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Effect of Wheat Flour Noodles with *Bombyx mori* Powder on Glycemic Response in Healthy Subjects

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ABSTRACT: Recent trial results suggest that the consumption of a low glycemic index (GI) diet is beneficial in the prevention of high blood glucose levels. Identifying active hypoglycemic substances in ordinary foods could be a significant benefit to the management of blood glucose. It has been hypothesized that noodles with *Bombyx mori* powder are a low GI food. We evaluated GI and changes in postprandial glucose levels following consumption of those noodles and compared them with those following consumption of plain wheat flour noodles (control) and glucose (reference) in healthy subjects. Thirteen males (age: 34.2±4.5 years, body mass index: 23.2±1.1 kg/m^2) consumed 75 g carbohydrate portions of glucose and the 2 kinds of noodle after an overnight fast. Capillary blood was measured at time 0 (fasting), 15, 30, 45, 60, 90, 120, and 180 min from the start of each food intake. The GI values were calculated by taking the ratio of the incremental area under the blood glucose response curve (IAUC) for the noodles and glucose. There was a significant difference in postprandial glucose concentrations at 30 and 45 min between the control noodles and the noodles with *Bombyx mori* powder: the IAUC and GI for the noodles with *Bombyx mori* powder were significantly lower than those for glucose and plain wheat flour noodles. The wheat flour noodles with *Bombyx mori* powder could help prevent an increase in postprandial glucose response and possibly provide an alternative to other carbohydrate staple foods for glycemic management.

Keywords: *Bombyx mori*, noodle, postprandial glucose, glycemic index, glycemic load

INTRODUCTION

Diabetes and its complications have become a major cause of morbidity and mortality worldwide. Similar to other countries, the prevalence of diabetes in South Korea has increased from 1.5% to 9.9% in the past 40 years (1). About 4 million Koreans (12.4%) aged 30 years or older had diabetes in 2011(2).

Diet management is a cornerstone of diabetes management (3). The glycemic index (GI), first introduced by Jenkins et al. (4) is a potential measure of the effect of a diet. The GI is a classification of the blood glucose response to the carbohydrates in foods (4). It is defined as the incremental area under the blood glucose curve (IAUC) of a 50 g carbohydrate portion of a test food expressed as a percentage of the response to 50 g of carbohydrates from a standard food (reference) taken by the same subject. It has long been reported that low GI foods provide several health benefits, such as improving glycemic control and hyperinsulinemia and reducing blood triglycerides and blood pressure levels (5-9). Moreover, consuming low-GI foods is associated with improved carbohydrate metabolism in type 2 diabetes patients (10,11).

A growing interest in dietary therapy has focused the attention of researchers on the functional aspects of foods (12). In the culture of Northeast Asia, traditional foods have been consumed with regional herbs or natural substances, reflecting this population's desire for health and longevity (13).

It is therefore unsurprising that diabetics are trying to regulate their blood glucose with functional foods and food additives. *Bombyx mori* (the silkworm), a sericulture-related material, has long been considered a healthy substance in South Korea (14). Recent studies have found that the silk peptide of *Bombyx mori* powder has hypoglycemic effects (15,16). Researchers have also found that glycine elements in the silk peptide of *Bombyx mori* powder lower cholesterol, reduce the excitometabolic effects of alcohol, and have anti-Alzheimer and antioxidant effects (17). Recent reports have considered sericultural products suitable as functional foods and food additives (18).

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Functional food products manufactured with mulberry leaves (*Folium mori*), such as beverages, snacks, noodles, and ice cream have been developed (19-21). Domestic *Bombyx mori* powder has been favored as a postprandial glucose management agent in South Korea (14,20). However, the powdered form of *Bombyx mori* is unpalatable. Therefore, *Bombyx mori* powder proponents have taken steps to improve its palatability, such as incorporating it into foods (rice cakes, bread, biscuits, noodles, and snack bars). However, few human clinical trials have been conducted to assess the efficacy of these foods.

Therefore we assessed the effect of wheat flour noodles containing *Bombyx mori* powder on postprandial glucose response to determine their GI value and glycemic load (GL).

MATERIALS AND METHODS

Study design and experimental protocol

The protocol used to measure GI was adapted from an internationally recognized GI methodology (22-24). Glucose was taken orally during the first 2 times on separate 4 test sessions. The 2 test foods (plain wheat flour noodles and wheat flour noodles with *Bombyx mori* powder) were taken on 2 subsequent visits over a 4-week period at 7-day intervals. In the morning, subjects visited the research center (Seoul, Korea) after a 12-h overnight fast. For the 3 days before each experiment, subjects were advised to take in more than 150 g of carbohydrates, to avoid alcohol consumption and any unusual amount or intensity of physical activity, and to eat 3 meals a day. The subjects were also asked to consume a similar evening meal before each test and refrain from consuming legumes to avoid a second-meal effect (25).

Study subjects

Subjects were recruited from April 2010 to October 2010 by advertisement at Kyung Hee University and local government community websites of metropolitan areas in Korea. Subjects were excluded if their body mass index (BMI) was less than 18.5 or more than 24.3 kg/m^2. The BMI standard applied in this study followed the fourth Korea National Health and Nutrition Examination Survey (KNHANES Ⅳ) (26) for the average BMI of a normal male aged 30~39 years. Subjects were also excluded if their fasting blood glucose concentration was greater than 110 mg/dL, if they were on a special diet, had a family history of diabetes, were suffering from any illness or food allergies, or were on any medications. A total of 13 subjects were included in the analysis. According to the recommendations of FAO/ WHO, 13 subjects was a sufficient sample size for the GI value estimations (24).

Subjects were given full details of the study protocol and the opportunity to ask questions. Informed consent was obtained from each subject before enrollment in the study. The present study was conducted according to the guidelines in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Institutional Review Board of Kyung Hee University (IRB No.2010-004).

Preparation of the reference and test noodles

A patented solution (Diasol-S Solution, Taejoon Pharm Co., Ltd., Seoul, Korea) (150 mL) containing 75 g of dextrose (glucose monohydrate), which is considered the portion size of one meal, was used as the reference (27).

Two test noodles

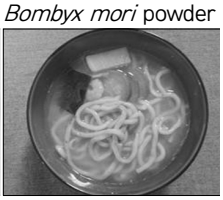
The test foods were wheat flour noodles (control noodle) and wheat flour noodles with *Bombyx mori* powder (functional noodle). The serving size of the test foods contained 75 g of available carbohydrate (defined as total carbohydrate minus dietary fiber), according to the information from the Computer Aided Nutritional Analysis Program (CAN Pro version 3.0, The Korean Nutrition Society, Seoul, Korea) (Table 1).

A licensed Korean chef measured each food ingredient the night before the test and made wheat flour noodles following a standard Korean recipe (28) (Table 2).

Bombyx mori powder

Branded commercial wheat flour (Daehan Flour Mills Corporation, Seoul, Korea) and 0.83 g of powdered *Bombyx mori* (silkworm, Sancheong Sericulture Agricultural Cooperative, Sancheong, Korea) were used in this study. As a highly effective α-glucosidase inhibitor, 1-deoxynojirimycin (DNJ) is known as a bioactive component of *Bombyx mori* powder (14,18). In addition to DNJ, more

Table 1. Nutritional information of the test foods[1)]

	Wheat flour noodles (control)	Wheat flour noodles with 0.83 g of *Bombyx mori* powder
		
Carbohydrate (g)	75.4	75.0
Energy (kcal)	379.2	378.4
Protein (g)	13.1	13.5
Fat (g)	5.2	6.0
Fiber (g)	4.6	4.6
Sodium (mg)	938.8	939.3
Serving weight (g)	158.0	159.0

[1)]Nutrient components of the test foods were analyzed using CAN Pro version 3.0.

Table 2. Ingredients and standard Korean recipe of the two test noodles

Ingredients	Noodles: wheat flour 93 g (add 0.83 g of *Bombyx mori* powder only for *Bombyx mori* noodles), salt 2 g, egg 5 g, and water 35 g Broth: water 1,100 g, radish 13 g, garlic 1 g, leek 1 g, anchovy 1 g, kelp powder 5 g, dried shrimp powder 1 g, soy sauce for soup 1 g, and sesame oil 3 g Garnish: potato 13 g, zucchini 13 g, shrimp 2 g, oyster 2 g, and short-neck clams 2 g
How to cook	1. Boil 1,100 g of water in a pot. Place anchovies, dried shrimp, and 2 pieces of kelp and put it in the boiling water. Once it starts to boil, boil with a lid for another 10 min on high heat then medium heat for 10 min. 2. Clean, dry, and dust the work surface with flour. Unwrap the dough and lightly dust your hands, dough and the rolling pin with flour. Pat down with your hands to make it flat. Roll the rolling pin to make the dough as thin as possible (about 2 mm). Fold the dough into thirds and cut it into 2 mm wide strips. 3. Cook potatoes in boiling broth on high heat for 3 min. Then add noodles, zucchini, seafood (shrimp, oyster, and clams), garlic, and leek. Cook for 15 min. 4. Serve in a noodle bowl. Add clamps and vegetables into the bowl then pour the broth. Sprinkle with sesame oil on top.

than 20 polyhydroxy alkaloids have been identified in the *Bombyx mori* powder (19). It is well known for blood glucose lowering effect and an effective chemical for the treatment of hyperglycemia. Powdered *Bombyx mori* was added when kneading the noodle dough. One gram of *Bombyx mori* powder contains 2.4 kcal of energy, 0.2 g of carbohydrate, 0.5 g of protein, 0.7 g of fat, and 0.1 g of fiber (CAN Pro version 3.0) (Table 1).

Blood measurements

In the first 10 to 15 min of the visit, fasting blood samples were obtained by collecting finger-prick capillary blood samples at −5 min and 0 min. The mean of the 2 values was used as the baseline value. After the fasting blood samples were obtained, subjects were given a fixed portion of a test food, which they consumed with 200 mL of plain water within 15 min. The subjects were then required to remain seated at the research center and refrain from any additional eating or drinking for the next 3 h. During the test sessions, additional finger-prick blood samples were taken at 15, 30, 45, 60, 90, 120, and 180 min after commencement of ingestion. Whole blood glucose concentration was measured using an automatic lancet device (Accu-Check Sensor^{TM}, Roche Diagnostics GmbH, Mannheim, Germany). Capillary blood samples were used for increased sensitivity and to remove the potential for variations in the measured GI due to fluctuations in factors such as ambient temperature (24,29-31).

Calculation of the GI and GL

The IAUC for the test foods and reference were calculated geometrically using the trapezoid rule, ignoring the area beneath the baseline. Therefore, the GI was calculated from the ratio of the "blood glucose IAUC value for the test food containing 75 g of available carbohydrate" to the "corresponding area after an equicarbohydrate portion of the reference food (mean IAUC for the reference glucose solution tested 2 times)" expressed as a percentage (30,32-34).

$$\text{GI value of test food (\%)} = \frac{\text{Blood glucose IAUC value for the test food}}{\text{Corresponding area after an equicarbohydrate portion of the reference food}} \times 100$$

GL values were calculated by multiplying the amount of available carbohydrate in a specified serving size (one serving size: 200 g; carbohydrate: 59.2 g by the Korean nutrition society) by the GI value, and dividing the result by 100 (5,35).

Statistical analysis

Data are shown as mean±standard error (SE). Statistical analyses were performed with SAS (version 9.2; SAS Institute Inc., Cary, NC, USA). The significance of the difference in GI and IAUC values for functional noodles compared to glucose or control noodles was determined using a one-sample *t*-test or student's *t*-test. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to determine whether significant differences existed among the mean blood glucose responses curves for 3 h following the consumption of glucose and the test foods. Statistical significance was set at $P<0.05$.

RESULTS

Postprandial glucose responses

The mean age of the 13 subjects was 34.2±4.5 years. The mean BMI (23.2 kg/m^2), waist circumference (82.2 cm), and blood pressure (123.1/80.0 mmHg) of the subjects were within normal ranges (data not shown). The mean blood glucose and serum insulin response curves and values for glucose and the 2 test foods are shown in Fig. 1. It can be seen that the post-prandial blood glucose responses after the consumption of glucose solution were comparable and higher than the blood glucose responses of functional noodles, whereas the control noodles produced an intermediate response. It should be

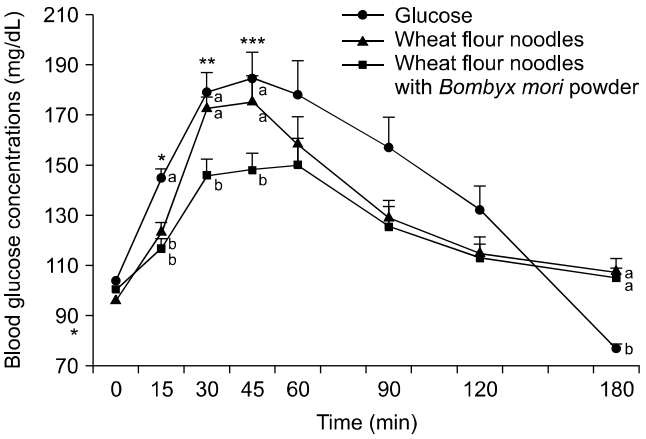


Fig. 1. Mean blood glucose responses curves for 3 h following the consumption of the test food. Values are mean±SE in mg/dL. Significant difference by the one way analysis of variance (ANOVA) test at $^{*}P<0.0001$ for 10 and 180 min, $^{**}P=0.002$ for 30 min, and $^{***}P=0.0284$ for 45 min. Different letters (a,b) are significantly different at $P<0.05$ by Duncan's multiple test.

noted that the peak glucose responses of functional noodles (60 min) occur 15 min later than the peak responses of glucose and control noodles (45 min). The responses of glucose and control noodles are similar, although the response of glucose is higher at the 15 min point ($P<0.0001$) but fall below at 180 min ($P<0.0001$). The differences in glucose levels between the post control vs. post functional noodles were statistically significant at 30 and 45 min ($P=0.0020$ and 0.0284, respectively).

IAUC, GI, and GI classification

Table 3 shows the GI values and GI classification of wheat flour noodles. The corresponding IAUC is presented in Fig. 2. The mean IAUC from the functional noodles was significantly lower than glucose or the control noodles. Likewise, the mean GI of the functional noodles was significantly lower than that of glucose or control noodles. The functional noodles produced a 38.01% decrease in the GI value compared to the control noodles. In addition, the functional noodles were in the "medium GI" range, whereas the control noodles remained in the "high GI" range (Table 3). The control noodles tended to have a higher mean GL value than the functional noodles.

DISCUSSION

The present study suggests that wheat flour noodles with *Bombyx mori* powder significantly lowered postprandial glycemic responses for 30 and 45 min and lowered the IAUC and GI values compared to the control noodles. Similar results were found in a report that indicated a 72% suppression in rising blood glucose levels when healthy subjects were given 830 mg of *Bombyx mori* powder (36).

Table 3. Glycemic index (GI), classification, and glycemic load (GL) of the test foods

Foods	GI	Classification[2)]	GL[3)]
Glucose[1)]	100	High	
Wheat flour noodles (control)	95.2±8.9	High	71.8±6.7
Wheat flour noodles with *Bombyx mori* powder	59.0±4.6***	Medium	44.25±3.5

[1)]75 g of dextrose solution (oral glucose tolerance test).
[2)]Low, ≤55; medium, 56~69; High, ≥70.
[3)]GI×carbohydrate/100.
***Significant difference between glucose and wheat flour noodles with *Bombyx mori* powder by one sample *t*-test at $P<0.0001$.

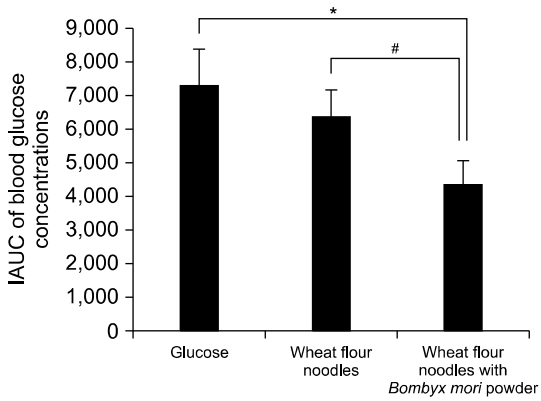


Fig. 2. Incremental area under the curve (IAUC) for 3 h following the ingestion of the test foods. Significant difference between *glucose and control by the one sample *t*-test at $P=0.0315$, and between #control and wheat flour noodles with *Bombyx mori* powder by the Student's *t*-test at $P<0.05$.

DNJ as a bioactive component of *Bombyx mori* is an alkaloid belonging to the polyhydroxylated piperidines and a potent α-glucosidase inhibitor (14,18,37,38). It is abundant in mulberry leaves and roots and has low cytotoxicity (14,18,39-41). Recent reports have considered sericultural products containing DNJ suitable for use as functional foods (18) Moreover, processing *Bombyx mori* powder by freeze drying seems to play a role in preserving its high DNJ content (14).

Our results showed that the peak glucose values of functional noodles are delayed 15 min as compared to control noodles and glucose. Given the effect of DNJ, oral administration of *Bombyx mori* powder is most effective before or just after meals (36). When these data are compared to published results, an experiment in which mice were given methanol extract derived from *Bombyx mori* powder, it was determined that *Bombyx mori* powder inhibited the transient rise of blood glucose levels 30 min after food intake (42). Therefore, adding *Bombyx mori* powder to wheat flour noodles shows the hypoglycemic effect of *Bombyx mori* powder.

The mean IAUC and GI for the functional noodles were

significantly lower than those for the glucose and control noodles. The GI-lowering effect of adding fiber to wheat-based products has been reported (44,45). The GI of atta mix containing Bengal gram, psyllium husk, and debittered fenugreek flour was considerably lower than whole wheat flour roti (45). The addition of whey protein to an oral glucose bolus reduced the IAUC in a dose-dependent manner (46). As recommended by the World Health Organization (WHO) Expert Committee on Diabetes Mellitus, it is important to investigate hypoglycemic agents of plant origin used in traditional medicine (47). These agents are directly or indirectly derived not only from plants, but also from functional foods that can provide therapeutic effects and are currently universally available.

It appears that the blood glucose responses of control noodles are similar to the responses of the glucose solution; however, functional noodles elicited much lower blood glucose responses than the consumption of glucose or control noodles. When *Bombyx mori* extract was injected into mice ingesting a high carbohydrate diet, hyperglycemia, and hyperinsulinemia disappeared (37). Moreover, the 2 h postprandial blood glucose level was slightly greater with the administration of *Bombyx mori* powder than with *Bombyx mori* powder with an oral hypoglycemic or *Bombyx mori* powder combined with insulin injection in type 2 diabetic patients (48). Thus, wheat flour noodles with *Bombyx mori* powder could be effective in Korean diets where energy is provided by carbohydrate-rich foods. Generally, noodles are well known as high GL foods according to the GL classification system (35). In the present study, a similar result was found in the reference noodles compared to the functional noodles. Therefore, adding the functional substance (*Bombyx mori* powder) to noodles could have lowered the GL to control the postprandial glucose response.

Scientific research on *Bombyx mori* as a functional food has been supported by the Sericultural and Apicultural Materials Division of the Rural Development Administration (RDA) in Korea since 1995 (46). The RDA conceived the hypoglycemic effect of mulberry leaves and *Bombyx mori* (36). Therefore, functional foods using freeze-dried *Bombyx mori* powder have been developed for glycemic management (14,36). As a national project, these scientific achievements have contributed to public health and satisfied the diverse needs of consumers.

This study had several strengths. Specifically, it is one of only a few attempts to examine blood glucose response to commonly consumed foods in Korea. In addition, we carried out this clinical study to investigate postprandial glycemic effects according to the protocol of a joint FAO/WHO expert consultant (24). Despite these strengths, our study had some limitations. We provided the wheat flour noodles a la carte without any side dishes. If the wheat flour noodles were served with other foods, the glucose response would change. As this research targeted healthy subjects, future studies are warranted to adapt the results for dietary education or dietary management of glucose intolerant or diabetic patients.

In this study, the hypoglycemic effect or lower GI was observed in subjects ingesting wheat flour noodles with *Bombyx mori* powder. Our results provide preliminary information on both, postprandial glycemic effects and the GI of a food containing a well-known natural functional substance (*Bombyx mori* powder). The results of this study will assist health professionals and consumers, particularly diabetic patients, with meal planning and dietary management.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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