Suppressive Effect of Peach Leaf Extract on Glucose Absorption from the Small Intestine of Mice

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The crude extract of peach leaves dose-dependently suppressed the postprandial elevation in the blood glucose level after an oral administration of soluble starch to mice. This study examines the mechanism for this suppressive effect in vivo. An oral carbohydrate-loading test on mice showed that the peach leaf extract suppressed the glucose-induced increase in the blood level of glucose, but without affecting the insulin level. An enteral soluble starch and glucose loading test on mice also showed that the crude extract (1,000 mg/kg) significantly suppressed the postprandial elevation of the blood glucose level and increased the amount of glucose that remained in the intestine to within the same range as that with phloridzin (500 mg/kg), a natural sodium-dependent glucose transporter (SGLT)-specific inhibitor. In contrast, the extract did not suppress the postprandial elevation of the blood triglyceride and cholesterol levels in mice, and did not affect the normal blood glucose level in a feeding test for 21 d. These results reveal that the extract of peach leaves suppressed the postprandial elevation of blood glucose level by inhibiting the absorption of glucose in the small intestine of mice.

Key words: peach leaf; Prunus persica Batsch; glucose absorption; food for diabetes therapy

Diabetes is a lifestyle-related metabolic disorder, and persistent hyperglycemia, the common characteristic of diabetes, can lead to such complications as diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy. Diabetes therefore impairs the quality of life, as well as posing a threat to life. Stabilization of the blood glucose level is important for diabetic patients, since this prevents hyperglycemia and the complications associated with diabetes. Medication that inhibits α-glucosidase such as acarbose, voglibose and miglitol are clinically used to treat diabetic patients. These medications are also useful for preventing or treating diabetes and are known to reduce postprandial hyperglycemia primarily by inhibiting carbohydrate-digestive enzymes and/or delaying glucose absorption in the small intestine, thereby controlling the blood glucose level. Materials that show anti-hyperglycemic activity have recently been screened and developed from natural sources to prevent diabetes and for preclinical control of the blood glucose level. Leaves of guava, wheat albumin and a Touchi extract are used for this purpose in Japan. We found in this preliminary study anti-hyperglycemic activity towards mice in an extract of the leaves of Prunus persica Batsch.

P. persica Batsch is a species of Prunus that is native to China. It bears an edible juicy fruit called a “peach,” the nucleus in the seed being used in Chinese medicine to improve the circulation of the blood. The bud is used as a diuretic and to treat constipation. Although the anti-inflammatory effects of seeds with fruit have been reported, previous studies have reported the anti-hyperglycemic activity in peach.

We found in the present study that a peach leaf extract showed anti-hyperglycemic activity toward mice. The inhibitory effect of this peach leaf extract on the postprandial elevation in blood glucose level and the glucose absorption in the small intestine of mice were evaluated in vivo to clarify the mechanism of action.

Materials and Methods

Chemicals. A Glucose C-II Test Wako Kit and Ultra Sensitive Mouse Insulin ELISA Kit were respectively purchased from Wako Pure Chemical Co. (Osaka, Japan) and Morinaga Institute of Biological Science (Japan). All other chemicals used in the experiments were of the highest purity commercially available.

Animals. Six-week-old male Slc:ddY mice from SLC (Hamamatsu, Japan) were housed in a room maintained at 24 ± 1 °C and 50 ± 2% humidity under a 12-h light/dark cycle. The animals had free access to water and standard laboratory feed (Labo MR Stock, Nusan, Tokyo, Japan) for one week to accustom them to their surroundings. All animal studies were conducted according to 2006 guidelines entitled Notification no. 88 of the Ministry of the Environment in Japan and Guidelines for Animal Experimentation of Tokyo University of Marine Science and Technology, with the approval of the Animal Care and Use Committee of Tokyo University of Marine Science and Technology.

Preparation of the crude extract from peach leaves. The leaves of peach Prunus persica Batsch ‘Shimizu-Hakotou’ were collected from a field in Okayama, Japan. An extract was prepared from leaves (100 g) that had been dried overnight at 70 °C in an oven and then soaked in water and boiled for 1 h. The extract was concentrated under reduced pressure to give a crude extract as an orange powder. The recovery rate from the leaves was 30.0% (w/w). This powder was stored at −80 °C and used in subsequent experiments as the crude extract of peach leaves. The peach leaf extract (subsequently referred to as “PLE”) was used in all experiments for evaluation as a food material.

Oral carbohydrate-loading test on mice. Mice were deprived of food for 24 h before the oral carbohydrate-loading test, and then orally administered with soluble starch, maltose, or glucose at 1,000 mg/kg.
or sucrose at 2,000 mg/kg, either alone or with PLE at 1,000 mg/kg dissolved in 1 mL of distilled water. Soluble starch was gelatinized by heating with boiled water in advance before administering to the mice. Blood samples were taken from the lateral tail vein at 0, 30, 60, 90 and 120 min after administering the carbohydrate. The blood was immediately centrifuged (4,500 × g for 2 min) to prepare plasma for measuring the blood glucose level with a Glucose C-II test kit, based on the mutarotase-glucose oxidase method.

**Postprandial blood insulin level on glucose-loaded mice.** To evaluate the postprandial blood insulin level, mice were deprived of food for 24 h and then orally administered with glucose at 1,000 mg/kg, either alone or with PLE at 500 and 1,000 mg/kg, dissolved in 1 mL of distilled water. Blood samples were taken from the lateral tail vein at 0, 5, 10, 30 and 60 min after administering the glucose to measure the blood glucose level as already described, together with the blood insulin level with an Ultra-sensitive Mouse Insulin ELISA kit which is based on the sensitive sandwich ELISA method.

**Enteral carbohydrate-loading test on mice.** Mice were deprived of food for 24 h, anesthetized with propofol (Mylan, Tokyo, Japan) at 10 mL/kg i.p., and an incision made in the abdomen. A 1,000 mg/kg amount of soluble starch or glucose with or without PLE (500 and 1,000 mg/kg) was injected by catheter from the pyloric region to the duodenal region. The small intestine was then ligated at two points, 15 cm apart, and returned to its original position. The contents of the intestinal segment were recovered after 30 min, washed with 5 mL of saline, and the remaining glucose was measured with a Glucose C-II test kit as already described. The glucose concentration of the infused solution measured by the same procedure is defined as 100%. Blood samples were taken from the tail vein at 0 and 30 min after the infusion to measure the blood glucose level and support the evaluation of glucose absorption in the intestine.

**Oral triglyceride- and cholesterol-loading test on mice.** Mice were deprived of food for 24 h for the oral triglyceride-loading test, and then orally administered with corn oil at 8 mL/kg, including cholesterol (146 mg/kg) and sodium cholate (36 mg/kg) with the additional ingestion of PLE (1,000 mg/kg) dissolved in 1 mL of distilled water. Blood samples were taken from the lateral tail vein at 0, 2, 4, 6, and 8 h after loading to measure the blood triglyceride level and the total blood cholesterol level with a Triglyceride E-Test Wako Kit and Cholesterol E-Test WAKO Kit, respectively.

**Feeding experiment with normal mice.** After receiving a standard laboratory diet (Labo MR Stock, Nosan) and water ad libitum for 1 week, mice were divided into two groups matched for body weight. The control group received the normal diet based on AIN-93M with minor modifications (10 g/100 g of food): 4% beef tallow, 14% casein, 15.5% alpha-corn starch, 46.6% beta-corn starch, 10% sugar, 5% cellulose, 1% vitamin mixture (AIN-93), 3.5% mineral mixture (AIN-93M), 0.18% l-cystine, 0.25% choline hydrogen tartrate, and 0.0008% r-butyldihydroquinone. The components of the diet were purchased from Oriental Yeast (Tokyo, Japan). The PLE group received a similar diet, but with part of the beta-corn starch replaced by 1% of PLE. The body weight and food intake were measured twice a week. After 21 d, blood was collected from the tail vein to measure the blood parameters, and some organs were removed for weighing.

**Statistical analysis.** Data are expressed as the mean ± SE. The statistical significance of differences between means was evaluated by Student’s t-test for Figs. 2 and 5, and by ANOVA followed by Tukey’s post-hoc analysis for Figs. 1, 3 and 4, where p < 0.05 is considered to be statistically significant.

**Results**

**Effect of PLE on the postprandial blood glucose level in the oral starch-loading test on mice**

The effects of PLE on the postprandial blood glucose level were examined with soluble starch-loaded mice, as shown in Fig. 1. The blood glucose level of the control group (soluble starch alone without PLE) reached a maximum value at 30 min after loading. When PLE (62.5, 125, 250 and 500 mg/kg) was orally administered simultaneously with the soluble starch, the blood glucose level at 30 min after administration was significantly suppressed in a dose-dependent manner. However, the elevation of the blood glucose level was not suppressed by treating with PLE at 15.6 mg/kg (data not shown).

**Effects of PLE on postprandial blood glucose level in the oral carbohydrate-loading test on mice**

The effects of PLE on the postprandial blood glucose level were examined in carbohydrate-loaded mice, as shown in Fig. 2. The blood glucose level of the control group reached a maximum value at 30 min after loading. When PLE (500 and 1,000 mg/kg) was orally administered simultaneously with soluble starch (A), maltose (B), sucrose (C) or glucose (D), the blood glucose level at 30 min after administration was significantly suppressed (p < 0.005).

**Effects of PLE on the postprandial blood insulin level in mice**

The effects of PLE on the postprandial blood insulin level were examined in glucose-loaded mice, as shown in Fig. 3. The blood insulin level of the control group reached a maximum value at 5 min after loading. When PLE (500 and 1,000 mg/kg) was orally administered simultaneously with glucose, the elevation of blood insulin level at 5 min after administration was significantly suppressed (p < 0.005). The blood glucose level of the PLE-treated group at that time was significantly suppressed when compared with the level of the control group (data are not shown).

**Effects of PLE on glucose absorption in enteral soluble starch-loaded mice**

The residual amount of glucose (i.e., not absorbed) in the recovered intestinal contents (Fig. 4A) and the blood
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Fig. 2. Effects of PLE on the Blood Glucose Level in Orally Carbohydrate-Loaded Mice.
Six-week-old male Slc:ddY mice were orally administered with soluble starch at 1,000 mg/kg (A), maltose at 1,000 mg/kg (B), sucrose at 2,000 mg/kg (C), and glucose at 1,000 mg/kg (D), either alone as a control (filled diamonds) or with PLE at 1,000 mg/kg (filled circles) after overnight fasting for 24 h. Blood samples were taken at 0, 30, 60, 90, and 120 min after loading. Each data value is presented as the mean ± SE (n = 6). Significant difference in the glucose level vs. the control group levels (C3/C3/C3/C3/C3) was indicated by *p < 0.05, **p < 0.01, and ***p < 0.005.

Fig. 3. Effects of PLE on the Blood Insulin Level in Orally Glucose-Loaded Mice.
Six-week-old male Slc:ddY mice were orally administered with glucose at 1,000 mg/kg either alone as a control (filled diamonds) or with PLE at 500 (unfilled circles) and 1,000 (filled circles) mg/kg after overnight fasting for 24 h. Blood samples were taken at 0, 5, 10, 30, and 60 min after glucose loading. Each data value is presented as the mean ± SE (n = 8). Significant difference in the glucose level vs. that in the corresponding control is shown by **p < 0.01.

The amount of glucose remaining in the small intestine at 30 min after glucose loading in the control group was 1.9 ± 0.3 mg and the blood glucose level (B-a) was 121.7 ± 14.0 mg/dL. These results reveal that almost all the loaded soluble starch had been digested to glucose and transported to vessels during the 30 min of the test.

Figure 4A shows that PLE at 500 and 1,000 mg/kg significantly suppressed the decrease in the amount of glucose remaining in the intestine (A-b and -c) and suppressed the elevation of the blood glucose level (B-b and -c) in a dose-dependent manner when compared with the control group. In a similar manner, the residual amount of glucose with 500 mg/kg of phloridzin (A-d), a natural SGLT-specific inhibitor, was significantly higher than that in the control group. The residual amount of glucose in the group treated with 10 mg/kg of acarbose (A-e), α-amylase and α-glucosidase inhibitor, was not significantly different from that in the control group. Phloridzin (B-d) and acarbose (B-e) significantly suppressed the elevation of blood glucose level when compared with that for the control group.

Effects of PLE on glucose absorption in the enteral glucose-loaded mice
The amount of glucose remaining in the small intestine at 30 min after glucose loading in the control group was 7.6 ± 0.6%, as shown in Fig. 5A. The residual amount of glucose (i.e., not absorbed) in the recovered intestinal contents after 30 min reflects the absorption of glucose from the intestinal mucous membrane. These results indicate that more than 90% of glucose was absorbed into the blood over 30 min under these experimental conditions. In contrast, the residual amount of glucose in the mice treated with 1,000 mg/kg of PLE (72.4 ± 2.3%) was significantly higher than that in the control group. PLE therefore had significantly suppressed the elevation of blood glucose level 30 min after glucose loading when compared with that in the control group of mice, as shown in Fig. 5B.

Effects of PLE on triglyceride and cholesterol absorption in mice
PLE had not significantly suppressed the postprandial elevation of triglyceride and cholesterol levels in the triglyceride- and cholesterol-loaded mice 4 h after loading vs. the control group levels (1164.3 ± 211.4 vs. 1330.4 ± 66.0 mg/dL, for triglyceride, and 214.1 ± 22.2 vs. 199.3 ± 20.4 mg/dL for cholesterol).

Effects of feeding PLE to normal mice
We confirmed the unanticipated reaction from continued ingestion by a feeding experiment with PLE to
normal mice for 21 d. The results are summarized in Table 1. The food intake data were not applicable to a statistical analysis because they were expressed as the total amount for each group, but no distinct difference was apparent between the two groups. The body weight and organ weights of the PLE group were not significantly changed by continued ingestion when compared with the control group. There was no significant difference in any data between the two groups, likewise for the blood glucose levels of the two groups.

**Discussion**

Various food materials to suppress the postprandial elevation of blood glucose level have been reported during the last few decades. The working mechanisms and active ingredients of these food materials need to be elucidated by fundamental research for their most effective application to maintain our health. Most materials have shown inhibitory activity against carbohydrate digestive enzymes as an active principle, i.e., in adzuki beans,14) paschaca,15) kiwifruit leaf,16) and sugar maple leaf.17) Part of our studies has shown PLE to significantly suppress the postprandial elevation of the blood glucose level in a dose-dependent manner after administering soluble starch (Fig. 1). This extract has been assumed to inhibit carbohydrate-hydrolyzing enzymes, inhibit glucose absorption in the small intestine, promote insulin secretion,18) and exhibit insulin-like effects.19,20) The effects of PLE (1,000 mg/kg) on the blood glucose level after orally administering other carbohydrates was studied to elucidate the mechanism of action (Fig. 2). PLE also showed an anti-hyperglycemic effect in the loading test with glucose, which require no enzymatic digestion for its absorption, so the PLE affect on any factors related to postprandial elevation would be likely to involve hydrolyzing steps of α-amylase and/or α-β-glucosidase. The results reveal PLE to be an interesting natural resource with the potential to regulate the postprandial blood glucose level. Inhibiting glucose absorption from the intestine can be one of the most effective approaches for suppressing postprandial elevation of the blood glucose level.21–25) Since the oral administration of PLE had no effect on the blood insulin level as shown in Fig. 3, PLE would not be likely to act on the pancreas to promote

### Table 1. Effects of 21 Days of Feeding on the Body Weight, Organ Weights, and Blood Glucose Level in Normal Mice

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>PLE group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total food intake (g/mouse)</td>
<td>84.9</td>
<td>87.2</td>
</tr>
<tr>
<td>Body weight (g/100 g BW)</td>
<td>31.6 ± 1.4</td>
<td>33.1 ± 0.8</td>
</tr>
<tr>
<td>Liver (g/100 g BW)</td>
<td>4.774 ± 0.197</td>
<td>4.510 ± 0.353</td>
</tr>
<tr>
<td>Kidney (g/100 g BW)</td>
<td>1.469 ± 0.055</td>
<td>1.394 ± 0.094</td>
</tr>
<tr>
<td>Spleen (g/100 g BW)</td>
<td>0.442 ± 0.016</td>
<td>0.440 ± 0.028</td>
</tr>
<tr>
<td>Blood glucose level (mg/dL)</td>
<td>209.6 ± 4.2</td>
<td>205.7 ± 6.4</td>
</tr>
</tbody>
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There was no significant difference for each item between the two groups.
insulin secretion. We therefore focused on the effects of PLE on glucose absorption in the small intestine of mice.

We accordingly studied the effects of PLE on the glucose uptake in the small intestine by using enterally starch-loaded mice, as shown in Fig. 4. The PLE-treated groups showed dose-dependent effects similar to those of phloridzin in respect of the residual glucose and elevated blood glucose level in mice 30 min after soluble starch loading. The rate-limiting step of the suppression in these groups is not thought to be glucose absorption, but to be the hydrolysis of carbohydrate. PLE also significantly suppressed the elevation of blood glucose level and increased the amount of residual glucose in the small intestine when compared with the values for the control group of glucose-loaded mice (Fig. 5).

These results reveal that PLE suppressed the postprandial elevation of the blood glucose level to inhibit glucose absorption from the small intestine to the blood vessels of mice. The result of little elevation of the blood glucose level supported the findings that the extract had no effect on insulin secretion and no insulin-like effects, as already discussed.

Natural products that have been reported to have suppressive effects on glucose absorption in the small intestine include phloridzin,24) ploretin,25) and gynemic acid.26) These compounds inhibit glucose transport from the intestinal lumen to blood vessels. The results from the enteral carbohydrate-loading test on mice indicate PLE to be a promising active compound for inhibiting the transport of glucose in the intestine. The results suggest that, as a crude extract, PLE should express stronger activity than the pure reagent, phloridzin. Phloridzin has been applied as a selective SGLT inhibitor,27) although the activity is known to have been lost during hydrolysis to release intramolecular glucose residues by digestive enzymes in the small intestine.28,29) If the molecule were to be protected from enzymatic decomposition, its inhibitory activity against glucose absorption could be expected to remain relatively longer without any loss of activity. Many kinds of the C-glycoside type of phloridzin,30,31) ploretin (the aglycon portion of phloridzin) derivatives,32) and the polymer type of phloridzin33) have been examined for application to developing a new SGLT inhibitor providing resistance to decomposition. The active ingredient in PLE is considered to be a molecule resistant to digestive enzymes and with a more enhanced effect than that of phloridzin. Further studies will be needed to purify and identify the inhibitors in the extract so that the detailed mechanism for the suppressive effect of PLE on the postprandial blood glucose level in mice can be elucidated.

We used 'Shimizu-hakutou' as the peach cultivar for experiments in this study, but confirmed that the leaves of other peach cultivars (‘Kawanakajima,’ 'Ougontou,' and 'Benishimizu') showed suppressive activity at a similar dosage in glucose-loaded mice (data not shown). None of the peach fruit extracts (1,000 mg/kg), however, show any suppressive activity in the same experiment. The active component would therefore be common in peach leaves (data not shown). Phytochemical studies revealed the peach leaves to contain such varied chemicals as prusiac acid, caffeic acid derivatives, pentacyclic triterpenes, flavonols and their glycosides, and essential oil constituents.34–36) Phloridzin, a dihydrochalcone glucoside characteristically found in the apple tree, has been not reported from the peach. A type of flavonol glycoside in peach could therefore be expected to have an effect on glucose absorption in the small intestine.

Peach leaves have been reported as an astringent folk medicine for treating constipation and dermatosis.37) The astringent action may be responsible for the effect on glucose absorption by degenerating the surface of intestinal epithelia in a non-specific fashion. However, no dyspepsia (diarrhea and constipation) nor any changes in behavior were apparent in the mice (male ddY, 6 weeks old) given excessive doses of 2,000 and 5,000 mg/kg of PLE for 24 h (n = 3). The results from the triglyceride- and cholesterol-loaded mice suggest that PLE (1,000 mg/kg) did not affect the triglyceride and cholesterol absorption in mice. It seems likely that the effect of PLE is not from a non-specific action against intestinal epithelia but more from a specific action against glucose transport.

The findings from these experiments indicate that PLE may be useful as a natural source in the daily diet for improving postprandial hyperglycemia and preventing type II diabetes mellitus by continuously suppressing postprandial elevation of the blood glucose level. We also investigated by feeding experiments on normal mice the possible negative aspect of reducing the intake of required energy due to the inhibitory effect of PLE on glucose absorption. However, the results showed no significant differences in the food intake, body weight, organ weights and the blood glucose level between the control and animals treated for 21 d. PLE can therefore be expected to suppress glucose absorption without any damage to the digestive organs at a suitable dosage. We will get more detailed information in a future study about the safety of the extract as a food, and will examine the pharmacological actions of PLE in comparison with those of other anti-diabetic agents after a long-term administration by using animal models for diabetes mellitus.

In conclusion, the peach leaf extract suppressed the postprandial elevation of blood glucose in mice, without affecting the blood insulin levels. The mechanism of action is believed to have involved the inhibition of glucose absorption from the small intestine to the blood vessels of mice. The leaves of peach may be a new natural resource for preventing the postprandial absorption of carbohydrates when used as a drug to treat diabetic patients and as a functional food for people who need to control their own blood glucose level.

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References


21) M. SHIROSAKI et al.


