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Effects of Buckwheat on the Insulin Sensitivity, Protein Digestibility and Utility in Diabetic Rats

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Abstract

An experiment was performed to determine if buckwheat intake would improve insulin sensitivity in normal healthy rats and streptozotocin-induced diabetic Sprague-Dawley rats. For four weeks, rats were fed either corn starch as a control diet or buckwheat as an experimental diet. As a result, the insulin sensitivity and plasma glucose levels in normal rats were not significantly affected by buckwheat feeding. The insulin sensitivity was lower in diabetic rats than in normal rats ($p<0.05$). Buckwheat tends to decrease the final plasma glucose level and increase insulin sensitivity in diabetic rats, but there was no significant difference. Another five-week experiment was conducted to determine protein digestibility and protein utility in normal healthy rats and streptozotocin-induced diabetic rats on a control diet or buckwheat diet. The diet composition in this experiment was the same as the preceeding experiment. In the control diet groups, the protein digestibility in diabetic rats was significantly lower than that in normal rats ($p<0.05$). Buckwheat reduced protein digestibility in both normal and diabetic rats ($p<0.05$). Interestingly, in buckwheat diet groups, protein digestibility in diabetic rats was similar to that in normal rats. Protein utility was significantly lower in diabetic rats than in normal rats. This phenomenon was observed as early as the first week of the feeding period. However, protein utility was not significantly altered in both normal and diabetic rats by buckwheat feeding. It follows that decreased protein digestibility and utility in diabetic rats are not further aggravated by buckwheat feeding, suggesting that buckwheat can be a feasible supplemental food for the diabetic therapeutic diet.

Key words: buckwheat, insulin sensitivity, protein digestibility and utility, diabetic rats

INTRODUCTION

The protein metabolism and insulin response are abnormal in diabetes mellitus with increased circulating levels of amino acids and glucose. Insulin exerts its anabolic effects mainly through an inhibition of protein breakdown and regulates the response to nutrient intake in achieving net protein retention (1). Therefore, insulin deficiency in the diabetes seems to produce profound changes in protein catabolism (2) which could be responsible for emaciation and some of the complications in diabetes. It has been reported that the altered protein metabolism in moderately hyperglycemic non-insulin-dependent diabetes subjects was improved with exogenous insulin doses sufficient to restore normoglycemia (3,4). Protein behaves as one of the important insulin secretagogues, which results in enhancement of the glycemic response (5). Recently, it was reported that a degree of lowering blood glucose levels in diabetic rats depends on the type and amount of protein in their diets (6). The diabetogenic effect in the animal could be reduced by a previously fed carbohydrate and protein source in the diet (7). Therefore, a qualified protein supply in the therapeutic diet seems to be crucial to treat diabetes (3,8).

Buckwheat has been commonly consumed as an ingredient in cold-noodle, mook custard, and bakery products in Korea. The buckwheat protein could be considered a good plant protein due to its balanced amino acid composition (9). Nutrient components in domestic buckwheat was analyzed (10-12) and found that it contains various amino acids including nine essential amino acids, and several minerals, especially high amounts of magnesium, selenium, sodium, and potassium (12). Some studies (10,13-16) reported that buckwheat is beneficial for the diabetic because it lowers blood levels of glucose, triglyceride, total cholesterol, and LDL-cholesterol in normal subjects (14), diabetic patients (13), normal rats (15, 16), and streptozotocin-induced diabetic rats (10). On the other hand, Thacker et al. (17) reported that the buckwheat diet increased the body weight, food intakes, and food conversion efficiency in weaning rats. These results may be due to a high lysine content in the buckwheat protein, suggesting that buckwheat may be a valuable supplement to other grain cereals with low concentration of lysine (18). Therefore, buckwheat could be a suitable compensate for the limited lysine content in rice which is the main staple in the Korean diet.

There is a relative paucity of information regarding effects of diets on protein metabolism and insulin sensitivity in the diabetic compared with knowledge in the dietary effect on carbohydrate and lipid metabolism. Therefore, this study has

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been conducted to investigate the effects of buckwheat on insulin sensitivity and protein metabolism in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats were fed the rodent laboratory pellet diet during a 7-day adaptation period under the environment of temperature of 22±2°C and the 12-hour light/dark cycle. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 40mg/kg Bwt, Sigma Chemical Co., St. Louis, USA) in 0.1 M citrate buffer of pH 4.5. After three days had elapsed, the rat were made to fast for 12 hours and blood samples were collected from the tail end, and plasma glucose levels was determined using a glucose analysis kit (Sigma Chemical Co, St. Louis, USA). Animals whose plasma glucose concentrations was greater than 200 mg/dl were considered as the diabetics in this study.

Diet

The composition of diet used in this study is shown in Table 1, and it is the same in both experiments for insulin sensitivity and for protein digestibility and protein utility. The control diet contained 20% casein and 46% corn starch. The experimental diet contained about 10% casein and 50% buckwheat by weight. Domestic buckwheat (Boeun, Chungchungbuk-do, Korea) was used since it was reported that nutrient values of domestically produced buckwheat were greater than those of the imported one (12). Buckwheat grains were peeled and roasted for 20 minutes on a hot plate on medium heat and ground twice with a break roller and smooth roller. All diets contained 60.25% carbohydrate, 19.75% protein, and 20.00% fat by kcal. Using a micro-kjeldhal method (a Kjeltec Auto System, Techator, Sweden), the quantity of crude protein in the roasted buckwheat powder was 18.2% by weight. As for the lipid source, beef tallow and corn oil were added to produce a 1 : 1 ratio of saturated to polyunsaturated fatty acid, since this ratio has been recommended in the human diet (19), and the type of fatty acids may change the post-prandial blood glucose and insulin levels (20).

Table 1. Composition of diet (g/100 g)

Ingredient	Control diet	Buckwheat diet[1)]
Buckwheat	0.00	50.00
Corn starch	46.00	11.87
Sucrose	15.00	15.00
Casein	20.00	9.96
Corn oil	4.98	3.45
Beef tallow	4.02	2.78
Carboxymethyl cellulose	5.00	3.20
Mineral mix (AIN-76)	3.50	2.16
Vitamin mix (AIN-76)	1.00	1.00
DL-methionine	0.30	0.30
Choline bitartrate	0.20	0.20
Total kcal	405	386
kcal/protein amount in diet (g)	20.25	20.25

[1)]Buckwheat contains 18.2% protein, 62.5% total sugar, 4.7% fat, 2.67% ash, and 3.44% dietary fiber.

Insulin sensitivity experiment

STZ-induced diabetic and normal healthy male Sprague-Dawley rats weighing about 400g were used in this experiment. They were fed corn starch as a control diet or buckwheat as an experimental diet over a 4-week period. On the last day of the experiment, rats were made to fast for 6 hours and insulin sensitivity was determined using an *in vivo* insulin suppression test (21). The infusate was freshly prepared and infused into the right internal jugular vein with an administration rate of 8mg/kg/min of glucose (Jung Woi Pharm. Co), 0.08μg/kg/min of epinephrine (Dai Han Pharm. Co.), 1.7μg/kg/min of propranolol hydrochloride (Inderal, imported by Dai Woong Pharm. Co.), and 2.5 mU/kg/min of insulin (Novolin R, Green Cross Co.). Body temperature was kept in the normal range with a heating pad. Blood samples were collected at 10 min intervals from 120 min to 180 min of the infusion period from the tip of the tail into heparinized microtubes.

Blood samples were centrifuged at 4°C, and plasma was separated and frozen at -20°C for the future analysis of glucose concentration. The plasma glucose concentration was determined by the glucose analyse kit. Insulin sensitivity was determined by the steady state plasma glucose levels (SSPG) during the 120~180 min infusion period.

Protein digestibility and utility experiment

Another experiment was carried out to determine protein digestibility and protein utility in the STZ-induced diabetic and normal healthy male Sprague-Dawley rats weighing about 220 g. They were fed the control diet or buckwheat diet as shown in Table 1 over a 5-week period. Body weight and food intakes were measured every week. To determine the true protein digestibility, additional normal rats (n=4, 229±6.5g Bwt) were fed the protein-free diet for 5 weeks. Feces were collected for 5 days during the fourth week period and stored at temperature of -20 °C. The combined fecal collections were dried in an air-circulated dry oven at 60°C for 72 hrs and ground using a laboratory mill. The Kjeldhal nitrogen analysis of the feces was conducted using a semi-auto nitrogen analyzer. Percent true protein digestibility was calculated as follows: % true protein digestibility = N intakes (g) - [N in feces (g) - endogenous N in feces (g)]/N intakes (g). The protein utility was calculated each week using body weight gain and protein intakes.

Statistical analysis

All data were analyzed using a two-way analysis of variance (ANOVA). When a statistical significance was found, the Scheffe F-test was applied to determine which mean values were significantly different.

RESULTS AND DISCUSSION

Plasma glucose and insulin sensitivity

Initial body weights were comparable among all four groups. In normal rats, average initial body weights were 410±22.3 g (n=6) in the control diet group and 399±23.8 g (n=6) in the buckwheat diet group. In diabetic rats, they were 352±10.8 g (n=6) in the control diet group, and 344±8.2 g (n=6) in the buckwheat diet group. After four weeks of feeding, the average final body weights were 438±25.7 g in normal rats on the control diet and 439±17.5 g on the buckwheat diet, and there was no significant difference between these two diet groups. The average final body weights were significantly lower in the diabetic rats than in the normal rats. In the diabetic groups, the average final body weights were 321±7.0 g under the control diet and 293±11.2 g under the buckwheat diet, but there was no significant difference between these two diet groups.

Table 2 shows the initial and final plasma glucose levels in normal and diabetic rats on the control or buckwheat diet. In normal rats, the initial and final plasma glucose levels in both the control diet group and buckwheat diet group were similar. In diabetic rats, the initial plasma glucose levels were 307±30.4 mg/dl in the control diet group and 322±30.0 mg/dl in the buckwheat diet group. The final plasma glucose level in diabetic rats on the buckwheat diet was not significantly different from the control diet, although the buckwheat diet tends to have a lower final plasma glucose level (433±67.4 mg/dl vs 463±26.1 mg/dl). Fig. 1 shows the SSPG in normal and diabetic rats on the control or buckwheat diet. In normal rats, there was no significant effect of the buckwheat diet on the SSPG. The SSPG was significantly greater in diabetic rats than in normal rats, indicating decreased insulin sensitivity. In diabetic rats, the buckwheat diet lowered the SSPG levels 11% compared to the control diet, but there exists no statistically significant difference. The data implicate that buckwheat may not enhance insulin sensitivity in diabetic rats.

Table 2. Initial and final plasma glucose levels in normal and diabetic rats on the control or buckwheat diet in the insulin sensitivity experiment

Plasma glucose (mg/dl)	Normal		Diabetic	
	Control diet	Buckwheat diet	Control diet	Buckwheat diet
Initial	119±7[a]	120± 7[a]	307±30[b]	322±30[b]
Final	115±4[a]	118±10[a]	463±26[b]	433±67[b]

Values are mean±SE (n=6 in all groups)
Values with the different superscripts within a row are significantly different ($p<0.05$)

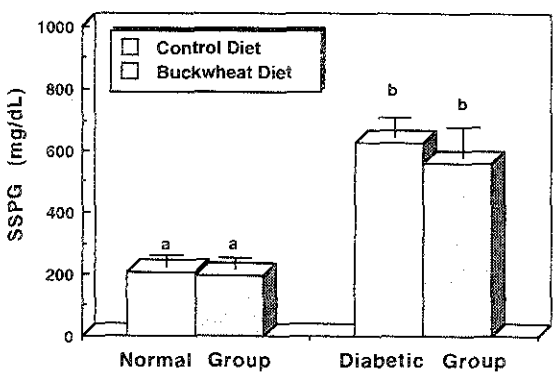

Fig. 1. Steady state plasma glucose level (SSPG, mg/dl) in normal healthy and diabetic rats on the control or buckwheat diet for a 4-week period. Values are mean±SE (n=6 in all groups). Values with the different superscripts are significantly different ($p<0.05$).

In our previous report (22), it was suggested that buckwheat diet alone may not significantly decrease the plasma glucose level during a 4-week of feeding period in sedentary diabetic rats. And these results are in good agreement with the results in our present study. On the contrary, Choe et al. (23) reported that glucose levels at 30 min, 60 min, and 90 min in oral glucose tolerance curve tested in normal rats on 50% raw-buckwheat were significantly lower than those on the control diet which contained 50% sucrose. According to Lee et al. (10), the fasting plasma glucose level in diabetic rats on the buckwheat diet for 2 weeks decreased by 18~37% compared with the control diet fed group. In their study, the control diet contained 50% sucrose, thereby the diet composition and duration of feeding time are much different from those of ours. Thus a possible glucose-lowering effect of buckwheat, as suggested by some investigators, is still in contradiction, and this effect may be dependent on the type of diet, feeding period, and other factors related to dietary behaviors. A further rigorous investigation which will include these factors is needed.

Protein digestibility and utility

The body weights during a 5 week-period are presented in Table 3. The results show that the body weights beginning the second week were significantly lower in diabetic rats than in normal rats. However, there was no effect of the buckwheat diet on the body weight in both normal and diabetic rats, and this phenomenon is in good accordance with our previous study (22). A food intake pattern during a 5-

Table 3. Body weights in normal and diabetic rats on the control or buckwheat diet during a 5-week feeding period in the protein digestion and utility experiment

Body Weight (g)	Normal		Diabetic	
	Control diet	Buckwheat diet	Control diet	Buckwheat diet
Initial	217± 4[a]	217± 5[a]	225± 8[a]	221±5[a]
1st week	247± 5[a]	248± 7[a]	250± 9[a]	245±7[a]
2nd week	282± 6[a]	282± 9[a]	243±11[b]	242±8[b]
3rd week	300± 7[a]	298±12[a]	241±10[b]	242±9[b]
4th week	323± 9[a]	322±13[a]	243±11[b]	235±8[b]
5th week	342±10[a]	334±16[a]	227±13[b]	223±7[b]

Values are mean±SE (n=6 in all groups)
Values with the different superscripts within a row are significantly different ($p<0.05$)

Table 4. Food intakes in normal and diabetic rats on the control or buckwheat diet during a 5-week feeding period in protein digestion and utility experiment

Food Intakes (g/week)	Normal		Diabetic	
	Control diet	Buckwheat diet	Control diet	Buckwheat diet
1st week	112±6[a]	125±3[a]	118±6[a]	106± 8[a]
2nd week	122±4[a]	126±4[a]	128±4[a]	123± 5[a]
3rd week	143±6[ab]	138±9[a]	187±9[b]	182±16[ab]
4th week	136±7[a]	136±5[a]	184±7[b]	195± 6[b]
5th week	146±5[a]	143±5[a]	193±4[b]	205± 2[b]

Values are mean±SE (n=6 in all groups)
Values with the different superscripts within a row are significantly different (p<0.05)

week period was shown in Table 4. It is shown that diabetic rats consumed more diet than the normal rats on the third week. Although a slight increase in food intake on the fourth and fifth week was also observed, there was no significant effect of the buckwheat diet on food intake in diabetic rats. It has been reported that there was no difference in food intake in diabetic rats between the sucrose diet and the buckwheat diet (10), and their results agree with the results from the present study.

Protein digestibility in normal and diabetic rats either on the control diet or on the buckwheat diet is presented in Fig. 2. In normal rats, the amount of daily protein intake and excretion in the feces was 3.99±0.43 g and 0.18±0.03 g in the control diet group, and 4.11±0.33 g and 0.39±0.05 g in the buckwheat diet group. In diabetic rats, the amount of daily protein intake and excretion in the feces was 5.41±0.39 g and 0.31±0.05 g in the control diet group, and 5.80±0.29 g and 0.57±0.06 g in the buckwheat diet group. In the protein free diet group, the amount of daily protein excretion in the feces was 0.11±0.01 g. As shown in Fig. 2, protein digestibility in normal rats was significantly lower on the buckwheat diet than on control diet. Protein digestibility in diabetic rats was also significantly lower on the buckwheat diet than on control diet. These data indicate that there is a diet effect

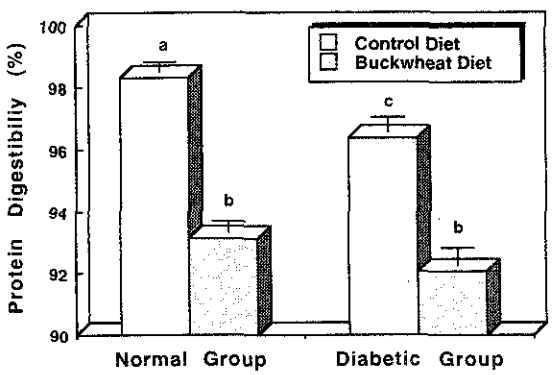

Fig. 2. Protein digestibility in normal healthy and diabetic rats on the control or buckwheat diet for a 5-week period. Values are mean ±SE (n=6 in all groups). Values with the different superscripts are significantly different (p<0.05).

on protein digestibility (p=0.0022, using two-way ANOVA). In rats on the control diet, diabetic rats had significantly lower protein digestibility than normal rats. This suggests that diabetic status itself also affects protein digestibility (p=0.0001, using two-way ANOVA). However, rats on the buckwheat diet, whether they are in diabetic or normal healthy states, had similar protein digestibility rates. And there was no interaction between diet and diabetes (p=0.3247, using two-way ANOVA).

In the study (24) with normal rats, apparent protein digestibilities of wheat flour noodle and buckwheat flour noodle were 94.1% and 93.9%, respectively. Their data are in good agreement with our results which produced 93.1% protein digestibility of the buckwheat diet in normal rats (Fig. 2). In a 2-week feeding study (25) with diabetic rats, the buckwheat diet increased to twice the fecal protein dry weight compared with the sucrose diet. This phenomenon may be attributed to low protein digestibility caused by the buckwheat diet. Even *in vitro* study reported that digestibility of buckwheat protein with pepsin and pancreatin was significantly lower than that of casein (26). Factors that inhibit protein digestion appear to be a protein trypsin inhibitor and tannin, which are highly contained in buckwheat (26-28). About 10% of polysaccharides in buckwheat is indigestible polysaccharides including crude hemicellulose, and these indigestible polysaccharides contain about 40% of protein (11). Ikeda et al. (27) reported that albumin and prolamin in buckwheat are not easily digested *in vitro* by pepsin and trypsin in comparison with gliadin in wheat or acid-precipitated protein in soybean. According to Son et al. (29), casein digestibility by trpsin and chymotrypsin *in vitro* was reduced to about 80~89% and 69~99% by low molecular weight insoluble- and high molecular weight soluble- polysaccharides isolated from buckwheat, respectively.

Protein utility during a five-week period are presented in Table 5. In general, protein utility was significantly lower in diabetic rats than in normal rats. Protein utility in diabetic rats was similar between the control diet group and the buckwheat diet group, suggesting that there is no deleterious

Table 5. Protein utility in normal and diabetic rats on the control or buckwheat diet during a 5-week feeding period in the protein digestion and utility experiment

Protein utility[1)]	Normal		Diabetic	
	Control diet	Buckwheat diet	Control diet	Buckwheat diet
1st week	1.33±0.30[a]	1.24±0.10[a]	1.01±0.27[b]	1.13±0.16[b]
2nd week	1.44±0.10[a]	1.34±0.07[a]	-0.28±0.13[b]	-0.09±0.30[b]
3rd week	0.60±0.13[a]	0.57±0.10[a]	-0.07±0.08[b]	-0.10±0.14[b]
4th week	0.83±0.16[a]	0.87±0.06[a]	-0.08±0.11[b]	-0.15±0.06[b]
5th week	0.63±0.05[a]	0.39±0.11[a]	-0.42±0.13[b]	-0.29±0.07[b]

[1)]Protein utility = body weight gain (g)/protein intakes (g)
Values are mean±SE (n=6 in all groups).
Values with the different superscripts within a row are significantly different (p<0.05)

Table 6. Organ weights in normal and diabetic rats on the control or buckwheat diet in the protein digestion and utility experiment

Variables	Normal		Diabetic	
	Control diet	Buckwheat diet	Control diet	Buckwheat diet
Liver (g)	11.35±0.64[a]	10.73±0.61[ab]	9.73±0.52[ab]	8.88±0.46[bc]
Stomach (g)	1.49±0.02[a]	1.32±0.12[a]	1.46±0.12[a]	1.50±0.06[a]
Pancreas (g)	1.23±0.06[a]	1.12±0.14[ab]	0.84±0.08[b]	0.86±0.02[b]
Spleen (g)	0.78±0.07[a]	0.68±0.04[a]	0.66±0.18[a]	0.44±0.04[a]
Kidney (g)	2.41±0.09[a]	2.33±0.14[a]	2.53±0.10[a]	2.56±0.09[a]
Liver (%/Bwt)	3.31±0.10[ab]	3.22±0.11[bc]	4.32±0.23[d]	3.97±0.14[ad]
Stomach (%/Bwt)	0.44±0.02[a]	0.40±0.03[a]	0.64±0.03[b]	0.68±0.03[b]
Pancreas (%/Bwt)	0.36±0.02[a]	0.33±0.03[a]	0.37±0.02[a]	0.39±0.02[a]
Spleen (%/Bwt)	0.23±0.02[a]	0.20±0.01[a]	0.29±0.07[a]	0.19±0.01[a]
Kidney (%/Bwt)	0.70±0.01[a]	0.70±0.01[a]	1.12±0.04[b]	1.15±0.05[b]

Values are mean±SE (n=6 in all groups)
Values with the different superscripts within a row are significantly different ($p<0.05$)

effect of buckwheat in diabetic rats.

Table 6 shows the organ weights of the liver, stomach, pancreas, spleen, and kidney. There was no significant effect of buckwheat feeding on the weights of these organs. The present results agree with the study by Lee et al. (10) in that the weights of the liver, pancreas, spleen, and kidney did not change by buckwheat feeding in diabetic rats. However, they reported that the pancreatic weight (g/100 g body weight) in diabetic rats on raw or roast buckwheat diet were significantly greater than those in diabetic rats on the sucrose diet (10,25). The present results show that the percent pancreatic weight per body weight of diabetic rats on the buckwheat diet (0.39±0.02%) tend to be greater than those of diabetic rats on the control diet (0.37±0.02%), but there was no significant difference. It has been reported (30) that trypsin inhibitor is correlated with pancreatic enlargement and the pancreatic weight of rats on raw buckwheat becomes heavy. The liver weight (8.88±0.46 g) in diabetic rats on the buckwheat diet was the lowest among the four groups, and significantly lower than that in normal rats on the control diet. However, when liver weight was expressed in terms of liver weight per body weight, there was no significant difference between those two groups. Kayashita et al. (15) reported that liver weight (g/100 g body weight) in normal rats fed buckwheat protein extract was significantly lower than that in rats fed casein or soybean protein. In the present study, stomach and kidney weights which were expressed by percent per body weight were significantly heavier in all diabetic rats than in all normal rats. A study by Lee et al. (10) shows that kidney weight (g/100 g of body weight) in diabetic rats is significantly greater than that in normal rats, which agrees well with our results.

In conclusion, the plasma glucose level and insulin sensitivity may not be significantly affected by buckwheat feeding in both normal and diabetic rats. Protein utility decreased in diabetic rats ($p<0.05$), but was not affected by the buckwheat feeding. Protein digestibility in diabetic rats on the control diet was significantly lower than that in normal rats on the same diet, indicating a decrease in protein digestibility in diabetic rats. The results suggest that buckwheat reduces protein digestibility in both normal and diabetic rats. However, in buckwheat diet fed groups, protein digestibility in diabetic rats was similar to that in normal rats, indicating that buckwheat may not deleteriously affect protein digestibility and utility in the diabetic. Therefore, roasted buckwheat could be recommended as a feasible supplemental food for the diabetic therapeutic diet.

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