cellulose, fructose, or psicose were used as experimental carbohydrates. [2]Based on the Harper's mixtures.

Experimental design. Rats were individually caged at 24±1°C, with lights

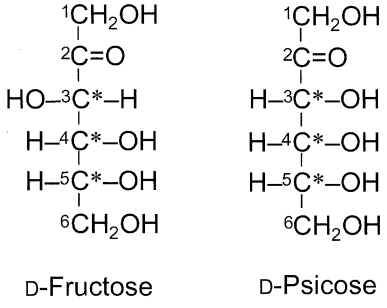

Fig. 1. Molecular structures of D-fructose and D-psicose. Asterisks designate chiral carbons.

from 08:00 to 20:00 h. Each group of rats ($n=6$/group) was given free access to the cellulose, D-fructose or D-psicose diet and water for 21 days. After 21 days of the experimental diet, the rats were fasted for 12 h and killed by exsanguination under ether anesthesia at 10:00 h. Blood was collected to obtain plasma, and interscapular BAT, liver, heart, soleus muscle and perirenal adipose tissue were quickly removed and stored at −40°C until analysis could be performed. Carcass samples were obtained by removing the head, digestive tracts, lungs, kidneys, testes, and abdominal adipose tissues. These were stored at −20°C prior to analysis of the carcass composition.

Analyses. Plasma glucose and triacylglycerol were determined by the method reported previously [21, 22]. Plasma insulin concentration was determined by using an enzyme immunoassay kit (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, UK).

Total RNA was extracted from the interscapular BAT by a guanidium thiocynate water-saturated phenol extraction method [23]. First-stand cDNA synthesis was performed on 5 μg total RNA using oligo(dT) as described in the manufacturer's instructions (GIBCO BRC Super Script Kit; Life Technologies, Gaithersburg, MD). The sequences of the primers used for polymerase chain

reaction (PCR) amplification are shown in Table 2. The PCR reactions were carried out in a DNA Thermal Cycler (PC-700, ASTEC, Fukuoka, Japan) using the following cycle conditions: initial denaturation at 94°C for 1 min, annealing at 58°C for 1.5 min and extension at 73°C for 8.5 min. PCR products (10 μl) were dyed with ethidium bromide and analyzed by electrophoresis in 2% agarose gels. The amounts of mRNA in each sample were quantified by densitometry using an analysis program for Macintosh (NIH image, U.S. National Institutes of Health, Springfield, VA). The numbers of PCR cycles were kept within their respective exponential and linear range.

Cytochrome *c* content in the interscapular BAT was measured by the Williams and Thorp method [24]. Cytochrome *c* was determined after reduction with dithionite ($E_{550}-E_{535}=20.4\ mM^{-1}$)[25]. Protein content in the interscapular BAT was measured by the Lowry method [26]. BAT triacylglycerol was determined according to the method of Fletcher [22] after total lipid extraction [27].

Lipoprotein lipase (LPL; EC3.1.1.34) activities of the heart, soleus muscle, and perirenal adipose tissue were measured as described previously [28]. The tissues were prepared by the method of Mori *et al.* [29]. The substrate for LPL was prepared according to the method of Nilsson-Ehle and Schotz [30], but unlabeled triolein was used instead of [^{3}H]triolein. 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 enzymatically with a NEFA C Test purchased from Wako Pure Chemical Co., Osaka. One unit of LPL activity was defined as that catalyzing the release of 1 μmol of free fatty acid/h.

Activities of the lipogenic enzyme fatty acid synthase (FAS; EC2.3.1.85) and glucose 6-phosphate dehydrogenase (G6PD; EC1.1.1.8) in the cytosolic fractions of liver, perirenal and subcutaneous adipose tissues were measured spectrophotometrically according to previously described methods [31, 32]. The enzyme activities were measured at 37°C and expressed as U/g wet tissue weight. One unit of the FAS enzyme oxidized 14 nmol NADPH/min, and 1 unit of G6PD converted 2 μmol $NADP^+$/min [31, 32].

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

Table 2. Sequences of polymerase chain reaction (PCR) primers.

cDNA	Primer	Nucleotide No.	Size of PCR product (b.p.)	GeneBank accession No.
UCP-1	5′-CAGAAGGATTGCCGAAACTG-3′	294–313	276	X03894
	5′-CATTGTAGGTCCCAGTGTAG-3′	550–569		
β_3-Adrenergic	5′-CTTGGGCGCAACTGGCTGCGA-3′	353–373	501	S73473
receptor	5′-CCATCCGATGCCGTGGGTGTC-3′	853–833		
β-Actin	5′-CCTAGCACCATGAAGATCAA-3′	2846–2865	377	J00691
	5′-AGCCATGCCAAATGTCTCAT-3′	3222–3203		

RESULTS

Body weight, body fat and food efficiency

Body weight gain, food intake, and food efficiency did not differ among the 3 groups (Table 3). No stool-softening effect was found on rats at the 5% carbohydrate supplement level. Although each group of rats consumed the same amount of food (Table 3), the type of dietary carbohydrate had a significant effect on the abdominal adipose tissue weight (Table 3). Abdominal adipose tissue weight (epididymal, perirenal, and mesenteric) was significantly lower in the D-psicose group than in the D-fructose group, whereas carcass fat and protein content were not affected by the dietary carbohydrate (Table 3).

Plasma glucose, insulin, and triacylglycerol concentrations

The plasma glucose concentration was not significantly different among the 3 diet groups (56.3±3.6, 57.6±4.6, and 55.5±2.1 mg/100 ml for cellulose, D-fructose, and D-psicose groups, respectively), nor were plasma insulin and triacylglycerol concentrations affected by the dietary carbohydrates (insulin, 9.6±2.0, 18.9±5.5, and 46.2±26.2 ng/ml; triacylglycerol, 117±11, 120±9, and 111±8 mg/100 ml for cellulose, D-fructose, and D-psicose groups, respectively).

Basic profile of interscapular BAT

BAT weight and triacylglycerol content were significantly lower in the D-psicose group than in the D-fructose group, whereas BAT total protein, cyto-

Table 3. Effects of dietary carbohydrates on rat body weight, food efficiency, tissue weights, and carcass composition.

		Cellulose	Fructose	Psicose
Body weight				
Initial	(g)	91±1	91±2	89±2
Final	(g)	187±3	190±8	176±7
Gain	(g)	96±3	100±7	87±6
Food intake	(g)	294±5	296±9	284±12
Food efficiency	(g/g)	0.33±0.01	0.33±0.02	0.31±0.01
Liver	(g)	7.24±0.28	7.28±0.53	7.60±0.42
Heart	(mg)	648±17	623±36	594±33
Soleus muscle	(mg)	169±7	166±10	153±11
Abdominal adipose tissue	(g)	8.5±0.4^{ab}	10.5±0.7^{a}	6.8±0.5^{b}
Carcass				
Weight	(g)	110±2	113±6	104±5
Fat	(g)	12.6±0.5	13.7±0.6	11.9±0.9
	(%)	11.4±0.4	12.2±0.6	11.4±0.9
Protein	(g)	21.8±0.7	23.3±1.3	22.1±1.0
	(%)	19.8±0.8	20.7±1.0	21.3±1.1

Values are means±SEM for 6 rats. Within a row, values with different superscripts are significantly different ($p<0.05$).

Table 4. Effects of dietary carbohydrates on basic profile of the interscapular brown adipose tissue.

		Cellulose	Fructose	Psicose
Weight	(mg)	245 ± 9^{ab}	277 ± 19^{a}	218 ± 9^{b}
Protein	(mg)	2.6 ± 0.2	2.9 ± 0.3	2.3 ± 0.2
Triacylglycerol	(mg)	142 ± 5^{ab}	162 ± 11^{a}	134 ± 6^{b}
Cytochrome *c*	(mg/g tissue)	21.7 ± 0.8	22.0 ± 0.9	20.7 ± 0.8
Total RNA	(mg/mg)	1.30 ± 0.06	1.23 ± 0.06	1.41 ± 0.06

Values are means±SEM for 6 rats. Within a row, values with different superscripts are significantly different ($p<0.05$).

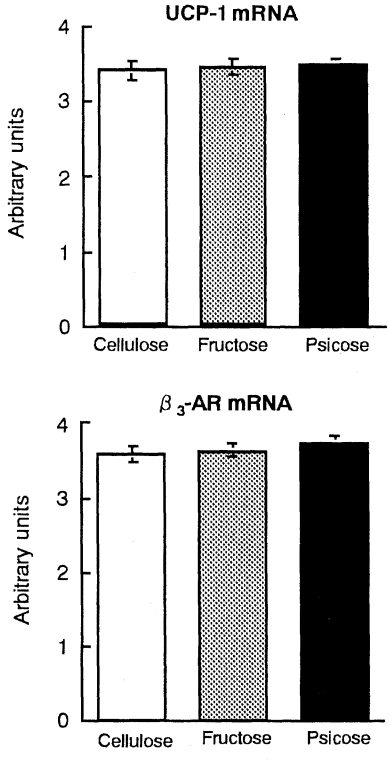

Fig. 2. Effects of dietary carbohydrates on uncoupling protein (UCP)-1 and β_3-adrenergic receptor (AR) mRNA levels normalized by β-actin mRNA content in the interscapular brown adipose tissue of rats. Values are means±SEM for 6 rats.

chrome *c*, and total RNA content were not affected by the dietary carbohydrates (Table 4).

Gene expression of UCP-1 and β_3-adrenergic receptor

UCP-1 and β_3-adrenergic receptor mRNA in the interscapular BAT were normalized by β-actin mRNA content, which did not differ among the groups

Table 5. Effects of dietary carbohydrates on lipoprotein lipase activity in the heart, soleus, and perirenal and subcutaneous adipose tissues of rats.

		Cellulose	Fructose	Psicose
Heart	(U/g tissue)	16.2±1.5	16.6±0.9	19.5±0.8
Soleus	(U/g tissue)	4.8±0.5[b]	4.9±0.6[b]	7.7±0.8[a]
Perirenal adipose tissue	(U/g tissue)	23.8±1.9	17.9±1.3	21.9±2.9
Subcutaneous adipose tissue	(U/g tissue)	14.1±2.3	13.1±1.7	14.5±0.9

Values are means±SEM for 6 rats. Within a row, values with different superscripts are significantly different ($p<0.05$).

Table 6. Effects of dietary carbohydrates on fatty acid synthase activity in the liver, and perirenal and subcutaneous adipose tissues of rats.

		Cellulose	Fructose	Psicose
Liver	(U/g tissue)	71.5±3.0[b]	91.0±4.3[a]	57.4±6.7[b]
Perirenal adipose tissue	(U/g tissue)	26.8±1.7	29.4±1.1	24.1±2.4
Subcutaneous adipose tissue	(U/g tissue)	23.7±3.5	28.9±1.5	22.5±1.6

Values are means±SEM for 6 rats. Within a row, values with different superscripts are significantly different ($p<0.05$).

Table 7. Effects of dietary carbohydrates on glucose 6-phosphate dehydrogenase activity in the liver, and perirenal and subcutaneous adipose tissues of rats.

		Cellulose	Fructose	Psicose
Liver	(mU/g tissue)	1,000±101[b]	1568±16[a]	872±139[b]
Perirenal adipose tissue	(mU/g tissue)	706±22	724±35	683±37
Subcutaneous adipose tissue	(mU/g tissue)	292±25	290±23	283±38

Values are means±SEM for 6 rats. Within a row, values with different superscripts are significantly different ($p<0.05$).

(data not shown). The mRNA levels of UCP-1 and β_3-adrenergic receptor were not affected by the dietary carbohydrate (Fig. 2).

Enzyme activities in various tissues

LPL activities in the heart, and perirenal and subcutaneous adipose tissues were not affected by the dietary carbohydrate, whereas soleus LPL activity was higher in the D-psicose group than in the other groups (Table 5). Hepatic FAS and G6PD activities were significantly lower in the D-psicose group than in the fructose group, whereas they were not significantly different between cellulose and D-psicose groups (Tables 6 and 7). FAS and G6PD activities in the perirenal and subcutaneous adipose tissues were not significantly different between the D-psicose group and the other groups (Tables 6 and 7).

DISCUSSION

We have shown here that dietary D-psicose, compared with D-fructose, appeared to promote less accumulation of abdominal fat during the 21-day experimental period and that D-psicose suppressed hepatic lipogenic enzyme activities. Because the 3 dietary groups of rats were offered and consumed the same amount of food throughout the experimental period, the difference in body fat accumulation among the 3 dietary groups can be ascribed to the different dietary carbohydrate supplements.

Diet-induced thermogenesis (DIT) in BAT plays an important role in resistance against dietary obesity by acting as an energy buffer [33, 34]. DIT in BAT is activated by the sympathetic nerves *via* β_3-adrenergic receptors [35, 36]. An increase in cAMP leads to increased lipolysis, providing a supply of oxidizable substrate for mitochondria. UCP-1, a mitochondrial protein transporter that uncouples oxidative metabolism from ATP synthesis, is activated, leading to the generation of heat involving free fatty acids as second messengers [37]. Besides these effects, stimulation by catecholamines causes a marked increase in UCP-1 mRNA and protein in BAT [38]. Due to the unique capacity of BAT for uncoupled metabolism, this tissue is ideally situated to play an important role in the regulation of energy balance. Various caloric carbohydrates in diets affect sympathetic activity. Walgren *et al.* [39] reported that a diet supplemented with sucrose, fructose or dextrose stimulated sympathetic activity in the interscapular BAT more than did commercial rat chow. Vander Tuig and Romsos [40] demonstrated that when rats were given a 10% sucrose solution to drink for 3 days, the norepinephrine turnover rate, an index of sympathetic activity, increased in the heart, BAT, liver, and pancreas. In this study, we examined UCP-1 and β_3-adrenergic receptor mRNAs in interscapular BAT components involved in sympathetic activity. The expression of these mRNAs did not differ between the cellulose and D-fructose groups. This disagreement concerning the effects of dietary carbohydrates on sympathetic activity in BAT may have been due to the dietary compositions of carbohydrates and other macronutrients. In addition, these disagreements may be caused by feeding periods (3 weeks in our case) or killing time (12 h fast prior to killing).

As higher lipogenesis in the liver and adipose tissues would promote hyperlipidemia and fat accumulation, the lipid-lowering effect of D-psicose is at least in part ascribable to lower lipogenic enzyme activity [41]. Since FAS [31] and G6PD [32] are lipogenic enzymes, we analyzed the activities of these enzymes in the liver and adipose tissues. The liver of rats fed the D-psicose diet appeared to have lower FAS and G6PD activities than those of rats fed the D-fructose 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 subcuta-

neous fat. The fat content of the carcass sample was not significantly different among the 3 dietary groups, but the abdominal fat weight was significantly less in the D-psicose group than in the D-fructose group. These results may indicate that dietary carbohydrate supplements affect body fat accumulation more in the abdominal adipose tissue than in the subcutaneous adipose tissue.

LPL 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, i.e., chylomicrons and very-low-density lipoproteins. This action provides free fatty acids for tissue utilization in the heart and skeletal muscles or for fat accumulation in the adipose tissues [42, 43]. Lipoprotein lipase plays a primary role in triacylglycerol metabolism as well as an important role in certain metabolic disorders, including obesity [44, 45]. Genetic and diet-induced obesity are each clearly associated with an increased LPL protein level in adipose tissue in humans [46] and rodents [47]. Since the LPL activities of the 3 groups were the same except for the soleus muscle, less body fat accumulation in rats fed a D-psicose diet may not be associated with the capacity to remove triacylglycerol from the bloodstream into adipose tissue, but with the level of blood triacylglycerol synthesized in the liver.

In spite of lower lipogenesis in the liver of rats fed the D-psicose diet, the plasma triacylglycerol concentration in the D-psicose group did not differ from that in the other diet groups. Plasma glucose and insulin concentrations were also approximately the same in the 3 dietary groups. We speculate that these findings may be caused by killing after 12 h of fasting.

Body weight gain, abdominal fat weight, and hepatic lipogenic enzyme activities of rats fed the cellulose diet were not significantly different from those of rats fed the D-psicose diet. Since cellulose is a non-metabolizable polysaccharide, these results suggest that the energy value of D-psicose might be effectively zero. However, it was suggested long ago that a large portion of D-psicose was metabolized in the rat's body [18]. If most of the D-psicose is absorbed, this sugar would 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 supplementation enhances mitochondrial oxidative capacity because the mRNA levels of UCP-1 and β_3-adrenergic receptor in the interscapular BAT did not differ among the 3 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 a supplement of D-fructose. 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.

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