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Original Paper

Effect of Dietary Adzuki Bean (*Phaseolus angularis*) on Serum Lipid Concentrations in Adult Rats

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Key words: adzuki bean, adult rat, serum cholesterol, glucose-6-phosphate dehydrogenase(G6PD)

Abstract

This study was designed to examine the effect of adding the adzuki bean to the diet (*Phaseolus angularis*) on the serum and liver lipid concentrations and metabolism of *ad libitum*-fed, adult male Sprague-Dawley rats. The rats were fed a control diet containing casein, α-starch and cellulose or a diet containing adzuki sarashi-an bean as the nutritional source. Fasting blood samples were collected seven times over 30 weeks. Serum triglyceride concentrations tended to be lower in the rats fed the adzuki bean diet for each sampling period. Serum total cholesterol concentrations were also significantly lower in the rats fed the adzuki bean diet at the end of the experimental period. Liver triglyceride and cholesterol concentrations, and glucose-6-phosphate dehydrogenase (G6PD) activity were significantly lower in the rats fed the adzuki bean diet compared with the control diet. These observations indicate that the adzuki bean is effective in lowering serum and liver lipid concentrations. The lipogenic effect of the adzuki bean may be through decreasing liver G6PD activity.

Introduction

Legumes are an important staple food in human nutrition. The nutrient composition of legumes makes them ideal for a healthy diet since they increase the intake of starch and complex carbohydrates and decrease the consumption of fat [1, 2]. Thus, the intake of legumes in the human diet can affect the incidence of coronary heart disease, diabetes mellitus, obesity and cancer [3, 4].

Many studies have shown that serum lipid concentrations and lipid metabolism can be greatly affected by consuming legumes, particularity soybean protein [5-8] and fiber [8-11], but also other bean fibers [2, 12, 13]. One such legume, the adzuki bean (*Phaseolus angularis*), is well-known in Japan, as are the soybean and kidney bean. However, few studies have investigated the effects of the adzuki bean in the diet [14], and no study has reported on its relationship with serum lipid concentrations and lipid metabolism. If the adzuki bean is indeed, like the soybean, a physiologically functional food source in terms of lowering serum lipid concentrations, it could be an important food source for reducing risk of hypercholesterolemia.

This study was designed to examine the effects of the adzuki bean on serum lipid concentrations in *ad libitum*-fed adult rats. We also measured changes in liver lipid concentrations and glucose-6-phosphate dehydrogenase (G6PD) activity, which play important roles in lipogenesis, to determine any correlation with changes in serum lipid concentrations.

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Methods

1. Animals, diets and experimental procedures

Forty-week-old, male Sprague-Dawley rats (Clea Japan Inc., Tokyo, Japan) were used. The animals were housed individually in stainless steel cages under controlled lighting and temperature conditions. The adzuki bean powder from the adzuki sarashi-an bean was obtained from Hakodate Nezu Seian (Hakodate, Hokkaido, Japan). This adzuki sarashi-an bean powder was made by steaming (90 minutes, 100 ℃), straining, soaking in water (40 minutes) and drying(260 ℃), and had an α-type starch content. The composition of the diets is shown in Table 1. At the start of the experimental period, the rats were divided into two groups. One group was fed the control diet, and the other was fed the adzuki bean diet. The diets and water were provided *ad libitum*. The experimental diets were given for 30 weeks with body weights and food intakes of each rat recorded three times a week.

Table 1 Composition of the diets

Ingredient	Control	Adzuki bean
	(g / 100 g)	
Adzuki bean[1]	―	90.0
Corn starch	64.0	―
Casein	23.5	―
Corn oil	5.0	4.1
Cellulose	3.0	1.4
Vitamin mix[2]	1.0	1.0
Mineral mix[3]	3.5	3.5
Total	100.0	100.0

[1]The adzuki sarashi-an bean used contained (g / 100 g); protein,23.5, fat,0.9, and dietary fiber, 1.6. Protein and fat contents were measured and fiber was quoted from a standard table of food composition in Japan.

[2]Oriental ™ vitamin mixture, obtained from Oriental yeast Co., Ltd., Tokyo. Composition per 100 g of vitamin mixture (mg); thiamine-HCl, 120; riboflavin, 400; pyridoxine-HCl, 80; vitamin B_{12}, 0.05; ascorbic acid, 3000; D-biotin, 2; folic acid, 20; calcium pantothenate, 500; p-amino benznic acid, 500; niacin, 600; inositol, 600; menadione, 520.

[3]Oriental ™ mineral mixture, obtained from Oriental yeast Co., Ltd., Tokyo, composition, % $CaHPO_4 \cdot 2H_2O$, 14.56; KH_2PO_4, 25.72; NaH_2PO_4, 9.35; NaCl, 4.66; Ca-lactate, 35.09; Fe-citrate, 3.18; $MgSO_4$, 7.17; $ZnCO_3$, 0.11; $MeSO_4 \cdot 4H_2O$, 0.12; $CuSO_4 \cdot 5H_2O$, 0.03; KI, 0.01.

After an overnight fast, fasting blood samples were collected from the tail vein under light ether anesthesia at the start of the experimental period, and then at 2, 4, 8, 12, 20 and 30 weeks. Serum samples were obtained by refrigerated centrifugation (700 × g for 15 minutes) and stored at -80 ℃. The rats were killed at the end of the experiment. Abdominal, back and epididymal fats were excised and weighed. The livers were quickly excised, weighed, rinsed with 0.9% saline and stored at -80 ℃.

2. Analytical methods

2.1 Serum triglycerides and total cholesterol

The serum triglyceride [15] and total cholesterol [16] concentrations were determined by enzymatic methods.

2.2 G6PD assay and protein determination

For the G6PD (EC 1.1.1.49) assay, a 0.5-g liver sample was homogenized in 4 volumes of medium containing 0.15 M KCl, 0.004 M $MgCl_2$ and 0.004M EDTA, pH 7.4. The homogenate was then centrifuged at 10,500 × g for 30 minutes at 4 ℃, and the supernatant fraction was used for the enzyme assay. The enzyme activity was determined in accordance with the method of Tsai and Dyne [17]. The protein content of the supernatant was determined by the method of Lowry *et al.* [18].

2.3 Liver lipids determination

For the determination of liver lipids, the liver was homogenized with chloroform and methanol (2:1) in a blender, and then extracted by the method of Folch *et al.* [19]. Aliquots of the homogenate were used for the determination of total fat, triglyceride and cholesterol concentrations. Triglyceride and cholesterol concentrations were determined by the same procedures used for the serum samples.

3. Statistical analysis

Values are expressed as mean ± SD, and where appropriate the significance of differences between mean values was determined by one-way ANOVA coupled with the Fisher's PLSD test or by the Student's t-test. Differences were considered significant at $P < 0.05$.

Results and Discussion

The body weight changes throughout the 30-week experimental period are shown in Fig. 1 and food intakes and body weights are summarized in Table 2. Food intake, final body weight and body weight gain did not differ significantly between the two diet groups, although body weights were slightly higher in the rats fed the control diet at all times. Abdominal and back fat weights were also higher in the rats fed the control diet but not significantly, while epididymal fat weights were significantly higher ($P < 0.01$). Randomization of the rats into two experimental groups resulted in initial body weights that were not significantly different. Thus, it can be assumed that the adzuki bean diet was not toxic to the rats,

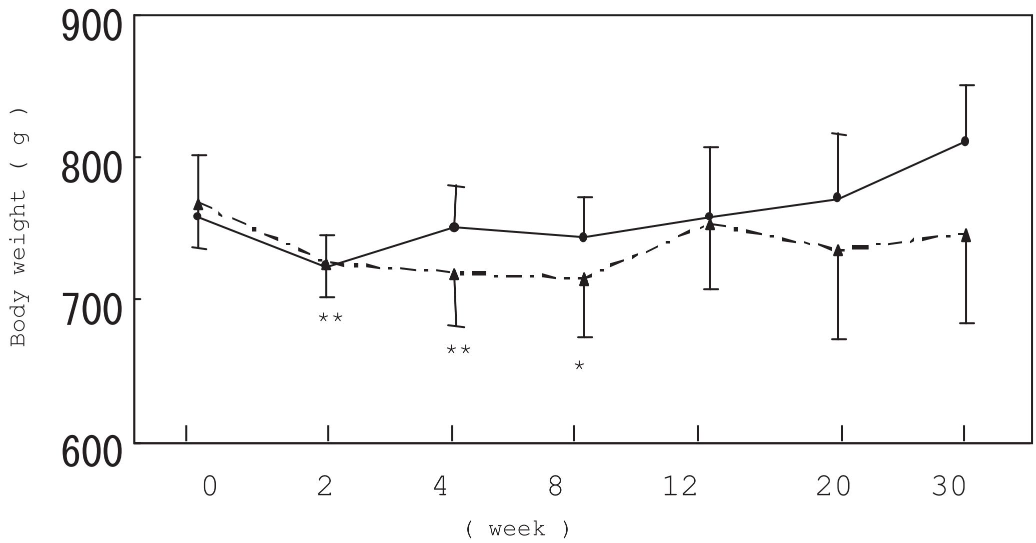

Fig. 1 Body weight change of rats fed experimental diets for 30 weeks. Data are mean ± SD of four rats per group. Control diet, ——— ; adzuki bean diet, -·-·-. $^{*}P<0.05$, $^{**}P<0.01$, compared with 0 week, for rats fed adzuki bean diet.

although final body weights and body weight gains tended to be lower in the rats fed the adzuki bean diet. It is suggested that the adzuki bean diet affected the lipid metabolism and body fat mass, since epididymal fat weight was significantly lower and abdominal and back fat weights tended to be lower in the rats fed the adzuki bean diet.

The time courses of serum lipid concentrations are shown in Fig. 2. The serum triglyceride concentrations were lower in the rats fed the adzuki bean diet at all times. Therefore, the effect of time was significant in the control diet. The serum triglyceride concentrations did not increase continuously in the rats fed the adzuki bean diet. However, they tended to gradually increase in the rats fed the control diet, and the values at 2, 4, 8 ($P < 0.05$) and 20 ($P < 0.01$) weeks were particularly significant. The serum total

Table 2 Daily food intake, body weight and body fat of rats fed experimental diets for 30 weeks.

		Control	Adzuki bean
Food intake	(g / day)	26.9 ± 2.2	25.3 ± 2.5
Initial body weight	(g)	758 ± 43	768 ± 31
Final body weight	(g)	810 ± 41	745 ± 61
Body weight gain	(g)	53 ± 43	-23 ± 34
Abdominal fat	(g)	19.0 ± 3.9	13.6 ± 6.0
Back fat	(g)	36.5 ± 4.6	30.4 ± 15.8
Epididymal fat	(g)	20.8 ± 1.7	15.2 ± 1.8 ##

Data are mean ± SD of four rats per group. ##P <0.01, for differences between groups fed control diet and adzuki bean diet.

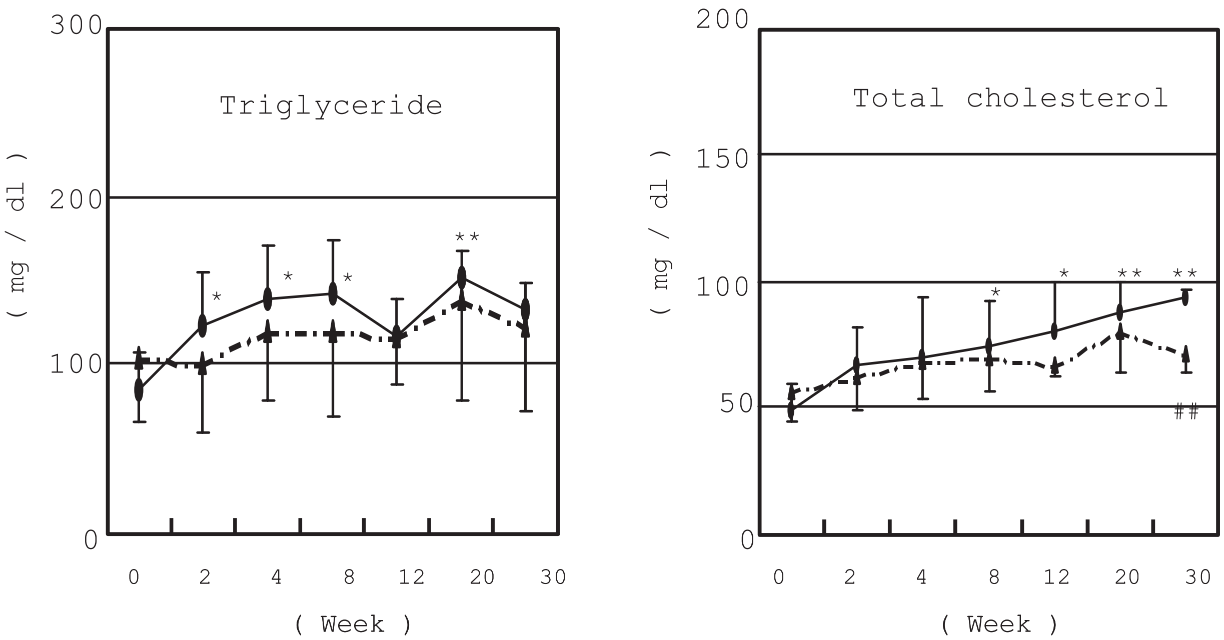

Fig. 2 Serum lipid concentrations of rats fed experimental diets for 30 weeks. Data are mean ± SD of four rats per group. Control diet, ———— ; adzuki bean diet, -.-.-.-. . *P<0.05, **P<0.01, compared with 0 week. ##P<0.01, for differences between groups fed control diet and adzuki bean diet.

cholesterol concentrations were significantly affected by both diets and time courses. The serum total cholesterol concentrations of the rats fed the control diet tended to continually increase with time, and were particularly significant at 8, 12, 20 and 30 weeks. However, those of the rats fed the adzuki bean diet did not increase continuously. The serum total cholesterol concentrations were higher in the rats fed the control diet at all times. Thus, at the end of the experimental period, the serum total cholesterol of the rats fed the control diet was significantly higher than that of the rats fed the adzuki bean diet ($P < 0.01$).

It is generally accepted that elevated levels of serum total cholesterol are positively correlated to the risk of coronary heart disease [20, 21], as to a lesser extent, are serum triglyceride concentrations [21, 22]. The serum cholesterol concentration has been reported to be affected by many components of diets, especially fatty acids in triglycerides [23-25], protein [5-8, 26], dietary fibers [11,12,17,27] and others [17,21]. In this study, the adzuki sarashi-an bean was used as the source of protein, carbohydrates and dietary fibers, and the control diet contained casein as the protein source and α-starch as the carbohydrate source. Protein was reported to affect serum cholesterol concentrations [5-8, 26], although carbohydrates did not [21]. The serum cholesterol concentrations of rats fed a casein diet were significantly higher than those fed soy protein diets [5, 6]. In the present study, the serum total cholesterol concentrations of the rats fed the experimental diet using the adzuki bean as the protein source, was significantly lower than that of the rats fed the control diet with casein as the protein source. The adzuki bean protein appears to have a hypocholesterolemic effect.

The effects of the diets on liver lipid concentrations and G6PD activities are shown in Table 3. The liver total lipid concentrations tended to be lower in the rats fed the adzuki bean diet, while liver triglyceride and cholesterol concentrations were significantly lower ($P < 0.05$). The G6PD activity was significantly higher in the rats fed the control diet ($P < 0.01$). This result does not correlate with the findings of Tsai and Dyer [17], who reported that liver G6PD activity in rats fed starch as a carbohydrate source was significantly lower than that of rats fed a sucrose diet. In the present study, the liver G6PD activity of the rats fed the adzuki bean diet while containing starch and oligosaccharide as the source of carbohydrate,

Table 3 Liver weight, lipid concentrations and glucose-6-phosphate dehydrogenase activity of rats fed experimental diets for 30 weeks.

		Control	Adzuki bean
Weight	(g)	17.1 ± 1.2	15.2 ± 1.4
Total lipids	(mg / g liver)	152.1 ± 61.1	114.3 ± 67.2
Triglycerides	(mg / g liver)	77.0 ± 31.6	35.0 ± 6.7 #
Cholesterol	(mg / g liver)	5.5 ± 1.4	3.5 ± 0.1 #
G6PD[1]	(U / g protein)	91.4 ± 20.1	28.3 ± 11.9 ##

[1]Glucose-6-phosphate dehydrogenase

Data are mean ± SD of four rats per group. #$P < 0.05$, ##$P < 0.01$, for differences between groups fed control diet and adzuki bean diet. One unit of G6PD activity is defined as that amount of enzyme which produces 1 μmol of measured product (NADPH) per minute under the conditions of the assay.

was lower than that of the rats fed the control diet containing α-starch. Since G6PD activity plays an important role in providing NADPH for lipogenesis, changes in this enzyme activity may have important physiological significance. The results found in this study might be correlated with the fact that rats fed the adzuki bean diet had significantly lower liver triglyceride and cholesterol concentrations.

The results of this study indicate that the adzuki bean is effective in preventing increases in serum and liver lipid concentrations. Its lipogenic effect may be found through decreasing liver G6PD activity. It may be that these changes in serum and liver lipid concentrations and G6PD activity were related to protein and dietary fiber in the diets. Therefore, although the dietary fiber content of the adzuki sarashi-an bean was taken from a standard Japanese table of food composition, a more detailed analysis should be done in a future study.

Furthermore, the mechanisms behind the hypocholesterolemic effects, such as changes in cholesterol and bile acids absorption and reabsorption, and whether cholesterol and bile acids are excreted in the feces, should be investigated further.

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