

## Effect of D-Psicose on Glycemic Response after Ingestion of Confectionery Foods in Healthy Subjects

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### Abstract

D-Psicose, a C-3 epimer of D-fructose, is a "rare sugar" present in small amounts in nature products. We investigated whether D-psicose suppresses the glycemic response after ingestion of various confections in healthy subjects. In Experiment 1, 20 male and 26 female subjects were randomly divided into 3 groups: marshmallow, fried cookie and chocolate groups. The test meals were these 3 confections containing 45 g of carbohydrate and hot coffee with 5 g of D-psicose or D-fructose. The increases in blood glucose concentration after intake of various confections were significantly lower with simultaneous intake of coffee and D-psicose than with that of coffee with D-fructose. In Experiment 2, 5 male and 5 female subjects ate the cake, almond jelly, baked cookie, fried cookie, and ganache containing 5 g of D-psicose or D-fructose as raw materials. The increases in plasma glucose concentration were significantly lower after intake of almond jelly and ganache containing D-psicose than those containing D-fructose. The postprandial plasma glucose concentration did not differ between intake of confections containing D-psicose and D-fructose cooked at high temperature. These results suggested that D-psicose is effective for the hypoglycemic response as a functional food material. However, this effect may be suppressed by high temperature cooking. Care is necessary when D-psicose is used as a food material.

**Key words :** D-psicose, plasma glucose, plasma insulin, confection, healthy subject

### Introduction

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<sup>(1)</sup>. D-Psicose is also present in processed cane and beet molasses<sup>(2)</sup>, and is found in wheat<sup>(3)</sup>, *Itea* plants<sup>(4)</sup>, and in the antibiotic psicofra-nine<sup>(5)</sup>. Due to the very small amounts of D-psicose in natural products, few studies have examined D-psicose as food or nutrient. In 2000s, we developed a new method for producing D-psicose enzymatically on a large scale<sup>(6)</sup>, making it possible to conduct such nutritional studies. We have since demonstrated that D-psicose is a sweet monosaccharide that provides no energy to growing rats<sup>(7)</sup> and that it has little toxicity in rats<sup>(8,9)</sup>. With respect to safety from a clinical viewpoint, the maximum non-effective level of D-psicose causing diarrhea in human subjects was estimated as 0.55 g per kg body weight<sup>(10)</sup>.

Examining the effects of D-psicose on glucose and lipid metabolism, we found that D-psicose leads to less intra-abdominal fat accumulation than D-glucose and D-fructose in rats<sup>(11)</sup>. In addition, we have suggested that supplemental

D-psicose can lower plasma glucose levels<sup>(12)</sup>. We have reported that D-psicose inhibits intestinal  $\alpha$ -glucosidase and suppresses the glycemic response after ingestion of disaccharides (sucrose, maltose or maltodextrin) in rats<sup>(13,14)</sup> and human<sup>(15)</sup>. Recently, Toyoda et al.<sup>(16)</sup> suggested that D-psicose can prevent postprandial hyperglycemia by improving the translocation of glucokinase from the nucleus to the cytoplasm in the liver of diabetic rats. D-Psicose is expected to have a beneficial effect in the control of blood glucose levels in type 2 diabetes.

One of the indicated effects D-psicose is suppression of postprandial blood glucose elevation<sup>(11-15)</sup>, which is helpful in reducing the risk of lifestyle-related diseases, such as type 2 diabetes. However, we have not examined the effects of D-psicose on blood glucose level after ingesting cooked meals in human subjects. In this study, we investigated whether D-psicose suppresses the glycemic response after ingestion of various confections in healthy subjects.

### Materials and Methods

Two experiments were conducted in 56 healthy volunteers aged 20-23 recruited from Kagawa University. The subjects

Table 1 Characteristics of subjects.

Groups	Experiment 1						Experiment 2	
	Marshmallow		Fried cookie		Chocolate		Male	Female
	Male	Female	Male	Female	Male	Female		
n	7	7	5	12	8	7	5	5
Age (y)	20.5±0.2	20.6±0.2	20.2±0.1	20.2±0.1	21.2±0.2	20.1±0.1	20.3±0.1	20.4±0.1
Height (cm)	169.0±1.1	153.0±0.9	168.0±1.1	157.8±1.2	169.2±1.0	156.2±0.9	170.1±1.0	155.2±0.9
Weight (kg)	60.2±1.2	50.1±1.0	62.8±1.5	50.6±1.3	61.8±1.6	50.2±1.2	63.1±1.2	49.5±1.0
BMI (kg/m <sup>2</sup> )	21.1±0.7	21.4±0.5	22.2±0.5	20.3±0.4	21.6±0.8	20.6±0.9	21.8±0.4	20.5±0.3

Values are means ± SE.

Table 2 Composition of test meals.

	Weight (g)	Fat (g)	Protein (g)	Carbohydrate (g)	Energy (kcal)
Experiment 1*					
Marshmallow	56.0	0.2	1.8	45.0	189.0
Fried cookie	62.0	9.5	4.1	45.0	283.0
Chocolate	80.0	28.0	4.2	45.0	449.0
Experiment 2 <sup>#</sup>					
Cake	117.5	7.8	5.1	50.0	290.6
Almond Jelly	96.0	10.0	20.0	50.0	370.0
Baked cookie	70.9	16.1	4.8	50.0	364.1
Fried cookie	79.2	21.5	5.6	50.0	415.9
Ganache	100.0	30.0	7.9	50.0	501.6

\*Each confection was taken with coffee (150ml) with D-fructose (5g) or D-psicose (5g).

<sup>#</sup>Each confection was made with D-fructose (5g) or D-psicose (5g) as a raw material.

were determined to be free of disease by a medical examination before the study. None of the subjects were using illegal drugs or taking medications that affect blood glucose level. Volunteers were fully informed of the objective of the study, the test methods, expected adverse reactions and other related matters. Before the study started, written consent was obtained from the subjects. The study protocol and the implementation complied with the spirit of the Declaration of Helsinki in 1995 as revised in Edinburgh 2000. This trial was carried out with the approval of the Ethical Committee of Kagawa University (Approval number: H20-33).

Experiment 1 Effects of D-psicose used as a sweetener on postprandial blood glucose level

Subjects and test meals

Twenty male and 26 female subjects were randomly divided into 3 groups: marshmallow, fried cookie and chocolate groups (Table 1). These confections were purchased from Maruyoshi Center Inc., Kagawa, Japan. The test meals were these 3 confections containing 45 g of carbohydrate and hot coffee with 5 g of D-psicose or D-fructose. The compositions of test meals are shown in Table 2. The hot coffee was made

from 2 g of powdered instant coffee (Nescafe, Nestle Japan, Hyogo, Japan) and an additional 150 ml of hot water.

Experimental design

Two meal load tests per subject were conducted under a randomized single blind study design. During the period of the study, each subject maintained a normal life style and ate *ad libitum* except for the day before the experiment, on which each subject ate the same dinner (800 kcal and 700 kcal for male and female subjects, respectively) at 19:00. After fasting for overnight, blood glucose concentration was first measured at 08:00 with a portable glucose analyzer (Glucocard G<sup>+</sup>, Arkray Inc., Kyoto, Japan). Soon after the first blood measurement, the each group of subjects took test meals within 5 min. The subjects then rested for 120 min. During the rest, the blood glucose concentrations were measured at 30, 60, 90 and 120 min after intake of test meals containing D-psicose or D-fructose. The 2 meal load tests (D-psicose or D-fructose) per subjects in each group were performed at intervals of at least 1 week. All procedures were performed in the experimental room under the same conditions (temperature: 22 degrees Celsius; humidity: 60%).

Table 3 Blood glucose concentrations (mg/dL) after ingestion of test meals with D-fructose or D-psicose (Experiment 1).

Test meals	Sweeteners	Time after ingestion (min)				
		0	30	60	90	120
Marshmallow	D-Fructose	76.2±2.0	115.7±4.0	119.6±5.7	103.7±4.8	89.7±4.3
	D-Psicose	74.5±1.6	107.3±3.4*	111.8±5.0	100.5±4.5	87.0±4.9
Fried cookie	D-Fructose	78.6±2.1	112.6±4.6	126.2±4.9	109.6±4.7	89.9±4.3
	D-Psicose	78.5±2.7	101.6±3.6*	116.3±3.0*	101.4±3.4	88.4±3.6
Chocolate	D-Fructose	74.8±1.5	95.2±2.2	102.1±3.1	97.8±2.8	83.6±2.3
	D-Psicose	77.8±2.0	94.3±2.7	91.8±2.5*	90.1±2.4*	79.1±2.4

Values are means ± SE for 14–15 subjects. \* $p < 0.05$ , vs. test meals with D-fructose (Student's paired  $t$ -test).

## Experiment 2 Effects of D-psicose used as a raw material on postprandial plasma glucose and insulin levels

### Subjects and test meals

Five male and 5 female subjects participated in this experiment, as shown in Table 1. The compositions of test meals are shown in Table 2. The cake, almond jelly, baked cookie, fried cookie and ganache were made with 5 g of D-psicose or D-fructose according to the recipe<sup>(17)</sup>. These confections were made by substitution of D-psicose or D-fructose in place of sucrose as a raw material.

### Experimental design

The 10 meal load tests per subject were conducted under a randomized single blind study design. During the period of the study, each subject maintained a normal life style and ate *ad libitum* except for the day before the experiment, on which each subject ate the same dinner (800 kcal and 700 kcal for male and female subjects, respectively) at 19:00. After fasting for overnight, blood was first collected at 08:00. Soon after the first blood collection, subjects ate the test meals within 5 min. Peripheral blood (180  $\mu$ L) was collected in heparin-coated capillaries to obtain plasma at 30, 60, 90 and 120 min after meal intake. All procedures were performed in the experimental room under the same conditions (temperature: 22 degrees Celsius; humidity: 60%).

### Measurements

Plasma glucose and insulin concentrations were determined by using commercial kits (Glucose CII-Test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan; Mercodia Rat Insulin ELISA kit, Mercodia Inc., Erling-Holmlund, Sweden) purchased from Shikoku Medical Instruments, Kagawa, Japan.

### Statistical analysis

Blood glucose (Experiment 1) and plasma glucose and insulin (Experiment 2) were analyzed statistically. For these tests, male and female subject data were combined. The values

for analysis were determined before the intake point as well as at 30, 60, 90 and 120 min after intake of each test meal. All measurements are expressed as means ± standard error. To examine the significance of differences, Student's paired  $t$ -test was employed with a level of significance of 5% or less. Statistical processing was performed using Excel Statistics 2008 (SSRI Co., Ltd., Tokyo, Japan).

## Results

### Experiment 1

There were no dropouts among the 46 subjects participating in this trial. The glycemic responses for the 3 test meals with D-fructose or D-psicose are shown in Table 3. The blood glucose concentrations after each test meal increased over time until 30–60 min and then decreased. The increases in blood glucose concentration after intake of various confections were significantly lower with the simultaneous intake of coffee with D-psicose than that of coffee with D-fructose. Statistically significant differences ( $p < 0.05$ ) between D-fructose and D-psicose were seen with marshmallow intake after 30 min, fried cookie intake after 30 and 60 min, and chocolate intake after 60 and 90 min. Increments of area under the curve of blood glucose were also lower in each test meal with D-psicose than with D-fructose (marshmallow, 3,514 vs. 3,070; fried cookie, 3,548 vs. 2,663; chocolate, 2,253 vs. 1,304  $\text{min} \cdot \text{mg/dL}$ , respectively).

### Experiment 2

There were no dropouts among the 10 subjects participating in this trial. The glycemic and insulinemic responses for the 5 test meals containing D-fructose or D-psicose are shown in Tables 4 and 5. The plasma glucose and insulin concentrations after each test meal increased over time until 30 min and then decreased. The increases in plasma glucose concentration were significantly lower after intake of almond jelly and ganache containing D-psicose than those containing D-fructose.

Table 4 Plasma glucose concentrations (mg/dL) after ingestion of test meals with D-fructose or D-psicose (Experiment 2).

Test meals	Additive sugars	Time after ingestion (min)				
		0	30	60	90	120
Cake	D-Fructose	82.9 ± 3.4	122.1 ± 7.1	97.7 ± 4.5	86.3 ± 2.9	83.6 ± 2.5
	D-Psicose	89.3 ± 1.3	119.9 ± 6.4	110.8 ± 9.6	92.3 ± 4.1	80.8 ± 3.7
Almond jelly	D-Fructose	80.5 ± 0.8	118.5 ± 1.3	114.4 ± 2.8	101.2 ± 2.3	86.7 ± 2.2
	D-Psicose	80.6 ± 1.4	101.4 ± 2.4*	110.2 ± 3.9	99.3 ± 1.9	84.1 ± 1.7
Baked cookie	D-Fructose	84.7 ± 2.2	159.6 ± 5.8	121.3 ± 6.9	103.3 ± 6.2	89.8 ± 2.8
	D-Psicose	80.1 ± 3.0	153.9 ± 7.3	110.6 ± 4.1	101.8 ± 3.7	89.6 ± 1.4
Fried cookie	D-Fructose	79.9 ± 4.0	116.5 ± 4.8	105.1 ± 4.8	99.9 ± 3.6	86.5 ± 2.8
	D-Psicose	79.1 ± 3.1	116.2 ± 3.9	101.4 ± 7.8	94.2 ± 3.3	83.6 ± 2.4
Ganache	D-Fructose	77.0 ± 0.5	107.7 ± 1.3	102.8 ± 0.8	92.5 ± 2.3	81.1 ± 2.1
	D-Psicose	77.9 ± 1.0	92.5 ± 1.9*	92.2 ± 1.8*	89.6 ± 1.8	79.6 ± 1.6

Values are means ± SE for 9 subjects. \* $p < 0.05$ , vs. test meals with D-fructose (Student's paired  $t$ -test).

Table 5 Plasma insulin concentrations (mU/L) after ingestion of test meals with D-fructose or D-psicose (Experiment 2).

Test meals	Additive sugars	Time after ingestion (min)				
		0	30	60	90	120
Cake	D-Fructose	3.8 ± 1.1	18.3 ± 3.5	10.7 ± 2.3	6.3 ± 1.6	4.6 ± 1.0
	D-Psicose	4.0 ± 0.5	18.1 ± 4.1	7.2 ± 1.4	7.3 ± 1.2	5.2 ± 0.7
Almond jelly	D-Fructose	6.5 ± 2.5	25.2 ± 4.0	15.1 ± 2.0	8.3 ± 1.3	5.9 ± 0.7
	D-Psicose	6.4 ± 0.8	15.5 ± 2.3*	9.4 ± 1.2*	9.1 ± 1.5	6.2 ± 1.2
Baked cookie	D-Fructose	6.5 ± 1.3	20.9 ± 4.2	11.8 ± 1.6	6.9 ± 1.2	6.0 ± 1.4
	D-Psicose	4.5 ± 1.3	13.1 ± 2.5	10.8 ± 3.3	5.8 ± 0.8	5.0 ± 1.0
Fried cookie	D-Fructose	4.1 ± 0.7	13.6 ± 2.1	10.3 ± 2.1	6.8 ± 1.9	4.3 ± 1.3
	D-Psicose	3.7 ± 0.4	14.7 ± 1.1	10.4 ± 1.3	6.9 ± 1.3	4.1 ± 0.7
Ganache	D-Fructose	2.9 ± 0.3	29.6 ± 7.5	13.1 ± 2.5	10.2 ± 2.6	5.2 ± 1.2
	D-Psicose	3.1 ± 0.4	10.5 ± 1.9*	12.4 ± 3.1	7.8 ± 1.3	5.3 ± 0.8

Values are means ± SE for 9 subjects. \* $p < 0.05$ , vs. test meals with D-fructose (Student's paired  $t$ -test).

Statistically significant differences ( $p < 0.05$ ) between D-fructose and D-psicose were seen with almond jelly intake after 30 min and ganache intake after 30 and 60 min. The postprandial plasma glucose concentration did not differ between intake of D-psicose and D-fructose containing confections cooked at high temperature (cake, baked and fried cookies). Increments of area under the curve of plasma glucose in the test meals containing D-psicose compared to those containing D-fructose were as follows: cake, 1,732 vs. 1,525; almond jelly, 2,871 vs. 2,125; baked cookie, 2,752 vs. 3,923; fried cookie, 2,553 vs. 2,628; ganache, 2,222 vs. 1,243  $\text{min} \cdot \text{mg/dL}$ , respectively).

The increases in plasma insulin concentration were significantly lower after intake of almond jelly and ganache containing D-psicose than those containing D-fructose. Statistically significant differences ( $p < 0.05$ ) between D-fructose and D-psicose were seen with almond jelly intake after 30 and 60 min and ganache intake after 30 min. The increases in plasma insulin concentration did not differ among the intake of cake,

baked and fried cookies. Increments of area under the curve of plasma insulin in the test meals containing D-psicose compared to those containing D-fructose were as follows: cake, 729 vs. 636; almond jelly, 864 vs. 441; baked cookie, 596 vs. 494; fried cookie, 555 vs. 633; ganache, 1,361 vs. 675  $\text{min} \cdot \text{mU/L}$ , respectively).

## Discussion

The present study suggested that D-psicose is effective for the hypoglycemic response as a functional food material in healthy subjects.

In our previous animal study, suppression of the increase in plasma glucose concentration with D-psicose showed significant decreases when maltose and sucrose were used as substrates, but no significant decreases were observed when glucose and soluble starch were used as substrates<sup>(13)</sup>. Another animal study proposed that D-psicose inhibited the hydrolysis

of maltose by  $\alpha$ -glucosidase prepared from the membrane of the rat small intestine<sup>(14)</sup>. It follows from these observations that one of the suppressive mechanisms of D-psicose on the elevation of plasma glucose concentration of rats after carbohydrate administration is the inhibition of  $\alpha$ -glucosidase. Suppression of the elevation of plasma glucose concentration in humans with D-psicose was expected when several types of sugars were used as a carbohydrate source. Iida *et al.*<sup>(15)</sup> reported dose-dependent effects of D-psicose on suppression of the elevation of plasma glucose and insulin concentration with concurrent administration of maltodextrin and D-psicose in healthy humans. They concluded that D-psicose is efficacious in suppressing of the elevation of blood glucose concentration after eating in humans.

As another hypothetical mechanism for suppression of the increase in plasma glucose concentration, absorbed D-psicose in small intestine, in which D-psicose was estimated to absorb at 25%<sup>(18, 19)</sup>, promoted uptake of glucose in the liver. It has been reported that D-fructose activates glucokinase and reduces plasma glucose concentration after being phosphorylated into fructose 1-phosphate by fructokinase in the liver<sup>(20, 21)</sup>. A similar mechanism of reducing plasma glucose concentration is also postulated for D-tagatose, an isometric form of D-psicose<sup>(22)</sup>. The same biochemical pathway as D-fructose and D-tagatose could accordingly enhance glucose tolerance.

The present applied study of D-psicose suggested that hypoglycemic and hypoinsulinemic responses to D-psicose used as raw material in foods were not found in those cooked at high temperature, *i.e.*, cake, baked and fried cookies, in Experiment 2. These confections were made at more than 180°C with relatively long cooking times (dozens of minutes), whereas the others, *i.e.*, almond jelly and ganache, were made at less than 90°C with short cooking times (several minutes). In Experiment 1, D-psicose was hardly heated because it was used as a coffee sweetener. These results suggest that the hypoglycemic effect of D-psicose may be suppressed by cooking at high temperature together with other raw materials.

D-Psicose is a reducing sugar that nonenzymatically glycosylates the amino groups of proteins or peptides, similar to D-glucose or D-fructose (amino-carbonyl reaction or Maillard reaction)<sup>(23-25)</sup>. This reaction proceeds through two stages. In the early stage, the sugar reacts with the amino groups of protein/peptide to form a stable Amadori and Heyn's product via a labile Schiff base<sup>(26)</sup>. In the advance stage, many different complex reactions occur, and consequently, brown, cross-linked fluorescent products are produced<sup>(27)</sup>. Amino-carbonyl

reaction, the subsequent modification of protein functionality, and the physiological and pathological consequences of protein glycation have been the subject of much investigation. Moreover, amino-carbonyl reaction modifies carbohydrate metabolism<sup>(27)</sup>. Amino-carbonyl products are hardly digested and absorbed in the mammalian intestine<sup>(27)</sup>. D-Psicose-protein/peptide conjugates may be excreted into feces without digestion and absorption. As amino-carbonyl reaction is promoted by heating at high temperatures, the disappearance of the hypoglycemic effect of D-psicose may be due to amino-carbonyl reaction with food proteins or peptides. However, detailed studies are required to clarify this mechanism. Care is required when using D-psicose as a food material.

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## D-プシコースが健常者における菓子食品摂取後の血糖反応に及ぼす影響

松尾達博

### 要 旨

D-プシコースは自然界に僅かしか存在しない希少糖の一つである。本研究では、D-プシコースが菓子食品摂取後の血糖反応を抑制するか否かについて、健常者を対象に検討した。実験1では、20名の男性および26名の女性被験者を摂取する試験食品によって3群（マシュマロ、かりんとう、チョコレート）に分けた。45gの糖質を含む試験食品と5gのD-プシコースあるいはD-フルクトースを含むコーヒーを各群の被験者に摂取させた結果、いずれの試験食品についても被験者の食後血糖値は、D-フルクトース入りコーヒーを摂取した場合に比べて、D-プシコース入りコーヒーを摂取した場合で低値であった。実験2では、男女各5名の被験者に5gのD-プシコースあるいはD-フルクトースを含むケーキ、杏仁豆腐、クッキー、かりんとう、生チョコレートを摂取させた。その結果、杏仁豆腐および生チョコレートについて、被験者の食後血漿グルコースおよびインスリン濃度はD-フルクトース入り食品を摂取した場合に比べて、D-プシコース入り食品を摂取した場合で低値であった。以上の結果から、D-プシコースは菓子食品摂取後の血糖値上昇抑制作用を有することが示唆された。