**Note**

**Extracts of Common Buckwheat Bran Prevent Sucrose Digestion**

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**Summary**  Buckwheat has been shown to have various health benefits such as reduction of hypertension and improvement of hypercholesterolemia; however, its effect on diabetes has not been fully elucidated. In this study, buckwheat bran extracts (BBE) inhibited sucrose activity in vitro more effectively than buckwheat. Balb/c mice pretreated with BBE showed dose-dependent reductions of blood glucose, greater than those observed with control mice, within 60 min following oral sucrose administration. Blood glucose levels in mice pretreated with buckwheat extracts were also significantly lower compared to those in control mice within 30 min following oral administration of sucrose. However, rutin, one of the abundant polyphenols of BBE, did not lower blood glucose level. Our data indicate that components of BBE other than rutin have inhibitory activity against sucrose in vivo. These results suggest that BBE could have beneficial effects on diabetes.

**Key Words**  blood glucose, buckwheat, buckwheat bran, rutin, sucrose

Type 2 diabetes mellitus is characterized by chronic hyperglycemia due to decreased insulin sensitivity in target tissues, including skeletal muscle, adipocytes and the liver, and/or impairment of insulin secretion (1, 2). In the early stage of type 2 diabetes postprandial glucose levels are increased, even though fasting glucose levels are normal. This is in part due to mild insulin resistance caused by factors such as obesity, hyperlipidemia, inadequate physical activity, and/or impaired glucose-stimulated insulin secretion. Recently, postprandial hyperglycemia has been shown to be an important risk factor for vascular complications (3–5).

Inhibitors of α-glucosidase, which prevent the hydrolysis of carbohydrates in the gastrointestinal tract, are commonly used to improve postprandial hyperglycemia in patients with type 2 diabetes and some have shown beneficial effects on both cardiovascular disease and progression to diabetes (6–10).

Buckwheat powder is used mainly for making noodles in Asia, and the remaining buckwheat bran is discarded. Recently, animal experiments have revealed that treatment with buckwheat improves hypertension (11, 12), obesity (12–14), and hyperlipidemia (14–20). In addition, treatment with concentrated buckwheat bran extracts (BBE) reduces plasma glucose in both streptozotocin-induced diabetic rats (21) and the KK-Ay mouse model for type 2 diabetes (22). The extracts used in those studies were rich in D-chiro-inositol, which acts in part as an insulin-mimetic via the glycosyl-phosphatidylinositol/inositol phosphoglycan signaling pathway, positively modulating insulin signal transduction (23, 24). Recently, rutin, a phenolic compound that is more abundant in tartary buckwheat than in common buckwheat, has been reported to inhibit α-glucosidase activity in vitro (25). However, there has been no investigation to determine whether buckwheat itself lowers postprandial blood glucose in vivo.

In this study, we investigated the role of common BBE in inhibiting sucrose activity as a measure of α-glucosidase activity in mice and found that BBE reduces this activity.

**Materials and Methods**

**Reagents.** Dimethyl sulfoxide (DMSO) was purchased from Nakalai Tesque, Inc. (Kyoto, Japan). Sucrose and glucose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of analytical grade.

**Preparation of extracts from buckwheat or buckwheat bran powder.**  Common buckwheat (Fagopyrum esculentum Moench) or buckwheat bran powder was manufactured by a milling machine at Tani Food Co., Ltd. (Ishii, Tokushima, Japan). Ten grams of buckwheat or buckwheat bran powder was added to 50 mL of 80% methanol (v/v) followed by stirring at room temperature for 1 h. The extracted solution was filtered through filter
paper (No. 5B, Advantec, Tokyo, Japan), and then concentrated and lyophilized at −80°C. Extracts were resolved individually with phosphate buffered saline (PBS) (for buckwheat extract) or 0.1% DMSO/PBS (for BBE) by sonication (Bioruptor Cosmo Bio, Tokyo, Japan) and used for in vivo animal experiments. The chemical composition of buckwheat bran powder (moisture, 4 g; protein, 2.2 g; lipid, 0.2 g; dietary fiber, 89.5 g; sugar, 2.4 g; ash, 1.7 g; rutin, 0.76 g; quercetin, 0.001 g) or buckwheat powder (moisture, 16.1 g; protein, 10.1 g; lipid, 2.3 g; carbohydrate, 69.9 g; ash, 1.6 g; rutin, 0.38 g; quercetin, undetectable) per 100 g before methanol extraction was determined according to AOAC (26). Dietary fiber composition of buckwheat bran powder was analyzed according to the method of Prosky et al. (27).

Inhibitory activities against sucrase. An in vitro sucrase enzymatic assay was performed using a crude α-glucosidase solution prepared from rat-intestinal acetone powder (Sigma, St. Louis, MO). BBE or buckwheat extracts purified by the same method as that described in the previous section were used for the assay. The assay mixture consisted of 200 μL of 100 mM maleate buffer (pH 6.0), 100 μL of 500 mM sucrose and 100 μL of BBE or buckwheat extracts. The mixture was pre-incubated at 37°C for 5 min and the reaction was initiated by adding 100 μL of the enzyme solution to the reaction mixture. The enzymatic assay was conducted for 60 min at 37°C and terminated by heating at 100°C for 5 min. The glucose generated was detected at 505 nm using the enzymatic test of glucose dehydrogenase (Glucose CHI-test, Wako). Inhibitory activity from a control sample without BBE or buckwheat extract was used to normalize inhibitory activity (%).

Animals. Male Balb/c mice (n = 12–16), 8- to 12-wk-old (Japan SLC, Inc., Shizuoka, Japan) were maintained under specific pathogen-free conditions with a 12-h light : dark cycle at 25°C and 55 ± 10% relative humidity. Mice were given a normal chow diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. All studies were approved by the institutional review board of the animal ethics committee of the Institution of Health Bioscience, University of Tokushima, and were performed in accordance with the ethical guidelines for animal experimentation.

Oral sucrose tolerance test. Balb/c mice were given oral BBE (0.1 or 1 mg), 1 mg buckwheat extract, 10 mg rutin, or vehicle (100 μL 0.1% DMSO/PBS) following overnight starvation (16–18 h). After a 30-min interval, the mice were orally administered 1 g of sucrose per kg of body weight. Blood samples were collected from the tip of the tail vein at 0, 15, 30, 60 and 120 min. Blood glucose levels were measured by the FAD-glucose dehydrogenase method with a GLUCOCARD GT-1820 device (Arklay, Tokyo, Japan). The average of three measurements at each time for each mouse was used for analysis. Theoral sucrose tolerance test was done in a crossover design on two different days with an interval of more than 1 wk between the 2 d.

Analysis of rutin, quercetin and total polyphenol in the BBE. One gram of the buckwheat bran extract was extracted with 50 mL of methanol, and stirred for 1 h. The extracted solution was filtered with a syringe (Minisart RC 15, pore size, 0.45 μm; Sartorius, Hanover, Germany) and 20 μL of the sample was injected

### Table 1. Sucrese inhibitory activity of BBE and buckwheat extracts.

<table>
<thead>
<tr>
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<th>Mean (n = 3)</th>
<th>SD</th>
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<tr>
<td>Buckwheat bran extract</td>
<td>56.95</td>
<td>±1.55</td>
</tr>
<tr>
<td>Buckwheat extract</td>
<td>7.15</td>
<td>±3.40</td>
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The assay mixture consisted of 200 μL of 100 mM maleate buffer (pH 6.0), 100 μL of 500 mM sucrose and 100 μL of BBE or buckwheat extracts. The reaction was initiated by adding 100 μL of the enzyme solution to the reaction mixture. Inhibitory activity from control sample without BBE or buckwheat extract was used to normalize inhibitory activity (%).

**Fig. 1.** BBE decreases sucrose-induced blood glucose. Balb/c mice were pretreated with 1 mg (black square) or 0.1 mg (gray triangle) of BBE or 0.1% DMSO/PBS control (white circle) and orally administered sucrose (1 g/kg of body weight) 30 min later. Blood glucose levels were measured at 0, 15, 30, 60 and 120 min (A) and incremental AUC of each pretreatment (white square: control, gray square: 0.1 mg BBE, black square: 1 mg BBE) was also calculated (B). Values are means ± SE (n = 8). *p < 0.05 (control vs. 0.1 mg, 1 mg BBE), **p < 0.01 (control vs. 1 mg BBE), †p < 0.05 (0.1 mg vs. 1 mg BBE) and N.S.: no significant difference (control vs. 0.1 mg, 1 mg BBE).
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into an HPLC system (LC-10A; Shimadzu Corporation, Kyoto, Japan) equipped with UV detection (350 nm). The separation of the compounds was carried out under isocratic reversed-phase conditions using a Nucleosil 7 C\textsubscript{18} column (4.6 × 150 mm; Cobert Associates, St. Louis, MO) with 2.5% acetic acid, methanol and acetonitrile (50 : 30 : 20) as the mobile phase at 1.0 mL/min. As for total polyphenol contents, they were measured according to the Folin-Denis method (28).

**Statistical analysis.** Data are expressed as means ± standard error of the mean (SE). Data were analyzed by ANOVA (Fig. 1) or the unpaired Student’s t-test (Figs. 2 and 3). When a significant difference was found with ANOVA, post-hoc analyses were performed with the Tukey-Kramer multiple comparison procedure. 

**Results**

Firstly, we examined whether BBE had inhibitory activity against \(\alpha\)-glucosidase in vitro. As shown in Table 1, BBE inhibited approximately 60% of sucrase activity, as well as that of maltase and amylase (data not shown), compared with buckwheat extract, which blocked only about 7% of sucrase activity. Next, we checked the effect of this inhibition in non-diabetic mice. Pretreatment with 1 mg BBE significantly decreased the level of blood glucose from 15 to 60 min (Fig. 1A) and tended to lower the incremental area under the curve (AUC) (Fig. 1B) after sucrose loading compared with that in control mice. Pretreatment with 0.1 mg BBE had a slight effect at 15 min (Fig. 1A) and also tended to lower the incremental AUC (Fig. 1B). Pretreatment with 1 mg of buckwheat extract also significantly lowered the elevation of blood glucose at 15 and 30 min (Fig. 2A) and tended to lower the incremental AUC after sucrose loading compared with that in control mice (Fig. 2B).

According to our analysis described in “Materials and Methods”, rutin is one of the abundant phenolic components of BBE (Table 2) and has well demonstrated inhibitory activity against \(\alpha\)-glucosidase in vitro (25). Therefore, we checked whether rutin itself decreases the level of sucrase-induced blood glucose. Despite the 714-fold greater dose of rutin used than is present compared with its content in BBE, there was no significant difference in blood glucose levels compared with control (Fig. 3). These results strongly suggest that the components of BBE other than rutin have inhibitory activity against sucrase in vivo.

**Discussion**

Previous studies have shown that BBE or buckwheat extracts can lower plasma cholesterol and triglycerides (14–19). D-\textit{chiro}-Inositol, which is a component of concentrated BBE, acts as an insulin-mimetic agent and can decrease blood glucose levels (21, 22). However, it
has not been determined whether BBE has inhibitory activity against \( \alpha \)-glucosidase. Therefore, in the present study, the effect of BBE on \( \alpha \)-glucosidase activity, which included sucrase, maltase and amylase (Table 1, Fig. 1 and data not shown) was examined both in vitro and in vivo. The results showed that treatment with BBE, which had an inhibitory effect on sucrase in vitro (Table 1), decreased the blood glucose level in non-diabetic Balb/c mice following sucrase administration but not after maltose or glucose administration (Fig. 1 and data not shown). In addition, the buckwheat extracts had a weak inhibitory effect on sucrose activity and their inhibitory effect on glucose levels following oral sucrase administration was lower than that of BBE (Figs. 1 and 2). The ability of BBE to promote reductions in blood glucose following a sucrose load compared with the control might make BBE an effective addition to snacks or cake, many of which usually contain sucrose rather than maltose as a sweetener.

Previous studies have shown that concentrated tartary or common BBE includes D-chiro-inositol and decreases blood glucose in diabetic animals (21, 22), believed to be due to its insulinotropic actions on target tissues (23, 24). The final amounts of BBE per mouse weight in our experiments were similar to those in a previous study (22). However, the BBE in that previous study was 20-times more concentrated than our BBE, which was extracted with a different hydrophobic reagent. We speculate that our BBE might have no D-chiro-inositol or much less D-chiro-inositol than in previous studies (21, 22).

The large amount of dietary fiber in carbohydrate-containing foods including buckwheat may trap carbohydrates to delay their adsorption. However, the levels of blood glucose after oral-glucose treatment were not changed by BBE pretreatment compared with those in control mice (data not shown). These results may not reflect the ability of BBE to slow the adsorption of carbohydrates as dietary fiber.

A recent study showed that rutin, a flavonoid, inhibited \( \alpha \)-glucosidase activity (25). Furthermore, quercetin, which is formed by hydrolysis of rutin, inhibited \( \alpha \)-glucosidase activity to an even greater extent than did rutin in an in vitro assay (25). However, their effects were not tested in mice in vivo. We found that our BBE was enriched with rutin and quercetin (Table 2). However, preincubation of 10 mg of rutin had no effect on sucrose-induced glucose elevation (Fig. 3) and the content of rutin or quercetin in 1 mg of BBE was much less than the amount of each of those that has been reported to be effective for \( \alpha \)-glucosidase inhibition (25). We are exploring which phenolic component(s) of BBE (Table 2) (29), other than rutin and quercetin, inhibit sucrase activity in Balb/c mice.

Improvement in postprandial hyperglycemia following treatment with an \( \alpha \)-glucosidase inhibitor in patients with type 2 diabetes has been shown to have beneficial effects for cardiovascular disease (6, 8–10). Moreover, acarbose, an \( \alpha \)-glucosidase inhibitor, delays progression of impaired glucose tolerance to diabetes and cardiovascular disease (7, 30). Results from these studies and ours suggest that foods made from BBE with buckwheat powder could be beneficial in patients with impaired glucose tolerance or a family history of diabetes. A combination of \( \alpha \)-glucosidase inhibitors and these BBE-enriched foods might be more effective in delaying the progression of diabetes in such persons.

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REFERENCES

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