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Effects of Dietary D-Psicose on Diurnal Variation in Plasma Glucose and Insulin Concentrations of Rats

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The effects of supplemental D-psicose in the diet on diurnal variation in plasma glucose and insulin concentrations were investigated in rats. Forty-eight male Wistar rats were divided into four groups. Each group except for the control group was fed a diet of 5% D-fructose, D-psicose, or psico-rare sugar (3:1 mixture of D-fructose and D-psicose) for 8 weeks. Plasma glucose levels were lower and plasma insulin levels were higher at all times of day in the psicose and psico-rare sugar groups than in the control and fructose groups. Weight gain was significantly lower in the psicose group than in the control and fructose groups. Liver glycogen content, both before and after meals was higher in the psicose group than in the control and fructose groups. These results suggest that supplemental D-psicose can lower plasma glucose levels and reduce body fat accumulation. Hence, D-psicose might be useful in preventing postprandial hyperglycemia in diabetic patients.

Key words: D-psicose; diurnal variation; glucose; insulin; glycogen

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 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. Previously we developed a specific method to produce D-psicose enzymatically on a large scale,[6,7] 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 as compared to D-glucose and D-fructose in rats.[8,9] In addition, we found that D-psicose is a sweet monosaccharaide that provides no energy to growing rats[10] and displays little toxicity.[11,12] Hence, D-psicose may be useful as a sweetener for obese people as an aid in weight reduction.

Recently, we reported that D-psicose inhibited intestinal α-glucosidase activity and suppressed the glycemic response after sucrose and maltose ingestion.[13,14] We found that 5% D-psicose supplementation increased liver weight and glycogen content in rats as compared with a 5% cellulose supplementation.[8,15] Moreover, Murao *et al.* found that D-psicose stimulated insulin secretion in a dose-dependent manner in INS-1 cells and that D-psicose might have a different insulin stimulating mechanism from glucose.[16] The blood glucose level is regulated by glucose uptake in several peripheral tissues, mostly the liver and skeletal muscles.[17] In addition, insulin secreted from the pancreas stimulated glucose uptake *via* glucose transporters (GLUT).[18] D-Psicose might facilitate glucose uptake in peripheral tissues resulting in a decrease in the blood glucose level in an insulin dependent or independent manner. It was suggested that supplemental D-psicose in the diet might reduce postprandial glycemic response and might have anti-diabetic effects. In this study, we examined the effects of 5% D-psicose supplementation in the diet on diurnal variation in plasma glucose and insulin concentrations in rats.

Materials and Methods

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

Animals and experimental diets. Forty-eight male Wistar rats (3 weeks old) were obtained from Japan SLC (Shizuoka, Japan). They were fed CE-2, a commercial rodent diet (CLEA, Tokyo) and water *ad libitum* until they were 4 weeks old. They were caged individually at $24 \pm 1\,^{\circ}C$, with light from 08:00 to 20:00 h. The rats were randomly divided into four groups (the control, fructose, psicose, and psico-rare sugar groups). The experimental diets are shown in Table 1. D-Psicose and psico-rare sugar (a mixture of D-fructose and D-psicose 3:1) were donated by the Rare Sugar Center of Kagawa University. Cellulose, sucrose, and D-fructose were

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Table 1. Compositions of Experimental Diets

Groups	Control	Fructose	Psicose	Psico-rare sugar
Ingredients		g/kg		
Casein	200.0	200.0	200.0	200.0
DL-Methionine	3.0	3.0	3.0	3.0
Cornstarch	550.0	500.0	500.0	500.0
Sucrose	100.0	100.0	100.0	100.0
D-Fructose	—	50.0	—	37.5
D-Psicose	—	—	50.0	12.5
Cellulose	50.0	50.0	50.0	50.0
Soybean oil	50.0	50.0	50.0	50.0
Mineral mixture*	35.0	35.0	35.0	35.0
Vitamine mixture*	10.0	10.0	10.0	10.0
Choline chloride	2.0	2.0	2.0	2.0
Butylhydroxytoluene	0.01	0.01	0.01	0.01
		kJ/g		
Metabolizable energy	15.5	15.5	14.8	15.3

*Based on AIN-76A mixture.

purchased from Wako Pure Chemical Industries (Osaka, Japan). Vitamin and mineral mixtures based on AIN-76A were used.[19,20] The metabolizable energy of each diet was calculated as digestible energy (gross energy fed minus energy in feces) multiplied by 0.96.[21] Each group of rats was meal-fed the diet at 8:30 to 9:30 and 20:30 to 21:30 and given free access to water for 8 weeks. Under meal-feeding conditions, one meal (within 2 h) a day decreased the food intake of the animals, but feeding two meals a day minimized the decrease in food intake.

Diurnal rhythm of plasma glucose and insulin levels. Diurnal variation of plasma glucose and insulin concentrations was measured in each diet group of rats after 7 weeks of feeding the experimental diets. Blood samples (about 150 µl) were obtained from a tail artery at 2:00, 6:00, 10:00, 14:00, 18:00 and 22:00 h into tubes coated with heparin and NaF for determination of plasma glucose and insulin levels.

Dissection. On the final day, half of the rats in each group were killed by decapitation at 08:00 h (preprandial) and the others were killed at 10:00 (postprandial). Liver and intra-adipose tissues (perirenal, epididymal and mesenteric) were quickly removed, weighed and stored at $-80\,^{\circ}C$ until analysis. Blood was collected to obtain serum. Carcass samples obtained by removing the head, tail, intra-pectral and intra-abdominal tissues were stored at $-20\,^{\circ}C$ until analysis of carcass composition.

Measurement. Concentrations of plasma and serum glucose were determined by methods reported previously.[22] Plasma insulin concentration was determined using kits (Rat Insulin EIA System, Amersham Bioscience, Tokyo). Evaluation of serum glucoalbumin was requested from SRL (Tokyo). Liver and soleus glycogen contents were determined according to Lo *et al.*[23] Carcass fat and protein were analyzed using the method reported by Mickelsen and Anderson.[24]

Data analysis. All values are expressed as mean ± SD. Data for diurnal variation of plasma glucose and insulin concentrations were analyzed by repeated measures of ANOVA and Scheffe's tests. Statistical differences in body and tissue weights, energy intake and carcass composition were analyzed by one-way ANOVA and Scheffe's tests. Statistical differences in serum glucose and tissue glycogen in rats killed before and after a meal were analyzed by two way (kill time × groups) ANOVA and Scheffe's tests. Differences were considered statistically significant at $p < 0.05$. All analyses were performed with a commercially available statistical package (StatView J-5.0, SAS Institute, Cary, NC).

Results

Diurnal rhythm of plasma glucose and insulin levels

Plasma glucose levels were lower and plasma insulin levels were higher at all times of day in the psicose and psico-rare sugar groups than in the control and fructose groups (Table 2). Compared with the levels in the control and fructose groups, glucose concentrations in the psicose and psico-rare sugar groups decreased significantly at 14:00 h, and insulin concentrations increased significantly at 06:00 and 18:00 h (Table 2). At 02:00 h, plasma insulin levels significantly higher in the psico-rare sugar group than in the control group (Table 2). Increments of area under the curve for plasma glucose were significantly lower and for plasma insulin significantly higher in the psicose and psico-rare sugar groups than in the control and fructose groups (Table 2).

Body and tissue weights, energy intake, and carcass composition

Final body weight and weight gain were significantly lower in the psicose group than in the control and fructose groups (Table 3). Weight gain in the psico-rare sugar group was significantly greater than in the psicose group, but was significantly lower than in the control and fructose groups (Table 3). Energy intake, energy efficiency, abdominal adipose tissue weight and carcass fat content were lower in the psicose group than in the other groups (Table 3). Supplementation with D-fructose significantly increased body fat accumulation compared with the other test sugars (Table 3). Carcass fat content and percentage of abdominal fat did not differ between the control and psico-rare sugar groups even though psico-rare sugar was consisted of 75% D-fructose (Table 3). Carcass protein content did not differ among the various groups (Table 3). Liver weight and relative liver weight were significantly greater in the psicose group than in the other groups, and were greater in the psico-rare sugar group than in the control and Fructose groups (Table 3).

Table 2. Diurnal Variation in Plasma Glucose and Iinsulin Concentrations

Groups	Time of day						Increment of area under the curve
	06:00	10:00	14:00	18:00	22:00	02:00	
			Glucose (mg/100 ml)				h·mg/100 ml
Control	121 ± 12	135 ± 10	130 ± 16^{a}	127 ± 16	141 ± 10	122 ± 10	2617 ± 117^{a}
Fructose	122 ± 9	133 ± 15	130 ± 13^{a}	126 ± 9	136 ± 24	122 ± 12	2607 ± 194^{a}
Psicose	115 ± 11	126 ± 11	117 ± 8^{b}	121 ± 5	134 ± 12	122 ± 11	2465 ± 129^{b}
Psico-rare sugar	116 ± 10	125 ± 14	117 ± 8^{b}	124 ± 10	131 ± 12	122 ± 10	2464 ± 159^{b}
			Insulin (ng/ml)				h·ng/ml
Control	5.5 ± 0.2^{c}	5.7 ± 0.1^{c}	6.0 ± 0.8	5.7 ± 0.4^{b}	6.1 ± 0.1	5.7 ± 0.1^{b}	116 ± 7^{b}
Fructose	5.7 ± 0.3^{b}	5.9 ± 0.5^{bc}	5.8 ± 0.2	5.8 ± 0.3^{b}	6.1 ± 0.4	5.8 ± 0.5^{ab}	118 ± 4^{b}
Psicose	6.2 ± 0.2^{a}	6.4 ± 0.2^{a}	6.3 ± 0.2	6.1 ± 0.3^{a}	6.4 ± 0.4	5.8 ± 0.2^{ab}	125 ± 3^{a}
Psico-rare sugar	6.1 ± 0.2^{a}	6.1 ± 0.3^{b}	6.2 ± 0.3	6.2 ± 0.2^{a}	6.5 ± 0.3	6.1 ± 0.2^{a}	124 ± 3^{a}

Values are means ± SD for 12 rats. Means with different superscripts within a column are significantly different ($p < 0.05$, ANOVA with repeated measures and Scheffe's tests).

Table 3. Body Weight, Tissue Weights and Carcass Composition

Groups		Control	Fructose	Psicose	Psico-rare sugar
Initial weight	(g)	85 ± 2	85 ± 2	85 ± 3	85 ± 2
Final weight	(g)	198 ± 11^{a}	197 ± 9^{a}	184 ± 10^{b}	191 ± 7^{ab}
Weight gain	(g)	113 ± 8^{a}	112 ± 6^{a}	99 ± 7^{c}	106 ± 4^{b}
Energy intake	(kcal/day)	38.2 ± 0.4^{a}	38.3 ± 0.4^{a}	35.2 ± 0.6^{b}	37.8 ± 0.4^{a}
Energy efficiency	(g/kcal)	2.96 ± 0.05^{a}	2.92 ± 0.04^{a}	2.76 ± 0.09^{b}	2.80 ± 0.05^{a}
Tissue weight					
Liver	(g)	5.7 ± 0.5^{c}	6.0 ± 0.3^{bc}	7.2 ± 0.5^{a}	6.3 ± 0.4^{b}
	(g/100 g)*	2.9 ± 0.3^{c}	3.0 ± 0.2^{c}	3.9 ± 0.3^{a}	3.4 ± 0.2^{b}
Abdominal adipose tissue	(g)	10.5 ± 1.5^{b}	11.5 ± 1.0^{a}	8.9 ± 1.0^{d}	10.1 ± 1.1^{c}
	(g/100 g)*	5.3 ± 0.8^{b}	5.8 ± 0.5^{a}	4.8 ± 0.6^{c}	5.3 ± 0.6^{b}
Carcass fat	(g)	17.9 ± 1.8^{b}	19.7 ± 1.8^{a}	17.3 ± 1.1^{b}	17.7 ± 1.8^{b}
	(%)	21.6 ± 2.1^{b}	23.6 ± 2.2^{a}	18.9 ± 1.4^{c}	20.6 ± 1.7^{b}
Carcass protein	(g)	28.0 ± 1.8	28.1 ± 1.1	27.0 ± 1.2	26.9 ± 1.6
	(%)	23.2 ± 1.0	23.4 ± 0.7	23.7 ± 0.9	23.1 ± 0.9

Values are means ± SD for 12 rats. Means with different superscripts within a row are significantly different ($p < 0.05$, one-way ANOVA and Scheffe's tests).
*Relative tissue weights per 100 g final body weight.

Table 4. Serum Glucose Concentration and Tissue Glycogen Contents in Rats Killed before and after a Meal

Groups	Kill time	Glucose (mg/100 ml)	Liver glycogen (mg/g)	Liver glycogen (mg/tissue)	Soleus glycogen (mg/g)	Soleus glycogen (μg/tissue)
Control	08:00 h	142 ± 16^{b}	7.6 ± 3.8^{b}	43 ± 21^{c}	0.21 ± 0.06	28 ± 10
	10:00 h	154 ± 8^{ab}	9.0 ± 4.4^{ab}	55 ± 29^{bc}	0.30 ± 0.16	40 ± 22
Fructose	08:00 h	144 ± 14^{b}	9.3 ± 3.8^{ab}	54 ± 23^{bc}	0.21 ± 0.08	26 ± 10
	10:00 h	160 ± 7^{a}	8.1 ± 4.2^{b}	51 ± 23^{bc}	0.38 ± 0.16	49 ± 21
Psicose	08:00 h	135 ± 16^{c}	9.9 ± 3.0^{ab}	70 ± 23^{b}	0.31 ± 0.15	36 ± 15
	10:00 h	150 ± 5^{ab}	13.5 ± 3.8^{a}	99 ± 28^{a}	0.35 ± 0.11	40 ± 13
Psico-rare sugar	08:00 h	129 ± 8^{d}	9.0 ± 3.6^{b}	55 ± 22^{bc}	0.24 ± 0.10	29 ± 14
	10:00 h	145 ± 7^{bc}	11.1 ± 3.9^{ab}	72 ± 25^{b}	0.36 ± 0.19	45 ± 26

Values are means ± SD for six rats. Means with different superscripts within a column are significantly different ($p < 0.05$, two-way ANOVA and Scheffe's tests).

Serum glucose and tissue glycogen in rats killed before and after a meal

Serum glucose levels and liver glycogen content increased after a meal (Table 4). Serum glucose concentrations both before and after a meal were lower in the psicose and psico-rare sugar groups than in the control and fructose groups (Table 4). The preprandial glucose level was significantly lower in the psicose group than in the other groups, but postprandial glucose in the psicose group did not differ significantly from that in the other groups (Table 4). Liver glycogen content before and after meals was higher in the psicose group than in the control and fructose groups (Table 4). Liver glycogen in the psico-rare sugar group tended to be higher than in the control and fructose groups, but the differences were not significant (Table 4). Glycogen

content in soleus muscle did not differ among the various groups either before or after meals (Table 4).

Percentage of serum glycoalbumin

Serum glycoalbumin was significantly higher in the psicose group than in the other group (16.0 ± 1.9, 16.0 ± 1.9, 24.1 ± 6.3, and $17.6 \pm 2.0\%$ for the control, fructose, psicose, and psico-rare sugar groups, respectively).

Discussion

In this study, we found that plasma glucose levels were lower and plasma insulin levels were higher at all times of day in rats fed D-psicose in diets (the psicose and psico-rare sugar groups) than in the other groups. We also found that D-psicose in diets increased liver glycogen but not muscle glycogen. The lower plasma glucose level observed in the psicose and psico-rare sugar groups might be caused by enhanced glucose uptake in peripheral tissues in rats fed D-psicose.[25] Because a large portion of the glucose taken from the blood is actively synthesized into hepatic glycogen and oxidized in the skeletal muscle, glycogen in soleus muscle was not increased by D-psicose. Glycogenesis in liver or skeletal muscle is known to be stimulated by insulin.[26] Thus the higher levels of plasma insulin in the psicose and psico-rare sugar groups are consistent with the higher levels of liver glycogen in those groups. The mechanism involved in the higher plasma insulin in the psicose and psico-rare sugar groups is unclear, but D-psicose might stimulate insulin secretion from pancreatic β-cells. Murao *et al.*[16] found that D-psicose stimulates insulin secretion in a dose-dependent manner in INS-1 cells and that D-psicose might employ a different insulin-stimulating mechanism than glucose. Our present findings support the results of Murao *et al.*[16]

On the other hand, we previously reported that D-psicose inhibits intestinal α-glucosidase activity and suppresses the glycemic response after sucrose and maltose ingestion.[13,14] In the present study, all experimental diets contained 10% sucrose. Therefore, the lower levels of plasma glucose in the psicose and psico-rare sugar groups might have been caused by decreased glucose absorption from the digestive tract resulting from inhibition of intestinal α-glucosidase activity induced by D-psicose.

Blood glycoalbumin is a known long-term control marker for blood glucose in diabetic patients.[27] Because dietary D-psicose decreased plasma glucose levels in the present study, we speculate that D-psicose has antidiabetic effects. Contrary to expectations, however, serum glycoalbumin was significantly higher in the psicose group than the other group. Son *et al.*[28] reported that D-psicose displays strong cross-linking with ovalbumin as compared with D-glucose or D-fructose. Consequently, glycated ovalbumin with D-psicose can improve gelling properties under certain controlled conditions. Previously we demonstrated that D-psicose occurred in serum when administrated orally (5 g/kg body weight) to rats.[12] The D-psicose concentration was about 70 mg/l 1 h after administration.[12] Serum albumin might be powerfully glycated by D-psicose, and if so, glucoalbumin is not an appropriate marker for diabetes mellitus.

In this study, D-psicose significantly decreased body fat accumulation as compared to D-fructose and the other sugars tested. Previously we reported that D-psicose supplements suppress hepatic lipogenic enzyme activity and reduce intra-abdominal fat accumulation as compared to D-glucose or D-fructose supplements in rats.[8,9] In addition, we found that D-psicose is a sweet monosaccharide that provides no energy to growing rats.[10] The present findings support our previous results. On the other hand, carcass protein content did not differ among the groups, which indicates that D-psicose did not inhibit rat growth. D-Psicose is a component of certain plant and agricultural products.[1–5] It has a potent, sweet taste and low toxicity; the LD_{50} value was 16 g/kg orally in rats in our previous tests.[11] D-Psicose caused no diarrhea at a dose of 10% of the diet in a rat study.[12] These findings suggest that D-psicose is a non-toxic sugar if taken in moderation.

Dietary D-psicose and psico-rare sugar produced liver enlargement (34.5 and 17.2% increase in relative liver weight *vs.* control group) in this study. Liver enlargement occurs in animals and humans under a variety of conditions and with different consequences for health.[29] For example, it can be the result of a physiological adaptation to an enhanced workload or metabolic demand, a metabolic abnormality, a toxic effect, an inflammatory process, or a proliferative disease. Bar *et al.*[30] found that D-tagatose, a rare sugar, at dietary levels of 5–20% increases liver glycogen deposition and relative liver weights in non-fasting rats. D-Tagatose, another rare sugar, is an incompletely absorbed ketohexose (stereo isomer of D-fructose) that has potential as an energy-reduced alternative sweetener. Bar *et al.* concluded that the liver enlargement seen in response to the consumption of D-tagatose was a physiological response to treatment-induced increased glycogen deposition. These results are similar to our present findings, but the relative liver weight of rats fed a 5% D-tagatose diet for 30 d was only 11.8% larger than that of control rats.[30] The mechanism of liver enlargement in rats might be different between that induced by D-psicose and that induced by D-tagatose.

In conclusion, the present study indicates that supplemental D-psicose decreases plasma glucose levels at all times of day and reduces body fat accumulation. Hence, D-psicose should be useful in preventing obesity or postprandial hyperglycemia in diabetic patients. More detailed study is required, however, to clarify the mechanism by which postprandial hyperglycemia is suppressed.

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