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Effects of Exercise on Plasma Glucose and Cholesterol Level in Streptozotocin-induced Diabetic Rats Fed Buckwheat Diet

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Abstract

The ability of exercise and buckwheat diet to modify plasma glucose and cholesterol levels in streptozotocin-induced diabetic rats has been studied. Diabetic rats were fed corn starch as a control diet or buckwheat as an experimental diet for 4 weeks. One group of rats were exercise-trained to swim for 60min/day, 6 days a week. Plasma glucose levels of sedentary rats both on the control diet and on the buckwheat diet were significantly increased from 367.0±33.6mg/dl to 545.0±38.7mg/dl and from 380.3±18.9mg/dl to 540.5±36.6mg/dl, respectively. However, this large increase of plasma glucose was not seen in exercised rats on the buckwheat diet(from 345.0±25.6mg/dl to 391.4±34.7mg/dl). The total plasma cholesterol level was not affected by either diet or exercise. The HDL-cholesterol level tends to increase due to the buckwheat diet or an exercise, but not with a significant increase. Our results suggest that the buckwheat diet is beneficial in lowering the plasma glucose level only when diabetic rats are exercised.

Key words: buckwheat diet, exercise, diabetic rats, plasma glucose

INTRODUCTION

Several studies have reported that an addition of buckwheat to usual diet causes to reduce the blood glucose and lipid in healthy animals(1-3). Lee et al.(4) have also reported that in diabetic rats, buckwheat lowered blood glucose, triglyceride, and total cholesterol levels. In addition, they observed that the buckwheat decreased total glycosylated hemoglobin, glycosylated protein, total cholesterol, and LDL-cholesterol levels in the noninsulin-dependent diabetes mellitus(NIDDM) patients, but it did not contribute to increase HDL-cholesterol as expected(5). A few researchers demonstrated that the buckwheat plays a significant role in controlling the glucose and lipid metabolism in the diabetics and recommended it as a therapeutic diet(4,5). Moreover, the buckwheat has long been recognized as a nutritious dietary source containing various amino acids(6). Besides, considering a traditional Korean dietary life style, the buckwheat can be an useful dietary substitute for the main carbohydrate source.

Exercise is one of the important therapies which should be accompanied with a diet therapy to effectively manage the diabetes. When NIDDM patients were treated with a diet therapy in parallel with an exercise program, the blood glucose level was more decreased than that with a diet therapy only(7). In order to improve the glucose intolerance, Schneider et al.(8) recommended 30min exercise a day, three days a week, with a 60% intensity of the maximum oxygen uptake. But, Rogers et al.(9) and Holloszy et al.(10) suggested the more intensive program that involves 50~60min exercise a day, 5 days a week, with 68% and 70~85% intensity of the maximum oxygen uptake, respectively. The same duration, but daily exercise with 68% intensity of the maximum oxygen uptake, was also suggested (10). Therefore, duration, frequency, and intensity of the training program should depend upon the physical condition of individuals. A study by Dall'aglio et al. (11) demonstrated that the blood glucose level in diabetic rats was reduced to 50% of the initial level when exercised on the voluntary running wheel. And levels of the blood triglyceride and free fatty acids were also decreased. Besides, according to Virtug et al.(12), the other benefit of the exercise is to delay a development of atherosclerosis in the insulin-dependent diabetes mellitus(IDDM) patients. The purpose of the present study is to evaluate the effects of exercise on plasma glucose

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and cholesterol levels in diabetic rats fed buckwheat diet.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats of about 300g body weight were used in the present study. They were fed rodent laboratory pellet diet during a 7 day period of an adaptation to temperature of 22±2°C and to a 12 hour light-dark cycle. It took 7 days to train rats to swim in a water tank for 60min a day. Water temperature was maintained at 28±2°C. Diabetes was induced by intraperitoneal injection of streptozotocin(STZ, 40mg/kg Bwt, Sigma Chemical Co., St. Louis, U.S.A.) in 0.1M citrate buffer of pH 4.5. After three days elapsed, blood(12 hours after food withdrawal) was sampled from the tail end and plasma glucose level was determined using a glucose analysis kit(Sigma Chemical Co., St. Louis, U.S.A.). Animals whose plasma glucose concentration was greater than 200mg/dl were used in this study. STZ of 18mg/kg was reinjected intraperitoneally to the rats that did not become diabetic by initial treatment.

Diets

The composition of diets is shown in Table 1. The control diet contained corn starch and the experimental diet contained 50% buckwheat by weight. Domestically produced buckwheat from Boeun(Chungbuk, Korea) was used since its nutritious values are greater than imported buckwheat(6). Peeled buckwheat grains were roasted for 20min on a hot plate and twice milled. Diets contained 60.25% of carbohydrate, 19.75% of protein, and 20% of fat by kcal. Although the casein amount was different between the control diet group and buckwheat diet group, all diets were isonitrogenous of 20.25 kcal/g protein. Using a micro-kjeldhal method(Kjeltec Auto System, Techator, Sweden), the quantity of crude protein in the roasted buckwheat was measured to be 18.2% by weight. As for the lipid source, beef tallow and corn oil were added to produce ratio of saturated to polyunsaturated fatty acid to become 1.0. Most recently, it has been reported that the fatty acids type in the diet can change the postprandial blood glucose and insulin levels(13). It has been known that an absolute prohibition of sugar intake is not necessary in the diabetic nutrition therapy(14-16). Therefore, the same amount of sugar(15% by weight) was added to all diets in order to reduce the strong taste of buckwheat.

Table 1. Composition of control and buckwheat diet (g/100g)

Ingredient	Control diet	Buckwheat diet[1)]
Buckwheat	0	50
Casein	20.00	9.96
DL-Methionine	0.3	0.3
Corn oil	4.98	3.45
Beef tallow	4.02	2.78
Corn starch	46	11.87
Sucrose	15	15
Mineral mix(AIN-76)	3.5	2.16
Vitamin mix(AIN-76)	1.0	1.0
Choline bitartrate	0.2	0.2
Carboxymethy cellulose	5.0	3.2

[1)]Buckwheat: Protein 18.2%, total sugar 62.5%, fat 4.7%, ash 2.67%, and dietary fiber 3.44%

Study design

Rats were divided into three experimental groups: sedentary rats on the control corn starch diet, sedentary rats on the buckwheat diet, and exercise-trained rats on the buckwheat diet. Rats in the exercise-trained group were trained to swim 15min on the first day, and a duration of exercise was gradually increased by 10 to 15min each day until they were able to swim 60min a day. They swimmed 6 days a week and this has been accomplished after the 4 weeks of training.

Body weight and food intake were measured every other day at the same hour. After a 4 week period, rats were fasted for 12 hours and anesthesized with ether, and blood was collected from the abdominal aorta. Samples were centrifuged at 4°C, and the plasma was separated and frozen at -70°C for the glucose and cholesterol analyses in the future. Plasma glucose, total cholesterol, and HDL-cholesterol levels were measured using enzymatic colorimetric kits(Wako Co., Japan). Organs including the stomach, liver, lung, kidney, spleen, epididymis, testis, and heart were removed and weighed after the moisture was blotted.

Data were analyzed by using one-way analysis of variance. When statistical significance was found, Scheffe F-test was applied to determine which mean values were significantly different.

RESULTS AND DISCUSSION

Body weights and food intake

Table 2 shows body weights of the sedentary and exercise-trained diabetic rats on control diet or buckwheat diet during the 4 week period. Initial body weights were roughly the same in all groups. Change in the body weight during that period was -5.7±9.4g in the sedentary rats on control diet, -26.3±14.2g in the sedentary rats on buckwheat diet, and -48.4±11.2g in the exercise-trained rats on buckwheat diet. Therefore, body weights in all the groups tend to decrease with time. The body weight loss in the exercised-trained rats on buckwheat diet seemed to be greater, but there was no significant difference when compared with other groups($p=0.0558$). In a study with healthy rats(17), the body weight was decreased by way of a daily treadmill exercise equivalent to running 1/4miles. It was suggested that the body weight reduction in exercise-trained rats may be related to an increase in cathecholamine levels(18), and an energy deficit induced by exercise(19). In the sedentary rats, there was no significant difference in the body weight between on control diet and on buckwheat diet. These results are in agreement with other studies that used diabetic rats on buckwheat(4,5).

Table 3 shows the food intake and food efficiency ratios in the sedentary and exercise-trained diabetic rats on control or buckwheat diet during a 4 week period. On the second week of the experiment, the food intake in the exercise-trained rats on buckwheat diet was significantly greater than in the other two groups. Three weeks after the experiment, a similar feeding behavior was observed although there was no statistical difference between groups. However, on the fourth week, the food intake in the exercise-trained rats on buckwheat diet was significantly less than in the sedentary rats on buckwheat. Ahrens et al.(17) reported in a study with healthy rats that the food intake was reduced by doing a 1/4mile daily running exercise on

Table 2. Body weight in sedentary and exercise-trained diabetic rats on control diet or buckwheat diet during a 4 week period

Body weight (g)	Sedentary control diet (n=6)	Sedentary buckwheat (n=6)	Exercised buckwheat (n=9)
Initial	311.5±6.0	307.3±2.8	308.1± 3.8
The first week	328.2±8.0	329.0±3.7	322.2± 5.8
The second week	309.5±7.8	300.8±5.7	296.6± 5.0
The third week	306.0±7.9	292.3±5.7	292.6± 8.1
The fourth week	306.2±9.1	274.0±9.3	270.4±10.9

Values are means±SE
Means in the same line do not differ significantly($p<0.05$)

Table 3. Food intake and food efficiency ratio(FER) in sedentary and exercise-trained diabetic rats on control diet or buckwheat diet during a 4 week period

Food intake (g/week)	Sedentary control diet (n=6)	Sedentary buckwheat (n=6)	Exercised buckwheat (n=9)
The first week	110.2±4.0	117.5±4.1	112.9±3.4
The second week	128.5±1.1[a]	131.0±9.5[a]	170.1±2.9[b]
The third week	175.0±0.7	172.3±1.5	199.0±0.9
The fourth week	210.0±3.4[a]	244.7±4.5[b]	200.0±4.7[a,c]
Total food intake	623.7±6.3[a]	666.0±8.3[b]	682.0±9.4[b]
FER	-0.01±0.02	-0.04±0.02	-0.07±0.02

Values are means±SE
FER: Total weight gain divided by total food intake during the experimental period
Means in the same line with different superscript differ significantly($p<0.05$)

the treadmill. It was indicated by Katch et al.(20) that the food consumption may be affected by the intensity of exercise in healthy rats. It has also been reported that the food intake in healthy rats on a 9 week-swimming program was decreased(18). This decrease is probably related to an increase in urinary excretion of epinephrine and norepinephrine.

Lee et al.(4) reported that there was no difference in the food intake in diabetic rats between on sucrose-corn starch diet and on buckwheat diet. It should be noted, however, that the feeding duration in their study was only 2 weeks. In the mean time, the food intake in sedentary diabetic rats in our study remains unchanged until the second week as Lee et al.(4) demonstrated. But, on the fourth week, the food intake in sedentary rats on buckwheat diet is 16% greater than in sedentary rats on control diet(see Table 3). The total food intake for 4 weeks in rats on buckwheat diet was significantly greater than in rats on control diet. Choi et al.(2) has shown in their 4 week-period feeding experiment that the food intake in healthy rats on buckwheat diet was also greater than on sucrose-corn starch diet.

Plasma glucose concentrations

Fig. 1 shows the initial and final plasma glucose levels in the sedentary and exercise-trained rats on control or buckwheat diet during a 4 week period. In the sedentary rats on control diet, the plasma glucose level was increased significantly from 367.0±33.6mg/dl to 545.0±36.6mg/dl(using Student's paired t-test).

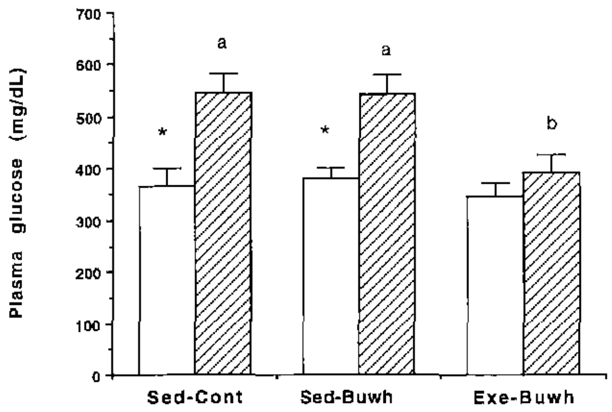

Fig. 1. Initial and final plasma glucose in sedentary(Sed) and exercise-trained(Exe) diabetic rats on control (Cont) or buckwheat(Buwh) diet for a 4 week period.

□ : Initial plasma glucose

▨ : Final plasma glucose

Means in the dashed bars not sharing a common letter differ significantly($p<0.05$).

*Means a significant difference between two groups ($p<0.05$).

In the sedentary rats on buckwheat diet, the plasma glucose level was also increased significantly from 380.3 ±18.9mg/dl to 540.5±38.7mg/dl. However, this significant increase in plasma glucose levels was not seen in the exercise-trained rats on buckwheat since the initial and final levels were 345.0±25.6mg/dl and 391.4 ±34.7mg/dl, respectively. The final plasma glucose level in this group was nearly 40% lower than that in the other two sedentary groups(ANOVA, $p<0.05$). It has been known that a dietary therapy plays an important role in controlling diabetes(21-25). Among various dietary therapies, the buckwheat diet has recently received an attention mainly due to its effectiveness in lowering the plasma glucose level as previously mentioned(1,4). However, the present study clearly demonstrated that the buckwheat diet alone can not effectively reduce the plasma glucose level in the sedentary diabetic rats, and these results are in good accordance with our other experiment that investigated the insulin sensitivity in diabetic rats on buckwheat diet(manuscript in preparation). These findings suggest that an exercise therapy should be paralleled with an appropriate dietary therapy to effectively reduce the plasma glucose level in the diabetics.

Plasma total- and HDL-cholesterol levels

Table 4 shows levels of total plasma cholesterol, HDL-cholesterol, and ratios of HDL to total cholesterol. There was no significant difference in the total plasma cholesterol level among three groups. The plasma HDL-cholesterol level in the sedentary rats on buckwheat diet is 20% greater than that in the sedentary rats on control diet, but it is not statistically significant due to a large standard error. In the exercise-trained rats on buckwheat diet, the HDL-cholesterol level tends to increase as opposed to the sedentary rats. However, this increase was not significant, either($p=0.0893$). Wood et al.(26) reported in a study with healthy human subjects that an 8 mile-running per week increased the HDL-cholesterol level and its increase was associated with the running distance.

Choi et al.(2) reported that a diet of 50% buckwheat noodle consisting of 30% buckwheat and 70% wheat flour did not alter the serum and liver cholesterol levels in the healthy rats. Lee et al.(4) also showed in a study with diabetic rats that the plasma total- and HDL-cholesterol levels in the 26% sucrose-50% buckwheat diet fed rats were not significantly different from those in the 50% sucrose-15% corn starch diet fed rats, but suggested that the buckwheat diet may have a tendency to reduce the total cholesterol level. On the other hand, He et al.(27) has demonstrated that an intake of high buckwheat diet may prevent a development of the hypercholesterolemia in that a daily 100g buckwheat consumption during a life long period caused a decrease in total- and LDL-cholesterol levels and an increase in the ratio of HDL to total cholesterol in one tribe of the Chinese people. In contrast, our results did not show a significant increase in the HDL-cholesterol level with buckwheat feeding. This may be attributed to not having an enough experimental time to reveal a notable response.

Table 4. Plasma total-cholesterol, HDL-cholesterol, and ratio of HDL-/total-cholesterol in sedentary and exercised diabetic rats on control diet or buckwheat diet for 4 weeks

	Sedentary control diet (n=6)	Sedentary buckwheat (n=6)	Exercised buckwheat (n=9)
Total-cholesterol (mg/dl)	106.65±11.39	96 47±9.34	101.41±5.50
HDL-cholesterol (mg/dl)	33.56± 3.92	40.42±4.19	54.17±8.03
HDL-/Total cholesterol(%)	31.76± 4.12	44.64±6.68	53.11±8.49

Values are means±SE

Means in the same line does not differ significantly($p<0.05$)

Organs weights of the animals

Table 5 shows weights of organ such as stomach, liver, lung, kidney, spleen, epididymis, testis, and heart. In the sedentary rats, the buckwheat diet feeding significantly increased weights of the stomach and testis as opposed to the control diet feeding. However, there was no significant change in weights of the liver, lung, kidney, spleen, epididymis, and heart due to the buckwheat diet feeding. Gallaher and Schaubert(28) reported that renal hypertrophy was seen in diabetic rats and was not diminished by feeding diet containing dietary fibers which are known to reduce the blood glucose level. It has been reported by Lee et al.(4) that weights of liver, kidney, and spleen were not changed by the buckwheat feeding in diabetic rats. In the present study, an exercise-training significantly decreased weights of stomach, spleen, epididymis, and testis in rats on buckwheat diet. The decrease in weights of these organs may be attributed to metabolic changes caused by an exercise. Since a prolonged moderate exercise-training could change a nerve system activity and hormone secretion(29), changes in the body mass and composition may occur(30) as with the weight change in organs.

In conclusion, the total plasma cholesterol level was affected by neither a buckwheat diet feeding nor an exercise-training during a 4 week period. The HDL-cholesterol level tends to increase with the buckwheat diet or the exercise-training, although they not show statistically significant difference. However, the plasma glucose level was significantly decreased by exercise-training along with the buckwheat diet. Our results suggest that the buckwheat diet may be beneficial in lowering the plasma glucose level only when diabetic rats are exercised.

Table 5. Weight of liver, lung, kidney, spleen, epididymis, testis, and heart in sedentary and exercised diabetic rats fed control diet or buckwheat diet for 4 weeks

Organs (g)	Sedentary control diet (n=6)	Sedentary buckwheat (n=6)	Exercised buckwheat (n=9)
Stomach	1.62±0.12[a]	1.91±0.03[b]	1.65±0.05[a,c]
Liver	11 48±0.79	9.65±0.50	11.49±0.79
Lung	1.35±0.08	1.19±0.04	1.22±0.05
Kidney	2.81±0.13	2.74±0.09	2.72±0.08
Spleen	0.57±0.04[a]	0.52±0.03[a]	0.45±0.04[b]
Epididymis	1.36±0.21[a]	0.94±0.07[a]	0.90±0.14[b]
Testis	2.68±0.21[a]	3.24±0.12[b]	3.13±0.11[a c]
Heart	0.93±0.03	0.90±0.02	0.96±0.03

Values are means±SE
Means in the same line does not sharing a common letter differ significantly($p<0.05$)

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