Influence of different dietary constituents of beans *(Phaseolus vulgaris)* on serum and biliary lipids in the rat

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Bean (Phaseolus vulgaris) *components were separated by physical and chemical methods to study their effect on serum and biliary lipids, and on hepatic cholesterol concentration in the rat. Experimental diets were prepared replacing each component of a basic casein diet by each of the constituents obtained from beans: protein, starch, non-soluble fiber, and lipid. Rats fed a whole-bean diet significantly decreased serum cholesterol by 20%, and markedly increased biliary cholesterol output by 200% and hepatic cholesterol esters by 100%. Rats fed the bean starch diet also decreased serum cholesterol by 20%. In these animals, biliary cholesterol saturation increased by 180%, biliary phospholipid output by 100%, and biliary cholesterol output by 400%; hepatic cholesterol esters also increased by 100%. The bean protein and bean lipids had no effect on these parameters. Non-soluble fiber fraction of beans significantly decreased biliary bile acids and biliary phospholipid output, but increased biliary cholesterol saturation. In contrast, serum cholesterol remained unchanged compared with the control group of rats. Saponins, which are known to have hypocholesterolemic effects, were only detected in the whole-bean and starch-bean diets. When 0.3% bean sapogenins were fed to rats for 3 days, biliary cholesterol output increased by 300%. These experiments indicated that the effect of beans on serum, biliary cholesterol, and phospholipid outputs, and on hepatic cholesterol ester concentration are dependent on the starch fraction of beans, presumably on its sapogenin content. Furthermore, this study suggests that increasing biliary cholesterol output by dietary manipulations may induce a significant hypocholesterolemic effect.*

Keywords: Bean intake; sapogenins; fiber; serum cholesterol; biliary lipids

Introduction

Bean intake has important hypocholesterolemic effects[1-3] and simultaneously it increases biliary cholesterol saturation in humans.[3] Similar effects have been found recently in the rat.[4] These studies suggested that legume consumption and specifically beans, may have protective effects for arteriosclerosis, and in addition, may represent a dietary risk factor for cholesterol gallstone formation by favoring biliary cholesterol saturation.

The mechanisms responsible for these effects is only partially understood.[4] Similarly, the chemical component(s) of beans responsible for these effects are unknown. Two sets of hypothesis have been proposed for the hypocholesterolemic effects of legumes. One relates to the action of their particular aminoacid composition;[5] and the other to the action of non-protein substances present in legumes, such as their fiber component, including saponins.[6] It has been hypothesized

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that saponins present in beans may be responsible for the stimulation of biliary cholesterol output in the rat.[7]

The present studies were undertaken to discover the dietary component(s) present in beans (*Phaseolus vulgaris*) that may be responsible for both the hypocholesterolemic and biliary cholesterol effects in the rat. Starch, protein, insoluble fiber, and lipids were separated from dry beans by physical and chemical methods. Each component was incorporated into a basic casein diet that represented the control diet. The experiments indicate that the starch fraction of beans has a potent hypocholesterolemic effect and markedly induces biliary cholesterol output.

Materials and methods

Experimental materials and animals

Casein, α-cellulose, DL-methionine, cholesterol, taurocholate, saponin, diosgenin, and hydroxysteroid dehydrogenase were purchased from Sigma Chemical Co (St. Louis, MO, USA). Choline chloride was obtained from Matheson Coleman (Ohio, NJ, USA), and polyethylene catheters were from Clay Admas Inc (Parsippany, NJ, USA). Vitamin mixture and mineral mixture were purchased from Veterquímica (Santiago, Chile). Beans were obtained from Menichetti (Santiago, Chile). Organic solvents were purchased from E. Merck (Darmstadt, Germany).

Male Wistar rats were housed five to a cage, in wire-bottomed cages, in a well-ventilated room. They were subjected to reversed light cycling for 3 weeks before use. The lights were turned off at 04:00 hr, so that the mid-dark phase of the diurnal cycle was at 10:00 hr. All experiments were initiated between 08:15 and 09:00 hr.

Diets and separation procedures of bean fractions

Animals were fed either casein or experimental diets for 5–6 days prior to the experiments, essentially as previously described.[4] Briefly, each diet contained by weight: protein 18%; carbohydrate 68%; fat 5%; fiber 4%; mineral mixture*, 3.5%; vitamin mixture†, 1%; DL-methionine, 0.3%; and choline chloride 0.2%. The experimental diets were compared with a casein diet containing corn flour for carbohydrates, casein for protein, α cellulose for insoluble fiber, and corn oil for fat. The experimental diets were prepared to match the casein diet, replacing the specific nutrient obtained from beans. The whole-bean diet had the same composition as previously described.[4]

The different components of beans used for the experimental diets were separated as previously described[8] with some modifications. Beans were soaked in water at 4° C (1:1 wt/wt ratio) for 18 hr, dehulled, and homogenized in a Waring blender (South Plainfield, NJ, USA) for 15 min in 0.1 M phosphate buffer, pH 7.0. The homogenate was extracted with 5% NaCl solution for 30 min and then filtered in a 45 μm screen to remove starch and fiber. The filtrate was centrifuged at 4.000 rpm, 4° C for 20 min, subjected to dialysis, freeze dried, and labeled "protein fraction." Solids from the screening step were washed several times with distilled water over the 45 μm screen. Material passing through was labeled "starch fraction" and corroborated to be starch by microscopy, while the solids remaining over the screen corresponded to the "insoluble fiber fraction." The "lipid fraction" was obtained after refluxing the bean flour with hexane in a Soxhlet (Pyrex, New York, USA) apparatus at 150° C for 24 hrs. The extract was then evaporated at 50° C and replaced the 5% corn oil in the basic casein diet for the experimental diet, "bean lipid." In some experiments 0.3% sapogenins, extracted from defatted-bean flour, or diosgenin were mixed in the casein diet.

The experimental diets were prepared to match the casein diet in its protein, carbohydrate, fat, and insoluble fiber content by replacing each specific component obtained from beans. The energy content of the casein and experimental diets was 3.930 kcal; 70% of calories were provided by carbohydrates, 12% by fats, and 18% by protein. The content of insoluble fiber was 5% in each diet.

Bile and serum specimens

Bile and serum specimens were obtained between 09:00–12:00 hrs as previously described.[9] Blood was collected by aortic puncture and serum was obtained after centrifugation.

Analytical methods

Cholesterol was quantitated by enzymatic methods.[10] Phospholipids were measured in the chloroform-methanol extracts by the colorimetric method of Baginsky, Fos, and Zack.[11] Bile acids were quantitated by the 3 α-hydroxysteroid dehydrogenase method of Talalay[12] as modified by Turley and Dietschy.[13] Serum triglycerides were measured enzymatically by the dissapearance of NADH after lipase hydrolysis.[14] Saponins were extracted from the different diets with methanol and their sapogenins were detected as previously described.[15] Sapogenins were extracted with ethylacetate from the methanolic extracts of the bean flour used in the experimental diets.[15] There was approximately 2 g × kg^{-1} of sapogenins in the defatted-bean diet as determined by gravimetry. The groups of sapogenins were separated by thin layer chromatography according to their polarity in a system of chloroform:methanol 96:4.

Statistics

Results are presented as the mean ± 1 SD. Statistical evaluation of the findings was carried out by one-way analysis of variance (ANOVA).

Results

The experimental diets were well tolerated by the animals. They looked healthy and their daily body weight increment varied between 4.4–5.1 g per day, as shown in *Table 1*. Rats fed the whole-bean diet, or the bean-starch diet significantly decreased serum cholesterol by 20% and serum triglycerides by more than 40% after 1 week of feeding the experimental diets.

*The mineral mixture was prepared in our laboratory according to the American Institute of Nutrition and provided (mg/kg of diet): calcium 5.200; phosphorus 4.000; sodium 1.020; potassium 3.600; magnesium 54; iron 35; copper 6; zinc 30; iodine 0.2; selenium 0.1; chloride 1.560; and sulfate 1.000.

†The vitamin mixture was prepared according to the American Institute of Nutrition recommendations, providing (mg or I.U./kg of diet): thiamine hydrochloride 6 mg; riboflavin 6 mg; pyridoxine hydrochloride 7 mg; nicotinic acid 30 mg; calcium pantothenate 16 mg; folic acid 2 mg; biotin 0.2 mg; cyanocobalamin 0.01 mg; vitamin A 4.000 I.U.; vitamin D_3 1.000 I.U.; vitamin E 50 I.U.; and vitamin K 0.05 mg.

Table 1 Effect of feeding different bean fractions on body and liver weights, and serum lipids

Diets		Final body weight	Body weight increment	Liver weight	Serum lipids concentration		Hepatic cholesterol concentration	
					Triglyceride	Cholesterol	Free	Ester
		g	g × day $^{-1}$	g	mg × dl^{-1}		mg × g^{-1} liver weight	
Casein	(6)	160 ± 14	4.8 ± 1.2	7.4 ± 1.1	108 ± 45	96 ± 11	2.0 ± 0.2	0.23 ± 0.04
Whole bean	(6)	158 ± 15	5.1 ± 1.5	6.2 ± 1.1	54 ± 22[a]	71 ± 6[a]	2.4 ± 0.2	0.42 ± 0.08[a]
Bean protein	(5)	161 ± 12	4.8 ± 1.6	6.9 ± 1.3	90 ± 6	127 ± 45	2.2 ± 0.4	0.27 ± 0.04
Bean starch	(6)	166 ± 12	4.6 ± 1.2	7.1 ± 0.8	66 ± 17[a]	76 ± 6[a]	2.5 ± 0.3[a]	0.43 ± 0.04[a]
Bean lipid	(5)	159 ± 18	4.9 ± 2.0	7.2 ± 0.9	112 ± 38	98 ± 14	2.1 ± 0.3	0.24 ± 0.06
Bean fiber	(5)	163 ± 10	4.4 ± 1.9	7.0 ± 0.9	97 ± 31[b]	94 ± 9[b]	1.9 ± 0.5	0.27 ± 0.05

All groups were fed different diets for 5–6 days prior to the experiments. The number of animals in each group is shown in parentheses. Values are the mean ± 1 SD.
[a]The value is significantly different from the casein group, $P < 0.01$
[b]The value is significantly different from the bean group, $P < 0.05$

Table 2 Biliary lipid concentration and molar percentages in rats fed different diets

Diets		Biliary lipid concentration			Biliary lipid molar percentages		
		Bile acids	Phospholipid	Cholesterol	Bile acids	Phospholipid	Cholesterol
			[mmol/L]			(%)	
Casein	(6)	39 ± 4	6.8 ± 1.2	0.64 ± 0.2	83.1 ± 12	15.0 ± 3	1.4 ± 0.3
Whole bean	(6)	45 ± 14	8.9 ± 1.2[a]	1.26 ± 0.3[a]	81.6 ± 25	16.1 ± 2	2.3 ± 0.3[a]
Bean protein	(5)	43 ± 4	6.9 ± 1.4	0.58 ± 0.1	85.2 ± 14	13.7 ± 3	1.1 ± 0.2
Bean starch	(6)	41 ± 9	8.2 ± 1.4	2.06 ± 0.5[a]	80.1 ± 15	16.0 ± 2	3.9 ± 0.9[a]
Bean lipid	(5)	35 ± 13	7.4 ± 1.5	0.54 ± 0.3	81.5 ± 19	17.2 ± 4	1.3 ± 0.2
Bean fiber	(5)	28 ± 10[a]	6.5 ± 0.9	0.84 ± 0.4	79.3 ± 28	18.4 ± 3	2.3 ± 0.3[a]

[a]The value is significantly different from the casein group, $P < 0.01$.

Table 3 Bile flow and biliary lipid output in rats fed different diets

Diets		Bile flow	Biliary lipid outputs		
			Bile acids	Phospholipid	Cholesterol
		μL/g^{-1} · min^{-1}		nmole/g^{-1} · min^{-1}	
Casein	(6)	1.4 ± 0.3	61 ± 17	10.6 ± 1.9	1.0 ± 0.3
Whole bean	(6)	1.9 ± 0.5	94 ± 39	18.9 ± 2.0[a]	2.6 ± 0.9[a]
Bean protein	(5)	1.7 ± 0.2	61 ± 17	11.7 ± 2.5	0.9 ± 0.2
Bean starch	(6)	1.6 ± 0.4	74 ± 21	15.8 ± 4.7[a]	4.1 ± 1.2[a]
Bean lipid	(5)	2.0 ± 0.5	70 ± 18	14.8 ± 3.4	1.1 ± 0.4
Bean fiber	(5)	1.2 ± 0.2	33 ± 11[a]	7.8 ± 1.3[a]	1.0 ± 0.5

[a]The value is significantly different from the casein group, $P < 0.01$.

Similarly, hepatic cholesterol ester concentration increased by almost 50% in the same groups. In contrast, the other diets maintained serum and hepatic lipid concentration in the range found in the casein group.

The effect of whole bean and the bean fractions on biliary lipids is shown in *Tables 2 and 3*. Biliary cholesterol concentration and the molar percentage of biliary cholesterol significantly increased by 80% and 180% in the whole bean and the bean starch groups, respectively. Biliary bile acid concentration significantly decreased from 39 ± 4 to 28 ± 10 mmol/L in the bean-fiber group. The molar percentage of biliary cholesterol increased from 1.4 ± 0.3 to 2.3 ± 0.3 ($P < 0.01$) in these animals. Bile flow remained in the normal range in all groups. As expected, biliary phospholipid and cholesterol outputs markedly increased in the rats fed the whole bean diet. The active component of beans was found in the starch fraction, because phospholipid outputs increased by 50% and cholesterol by 400%. Biliary bile acid secretion significantly decreased by 50% and phospholipid output by 27% in the bean fiber group as compared with both the casein and whole-bean diets.

Because we previously demonstrated that saponins

and sapogenins may increase biliary lipid output in the rat,[7] aliquots of the different experimental diets were extracted with methanol. After thin layer chromatography, saponins were only detected in the whole-bean diet and in the bean-starch diet. In the final series of experiments, sapogenins were extracted from the bean flour and separated by thin layer chromatography according to polarity in three groups of compounds. As shown in *Table 4*, sapogenins with intermediate polarity had a profound effect on biliary cholesterol output with a comparable effect to diosgenin, a well known inducer of biliary cholesterol secretion. In contrast, the other two groups of sapogenins, I and III, had no effect, indicating that only some sapogenins are active as inducers of biliary lipid secretion.

Discussion

The major finding of these experiments was that the starch component of beans reproduced the same effects of the whole-bean diet on serum and hepatic cholesterol concentration and on biliary lipid secretion, and also on serum triglyceride concentration. In addition, this study shows for the first time that some bean sapogenins can markedly induce biliary cholesterol secretion, suggesting that these compounds may be the principal effector on cholesterol metabolism present in beans. It was also apparent that the diet prepared with the non-soluble fraction of bean fiber significantly decreased biliary bile acids and phospholipid secretion as compared with both the casein and the whole-bean diet.

It is important to emphasize that all the experimental diets were designed to supply identical quantities of energy, protein, fat, and non-soluble fiber. Thus the casein and the experimental diets apparently differed in the amount, the quality, or both of the non-soluble fiber and the carbohydrate fraction isolated from beans. In fact, it was shown that the bean-starch diet contained saponin, a compound also found in the whole-bean diet, but not in the bean-protein or the bean-fiber diets. It is known that saponins are commonly found in legumes and have potent effects on both serum cholesterol concentration and biliary cholesterol output. The presence of other active components on serum cholesterol, such as soluble fibers commonly found in legumes,[6] in the starch fraction cannot be discarded. The method used to separate the different components of beans in this study in fact permitted that soluble material, such as soluble fiber, would be included in the fraction labeled "starch fraction." It is possible therefore, that besides the saponins detected in the bean starch diet, i.e., soluble fiber, also may have contributed to the profound effects found on serum cholesterol and triglyceride concentrations in the group of animals fed the active diets, as previously described.[4]

The finding of decreased bile acid secretion into the bile in the rats fed the non-soluble bean-fiber diet is likely to be the consequence of a depleted pool of bile acids, secondary to fecal wastage of these molecules. The effects of certain fibers on fecal bile acid excretion is well documented.[16] It is interesting to note however that the rats fed the bean-fiber diet maintained serum cholesterol concentrations in the normal range, supporting the hypothesis that the hypocholesterolemic effect of beans is related to the important stimulation of biliary cholesterol secretion induced by one or several dietary effectors. Our experiments clearly showed that bean sapogenins may markedly induce biliary cholesterol secretion, suggesting that these compounds may also be responsible for the hypocholesterolemic effect of beans through this mechanism.

The present studies do not add to the understanding of the changes of the basic mechanisms of hepatic cholesterol metabolism modified by the bean components. We previously postulated[4] that some component(s) of beans may inhibit VLDL production, explaining both the high concentration of hepatic cholesterol esters and the stimulation of biliary cholesterol output, by increasing the availability of free cholesterol in the pre-canalicular pool for recruitment by the bile acid-dependent secretory mechanism.[17] A second possibility is that both high hepatic cholesterogenesis and low hepatic cholesterol esterification induced by beans[4] represent the consequence of the high biliary cholesterol secretory state. With this interpretation we should postulate then that the hypocholesterolemia induced

Table 4 The effect of different sapogenins extracted from bean flour on bile flow and biliary lipid secretion

Group		Bile flow $\mu L/g^{-1} \cdot min^{-1}$	Biliary lipid outputs		
			Bile acids	Phospholipid $nmol/g^{-1} \cdot min^{-1}$	Cholesterol
Control I	(6)	1.8 ± 0.3	80 ± 16	11.2 ± 2.3	1.8 ± 0.5
Fraction I	(4)	1.9 ± 0.2	64 ± 11	12.0 ± 4.0	1.0 ± 0.1
Fraction II	(5)	2.2 ± 0.4	96 ± 19	14.1 ± 3.9	3.3 ± 1.6[a]
Fraction III	(6)	1.8 ± 0.2	80 ± 28	11.3 ± 1.4	1.7 ± 0.2
Diosgenin	(4)	1.8 ± 0.3	75 ± 34	12.4 ± 2.5	4.8 ± 1.4[a]

Control rats were fed a casein diet. The contents of fractions I, II, III, and Diosgenin were dissolved in chloroform and mixed with the casein diet at a concentration of 0.3 g × 100 g^{-1} of casein diet (weight/weight). The diets were fed for 3–4 days prior to the experiments. Thin layer chromatography of fraction I (less polar) to fraction III (more polar) and diosgenin gave a positive reaction characteristic for steroids-triterpenes when sprayed with p-anisaldehyde: glacial acetic acid: concentrated sulphuric acid reagent (1:100:2).
[a]The value is significantly different from the control group, $P < 0.01$.

by beans is the consequence of a stimulation on the activity of the sinusoidal lipoprotein receptors, which in turn would explain the high concentration of hepatic cholesterol ester found in the rats fed the whole bean and starch bean diets. Preliminary unpublished data from this laboratory strongly supports this second interpretation for the complex effects of bean intake on hepatic, serum, and biliary cholesterol. This second interpretation necessarily would imply that triterpenoid or steroidal sapogenins have a direct effect on the canalicular secretory mechanisms of biliary lipids. In fact, these compounds share similar molecular and physicochemical characteristics with bile acids. It is known that diosgenin may be absorbed in the intestine, biotransformed in the liver, and secreted into the bile in significant amounts;[18] therefore it may be postulated that some dietary sapogenins have bile acid-like effects in the co-secretory mechanism regulating biliary cholesterol and phospholipid secretion.

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References

1 Nallendoom, E.W. (1976). Beneficial physiologic action of beans. *J. Amer. Diet Assoc.* **69,** 248–253

2 Anderson, J.W., Story, L., Sieling, B., Lin Chen, W.J., Petro, M.S., and Story, J. (1984). Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *Amer. J. Clin. Nutr.* **40,** 1146–1155

3 Nervi, F., Covarrubias, C., Bravo, P., Velasco, N., Ulloa, N., Cruz, F., Fava, M., Severín, M.C., and del Pozo, R. (1988). Influence of legume intake on biliary lipids and cholesterol saturation in Chilean men. *Gastroenterology* **96,** 825–830

4 Rigotti, A., Marzolo, M.P., Ulloa, N., González, O., and Nervi, F. (1989). Effect of bean intake on biliary lipid secretion and on hepatic cholesterol metabolism in the rat. *J. Lipid Res.* **30,** 1041–1048

5 Gibney, M.J. and Kritchevsky, D. (1983). Animal and vegetable proteins in lipid metabolism and atherosclerosis. In *Current Topics in Nutrition and Disease,* (A.A. Albanese and D. Kritchevsky, eds.), p. 1–148 Alan R. Liss, Inc., New York, NY, USA

6 Anderson, J.W. and Lin Chen, W. (1979). Plant fiber. Carbohydrate and lipid metabolism. *Am. J. Clin. Nutr.* **32,** 346–363

7 Ulloa, N. and Nervi, F. (1985). Mechanisms and kinetic characteristics of the uncoupling by plant steroid of biliary cholesterol from bile salt output. *Biochim. Biophys. Acta* **837,** 181–189

8 Hohlberg, A.I. and Stanley, D.W. (1987). Hard-to-cook defect in black beans. Protein and starch considerations. *J. Agric. Food Chem.* **35,** 571–576

9 Nervi, F., del Pozo, R., Covarrubias, C., and Ronco, B. (1983). The effect of progesterone on the regulatory mechanisms of biliary cholesterol secretion in the rat. *Hepatology* **3,** 360–367

10 Roschlau, P., Bernt, E., and Gruber, W. (1974). Enzymatic determination of total cholesterol in serum. *Z. Klin. Chem. Klin. Biochem.* **12,** 403–408

11 Baginsky, E.S., Fox, P.P., and Zack, B. (1967). Microdetermination of inorganic phosphate, phospholipids and total phosphate in biological material. *Clin. Chem.* **13,** 326–332

12 Talalay, P. (1960). Enzymic analysis of steroid hormones. *Methods Biochem. Anal.* **8,** 119–143

13 Turley, S.D. and Dietschy, (1978). Re-evaluation of the 3 α -hydroxysteroid dehydrogenase assay for total bile acids in bile. *J. Lipid Res.* **19,** 924–928

14 Bucolo, G. and David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19,** 476–482

15 Price, K.R., Caralyn, L., and Fenwick, G.R. (1986). The saponin content and sapogenol composition of the seed of 13 varieties of legume. *J. Sci. Food Agric.* **37,** 1185–1191

16 Miettinen, T.A. and Tarpila, S. (1977). Effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. *Clin. Chim. Acta* **79,** 471–477

17 Marzolo, M.P., Rigotti, A., and Nervi, F. (1990). Secretion of biliary lipids from the hepatocyte. *Hepatology* **12,** 134S–142S

18 Cayen, M.N., Ferdinandi, E.S., Greselin, E., and Doornik, D. (1979). Studies on the disposition of diosgenin in rats, dogs, monkeys and man. *Atherosclerosis* **33,** 71–87