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Note

Addition Ratio of Palatinose and Body Fat Accumulation in Mice

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In the present study, the relationship between the addition ratio of palatinose in feed and body fat accumulation was investigated in mice. Thirty eight-week-old male mice (C57BL/6CrSlc) were divided into three groups: a 0% palatinose group (control group), a 18% palatinose group, and an 40% palatinose group. The 3 groups were then fed their respective diets for 8 weeks. Following the conclusion of the feeding period, fat tissues in the perirenal, periepididymal, and perimesenteric regions were removed to determine their wet weight. The weight of the visceral fat was clearly lower in mice of groups fed with feed containing palatinose than in the mice of the control group. In particular, the weight of perirenal fat was significantly lower in the 40% palatinose group than in the control group ($p < 0.05$).

Keywords: blood glucose, insulin, enzyme inhibition, internal organ fat, lipo-protein lipase

Introduction

Palatinose (6-O-D-glucopyranosyl-D-fructofuranose), also known as isomaltulose, has been used in a variety of food products as a noncariogenic (Ooshima *et al.*, 1983) natural sugar with sucrose-like superior taste (Kaga and Mizutani, 1985). The calorie count of palatinose is 4 kcal/g; the same as that of sucrose. Palatinose is characterized by its slow rate of digestion and absorption. It is digested at a rate approximately 1/5 that of sucrose (Tsuji *et al.*, 1986), resulting in only gradual increases in blood glucose levels (Kawai *et al.*, 1985, 1989). The glycemic index (GI) of palatinose is estimated to be 44. Despite the slow rate of digestion, there are numerous enzymes in the small intestine that are able to digest palatinose, and ingestion of a large amount of the sugar does not cause diarrhea. Thus, palatinose is a safe sugar to consume. Recently, several reports have been published on the functions of palatinose associated with gradual increases in blood glucose levels (Kashimura *et al.*, 2003; Nagai *et al.*, 2003).

Meanwhile, GI has recently been drawing worldwide attention with relation to health (Jenkins *et al.*, 2002; Willet *et al.*, 2002), particularly with that of obesity (Brand-Miller *et al.*, 2002). A mechanism is postulated whereby increased blood glucose level leads to an increase in insulin level and lipoprotein lipase (LPL) activity in fat tissues, resulting in lipid uptake in fat tissues (Suzuki, 1987).

In a study in which palatinose was fed to rats in place of sucrose, it was reported that accumulation of visceral fat was suppressed compared with animals fed with sucrose, and that there was little increase in LPL activity in fat tissues of rats fed with feed containing palatinose (Mizutani, 1989). In recent years, we have discovered that palatinose suppresses the increase in blood glucose level caused by sucrose or glucose ingestion (Kashimura *et al.*, 2003). These results suggest the possibility that palatinose may suppress blood glucose increase, thereby suppressing obesity and accumulation of visceral fat associated with lifestyle-related diseases. Here we investigate the relationship between the addition ratio of palatinose in feed and fat accumulation in mice.

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Materials and Methods

Animals Thirty seven-week-old male mice (C57BL/6 CrSlc) were acclimatized for one week and fed commercial powder feed (CRF-1, Oriental Yeast) and given water in a room maintained at 22±3℃ and 50±20% relative humidity with ventilation 13 to 17 times/hour and illumination from 8:00 to 20:00 (12-hour light and dark cycles). After acclimatization, the mice were divided into 3 groups of 10 (0% palatinose group [control group], 18% palatinose group, and 40% palatinose group) by stratified continuous randomization method using body weight as the index, and then housed individually in polycarbonate cages. At group assignment, it was confirmed that there was no significant difference in body weight among groups. Mice in each group were subjected to pair feeding using the feed shown in Table 1, with tap water ad libitum, for 8 weeks.

Test parameters: Feed consumption was measured everyday, during the feeding period, and body weight was measured every week. The animals had been fed with feed and tap water until the amount of intake of feed was measured on the day when the anatomy was to be performed. Following that, the abdominal cavity of the mice was opened under a subnarcotic condition with ether and 1 ml of blood was collected from the postcava, and subsequently left to exsanguinate. Subsequently,

Table 1. Composition of Experimental Diets.

Component/group	0% palatinose (control group)	18% palatinose	40% palatinose
Corn starch	14.95	14.95	14.95
Sucrose	40.00	22.00	0.00
Palatinose	0.00	18.00	40.00
Cellulose	5.00	5.00	5.00
Soybean oil	15.00	15.00	15.00
Mineral mixture(AIN93G)	3.50	3.50	3.50
Vitamin mixture(AIN93G)	1.00	1.00	1.00
L-cystine	0.30	0.30	0.30
Choline hydrogen tartrate	0.25	0.25	0.25
Casein	20.00	20.00	20.00

Table 2. Body Weight Gain.

	Intial weights	Final wieghts
0% palatinose group (control group)	24.0±0.3	36.2±0.8
18% palatinose group	23.7±0.3	32.8±0.9*
40% palatinose group	23.8±0.3	33.5±0.8*

Data were expressed as Mean (g)±SD

*Statistically significant at the $p<0.05$ level when compared with the data obtained in the control group.

Table 3. Effects of Palatinose Administration on Body Fat Accumulation in Male Mice.

	0% palatinose group (control group)	18% palatinose group	40% paltinose group
Fat tissue in the perirenal region	1.98±0.22	1.73±0.43	1.61±0.46*
Fat tissue in the periepididymal region	3.91±0.72	3.58±0.65	3.48±0.64
Fat tissue in the perimesenteric region	1.46±0.28	1.46±0.43	1.40±0.47

Data were expressed as Mean (wet g/100g B.W.)±SD

*Statistically significant at the $p<0.05$ level when compared with the data obtained in the control group.

fat tissues (perirenal, periepididymal, and perimesenteric regions) were removed and measured for wet weight. Collected blood was centrifuged to obtain serum, which was measured for free fatty acids, triglycerides, phospholipids, and total cholesterol using a Hitachi 7070 automatic analyzer.

Statistical method: Data obtained were subjected to testing for homogeneity of variance and, if found to be homogenous, subjected to unpaired t-test. Significance levels were set at 5% and 1%, respectively.

This study was done in accordance with the guidelines of the Japanese Association for Laboratory Animal Science, 1987.

Results

General conditions: Throughout the study period, none of the animals in any group exhibited evidence of diarrhea or loose stools, symptoms often observed when non- or low- digestible sugars are ingested. No other abnormalities in general conditions were observed either.

Feed consumption: No difference was observed in feed consumption among groups throughout the feeding period.

Body weight: Changes over time of body weight in each group during the feeding period are shown in Table 2. Body weight gain was slightly slower in the 18% palatinose and 40% palatinose groups when compared with the control group. As a result, significantly lower body weight in both palatinose and control groups were observed at the end of the feeding period ($p<0.05$).

Fat tissue weight: Table 3 shows the weight per 100 grams of body weight for fat tissues in the perirenal, periepididymal, and perimesenteric regions of animals in each group. It was observed that there was a general tendency of the weight of the visceral fat becoming lower in the mice of the groups fed with feed containing palatinose.

In particular, the weight of perirenal fat was significantly lower in the 40% palatinose group when compared with that of the control group ($p<0.05$). The weight of the visceral fat in the 40% palatinose group was lower than the weight in the 18% palatinose group, however the difference was insignificant.

Blood biochemistry: Changes over time of blood bio-

Table 4. Effect of Palatinose Administration on Blood Chemistry in Male Mice.

	0% palatinose group (control group)	18%palatinose group	40%palatinose group
Triglyceride (mg/dL)	18±2	21±2	24±2*
Phospholipid (mg/dL)	249±14	229±6	244±6
Total cholesterol (mg/dL)	144±10	130±5	143±4
NEFA(μEq/L)	410±37	460±50	397±26

Data were expressed as Mean±SE

*Statistically significant at the $p<0.05$ level when compared with the data obtained in the control group.

chemistry are shown in Table 4. Triglycerides showed a tendency to increase with palatinose consumption, being significantly higher in the 40% palatinose group than in the control group ($p<0.05$). No marked difference was observed in free fatty acids, phospholipids or total cholesterol level among groups.

Discussion

In this study, the body weight of animals in the 18% and 40% palatinose groups was significantly lower than the body weight of animals in the control group despite the fact that there was no difference in feed consumption among the groups throughout the study period. A possible cause of this result is water of crystallization contained in the palatinose. Since palatinose contains approximately 5% water as crystallization water, the net amount of palatinose is 5% less than that of sucrose. However, in terms of the entire amount of feed, this difference is only 2% for the 40% palatinose group and 0.9% for the 18% palatinose group, whereas a difference of approximately 10% was observed in body weight between the palatinose groups and the control group at the end of the feeding period. This suggests that the difference was not due to the water content of palatinose but to changes induced by the difference in digestibility and absorption between the two types of sugars.

It was reported that when palatinose was consumed together with sucrose or glucose, the increase in blood glucose level was smaller than that observed when sucrose or glucose was consumed alone (Kashimura *et al.*, 2003). This blood glucose-suppressing effect of palatinose is postulated to be caused by inhibition of enzymes such as sucrase, maltase and glucoamylase (Kashimura *et al.*, 2005). The fact that blood glucose increase induced by glucose is also suppressed suggests that palatinose inhibits, not only the activity of the above enzyme, but also the absorption of glucose. From these results, it is thought that palatinose, when consumed together with sucrose or starch, may suppress the increase in blood glucose level induced by sucrose or starch. In fact, 18% palatinose was as equally effective as 40% palatinose in suppressing fat accumulation. This is because palatinose is active in suppressing the increase in blood glucose induced by sucrose or glucose in addition to its function as a low glycemic substance. It is postulated that the reason why blood triglyceride levels were higher in the palatinose groups than in the control group is because, in the present study, animals were fed with feed until the day an anatomy was performed, thereby bringing about high triglyceride values. There was a report concerning SD rats where blood triglyceride levels were found rather lower in a group of animals fed with a fluid diet containing palatinose as the main ingredient for 2 months than in a group fed with commercially available solid feed (Arai *et al.*, 2003).

With regard to the effective addition ratio of palatinose in suppressing fat accumulation, the ratio of 10% or more, relative to sucrose or starch, has been found to be effective in inhibiting enzyme activities (Kashimura *et al.*, 2005). In the present study, the percentage of palatinose to total carbohydrate was approximately 32% in the 18% palatinose feed group. Since fat accumulation is directly regulated by insulin, and blood glucose level should therefore change to an extent sufficient to induce insulin secretion, the required addition ratio of palatinose may be higher than that sufficient for inducing enzyme inhibition.

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