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Dietary phytic acid lowers the blood glucose level in diabetic KK mice

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Abstract

Phytic acid, myo-inositol hexaphosphate, is a plant component existing in most grains and legumes. Although much attention has been focused on the biologic actions of phytic acid in human beings and animals, its effect on the blood glucose level in diabetic models has not been evaluated. This study was conducted to examine the supplementary effect of phytate on the blood glucose level in a diabetic rodent model. Thirty male diabetic KK mice were fed with purified diets supplemented with 0% (P0), 0.5% (P5), or 1.0% (P10) sodium phytate for 8 weeks. Diet intake, body and organ weights, and levels of fasting and random blood glucose, hemoglobin A_{1c}, as well as insulin were measured. A glucose tolerance test was conducted. There was no significant difference in diet intake, body weight, and organ weight among the experimental groups. The concentrations of fasting and random blood glucose were lower in the groups fed with the phytate diets, and the significant ($P < .05$) difference from P0 was found only in the P10 group. Hemoglobin A_{1c} levels were significantly ($P < .05$) lower in the P5 and P10 groups as compared with those in the P0 group. There was no significant difference in insulin levels among the experimental groups. The blood glucose levels after 30 minutes of glucose injection were significantly lower in the P5 and P10 groups than in the P0 group. These results suggest that phytate reduced the blood glucose levels of diabetic mice. Effective blood glucose control by phytate may be an alternative for the management of diabetes and disorders of carbohydrate metabolism.
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Keywords: Phytic acid; Diabetic mice; Blood glucose; HbA_{1c}; Insulin

1. Introduction

Diabetes mellitus is the most significant chronic disease and cause of death in the modern society [1]. Diabetes mellitus is divided into 2 major categories: type 1 and type 2. These 2 types of diabetes have a distinct pathogenesis, but hyperglycemia and various life-threatening complications resulting from long-term hyperglycemia are their most common features [1]. The prevalence of diabetes is approximately 10% in Koreans, of whom 90% represent type 2 diabetes [2,3]. Diabetes is a metabolic disorder caused by an absolute or relative lack of insulin. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving the quality of life in diabetic patients [1]. Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely will reduce the risk associated with microvascular complications [4,5].

Throughout the world, many types of traditional food treatments for diabetes exist [6-8]. However, few have received scientific or medical scrutiny to validate their effects [9-11], and the World Health Organization has

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Table 1
Composition of the experimental diets (g/kg diet)[a]

Content	P0	P5	P10
Cornstarch	570.8	571	572.1
Phytate[b]		5	10
$CaCO_3$	12.5	12.5	12.5
KH_2PO_4	17.4	12.2	6.1
Casein	140	140	140
Soybean oil	75	75	75
Lard	75	75	75
Cholesterol	10	10	10
α-Cellulose	50	50	50
AIN-93 mineral mix[c] (Ca, P-free)	35	35	35
AIN-93 vitamin mix[d]	10	10	10
L-Cystine	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5

[a] P0, 0% phytic acid; P5, 0.5% phytic acid; P10, 1.0% phytic acid.
[b] Phytic acid (sodium phytate from corn) was obtained from Sigma.
[c] Obtained from ICN (Aurora, Ohio).
[d] Obtained from ICN.

recommended that traditional plant treatments for diabetes warrant further evaluation [12]. Recently, the search for appropriate antihyperglycemic agents has been focused on the types of food used in traditional medicine because these are natural products, not drugs [13]. Grains and cereals are the main source of energy for Koreans, and whole grains and cereals are recommended for diabetes to control blood glucose [14,15]. Phytic acid, myo-inositol hexaphosphate, is a plant component existing in most grains and legumes [16,17]. Although much attention has been focused on the biologic effects of phytic acid in human beings and animals [18-20], its effect on the blood profiles in diabetic models has not been completely evaluated. Therefore, the present investigation was undertaken with the objective of examining the effects of feeding diets containing different levels of phytate on the blood glucose concentrations of KK mice.

2. Methods and materials

2.1. Experimental diets

The composition of the diets fed to the mice is shown in Table 1. The P0 (control) diet was composed of casein and cornstarch with no added phytate, whereas the P5 and P10 diets were supplemented with 0.5% and 1.0% sodium phytate (Sigma, St Louis, Mo), respectively. The P5 diet contained a phytate content equivalent to regular human consumption [21]. The P10 diet contained 2 times more phytate than the P5 diet. All diets contained 15% of energy from fat and were isonitrogenous with a slight change in the amount of cornstarch. Diets and deionized water were provided ad libitum for 8 weeks. Daily feed intake and water consumption of individual animals were recorded throughout the experiment.

2.2. Animals

Diabetic KK mice, frequently used as an animal model for non–insulin-dependent diabetes, were used in this study [22-25]. Thirty male KK mice were purchased from the Laboratory Animal Center (Daehan Biolink Ltd, Daejeon, Korea). All mice were housed individually in stainless-steel wire-bottom cages in an air-conditioned room kept at approximately 22° of temperature and 60% of humidity with a 12-hour light/dark cycle (light from 6 AM to 6 PM) and were allowed free access to the control diet for 8 weeks before the experiment. After that, blood was drawn from their tail vein; the mice were considered diabetic only if their blood glucose levels exceeded 200 mg/dL [26]. Diabetic mice were not treated with insulin in this study. The diabetic mice were classified into 3 groups according to their weight and blood glucose level to make the average weights and blood glucose levels similar among the groups. Body weights were determined weekly for each mouse throughout the experiment. The experiment was carried out according to the approved guidelines established by the Rural Resources Development Institute Experimental Animal Care Committee.

2.3. Fasting and nonfasting blood glucose levels and glucose tolerance

Fasting blood glucose levels of mice fasted overnight and nonfasting blood glucose levels were measured at the 8th week of the feeding experiment. Blood glucose levels were determined at 9 AM using a glucose analyzer (Medisense, Abbott Park, IL). An intraperitoneal glucose tolerance test was performed after 8 weeks of diet supplementation. On the test day, animals were fasted for 15 hours, followed by an intraperitoneal administration of glucose (3 g/kg body weight; Sigma). Blood glucose levels were measured at 0 (before glucose administration), 30, 60, and 120 minutes after glucose administration [27,28].

Table 2
Feed and water intake as well as initial and final body weights of the mice

Group	Food intake (g/d)	Water intake (mL/d)	Body weight (g)		Food efficiency ratio[1]
			Wk 0	Wk 8	
P0	3.79 ± 0.11[NS]	19.2 ± 1.0[NS]	28.7 ± 0.3[NS]	30.3 ± 0.5[NS]	0.8 ± 0.1[b]
P5	4.01 ± 0.09	20.6 ± 1.6	29.1 ± 0.4	32.3 ± 0.5	1.4 ± 0.2[a]
P10	4.12 ± 0.13	18.9 ± 1.7	29.1 ± 0.5	31.9 ± 0.6	1.2 ± 0.1[a]

NS indicates not significant. Values are expressed as mean ± SEM. Values in the same column with different superscript letters are statistically significant, $P < .05$.
[1] Calculated as: [body weight gain (g/d)/food intake (g/d) × 100].

Table 3
Comparison of the liver, kidney, heart, spleen, and epididymal fat pad weights (g)

Group	Liver	Kidney	Heart	Spleen	Epididymal fat pad
P0	1.23 ± 0.05^{NS}	0.34 ± 0.01^{NS}	0.14 ± 0.01^{NS}	0.08 ± 0.01^{NS}	0.88 ± 0.01^{NS}
P5	1.22 ± 0.05	0.38 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.90 ± 0.01
P10	1.25 ± 0.03	0.38 ± 0.01	0.14 ± 0.01	0.08 ± 0.01	0.89 ± 0.01

Values are expressed as mean ± SEM.

2.4. Operation procedures

After 8 weeks of the feeding experiment, mice were fasted overnight, and blood was drawn from the venous blood of their eye to measure differences in hemoglobin A_{1c} (HbA_{1c}) and serum insulin levels among groups under a fasting condition. After bleeding, mice were euthanized, and their liver, kidney, heart, spleen, and epididymal fat pad were rapidly excised and weighed. Serum collected after centrifugation of the blood for 10 minutes at 3000 rpm was stored at −70°C for the measurement of insulin concentration.

2.5. Hemoglobin A1c and insulin analyses

Hemoglobin A_{1c} content was measured by a Micromat II Hemoglobin A_{1c} Test Cartridge (Bio-Rad, Hercules, CA). Serum insulin level was analyzed by a rat insulin–specific radioimmunoassay kit (Linco, St. Charles, MO) using a γ counter (Cobra, Ramsey, MN).

2.6. Statistical analysis

Data analyses were performed using SPSS software (version 10.0 for Windows, SPSS, Chicago, Ill). All data were expressed as mean ± SEM. Analysis of variance was used to test for differences between the groups. Duncan's multiple range test was used to determine significant differences among the mean values at $P < .05$ [29].

3. Results

3.1. Feed and water intake as well as body weight change

Table 2 shows the feed and water intake as well as body weight change of the mice. There was no significant difference in feed and water intake, initial body weight, and final body weight among all the groups during the experiment. The feed efficiency ratio (calculated by body weight gain over food intake) was significantly higher in the P5 (1.4) and P10 (1.2) groups as compared with the P0 control (0.8) group.

3.2. Organ and fat weight

Table 3 shows a comparison of the animals' liver, kidney, heart, spleen, and epididymal fat pad weights. There was no significant difference in organ weights among the groups.

3.3. Fasting and random blood glucose levels

The effect of treatment with phytate on blood glucose levels in diabetic KK mice is shown in Fig. 1. Fasting blood glucose concentrations of diabetic mice after treatment with the phytate were lower in the P5 and P10 groups (185.9 ± 2.9 and 154.7 ± 2.0 mg/dL, respectively) as compared with the P0 control group (195.8 ± 10.0 mg/dL). Nonfasting blood glucose concentrations of diabetic mice after treatment with phytate were lower in the P5 and P10 groups (197.6 ± 13.0 and 182.5 ± 10.8 mg/dL, respectively) as compared with the P0 control group (214.0 ± 9.6 mg/dL). Diabetic mice treated with 0.5% phytate in the diet showed a weak hypoglycemic effect in the 8th week. Fasting and nonfasting blood glucose levels decreased significantly ($P < .05$) in the P10 group.

3.4. Blood glucose tolerance

Glucose tolerance was evaluated after 8 weeks of treatment with phytate supplementation. Fig. 2 shows the supplementary effect of phytate on the change in blood

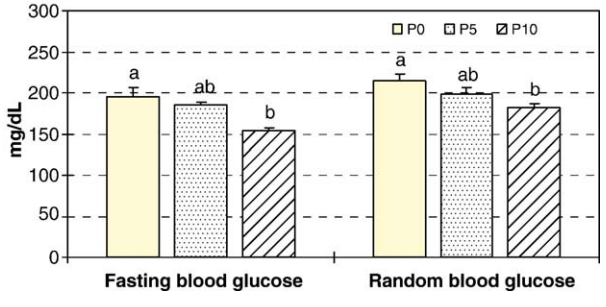

Fig. 1. Concentration of fasting and random blood glucose levels of the diabetic KK mice after consuming an experimental diet for 8 weeks (P0 [0% phytic acid], P5 [0.5% phytic acid], or P10 [1.0% phytic acid]). Values are expressed as mean ± SEM. Values with different superscript letters are statistically significant, $P < .05$.

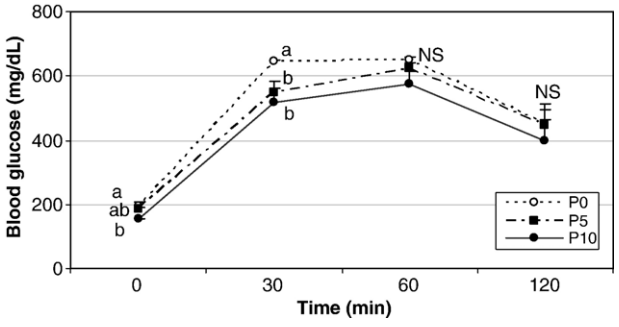

Fig. 2. Blood glucose responses of the mice to an intraperitoneal glucose challenge (3 g/kg body wt) after consuming an experimental diet for 8 weeks (P0 [0% phytic acid], P5 [0.5% phytic acid], or P10 [1.0% phytic acid]). Values are expressed as mean ± SEM. Values with different superscript letters are statistically significant, $P < .05$. NS indicates not significant.

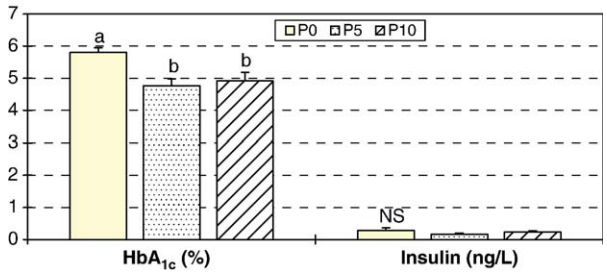

Fig. 3. Concentration of HbA_{1c} and insulin levels in the diabetic KK mice after consuming an experimental diet for 8 weeks (P0 [0% phytic acid], P5 [0.5% phytic acid], or P10 [1.0% phytic acid]). Values are expressed as mean ± SEM. Values with different superscript letters are statistically significant, $P < .05$.

glucose levels after the intraperitoneal glucose load. The blood glucose levels before the glucose injection were lower in the P5 and P10 groups as compared with the P0 control group. A significant difference in blood glucose from the P0 control (645.0 ± 5.0 mg/dL) was found in both the P5 (546.9 ± 34.8 mg/dL) and P10 (519.3 ± 41.6 mg/dL) groups after 30 minutes of the intraperitoneal glucose load. These blood glucose levels reached a peak (572.8–650.0 mg/dL) at 60 minutes after glucose administration and then declined. The blood glucose levels of the P0, P5, and P10 groups remained high (450.8 ± 42.6, 446.6 ± 65.6, and 395.7 ± 67.3 mg/dL, respectively) until 120 minutes after glucose administration. The blood glucose level of the P10 group was the lowest among the 3 groups, although there was no significant difference among the groups fed with experimental diets with or without phytic acid.

3.5. Hemoglobin A1c and insulin levels

As shown in Fig. 3, HbA_{1c} levels were significantly ($P < .05$) lower in the P5 (4.77% ± 0.21%) and P10 (4.92% ± 0.26%) groups as compared with the P0 (5.80% ± 0.28%) group after 8 weeks of phytate treatment. The insulin levels for P0, P5, and P10 were 0.28 ± 0.08, 0.17 ± 0.03, and 0.23 ± 0.08 ng/L, respectively, and the level was slightly decreased in the P5 and P10 groups as compared with the control group.

4. Discussion

Sustained reductions in hyperglycemia decrease the risk of developing microvascular complications [4,5]. However, the powerful inhibitory actions of drugs on high blood glucose levels cause side effects such as flatulence, diarrhea, serious hepatic injury, and renal failure [30-33]. As an alternative approach, natural food with antihyperglycemic activities have been increasingly used by diabetic patients and health care professionals [24,25].

An increase in body weight was far more likely in well-controlled diabetic rats as compared with poorly controlled ones [34]. In the present study, there was no significant difference in feed and water intake as well as initial and final body weights among all groups of mice. The feed efficiency ratios of the P5 and P10 group mice were significantly ($P < .05$) higher at 75% and 50%, respectively, as compared with the ratio for the control mice. This may be explained by the results of Rasch [34], who reported improved glycemic control and/or adipogenesis induced by the diabetes treatment.

Diabetic KK mice have high fasting and nonfasting blood glucose levels similar to those of diabetic patients. These animals, which are homozygous for the mutation, exhibit metabolic abnormalities such as hyperglycemia and glucose intolerance that phenotypically resemble human type 2 diabetes [23-25]. In the present study, the fasting blood glucose levels of the P5 and P10 groups decreased remarkably (5% and 21%, respectively) after 8 weeks of treatment with 0.5% and 1.0% phytate, respectively. Nonfasting blood glucose levels were reduced in the P5 (8%) and P10 (15%) groups as compared with the level in the control group. However, the significant reducing effects of phytate on fasting and nonfasting blood glucose levels were found only in the P10 group. The reducing effect of phytate on blood glucose was similar to the results of Dilworth et al [35], who suggested that the effect of sweet potato on the levels of blood glucose in normal rats was caused by phytate. Moreover, the glycemic index was found to correlate negatively with the phytate content of food tested in healthy volunteers [36]. It has been reported that phytate slowed down the rate of starch digestibility in vitro and glycemic response to legumes in vivo [37]. These effects of phytate appear to be related to reducing starch digestibility by the interactions of phytate with the starch or by inducing hormonal changes [38,39].

The diabetic KK mice used in this study showed hyperglycemia, and this hyperglycemia was exacerbated by the intraperitoneal glucose load. Phytate-treated mice (P5 and P10 groups) showed a significant ($P < .05$) decrease at 30 minutes after glucose administration when compared with the diabetic control mice (P0 group). For the P5 and P10 groups, the blood glucose levels decreased by approximately 15% and 19%, respectively, as compared with the control group. This means that the glucose tolerance at 30 minutes after glucose injection significantly ($P < .05$) improved in the P5 and P10 groups. The blood glucose values (395.7–450.8 mg/dL) at 120 minutes after glucose administration continued to be high and did not return to the fasting level (154.7–195.8 mg/dL), indicating glucose intolerance [15,25]. However, the blood glucose level of the P10 group decreased by 11% to 12% as compared with the levels of the P0 and P5 groups. Glycosylated HbA_{1c} levels were significantly ($P < .05$) decreased in the P5 (18%) and P10 (15%) groups as compared with the level in the P0 group, although there was no significant difference between the P5 and P10 groups. The levels of HbA_{1c} decreased before the fasting blood glucose levels did [40].

It has been shown that the ability of insulin to mediate tissue glucose uptake is a critical step in maintaining

glucose homeostasis and in clearing the postprandial glucose load [41,42]. Patients with type 2 diabetes exhibit a marked reduction in insulin-mediated glucose disposal [43,44]. An insulin secretagogue effect has been reported with the ingestion of plant-based products [45,46]. The hypoglycemic effect may be caused by improved insulin activity rather than by its increased secretion. A similar result was observed in an experiment with patients with type 2 diabetes mellitus fed with chromium-enriched yeast [47].

In summary, the data from this study show that the administration of phytate reduced high blood glucose levels in diabetic KK mice. Our results support an overall in vivo antihyperglycemic activity of phytate that may be of clinical importance in improving the management of type 2 diabetes. Identification of active compounds with significant antihyperglycemic activities from different phytate sources may provide an opportunity to develop a novel class of antidiabetic agents. The effects of a longer treatment period and higher concentrations of phytate supplement on the hypoglycemic mechanism needs to be studied.

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