Hypocholesterolemic Effects of Protein Concentrates from Three Chinese Indigenous Legume Seeds

Chi-Fai Chau, Peter C. K. Cheung,* and Yum-Shing Wong

Research Laboratory for Food Protein Production, Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

The hypocholesterolemic effect of the protein concentrates (PCs) prepared from *Phaseolus angularis*, *Phaseolus calcaratus*, and *Dolichos lablab* seeds relative to that of casein were studied in hamsters. Male Golden Syrian hamsters were fed test diets containing these proteins at 12% level for 30 days. As compared with casein, the three legume PCs produced significantly lower ($p < 0.05$) levels of triglyceride and total and low-density lipoprotein cholesterol in the blood serum as well as lower liver total lipids and cholesterol contents, except that *P. angularis* PC gave a only nonsignificantly slight decrease in the serum total cholesterol. Moreover, only the PC of *P. calcaratus* gave a significantly higher ($p < 0.05$) level of serum high-density lipoprotein cholesterol. Generally, the PCs of *P. calcaratus* and *D. lablab* seemed to be more potent at lowering raised serum cholesterol levels than that of *P. angularis*.

Keywords: *Phaseolus angularis; Phaseolus calcaratus; Dolichos lablab; protein concentrate; hypocholesterolemic effect; cholesterol; hamster*

INTRODUCTION

Evidence for a hypocholesterolemic effect of grain legumes on raised cholesterol levels in animal models and man has accumulated in recent years (Kingman, 1991). Consumption of dietary soy protein was effective in lowering the levels of serum triglycerides and cholesterol in humans (Carroll, 1991; Potter et al., 1993; Bakhit et al., 1994; Anderson et al., 1995). Such hypocholesterolemic effect is thought to be at least partially attributable to the amino acid profile of the plant protein itself (Carroll, 1991). Seed proteins of jackbean and golden pea have also been reported to lower plasma cholesterol levels effectively in animals and humans (Marfo et al., 1990; Lasekan et al., 1995).

The legume seeds of *Phaseolus angularis*, *Phaseolus calcaratus*, and *Dolichos lablab* indigenous to China are found to be rich in proteins (ranging from 24.9 to 26.5%, dry weight) (Chau et al., 1998). These three seeds are traditionally used as soup ingredients for therapeutic purposes such as preventing edema and relieving diarrhea (Li, 1973).

The objective of the present study was to investigate the cholesterol-lowering potential of the protein concentrates (PCs) prepared from these three seeds on hamsters fed on hypercholesterolemic semipurified diets supplemented with 20 g of cholesterol/kg. To ascertain the effect of legume PCs on serum and hepatic cholesterol levels, all of the test diets were similar in all aspects with the exception of provision of protein from either isolated PCs of the three legume seeds or casein.

MATERIALS AND METHODS

Preparation of Protein Concentrates. Fully grown seeds of *P. angularis*, *P. calcaratus*, and *D. lablab*, imported from mainland China, were cleaned and ground in a Cyclotec mill (Tecator, Hoganas, Sweden) to pass through a 0.5 mm screen. Procedures for the preparation of legume protein concentrates have been described elsewhere (Chau et al., 1997). In brief, the PCs of these three seeds were obtained first by stirring the three meals in distilled water with the pH adjusted to 9.5 by sodium hydroxide solution. The solubilized proteins were precipitated from the alkali solutions by adjusting the pH to the isoelectric points of the legume proteins, which were 4 for *D. lablab* and 5 for both *P. angularis* and *P. calcaratus*. The precipitates were then redispersed in distilled water, and the pH was adjusted to 7 prior to freeze-drying. Protein contents of the PCs determined by Kjeldahl method using a factor of 6.25 were 71.2, 71.5, and 71.4%, for *P. angularis*, *P. calcaratus*, and *D. lablab*, respectively. The nonprotein substances in these PCs were mainly carbohydrates and ash.

Diet Preparation. With some modifications in the AIN-76 purified diet (AIN, 1977), the formulations of the semipurified test diets are shown in Table 1. Each diet was made up in a single batch of ~3 kg. The diets that contained casein (Teklad, Madison, WI) (~90% protein), *P. angularis* PC, *P. calcaratus* PC, and *D. lablab* PC as the sole source of protein

Table 1. Formulations of the Test Diets

	test diet			
ingredient[a]	control	*P. angularis*	*P. calcaratus*	*D. lablab*
casein	133			
P. angularis PC		169		
P. calcaratus PC			168	
D. lablab PC				168
cellulose	50	50	50	50
sucrose	100	100	100	100
cornstarch	578	542	543	543
corn oil	70	70	70	70
choline chloride	2.5	2.5	2.5	2.5
L-cystine	1.8	1.8	1.8	1.8
AIN-76 vitamin mix	10	10	10	10
AIN-76 mineral mix	35	35	35	35
cholesterol	20	20	20	20

[a] The ingredients are expressed as grams per kilogram of diet.

* Author to whom correspondence should be addressed [telephone (852) 26096144; fax (852) 26035646].

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Table 2. Food Intake and Liver and Serum Cholesterol Concentrations in Hamsters Fed the Casein and Legume Protein Diets for 30 Days[a]

diet	food intake (g/30 days)	final body wt (g)	triglyceride (mg/dL)	total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	HDL/LDL cholesterol ratio	HDL/total cholesterol ratio
control	252^{w}	150^{w}	204^{w}	251^{w}	123^{w}	105^{w}	1.17^{w}	0.49^{w}
P. angularis	244^{w}	152^{w}	172^{x}	243^{w}	130^{wx}	97.9^{x}	1.33^{x}	0.53^{wx}
P. calcaratus	246^{w}	149^{w}	173^{x}	225^{x}	134^{x}	83.6^{y}	1.60^{y}	0.60^{x}
D. lablab	252^{w}	151^{w}	129^{y}	217^{x}	125^{wx}	85.0^{y}	1.47^{xy}	0.58^{x}

[a] Data are expressed as means ($n = 8$). Values in the same column with different superscripts are significantly different (Tukey, $p < 0.05$).

were named control, *P. angularis*, *P. calcaratus*, and *D. lablab* diets, respectively. Two percent (w/w) of cholesterol was added to the control and the three legume PC diets to induce an alimentary hypercholesterolemia in the hamsters.

Experimental Design. Thirty-two fully mature male Golden Syrian hamsters (15 weeks old) with average initial body weight of 150 ± 11.5 g were obtained from the Animal House of the Chinese University of Hong Kong. The hamsters were randomly divided into four diet groups of eight animals each and were housed in screen-bottomed, stainless steel cages in a room maintained at 24 ± 1 °C with a 12 h light/dark cycle. The animals had free access to food and water for 30 days. Food intakes of each group during this period were recorded weekly. At the end of the experiment, the hamsters, which had been deprived of food for 18 h, were weighed and then anesthetized using diethyl ether. After anesthesia, blood samples taken by heart puncture were collected in test tubes without anticoagulant and were centrifuged at 3000*g* for 30 min to obtain serum. The liver was removed, washed with saline, blotted to remove excess solution and blood, weighed, and kept in a plastic bottle at −70 °C for liver analysis.

Analytical Methods. Using commercially available kits (Sigma Chemical Co., St. Louis, MO), serum total (Catalog No. 352), high-density lipoprotein (HDL) (Catalog No. 352-4), and low-density lipoprotein (LDL) cholesterol (Catalog No. 353), as well as triglyceride (Catalog No. 336), were measured enzymatically.

The total liver lipid was extracted from ∼1−2 g of liver with chloroform/methanol (2:1, v/v) according to the method of Folch et al. (1957). After the lipid extraction, the lipid solution volume was adjusted to 10 mL with the same solution. Using $FeSO_4$ in acetic acid and H_2SO_4, the total cholesterol in the liver lipid extract was determined colorimetrically at 490 nm (Searcy and Bergquist, 1960). The total lipids in the liver was determined gravimetrically by evaporating off the organic solvents in the lipid extract.

Statistical Analysis. All data were analyzed by one-way analysis of variance and Tukey's pairwise means comparisons test to detect differences among groups (Ott, 1988). An α level of 0.05 was set to determine statistical significance.

RESULTS AND DISCUSSION

Composition of the test diets is shown in Table 1. The quantity of protein source used in each diet was based on the amount required to give 12% protein. Because of the differences in the nitrogen contents (14.4% for casein and ∼11.4% for each PC), the amount of protein source used in the test diets varied from 13.3% for casein to 16.9% for *P. angularis* PC.

All animals remained healthy and active throughout the experiment. At the beginning of the feeding period, the mean body weights of the mature hamsters fed the control, *P. angularis* PC, *P. calcaratus* PC, and *D. lablab* PC diets, which were 148, 149, 152, and 151 g, respectively, were similar to each other. After 30 days of feeding, there was also no significant difference in the body weights between hamsters fed the legume PC diets and those fed the control diet (Table 2). The total food intakes (for 30 days) among the four diet groups, which were similar (Table 2), might explain the similar final body weight of the animals in the four dietary groups. In this study, there was no significant change in the body weights of the mature hamsters in each diet group after 30 days of feeding.

The serum triglyceride levels of hamsters fed casein and PC diets are shown in Table 2. The serum of hamsters fed casein diet contained significantly higher ($p < 0.05$) levels of triglyceride than did the serum of hamsters fed the three legume PC diets. Moreover, the serum triglyceride levels of hamsters fed *P. angularis* and *P. calcaratus* PCs, which were comparable to each other, were significantly higher ($p < 0.05$) than those of hamsters fed *D. lablab* PC. It has been found that the consumption of golden pea protein-containing diet could also result in a reduction of serum triglyceride levels in rats (Lasekan et al., 1995). Some recent clinical studies have supported the concept that a decreased plasma triglyceride level was associated with a lower risk of coronary heart disease (Davignon and Cohn, 1996).

The total, HDL, and LDL cholesterol levels in the serum of hamsters fed the four different diets are shown in Table 2. As compared with casein, only *P. calcaratus* PC was found to give a significantly higher ($p < 0.05$) level of serum HDL cholesterol, whereas the other two PCs gave a slightly nonsignificant increase in the levels of HDL cholesterol. On the other hand, the three legume PC diets were found to produce significantly lower ($p < 0.05$) levels of serum total and LDL cholesterol than casein, except that *P. angularis* PC gave only a slightly nonsignificant decrease in the total cholesterol level. In general, a combined reduction in the levels of both the total and LDL cholesterol would reduce the risk of coronary heart disease in humans (Schaefer et al., 1995). With respect to the differences in the serum lipoprotein profiles among the hamsters fed the four diets, the observed reduction in the total cholesterol was associated with a lowering of LDL cholesterol levels. These results were consistent with previous findings on pigs fed diets containing *Phaseolus vulgaris* and *Phaselous lunatus* (Kingman et al., 1993). Generally, the proteins from *Canavalia* seed (Marfo et al., 1990), soybean (Carroll, 1991), golden pea seed (Lasekan et al., 1995), and some other legume seeds (Dabai et al., 1996) were also found to have hypocholesterolemic properties when incorporated into animal and human diets. In this study, all three legume PCs seemed to have some differences in their cholesterol-lowering capacities as compared with casein. Basically, the variations in the legume species, the quantity, and the amino acid profile of legume seed protein in the diets might affect the cholesterol-lowering effect of legume protein (Nagata et al., 1982; Shutler et al., 1987; Dabai et al., 1996).

Table 3. Liver Lipids of Hamsters Fed the Casein and Legume Protein Diets for 30 Days[a]

diet	liver wt (g)	liver/body wt ratio	liver total lipids (g/g of liver)	liver cholesterol (mg/g of liver)
control	7.90[w]	0.05[w]	0.25[w]	85.7[w]
P. angularis	8.13[w]	0.05[w]	0.22[wx]	84.5[w]
P calcaratus	7.93[w]	0.05[w]	0.19[x]	82.2[x]
D. lablab	7.74[w]	0.05[w]	0.15[y]	80.6[x]

[a] Data are expressed as means ($n = 8$). Values in the same column with different superscripts are significantly different (Tukey, $p < 0.05$).

The value of the HDL/LDL cholesterol ratio for casein (1.17) was significantly lower ($p < 0.05$) than those for the three legume PC diets (ranging from 1.33 to 1.60) (Table 2). The significantly higher ($p < 0.05$) values of the HDL/LDL cholesterol ratios with the three legume PC diets relative to the casein diet further support the suggestion made earlier that the LDL cholesterol fractions were selectively reduced by the three PCs via specific mechanisms. The reduction in serum LDL cholesterol might be due to either an increase in their catabolism or a reduction in their synthesis. Generally, the lower level of serum LDL cholesterol in animals fed legume protein diets, compared with those fed casein, might be the result of relatively lower levels of sulfur-containing amino acid in the legume proteins, leading to limited numbers of LDL particles being available for the transport of cholesterol in the plasma (Kingman et al., 1993). The methionine contents of the casein, *P. angularis* PC, *P. calcaratus* PC, and *D. lablab* PC were 25.9 (Morita et al., 1997), 15.8, 15.3, and 12.5 mg/g of protein (Chau et al., 1998), respectively, whereas their methionine/glycine ratios were 1.57 (Morita et al., 1997), 0.42, 0.37, and 0.31 (Chau et al., 1998), respectively. From the above results, a positive correlation was also observed between the serum cholesterol levels (Table 2) and the methionine contents ($r = 0.79$, $p < 0.05$) or methionine/glycine ratios of the four dietary proteins ($r = 0.69$, $p < 0.05$). Moreover, the lysine/arginine ratios of the casein (2.17) (Morita et al., 1997), *P. angularis* PC, *P. calcaratus* PC, and *D. lablab* PC (1.17, 1.19, and 1.13, respectively) (Chau et al., 1998) also showed a significant correlation with serum cholesterol levels ($r = 0.74$, $p < 0.05$). This was consistent with previous findings that the methionine content, methionine/glycine ratio, and lysine/arginine ratio in dietary proteins were considered to be the responsible factors in lowering the serum cholesterol levels in rats (Sharma, 1987; Tanaka and Sugano, 1989; Morita et al., 1997). Generally, it should be pointed out that the above results hold true when the particular legume protein being tested was the only protein being fed. Moreover, it was observed that the reduction in serum total cholesterol levels with legume PC diets was accompanied by a significantly ($p < 0.05$) higher HDL/total cholesterol ratio (ranging from 0.53 to 0.6) compared with that of casein diet (0.49) (Table 2). A high HDL/total cholesterol ratio was considered to reduce the risk of coronary heart disease in humans, even when the total cholesterol level was elevated (Malaspina et al., 1981). In addition, human studies indicated that a higher proportion of HDL cholesterol in serum was antiatherogenic (Barter and Rye, 1996).

Table 3 shows that there were no significant differences in the mean liver weight and liver/body weight ratio of hamsters fed the control and experimental diets. Previous findings indicated that variations in the weight and appearance of livers in rats fed different diets were related to the difference in the amount of cholesterol and oil added into the diets (Beynen et al., 1986; Dabai et al., 1996). Therefore, the above observations could be explained by the fact that the amounts of cholesterol and corn oil added in each test diet were identical with the sources of protein as the only variation (Table 1).

The levels of liver total lipids obtained with hamsters fed the casein diet were significantly higher ($p < 0.05$) than those of hamsters fed the *P. calcaratus* and *D. lablab* diets (Table 3). Among the three PCs, the *D. lablab* PC was found to give a significantly lower ($p < 0.05$) level of liver lipid than the other two PCs. As shown in Table 3, the levels of liver cholesterol of hamsters fed the casein and *P. angularis* diets, which were comparable to each other, were significantly higher ($p < 0.05$) than those of hamsters fed the *P. calcaratus* and *D. lablab* diets. Among the four diet groups, the trend of changes in the levels of hepatic cholesterol (Table 3) paralleled those of serum total cholesterol (Table 2). In general, the levels of total liver lipids and liver cholesterol in rats had been reported to be lowered by the legume seed proteins of *Canavalia ensiformis*, *Pisum sativum*, and *P. lunatus* but not by those of *P. vulgaris* and red lentils when compared with casein (Marfo et al., 1990; Lasekan et al., 1995; Dabai et al., 1996).

CONCLUSION

The inclusion of the three legume PCs in the diets of hamsters with elevated serum cholesterol levels produced a very pronounced hypocholesterolemic effect. Compared with casein, the three legume PCs were found to give significantly lower levels of serum triglyceride and total and LDL cholesterol, as well as liver total lipids and cholesterol. Only *P. calcaratus* PC gave a significantly higher level of serum HDL cholesterol in the animals. The three legume PCs, especially the *P. calcaratus* and *D. lablab* PCs, seemed to be potential cholesterol-lowering ingredients in human diets. Further in vivo studies on the mechanisms of the hypocholesterolemic properties of these three legume PCs are underway.

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