

Effect of bean intake on biliary lipid secretion and on hepatic cholesterol metabolism in the rat

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Abstract We studied the effect of a bean diet on biliary lipid secretion, serum cholesterol concentration, and hepatic cholesterol metabolism in the rat. Rats fed a bean diet for 10–12 days had increased biliary cholesterol output and molar percentage by 300% and 200%, respectively, compared to rats fed an isocaloric and isoproteic casein diet. Biliary phospholipid output increased 180%. Bile flow and biliary bile salt output remained in the normal range. Total serum and VLDL cholesterol concentration significantly decreased 27% and 50%, respectively, in the rats fed the bean diet. Hepatic cholesterogenesis was increased 170% in the bean-fed animals. The relative contribution of newly synthesized hepatic cholesterol to total biliary cholesterol increased 200%, and that of endogenous origin only 50%. These results suggested that newly synthesized hepatic cholesterol was preferentially channelled to the biliary cholesterol secretory pathway in bean-fed rats. Although hepatic cholesteryl ester concentration increased 240%, the incorporation of [¹⁴C]oleate into hepatic cholesteryl esters was significantly decreased by 30% in isolated hepatocytes of bean-fed animals. These results were consistent with the possibility that the availability of hepatic free cholesterol for biliary secretion was increased in the bean-fed animals. ■ This study demonstrates that bean intake has a profound effect on the metabolic channelling and compartmentalization of hepatic cholesterol, resulting in a significant decrease in total serum and very low density lipoprotein cholesterol concentrations and a high biliary cholesterol output. —Rigotti, A., M. P. Marzolo, N. Ulloa, O. González, and F. Nervi. Effect of bean intake on biliary lipid secretion and on hepatic cholesterol metabolism in the rat. *J. Lipid Res.* 1989. 30: 1041–1048.

Supplementary key words biliary cholesterol • hepatic cholesterogenesis • serum cholesterol • beans

Recent studies from this laboratory indicate that a legume diet, particularly beans, significantly increases biliary cholesterol saturation and decreases total serum cholesterol and LDL cholesterol concentration in young Chilean men (1). These studies suggested that legume intake may represent a dietary risk factor for cholesterol gallstone formation. Similarly, legumes may have important protective effects against cardiovascular disease because of their serum cholesterol-lowering effects. It has been shown that bean intake and water-soluble fiber extracts from beans have significant hypocholesterolemic effects (2–4).

The present studies were undertaken to characterize the effects of a bean diet on biliary lipid secretion and cholesterol saturation in the rat. We also studied the mechanisms of bean-induced biliary cholesterol secretion. The results indicate that bean intake markedly induces biliary cholesterol output and significantly increases the contribution of newly synthesized hepatic cholesterol to total biliary cholesterol output.

METHODS

Experimental materials and animals

Casein, taurocholate, hydroxysteroid dehydrogenase, α -cellulose, Metrizamide, DL-methionine, and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO). Choline chloride was obtained from Matheson Coleman (Chio, NJ) and polyethylene catheters were from Clay Adams Inc. (Parsippany, NJ). Vitamin mixture and mineral mixture were purchased from Veterquímica (Santiago, Chile). Bean flour was obtained from Campex (Gorbea, Chile). All organic solvents were obtained from E. Merck (Darmstadt, Federal Republic of Germany).

Male Wistar rats in the weight range 80–140 g 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 h, so that the middark phase of the diurnal cycle was at 10:00 h. All experiments were initiated between 08:15 and 09:00 h.

Diets

Animals were fed with either casein or a bean diet with the following composition by weight: protein, 18%; carbo-

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein.

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hydrate, 68%; fat, 5%; fiber, 4%; mineral mixture,² 3.5%; vitamin mixture,³ 1%; DL-methionine, 0.3%; choline chloride, 0.2%. The components of the control diet were: casein for protein; corn flour for carbohydrates; α -cellulose for fiber; corn oil for fat. The experimental diet was prepared to match the control diet in its protein, carbohydrate, and total fat content. The bean diet had the following composition by weight: bean flour, 64.5%; corn flour, 25%; α -cellulose, 1.4%. According to Campex, the bean flour contained 1.4% fat (w/w), therefore the final fat content of the experimental diet was adjusted to 5% by adding 4.1% corn oil. The energy content of both the control and bean diets was 3930 kcal; 70% of calories were provided by carbohydrates, 12% by fats, and 18% by protein. The digitonin-precipitable sterol concentration of the diets was 24 and 35 mg/100 g for the control and the bean diet, respectively.

Biliary secretion studies

Bile specimens were obtained as previously described (5). Acute depletion of the bile salt pool was obtained by ligating the ileal and caecal blood vessels and perfusing the small intestine from the duodenum to the ileum with Krebs-bicarbonate buffer at 37°C for a period of 30 min at a rate of 3 ml/min. This procedure decreases the rate of biliary bile salt output by approximately one order of magnitude compared with the initial rates (5). In addition, some rats received an intravenous infusion of 45 mM taurocholate in 0.15 M NaCl at a rate of 0.075 ml/min for 40–60 min.

Simulated curves were derived with the experimental data by standard computer procedures (B.M.D. PAR Subroutine, Biomedical Computer Programs P-Series, University of California, Los Angeles, CA) as previously described (6). The best curve fitting for the experimental data was a nonlinear regression of the form of the rectangular hyperbola $y = ax/b + x$ for the relationship bile salts–cholesterol and bile salts–phospholipid. In this equation “a” represents the maximal theoretical output of biliary lipid obtained with a specific diet.

Determination of newly synthesized biliary cholesterol and hepatic cholesterogenesis

The contribution of newly synthesized hepatic cholesterol to biliary cholesterol was measured according to the method of Turley and Dietschy (7). Briefly, the rats were anesthetized with diethyl ether and a polyethylene catheter was placed into a tail vein. A bolus of 60 mCi of [³H]water contained in 0.5 ml of 0.9% NaCl was infused between 08:15 h and 09:15 h under a hood. The animals were left there for 6 h to allow equilibration of the tritiated water in the body compartments of the rats. At the end of this period, a bile fistula was performed under pentobarbital anesthesia. Blood was collected by aortic punc-

ture and plasma was obtained after centrifugation in sealed tubes for determination of water specific activity. The mass and radioactivity of biliary digitonin-precipitable sterols were determined after digitonin precipitation and pyridine solubilization (8).

The amount of newly synthesized cholesterol excreted into bile was calculated according to the formula (dpm ³H-labeled sterol) \times (1.45)/(sp act of plasma water) \times 18. The number 1.45 represents the nmol of acetyl-CoA units incorporated into sterols for each nmol of [³H]water. Since 18 nmol of acetyl-CoA is needed for the synthesis of 1 nmol of cholesterol, the number of acetyl-CoA units incorporated into cholesterol was converted to nmol of cholesterol synthesized by dividing the value by 18 (7).

Hepatic cholesterogenesis was determined in vivo using [³H]water as the radioactive precursor (9). A tail vein catheter was inserted under ether anesthesia and a bolus of 15 mCi of [³H]water contained in 0.5 ml of 0.9% NaCl solution was rapidly infused through the catheter. During the following hour, no food or water was given to the rats. At the end of this period, the animals were anesthetized again. Approximately 5 ml of blood was withdrawn from the abdominal aorta into a heparinized syringe. The livers were rinsed in situ through the portal vein with 10 ml of cold 0.9% NaCl solution, and then excised. Hepatic lipids were extracted with 20 volumes of chloroform-methanol 2:1. After evaporation of the chloroform phase, lipids were saponified with 0.625 N alcoholic KOH at 80°C for 60 min. Nonsaponifiable lipids were extracted with petroleum ether, and the free sterols were isolated as previously described (8). The rate of hepatic cholesterol synthesis was expressed as the content of ³H-labeled digitonin-precipitable sterols found in 1 g of liver per h.

Determination of hepatic cholesterol esterification in isolated hepatocytes

Isolated hepatocytes were prepared as described by Berry and Friend (10). The rates of [¹⁴C]oleic acid incorporation into hepatocytic cholesteryl esters were measured as previously described (11). More than 87% of isolated hepatocytes used in these experiments excluded Trypan Blue. Determinations were performed in triplicate. The casein-fed rats were studied parallel with the legume-fed animals.

²The 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 D₃ 1,000 I.U.; vitamin E 50 I.U., and vitamin K 0.05 mg.

³The mineral mixture was prepared in our laboratory according to the American Institute of Nutrition and provided (mg/kg of diet): Calcium 5200; phosphorus 4000; sodium 1020; potassium 3600; magnesium 54; iron 35; copper 6; zinc 30; iodine 0.2; selenium 0.1; chloride 1560, and sulfate 1000.

Fractionation of plasma lipoproteins

Blood was obtained by aortic puncture and collected in 1.5 mM EDTA; 0.05 mg/ml chloramphenicol and 0.01% thimerosal were added to the plasma specimens.

A continuous 3.5 ml Metrizamide density gradient was made with a gradient former in a 5-ml QuickSeal polyallomer centrifugation tube (Beckman, Palo Alto, CA), using 0.9% NaCl and 27% Metrizamide in 0.9% NaCl. The concentration of Metrizamide varied between 12% and 23% in the gradient. Immediately after gradient preparation, 1.5 ml of plasma containing 1% Metrizamide was layered over the gradient. Centrifugation was performed in a Beckman vertical VTI 65 rotor for 5 h at 10°C and 50,000 rpm in a L5-65 Beckman ultracentrifuge. After centrifugation, the density of the gradient was between 1.008 and 1.230 g/ml.

In preliminary experiments several fractions were collected from the 5-ml tubes by tube puncturing. Cholesterol and density (12) were measured in each fraction. Three major fractions were obtained from bottom to top: $d > 1.060$ g/ml (1.9 ml), HDL; $1.020 < d < 1.060$ g/ml (1.6 ml), LDL; $d < 1.020$ g/ml (1.5 ml), VLDL. Electrophoretic migration of intact lipoprotein fractions was done on cellulose-acetate paper, pH 8.6, according to the method of Kohn (13).

Analytical methods

Phospholipids were measured in the chloroform-methanol extracts by the colorimetric method of Baginsky, Fos, and Zack (14). Cholesterol was quantitated by the colorimetric method of Zack et al. (15). The free and esterified fractions were separated as previously described (11). Bile salts were quantitated by the 3α -hydroxysteroid dehydrogenase method of Talalay (16) as modified by Turley and Dietschy (17). Biliary bile acid composition was estimated by high pressure liquid chromatography (1). Serum triglycerides were measured enzymatically by the disappearance of NADH after lipase hydrolysis (18).

Statistics

Results are presented as the mean \pm 1 SD. The values of a and b of the mathematical formulas obtained from

the kinetic studies of biliary lipid secretion represent the mean \pm 1 SE. Differences in mean values were tested using the unpaired *t* test.

RESULTS

Effect of feeding a bean diet on body weight and bile secretion

Animals fed the casein or bean diets had a similar daily weight increment during the period of observation, as shown in **Table 1**. The more striking effects of beans on bile secretion were related to output of biliary phospholipid and cholesterol and to the biliary cholesterol molar percentage. Biliary phospholipid output increased by 178%. Biliary cholesterol output and the cholesterol molar percentage increased by 294% and 219%, respectively. Bile flow and biliary bile salt output remained in the normal range in rats fed the bean diet.

The biliary cholesterol output and molar percentage as a function of time and bean diet intake are shown in **Fig. 1**. The effect was already apparent by the 4th day of diet, reaching a maximum by the 12th day (panels A and B). It is also apparent that replacement of the bean diet with 50% and 75% casein diet resulted in a progressively lower biliary cholesterol output and molar percentage (panels C and D).

Because output of biliary cholesterol and phospholipid is tightly coupled to bile salt output, we studied biliary lipid output as a function of biliary bile salt output, as shown in **Fig. 2**. In both casein- and bean-fed rats, the best mathematical expression of the experimental data was the rectangular hyperbola, as previously found for man, dog, and rat (5, 6). The calculation of the theoretical maximal cholesterol output (**Fig. 2**, panel A) represented by the term "a" of the equation $y = \frac{a \cdot x}{b + x}$ demonstrated that this parameter was significantly increased by 720%. It is also evident that the term "b" of the same curve was increased in the animals fed the bean diet. This parameter indicates the dependence of cholesterol secretion on bile salt secretion; the half-maximal rate of secretion was reached at a greater bile salt secretion rate in the bean-fed

TABLE 1. Effect of bean intake on body and liver weight, and biliary lipid outputs

Diet	Final Body Weight g	Weight Increment g/day	Liver Weight g	Bile Flow μ l/g/min	Biliary Lipid Output			Biliary Cholesterol Molar Percentage %
					Bile Salts	Phospholipids nmol/g/min	Cholesterol	
Casein (8)	195 \pm 15	4.9 \pm 2.0	7.7 \pm 0.8	2.1 \pm 0.4	95 \pm 26	14 \pm 3	1.7 \pm 0.5	1.6 \pm 0.3
Bean (10)	185 \pm 25	4.3 \pm 1.6	7.1 \pm 1.7	2.5 \pm 0.5	112 \pm 10	25 \pm 3*	5.0 \pm 0.9*	3.5 \pm 0.5*

All groups were fed the different diets for 10 to 12 days prior to the experiments. The number of animals in each group is shown in parentheses. Values are the mean \pm 1 SD.

*The value is significantly different at the $P < 0.05$ level.

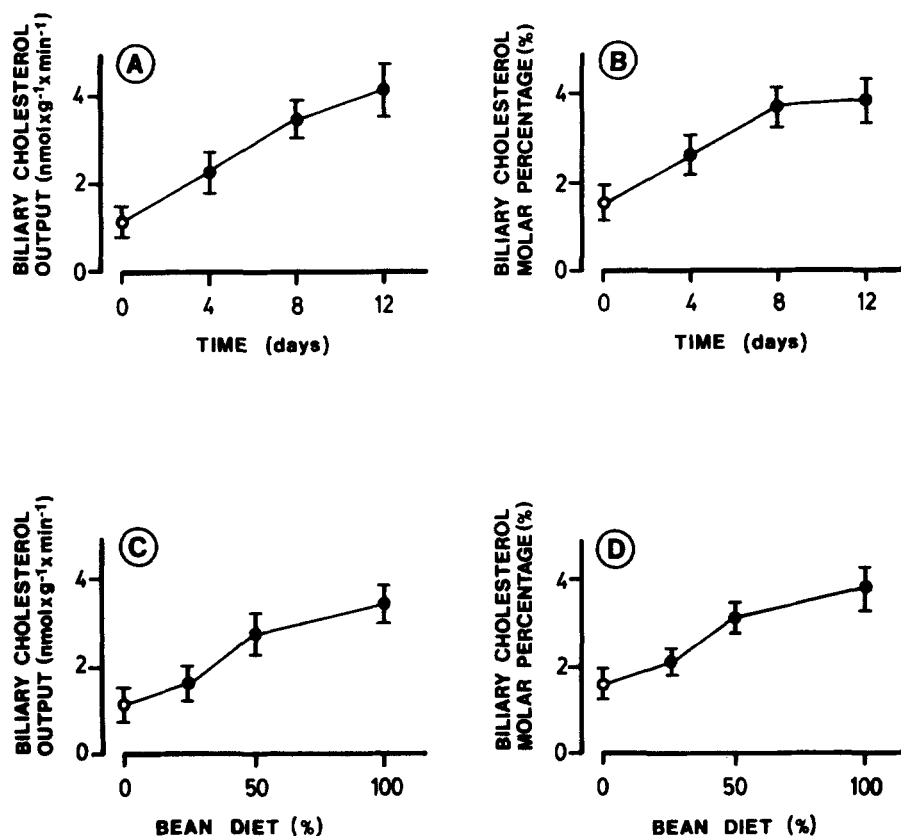


Fig. 1. Panels A and B show the relationship between biliary cholesterol output and the molar percentage of cholesterol in bile as function of the time of bean intake. Panels C and D show the effect of the concentration of the bean diet on biliary cholesterol output and the molar percentage of cholesterol in bile. The bean diet was mixed with the casein diet consumed by the control animals (represented by the open circles). These diets were fed for 10 to 12 days prior to the experiments. Values represent the mean \pm SD.

rats. Similar kinetics were found for the relationship between biliary phospholipid and bile salt output. The theoretical maximal phospholipid output increased only by 493%. It was also apparent that relatively more cholesterol than phospholipid was secreted into the bile per mol increment in bile salt output.

Biliary bile acid composition was modified by the bean diet, as shown in Table 2. In general, tauro-conjugated bile acids were markedly reduced in bean-fed animals. The glycine/taurine ratio increased from 0.1 to 1.5 ($P < 0.01$) in the bean-fed group. Taurocholic decreased from 61 to 22%, whereas glycocholic increased from 3 to 29% in the bean-fed rats. In contrast, the proportion of taurochenodeoxycholic remained essentially similar in both casein- and bean-fed rats.

Effect of feeding a bean diet on serum cholesterol and triglycerides and on hepatic cholesterol concentration

Serum cholesterol concentration significantly decreased by 27% ($P < 0.05$). Serum triglyceride concentration also significantly decreased from 129 to 70 mg/dl, as

shown in Table 3. Fig. 3 shows the effect of feeding a bean diet on lipoprotein cholesterol concentration. Lipoprotein cholesterol decreased in all fractions, but reached a significant difference only in VLDL cholesterol concentration. Hepatic free cholesterol concentration remained in the normal range; however, the ester fraction significantly increased from 0.17 to 0.41 mg/g.

Effect of feeding a bean diet on hepatic cholesterol synthesis and esterification and on the contribution of newly synthesized hepatic cholesterol to biliary cholesterol

The final series of experiments was designed to relate several parameters of hepatic cholesterol metabolism to the increment of biliary cholesterol output induced by the bean diet. As shown in Table 4, [³H]water incorporation into hepatic digitonin-precipitable sterols significantly increased by 171% ($P < 0.05$) in the bean-fed animals compared to the casein group. In contrast, [¹⁴C]oleate incorporation into hepatocyte cholesterol esters significantly decreased by 30% in the bean-fed rats.

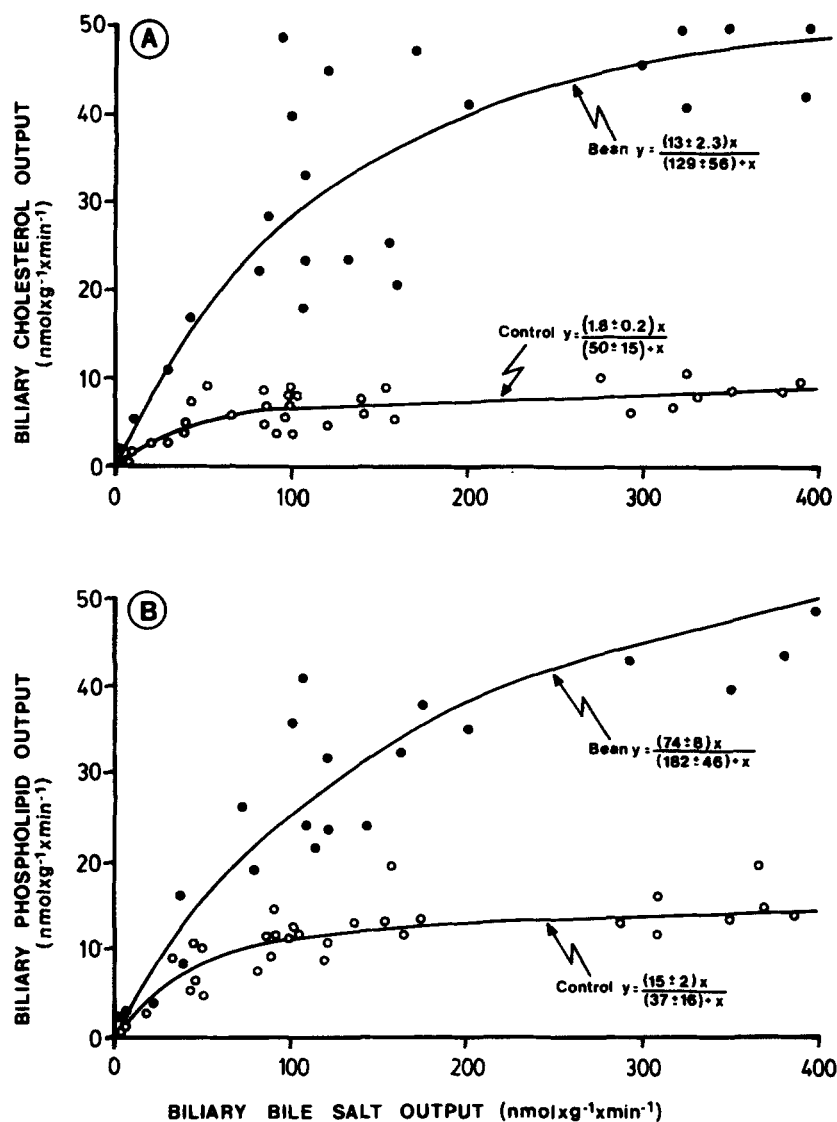


Fig. 2. Relationship between biliary cholesterol (panel A), biliary phospholipid (panel B), and bile salt output. The control bile samples are represented by open circles. The bile specimens from bean-fed rats are represented by solid circles. Each group was formed with the rats of Table 1, three rats infused intravenously with 45 mM taurocholate in 0.9% NaCl at a rate of 0.075 ml/min for a period of 20 min (equivalent to the period of bile collection), and a third group of two rats subjected to acute depletion of the bile acid pool as previously described (5). Five to six bile samples were obtained from each depleted animal. The best fitting curve for the experimental values was represented by the rectangular hyperbola $y = ax/b + x$. The term "a" represents the maximal theoretical output of y, and the term "b" represents the bile salt output at which the half maximal cholesterol or phospholipid output is obtained. Parameters a and b were both significantly increased in the bean-fed rats ($P < 0.05$).

To elucidate whether the high rate of biliary cholesterol output found in the bean-fed rats was the result of a higher rate of hepatic cholesterologenesis in these animals, we quantitated the contribution of newly synthesized hepatic cholesterol to biliary cholesterol output as shown in Fig. 4. The bean-fed rat group secreted 700% more newly synthesized cholesterol than the casein-fed group. It was also apparent that biliary cholesterol of preformed origin was also significantly increased by approximately 50% ($P < 0.05$), as shown in the left panel of Fig. 4. The

relative contribution of newly synthesized cholesterol to biliary cholesterol significantly increased from 10% in the casein-fed rats to 27% in the bean-fed animals.

DISCUSSION

The most striking finding of this study was the marked stimulation of biliary cholesterol output by nearly 300%

TABLE 2. Effect of bean intake on biliary bile acid composition (%)

Diet	Taurocholic	Glycocholic	Taurochenodeoxycholic	Glycochenodeoxycholic	Taurodeoxycholic	Glycodeoxycholic	Glycoderivatives
							Tauroderivatives
Casein (6)	61 ± 6.3	3.0 ± 3.5	30 ± 6.8	4.0 ± 5.3	4.0 ± 1.8		0.1 ± 0.1
Bean (6)	22 ± 13 ^a	29 ± 8.0 ^a	21 ± 6.5	23 ± 12 ^a	4.0 ± 0.9	2.0 ± 0.8	1.5 ± 1.1 ^a

Bile specimens were obtained from rats represented in Table 1. The number of animals in each group is shown in parentheses. Values are the mean ± SD.

^aValue is significantly different at the $P < 0.05$ level.

in rats fed a bean diet, as compared to rats fed an isocaloric and isoproteic casein diet. Biliary secretion of phospholipid was also increased 180% in the bean-fed animals. As a result of these changes in bile composition, the molar percentage of biliary cholesterol increased 200% in the animals fed the bean diet. The contribution of newly synthesized hepatic cholesterol to total biliary cholesterol increased 200% and that of preformed origin increased 50% in the rats fed the bean diet, indicating a preferential channelling of newly synthesized hepatic cholesterol into the bile in these animals. In addition, the experimental diet decreased the concentration of total serum cholesterol by 27% and VLDL cholesterol by 50%.

Biliary cholesterol originates from an intrahepatic free cholesterol pool derived from newly synthesized hepatic cholesterol and from the uptake of chylomicron and VLDL remnants, LDL, and HDL (19). The co-secretion of both biliary cholesterol and phospholipid are tightly coupled and largely dependent on the rate of biliary bile salt output (20). In this study, biliary bile salt output remained in the range found in casein-fed rats. Another factor that may influence biliary lipid output is the composition of the bile salt pool. The amount of cholesterol secreted into the bile is directly proportional to the hydrophobicity of secreted bile salts (21). Glyco-conjugated bile salts markedly increased in the bile of bean-fed rats. Glycocholic acid may increase biliary cholesterol output

by 30% compared to taurocholic acid (21). However, this factor is not sufficient to explain the effect of the bean diet on biliary cholesterol output.

It is known that several dietary maneuvers in the rat directly increase biliary cholesterol output without modifications of the rate of biliary bile salt secretion. The effect of these dietary maneuvers such as the addition of spirinolactone and pregnenolone (22), β -sitosterol, digitonin, diosgenin, or saponin (23), n-6 fatty acids (24), and n-3 fatty acids (25) apparently occur through modifications of the intrahepatic determinants of biliary cholesterol output. These determinants are related to hepatic anabolic processes such as the rate of hepatic cholesterol esterification (5, 11) and hepatic VLDL production (26). Hepatic cholesterol esterification was inhibited in the rats fed the bean diet. It is unknown whether the low serum concentration of VLDL cholesteryl esters found in the bean-fed animals represents an inhibition of hepatic VLDL synthesis and/or secretion. Theoretically, the bean diet could have some component(s) with the capacity to inhibit hepatic cholesterol esterification and VLDL production, and through this mechanism, increase the availability of free cholesterol in the pre-canalicular pool for recruitment by the bile salt-dependent mechanism. Alternatively, it may be postulated that both high hepatic cholesterogenesis and low hepatic cholesteryl esterification mainly represent the consequence, rather than the

TABLE 3. Effect of bean intake on serum cholesterol and triglyceride and on hepatic cholesterol concentration

Diet	Serum Triglyceride	Serum Cholesterol	Hepatic Cholesterol	
			Free	Ester
	mg/dl		mg/g	
Casein (10)	129 ± 40	99 ± 20	2.0 ± 0.18	0.17 ± 0.05
Bean (10)	70 ± 13 ^a	72 ± 9 ^a	2.0 ± 0.13	0.41 ± 0.10 ^a

Both groups of rats were fed the diets for 11 days prior to the experiments. The number of animals in each group is shown in parentheses. Values are the mean ± SD.

^aThe value is significantly different at the $P < 0.05$ level.

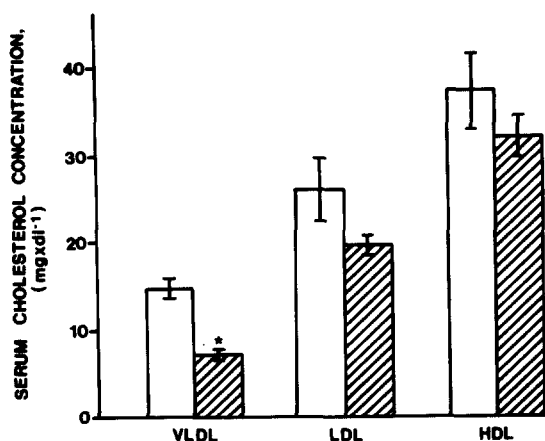


Fig. 3. Effect of the bean diet on serum lipoprotein cholesterol concentration. The hatched bars represent the animals fed the bean diet for 12 days prior to the experiments. There were six rats in each group. Bars represent the mean \pm SD. The asterisk indicates a significant difference at the $P < 0.05$ level.

cause, of the high biliary cholesterol secretory state induced by the bean diet.

There is now considerable evidence that cholesterol and phospholipids are secreted together as unilamellar cholesterol-phospholipid vesicles which interact with bile salts along the biliary tract (27, 28). It is possible that some component(s) of the bean diet could directly stimulate the formation and secretion of these vesicles, in addition to the clear effect of the experimental diet on hepatic cholesterol compartmentalization and serum lipoprotein cholesterol metabolism.

We designed the casein and bean diets to supply identical quantities of energy, carbohydrates, protein, total fat, and fiber. It is interesting to note that the bean diet had a similar hypocholesterolemic effect in both rats and humans (1). The hypocholesterolemic effect of beans has

TABLE 4. Effect of bean feeding on $[^3\text{H}]\text{H}_2\text{O}$ incorporation into digitonin-precipitable sterols and $[^{14}\text{C}]\text{oleate}$ incorporation into cholesteryl esters

Group	$[^3\text{H}]\text{H}_2\text{O}$ Incorporation into Sterols	$[^{14}\text{C}]\text{oleate}$ Incorporation into Cholesteryl Esters
	nmol/g/h	pmol/mg protein/h
Casein (6)	306 \pm 85	53.3 \pm 5
Bean (6)	830 \pm 186*	36.1 \pm 4*

All experiments were performed between 9 and 12 AM (mid-dark point at 10 AM). Rates of hepatic cholesterogenesis were measured in vivo 1 h after the intravenous injection of 15 μCi $[^3\text{H}]\text{water}$. Rates of cholesteryl ester synthesis were measured in isolated hepatocytes. The cells were resuspended in Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 2.5 mM CaCl_2 . Each incubation flask contained 0.2 ml of cell suspension (7–10 mg of protein). The flasks were incubated at 37°C and the reactions were stopped with 20 ml chloroform-methanol (2:1).

*Significant difference at the $P < 0.05$ level.

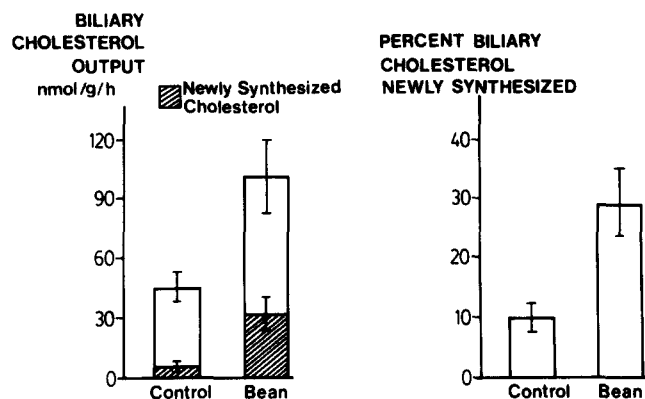


Fig. 4. Relationship between newly synthesized and total biliary cholesterol in casein- (control) and bean-fed rats. There were six rats in each group. All parameters of the bean-fed animals were significantly higher at the $P < 0.05$ level.

been attributed to their specific water-soluble fiber content (29).

One of the nondigestible water-soluble components of beans and other legumes is saponin, which has several effects on cholesterol metabolism besides the capacity to decrease serum cholesterol in experimental animals (30). Saponins may decrease cholesterol absorption (31), increase fecal steroid excretion, and increase biliary cholesterol secretion and saturation in the rat (24). It is conceivable that β -glycosidase activity in the intestinal lumen (32) may release sapogenins, which in part may be absorbed (33) and have a direct effect on the liver. It is known that the intraperitoneal injection of diosgenin, a sapogenin of the cholane group, increases biliary cholesterol secretion and saturation in the rat (24).

The present experiments are relevant to humans in that legumes, particularly beans, have a significant hypocholesterolemic effect in both rats and humans (1). In addition, biliary cholesterol saturation also increases in both species. It is unknown whether the mechanism of biliary cholesterol supersaturation described in this study in the rat is also applicable to humans fed a legume-rich diet, a common diet for Pima Indians and Chileans, who also have in common a high prevalence of cholesterol gallstone disease (1).

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