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Dietary D-psicose enhances gene expression of GLUT2 and glucokinase in rat liver

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ABSTRACT

The effect of long-term intake of D-psicose on the gene expressions related to glucose utilization was studied. Expressions of mRNAs for GLUT2 and glucokinase in the liver were markedly enhanced by the intake of dietary D-psicose. Serum glucose concentration was lower and liver glycogen content was higher in rats fed D-psicose diet than in rats fed control diet. These results suggest that the long-term feeding of D-psicose enhances the glucose transport action and phosphorylation followed by the glycogen synthesis in hepatocyte, which prevents the elevation of the blood glucose level.

KEYWORDS

D-psicose;
GLUT2;
Glucokinase;
Glycogen.

A previous study reported that rare sugar D-psicose, a C-3 epimer of D-fructose, has approximately 70% sweetness of sucrose and provide no energy for growing rats[1]. Another previous study showed that dietary D-psicose attenuates the increase of postprandial glucose levels in plasma[2]. Thus, it is expected that D-psicose can be applied to the foods with health-promoting benefits if it is confirmed that hose inhibitory effects on the postprandial increase of blood glucose continue for a long period. The blood glucose levels is regulated by the uptake of glucose into several peripheral tissues, large partly liver[3]. It seemed that the glucose reduction in serum owing to dietary D-psicose was induced by the increase of glucose utilization in liver. However, those details, especially of molecular basis remain unknown. The first step of glucose metabolism is controlled by glucose transporter (GLUT) and GLUT2 is predominantly expressed in liver[4]. Glucokinase, key enzyme for liver glycolysis, also enhances the glycolic system under the control of insulin and stimulates the utilization of glucose indirectly[5]. Therefore, in this study, we have examined the effect of long-term intake of D-psicose on hepatic glucose utilization in rats. Especially we focused whether the gene expressions related to the glucose utilization, such as GLUT2 and glucokinase in the liver, involved in the suppression of the level of serum glucose by dietary D-psicose.

All procedures involving rats were approved by the Experimental Animal Care Committee of Kagawa University. Eighteen Wistar rats (mean body weight: 50 ± 1 g) obtained from Japan SLC (Shizuoka, Japan) were divided into two groups. One-half of the animals were fed a control diet; the other half were fed a D-psicose diet. Control diet was contained the following ingredients, in grams per kilogram: corn starch, 550; sucrose, 100; casein, 200; soybean oil, 50; cellulose, 50; vita-

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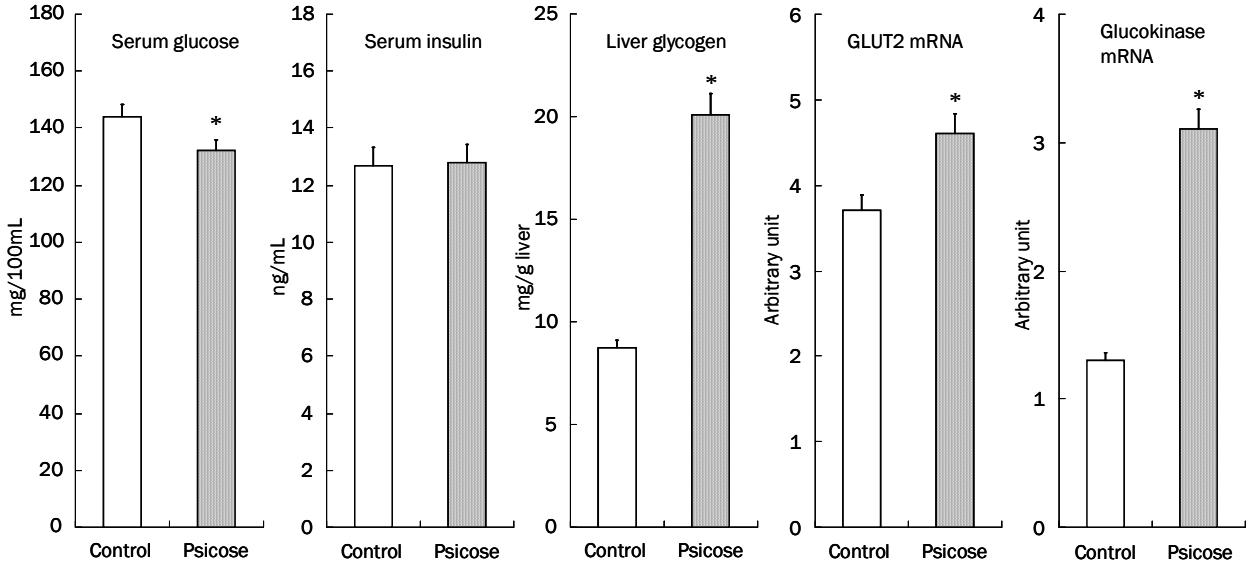

***Statistically significant difference ($p<0.05$, Student's t-test) from the control group.**

Figure 1 : Serum glucose and insulin concentrations, liver glycogen content, and real-time PCR analyses of GLUT2 and glucokinase in liver. Values are means ± SE for 9 rats.

min mixture, 10; mineral mixture, 35; DL-methionine, 3; choline chloride, 2; butylhydroxyltoluene, 0.01. D-Psicose diet was replaced by D-psicose in 5% of starch of the control diet. Each group of rats was meal-fed the diet at 8:30-9:30 and 20:30-21:30 and given free access to water. After 8 weeks, all rats were sacrificed following 6h fasting, whose serum levels of insulin and glucose as well as glycogen concentration in liver were measured.

Serum glucose and insulin concentrations were determined using kits, Glucose E-Test (Wako Pure Chemical Industry, Osaka) and Rat Insulin EIA System (Amersham Bioscience, Tokyo). Glycogen content in liver was determined according to Lo et al.[6].

Total RNA was extracted with a guanidium thiocynate water-saturated phenol extraction method using ISOGEN (Nippon Gene). The cDNAs for PCR were synthesized by reverse transcription of total RNA with random hexamer primers and Super Script II reverse transcriptase (Invitrogen CA, USA) according to the manufacturer's instruction. Rat GLUT2, glucokinase, and β-action genes were cloned into the TOPO vector (Invitrogen CA, USA) and used as a standard. After propagation and purification of the plasmid, the concentration of GLUT2, glucokinase, and β-action genes were obtained from A260 optical density measurement and the plasmid molecular weight. Ten fold serial dilutions (10^{-2} to 10^{2} gene copies/μL) of the linearized plasmid in water were prepared.

Quantitative real-time PCR was performed according to the standard method[7] using an ABI7700 system (Applied Biosystems, CA, USA). Reaction volume was 20 μL (0.5 μM primer dNTPs, Taq DNA polymerase, and reaction buffer were provided in the SYBR Green PCR Master Mix, Applied Biosystems, CA, USA). All real-time assays included 40 cycles of a denaturation step at 95 °C for 15 sec followed by annealing at 60 °C for 1 min after initial incubation at 95 °C for 10 min. The oligonucleotide primers were for GLUT2 (sense) 5'-GGCACTGGCTGCCTTCAG-3', (antisense) 5'-CCAAGGAAGTCCGCAATGTAC-3', glucokinase (sense) 5'-CACCCTCAGTGTCCCTCCAA-3', (antisense) 5'-CCAGGTAGAGAGGCTGGCATT-3'. Expected sizes of the products were 78 bps for GLT2 and 75 bps for glucokinase.

All values are expressed as means ± SE. The data was assessed by unpaired Student's t-test. All analyses were performed with a commercially available statistical package (Stat View J-5.0, SAS Institute Inc., Cary, NC).

Weight gain and food intake were similar between the control and D-psicose groups (118 ± 2 vs. 135 ± 2 g and 30.2 ± 0.2 vs. 30.0 ± 0.1 kcal/day, respectively). Whereas the liver weight was significantly higher in the D-psicose group than in the control group (4.7 ± 0.1 vs. 6.1 ± 0.1 g, $p<0.05$). Serum glucose concentration was

lower and liver glycogen content was higher in the D-psicose group than in the control group (Figure 1). On the other hand, there was no significant different in serum insulin concentration (Figure 1). Expression of mRNA for GLUT2 and glucokinase markedly increased for the D-psicose group than for the control group (Figure 1).

The aim of the present study was to examine the effect of long-term intake of D-psicose on liver glucose utilization in rats. Especially we focused the gene expression related to the liver glucose utilization such as GLUT2 and glucokinase involved in the suppression of the level of serum glucose by dietary D-psicose group. The serum glucose level is regulated by the uptake of glucose into several peripheral tissues, mostly liver and skeletal muscle[3] and the first step of glucose metabolism is controlled by the GLUT. GLUT is the final substrate of the insulin signal transduction pathway and the amount of glucose transported by GLUT depends on hormonal and metabolic conditions[4]. Glucokinase catalyzes the formation of glucose 6-phosphate, known as an activator of glycogen synthase[8]. The present study showed that expression of mRNA for GLUT2, the hepatocyte specific subtype of GLUT, and that for glucokinase in the liver of D-psicose-fed rats was markedly higher than that for the control rats. Furthermore, hepatic amount of glycogen for the D-psicose group was larger than for the control group. These findings suggested that the suppression of blood glucose level by the dietary D-psicose was due to the increase of the conversion of glucose into glycogen in the liver.

In the present study, the increase of glycogen content in the liver and the hypertrophy of liver were observed for D-psicose fed group, which were consist with some previous studies[2,9]. It is also reported that the hypertrophy of liver by dietary D-psicose is not pathological[9]. In addition, the investigation on the mechanism of liver enlargement induced by D-tagatose, another rare sugar, was revealed that the formation of live glycogen from dietary starch is enhanced when the rats ingests D-tagatose[10]. Significant enlargement of the liver is observed when the rats are fed with a diet containing 10 to 20% of D-tagatose for two weeks or more[11]. Since the liver enlargement is not accompanied by the increase of hepatic enzyme activities, no toxicological relevance is attributed to this phenomenon, which is supported by the fat that both D-fructose and sucrose influence on the size of liver in rats[12]. Earlier studies suggest that this effect is mediated by D-tagatose 1-phosphate which induces the translocation of glucokinase from the nucleus to the cytosol[13]. The present study showed that the hepatic glucokinase expression for the D-psicose fed rats was significantly higher than that for the control rats, which was strongly supported by Agius et al.[8]. The liver enlargement occurs in animals and humans under a variety of conditions with different consequences for health[14]. It is likely that the enlargement of liver is the result of a physiological adaptation to an enhanced workload.

D-Psicose is known for having suppression effect of blood glucose level by inhibiting the activity of intestinal α-glucosidase[15]. We demonstrated that intake of D-psicose suppressed blood glucose level, which was consistent with conforming to the previous studies[1,2]. Because considerable amount of D-psicose are excreted in the urine or feces[16], the improvement of the absorption rate into the small intestine were required for the application as a functional food in future.

In conclusion, it was suggested that the long-term feeding of D-psicose enhances the glucose transport action and phosphorylation followed the glycogen synthesis in hepatocyte, which prevent the elevation of the blood glucose level.

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