Hepatic production of very low density lipoprotein, catabolism of low density lipoprotein, biliary lipid secretion, and bile salt synthesis in rats fed a bean (*Phaseolus vulgaris*) diet

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Abstract Rats fed a bean diet develop a significant hypocholesterolemia. The catabolism of low density lipoprotein (LDL; d 1.019–1.063 g/ml) was studied in vivo and in vitro in the isolated perfused liver of rats fed either a casein or a bean diet. The clearance of LDL was significantly increased by 100% from 0.38 ± 0.04 to 0.63 ± 0.04 ml/h × 100 g body wt in vivo in the bean-fed rat. Similarly, the clearance of homologous and heterologous (human) LDL was also increased by 100% in the isolated perfused liver of bean-fed animals. Spleen, kidney, and hepatic cholesterogenesis was increased by 150% in these animals. Bile salt synthesis was increased from 1.54 ± 0.02 to 2.84 ± 0.09 nmol/min × g liver wt ($P < 0.02$) and biliary cholesterol output by 200% from 0.81 ± 0.03 to 2.18 ± 0.04 nmol/min × g ($P < 0.02$) in the isolated perfused liver of rats fed a bean diet. These results explained the depletion of hepatic cholesterol and were consistent with the LDL turnover studies, suggesting that apoB/E receptor activity was increased in these animals. ApoB and triglyceride secretion in the d < 1.060 g/ml lipoprotein fraction of liver perfusates remained normal in the bean-fed rats. In contrast, total sinusoidal cholesterol output isolated in the d < 1.060 g/ml fraction significantly decreased by 100% after 90 min of perfusion. Cholesterol output in the d > 1.060 g/ml lipoprotein fraction was unmodified by the bean diet. These data demonstrate that key metabolic pathways of hepatic cholesterol are modified in the bean-fed rat. These modifications are consistent with hypocholesterolemia induced by this legume. The marked excretion of hepatic cholesterol into the bile associated with a decreased output of sinusoidal cholesterol in apoB-containing lipoproteins suggest a functional reciprocal interrelationship between both cholesterol secretory pathways in the bean-fed animals.—**Marzolo, M. P., L. Amigo, and F. Nervi.** Hepatic production of very low density lipoprotein, catabolism of low density lipoprotein, biliary lipid secretion, and bile salt synthesis in rats fed a bean (*Phaseolus vulgaris*) diet. *J. Lipid Res.* 1993. **34:** 807–814.

Supplementary key words lipoprotein production and catabolism • biliary cholesterol

Chronic feeding of beans lowers total serum cholesterol concentration and increases biliary cholesterol output in the rat (1). Bean consumption decreases total serum and LDL cholesterol in humans (2–4) and, therefore, may have protective effects against cardiovascular disease. It has been shown that legume intake significantly increases biliary cholesterol saturation in young Chilean men, suggesting that legumes may represent a risk factor for cholesterol gallstone disease (4).

The mechanisms of the hypocholesterolemic effect of beans are unknown. One possibility is that the important induction of cholesterol secretion into the bile decreases the availability of hepatocytic cholesterol for lipoprotein production and secretion into the sinusoids. Alternatively, the depletion of metabolically active hepatic cholesterol secondary to the increment of biliary cholesterol in bean-fed rats induces the activity of sinusoidal LDL receptors, increasing the catabolism of apoB-containing lipoproteins. These possibilities are now examined experimentally by studying the catabolism of LDL and the production of hepatic lipoproteins in rats fed a bean diet. These parameters are correlated with measurements of tissue cholesterogenesis, biliary lipid secretion, and bile salt synthesis in the isolated perfused liver.

METHODS

Experimental materials and animals

Bean flour was obtained from Campex (Santiago, Chile). Vitamin mixture and mineral mixture were purchased

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; h-LDL, human LDL; r-LDL, rat LDL; PBS, phosphate-buffered saline; FCR, fractional catabolic rate.

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from Veterquímica (Santiago, Chile). Casein, cholesterol taurocholate, α cellulose, Metrizamide, DL-methionine, hydroxysteroid dehydrogenase, and NAD were purchased from Sigma Chemical Co. (St. Louis, MO). Triglyceride enzymatic colorimetric test for automated analysis was obtained from Boehringer Mannheim GmbH (Mannheim, Germany). Choline chloride was obtained from Matheson Coleman (Chio, NJ) and polyethylene catheters were from Clay Adams (Parsippany, NJ). Silastic tubes were purchased from Dow Corning Co. (Midland, MI). [24-^{14}C]taurocholic acid, [^{3}H]cholesteryl linoleyl ether, ^{125}ICl, and tritiated water were purchased from New England Nuclear (Boston, MA). All organic solvents were obtained from E. Merck (Darmstadt, Germany). Rats weighed between 180 and 240 g at the time of the experiments and were maintained as previously described (1).

Diets

The experimental diets were identical to those used in previous studies from this laboratory (1, 5). Diets were fed for 1 week prior to the experiments. Each diet contained by weight: protein, 18%; carbohydrate, 68%; fat, 5%; fiber, 4%; mineral mixture,[2] 3.5%; vitamin mixture,[3] 1%; DL-methionine, 0.3%; choline chloride 0.2%. The bean diet was compared with a casein diet containing corn flour for carbohydrates, casein for protein, α cellulose for insoluble fiber, and corn oil for fat. The energy content of the casein and bean diets was 3.930 kcal; 70% of calories was provided by carbohydrates, 12% by fats, and 18% by protein. The content of fiber was 5% in each diet.

Bile and serum specimens

Bile and serum specimens were obtained between 09:00 and 12:00 h as previously described (6). Blood was collected by aortic puncture and serum was obtained after centrifugation.

Synthesis of cholesterol from [^{3}H]H_2O in vivo

Hepatic, spleen, and kidney cholesterol synthesis was assayed in vivo based on the method of Jeske and Dietschy (7). Briefly, a tail vein catheter was inserted while the rats were under ether anesthesia and a bolus of 15 mCi [^{3}H]water contained in 0.5 ml of 0.9% NaCl solution was rapidly infused through the catheter. After 1 h the animals were again anesthetized. Five ml of blood was withdrawn from the abdominal aorta. The circulatory system of the animal was flushed in situ through the portal vein and vena cava with 10 and 30 ml of cold 0.9% NaCl solution, respectively, before the liver, spleen, and kidney were removed. Organ 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).

Isolation and administration of human (h) LDL and rat (r) LDL

Human and rat LDL (d 1.019 < d 1.060 g/ml) were isolated as previously described (1). Human LDL were radiolabeled with ^{125}ICl by the method of Bilheimer, Eisenberg, and Levy (9) for the experiments in vivo. LDL were also labeled with [^{3}H]cholesteryl linoleyl ether by the method of Stein, Halperin, and Stein (10) to study the catabolism of LDL in the isolated perfused liver.

Radiolabeled LDL were biologically tested before using them in the experiments. An aliquot of radiolabeled LDL (200,000 cpm) was injected intravenously into a rat. One hour later the animal was bled. Three ml of plasma was obtained and a Metrizamide gradient was performed to reisolate the radiolabeled LDL. Approximately 95% of the radioactivity of both ^{125}I-labeled LDL and [^{3}H]cholesteryl ether-LDL were found in the density range 1.030–1.060 g/ml, suggesting that the radiolabeling procedure did not change the physical characteristics.

Catabolism of LDL in intact rats

One day prior to the experiments rats received drinking water containing KI. Approximately 480 μg of radioiodinated apo-h-LDL was injected into fed rats through a jugular vein during diethyl ether anesthesia. Blood samples were obtained from a tail vein at intervals during the next 24 h. ^{125}I was determined in 100-μl samples of serum after precipitation in 20% trichloroacetic acid and the results were expressed as percentage of the ^{125}I in the sample taken 2–3 min after the injection (time 0). The kinetic parameters of the system were calculated by the two-compartment model of Cazin and Luyckx (11).

Catabolism of LDL in the isolated perfused liver

Liver perfusions were performed as previously described (12), except that Essential Medium Eagle containing nonessential amino acids was used in these experiments. The perfusion medium also contained 25 mM $NaHCO_3$, 2 mM L-glutamine, and 1% bovine fatty acid-free albumin. The system was continuously infused with 400 nmol sodium taurocholate per min at a rate of 0.017 ml/min. Liver perfusions were carried out for 3 h. Small samples of perfusate were removed from the circuit every

[2]The mineral mixture provided (mg/kg diet): calcium, 5,200; phosphorus, 4,000; sodium, 1,020; potassium, 3,600; magnesium, 500; manganese, 54; iron, 35; copper, 6; zinc, 30; iodine, 0.2; selenium, 0.1; chromium, 2; chloride, 1,560; sulfate, 1,000.

[3]The vitamin mixture was prepared according to American Institute of Nutrition recommendations, providing (mg or I.U./kg 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.; vitamin K, 0.05 mg.

20 min during the perfusion. The disappearance rate of [^{3}H]cholesteryl linoleyl ether-LDL from the perfusate was used to calculate the hepatic clearance of human and rat LDL (11).

Isolation of perfusate lipoproteins

Lipoprotein production by the isolated perfused liver was determined essentially as previously described (12). Perfusions were maintained for 90 min. Two lipoprotein fractions were separated by ultracentrifugation: d < 1.060 g/ml and d > 1.060 g/ml. Nascent triglyceride-rich apoB lipoproteins migrate in the d < 1.060 g/ml density fractions of perfusates and contain VLDL and LDL particles (13).

Enzyme-linked immunosorbent assay (ELISA) of apoB

The VLDL-LDL fractions isolated from the liver perfusates were assayed for apoB using an ELISA method. The standard samples were rat plasma VLDL-LDL fractions prepared by ultracentrifugation at d < 1.063 g/ml in a Beckman VTi 65 rotor at 50,000 rpm for 6 h at 10°C. The apoB concentration was measured according to the method of Egusa et al. (14). The standard samples were adsorbed overnight at 4°C to the wells of 96-well microtiter plates (Dynatec Immunolon I, Vienna, VA) containing 2.5 μg of apoB in 50 μl PBS. The plates were washed four times with a solution of PBS-0.05% Tween 20. Samples and standards, in the range of 0–0.5 μg/ml, were mixed in an Eppendorf tube with a monoclonal antibody DB-11 and then they were incubated for 2 h at 25°C in the wells of the plates. After washing four times with PBS-0.05% Tween 20, anti-mouse IgG alkaline phosphatase conjugate was added to each well 1/500, 50 μl per well. The plates were again incubated for 2 h at 25°C and then washed four times with PBS-0.05% Tween 20. Controls without standard VLDL-LDL coating plus first and second antibody and wells without the first antibody were also included. After washing, the substrate (50 μl per well of *p*-nitrophenyl phosphate, 1 mg/ml, in 0.5 M carbonate-bicarbonate buffer, 1 mM $MgCl_2$, pH 9.6) was added and the plates were incubated for 30 min. The reaction was stopped by adding 50 μl 3 M NaOH to each well. Reading was performed at 405 nm in an automated ELISA plate reader. The displacement curves of standard VLDL-LDL were similar to the lipoprotein fraction prepared from rat liver perfusates.

Bile salt synthesis in the isolated perfused liver

Total bile salt synthesis was measured between 9:30 and 11:00 AM in isolated perfused livers. Twelve to 15 h before the experiments the animals received an intraperitoneal dose of 0.4 μCi [24-^{14}C]taurocholic acid. The initial 10 min of bile collection with the liver in situ was used to calculate the specific activity of the total bile salt pool. After portal vein cannulation and transfer of the liver to the perfusion apparatus, the radioactivity measured in bile rapidly declined. At the end of the first 20 min of bile collection, <5% of bile salts secreted into the bile originated from the initial bile salt pool. Total bile salt secreted after this period for 1 h was considered newly synthesized. Bile salt synthesis was expressed as nmol per min per g liver.

Analytical methods

Cholesterol was quantitated by enzymatic methods (15). Bile salts were quantitated by the 3α-hydroxysteroid dehydrogenase method of Talalay (16). Phospholipids were measured in the chloroform–methanol extracts by a colorimetric method (17). Perfusate triglycerides were measured enzymatically by the disappearance of NADH after lipase hydrolysis (18).

Statistics

Results are expressed as the mean ± 1 SD. Statistical evaluation of the experimental results was carried out by using the unpaired *t* test.

RESULTS

Effect of bean intake on serum and hepatic cholesterol concentration, h-LDL turnover, and tissue cholesterogenesis

After 2 weeks of consuming the bean diet, the rats

TABLE 1. Serum and hepatic cholesterol and catabolism of ^{125}I-labeled h-LDL in bean-fed rats

Diet	Body Weight	Liver Weight	Serum Cholesterol	Hepatic Cholesterol		FCR	Clearance	$t_{½}$
				Free	Ester			
	g	*g*	*mg · dl^{-1}*	*mg · g^{-1}*		*h^{-1}*	*mg · h^{-1} · 100 g^{-1}*	*h*
Casein (6)	204 ± 20	7.6 ± 1.0	75 ± 11	1.9 ± 0.27	0.19 ± 0.02	0.072 ± 0.01	0.38 ± 0.04	12.4 ± 1.0
Bean (6)	200 ± 15	7.1 ± 0.8	56 ± 9[a]	2.1 ± 0.25	0.48 ± 0.05[a]	0.93 ± 0.01[a]	0.63 ± 0.04[a]	9.8 ± 0.8[a]

Rats were fed the casein and bean diets for 2 weeks prior to the experiments. The number of animals in each group is shown in parentheses. Each animal received 485 μg of ^{125}I-labeled h-LDL protein as an intravenous bolus. Values are the mean ± 1 SD. FCR represents the fractional catabolic rate of the ^{125}I-labeled h-LDL.

[a]Significant difference at the $P < 0.05$ level.

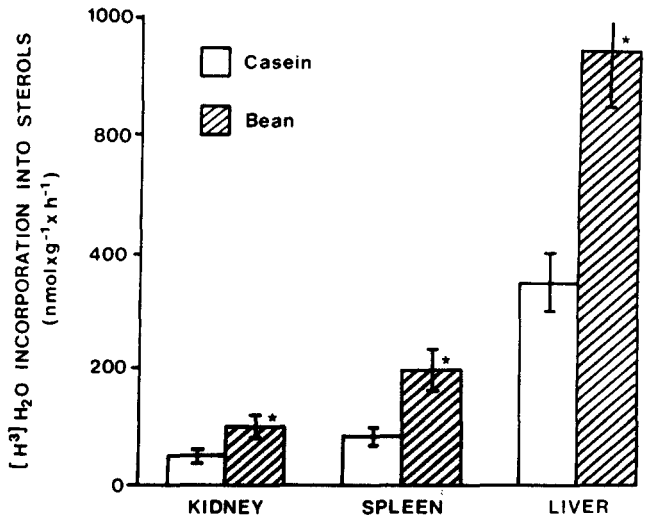

Fig. 1. Effect of bean intake on ^3H_2O incorporation into digitonin-precipitable sterols. All experiments were performed between 9 and 12 AM (mid-dark point at 10 AM). Rates of kidney, spleen, and hepatic cholesterogenesis were measured in vivo 1 h after the intravenous injection of 15 μCi [^{3}H]water. There were five casein- and six bean-fed rats. The column represents the mean ± 1 SD. The asterisks indicate a significant difference at the $P < 0.05$ level.

looked healthy. The final body weights at the time of the experiments were in the normal range, in rats fed either the casein or the bean diet. Serum cholesterol concentration decreased by 25% and hepatic cholesteryl ester concentration increased by 250% in the rats fed the bean diet as shown in **Table 1.** The first series of experiments was designed to study the effect of the bean diet on heterologous h-LDL clearance (turnover) and the rates of hepatic, kidney, and spleen cholesterogenesis in the whole animal. The clearance of h-LDL increased from 0.38 ± 0.04 to 0.63 ± 0.04 ml per h per 100 g body weight ($P < 0.05$) (Table 1). Tissue cholesterogenesis also significantly increased by more than 150% in the three organs, as shown in **Fig. 1.**

Effect of bean intake on biliary lipid secretion, homologous and heterologous LDL clearance, and bile salt synthesis in the isolated perfused liver

The rate of bile flow and biliary bile salt output by the perfused liver remained constant during the first 100 min of perfusion, declining slightly during the next 30 min. As shown in **Table 2,** the average bile flow rate remained in the normal range of approximately 1 μl per min per g of liver weight in the two groups of rats. Bile salt output was similar in the casein- and bean-fed animals. As previously shown in the whole animal (1), biliary phospholipid output increased more than 180% and biliary cholesterol output increased more than 250% in the rats fed the bean diet.

An estimate of the effect of the bean diet on total bile salt synthesis was obtained with the isolated perfused liver. In this case, exogenous taurocholate was not infused in the perfusate. Essentially all preformed bile salt molecules were washed out during the first 15 min of bile collection. Total bile salt synthesis significantly increased from 1.54 ± 0.2 to 2.84 ± 0.09 nmol per min per g liver ($P < 0.02$) in the bean-fed group of animals (results not shown).

The clearance of heterologous h-LDL and homologous LDL was studied in the isolated perfused liver; the results are shown in **Table 3.** The amount of h-LDL added to the perfusates was in the range of 0.5 mg of apoB. The fractional catabolic rate (FCR) of this lipoprotein significantly increased by 100% from 1.2% to 2.6% per h in the isolated perfused liver of the bean-fed rat. Similarly, the clearance of h-LDL was significantly increased from 1.6 ± 0.3 to 4.4 ± 1.4 ml/h · g^{-1} liver in these experiments. Because the mass of circulating r-LDL is very low, a large number of donor animals must be killed for this type of study. Therefore, we chose a lower mass of r-LDL, in the range of 0.15 mg per perfusion, as compared with the 0.5 mg of heterologous h-LDL. Under these conditions, the clearance of r-LDL increased by 300%, as compared with the h-LDL studies. The clearance of r-LDL increased from 7.2 ± 1.4 to 12.2 ± 0.8 ml/h · g^{-1} liver in the rats fed the bean diet.

Effect of a bean diet on lipoprotein production in the isolated perfused liver

As previously shown in this laboratory (12), control livers secreted triglyceride actively at the beginning of the perfusion, although net triglyceride output subsequently declined (**Fig. 2**). A similar pattern was observed in the

TABLE 2. Effect of bean intake on bile flow and biliary lipid output in the isolated perfused liver

Diet	Liver Weight	Bile Flow	Biliary Lipid Output		
			Bile Salts	Phospholipid	Cholesterol
	g	*μl · g^{-1} · min^{-1}*		*nmol · g^{-1} · min^{-1}*	
Casein (6)	7.3 ± 1.5	1.02 ± 0.23	39.4 ± 4.8	6.0 ± 0.8	0.81 ± 0.30
Bean (4)	7.5 ± 1.9	1.07 ± 0.26	38.6 ± 6.6	11.8 ± 2.7[a]	2.18 ± 0.4[a]

Liver perfusates received a continuous infusion of 0.4 μmol per min of sodium taurocholate throughout the experiment. Values of biliary lipid output correspond to the bile collected during the first hour of perfusion. Values are the mean ± 1 SD.
[a]Significant difference at the $P < 0.02$ level.

TABLE 3. Effect of bean diet on the uptake of [^{3}H]cholesteryl linoleyl ether human LDL and rat LDL by the liver

Diet	LDL Pool	FCR	Clearance	$t_{½}$
	mg	h^{-1}	$ml \cdot h^{-1} \cdot g^{-1}$ *liver*	*h*
Human LDL				
Casein (6)	0.52 ± 0.2	0.012 ± 0.002	1.6 ± 0.3	4.6 ± 0.9
Bean (4)	0.56 ± 0.3	0.026 ± 0.011[a]	4.4 ± 1.4[a]	2.4 ± 0.7[a]
Rat LDL				
Casein (3)	0.16 ± 0.03	0.032 ± 0.008	7.2 ± 1.4	1.1 ± 0.2
Bean (3)	0.15 ± 0.03	0.029 ± 0.005	12.2 ± 0.8[a]	0.7 ± 0.1[a]

Liver perfusates received a continuous infusion of 0.4 μmol per min of sodium taurocholate throughout the experiment (150 min). A bolus of the radiolabeled lipoprotein was added 10 min after the beginning of the perfusion. Values are the mean ± 1 SD.

[a]Significant difference at the $P < 0.05$ level.

liver of bean-fed rats. The bean diet did not modify apoB and triglyceride outputs, but total cholesterol output significantly decreased after 90 min of perfusion, by almost 100% in the d < 1.060 g/ml lipoprotein particles. In contrast, cholesterol output in the d > 1.060 g/ml lipoprotein particles remained constant. These experiments indicated that hepatic VLDL production remained essentially normal in the bean-fed rats; only total VLDL cholesterol output significantly decreased in these animals.

DISCUSSION

These studies demonstrate that the hypocholesterolemia

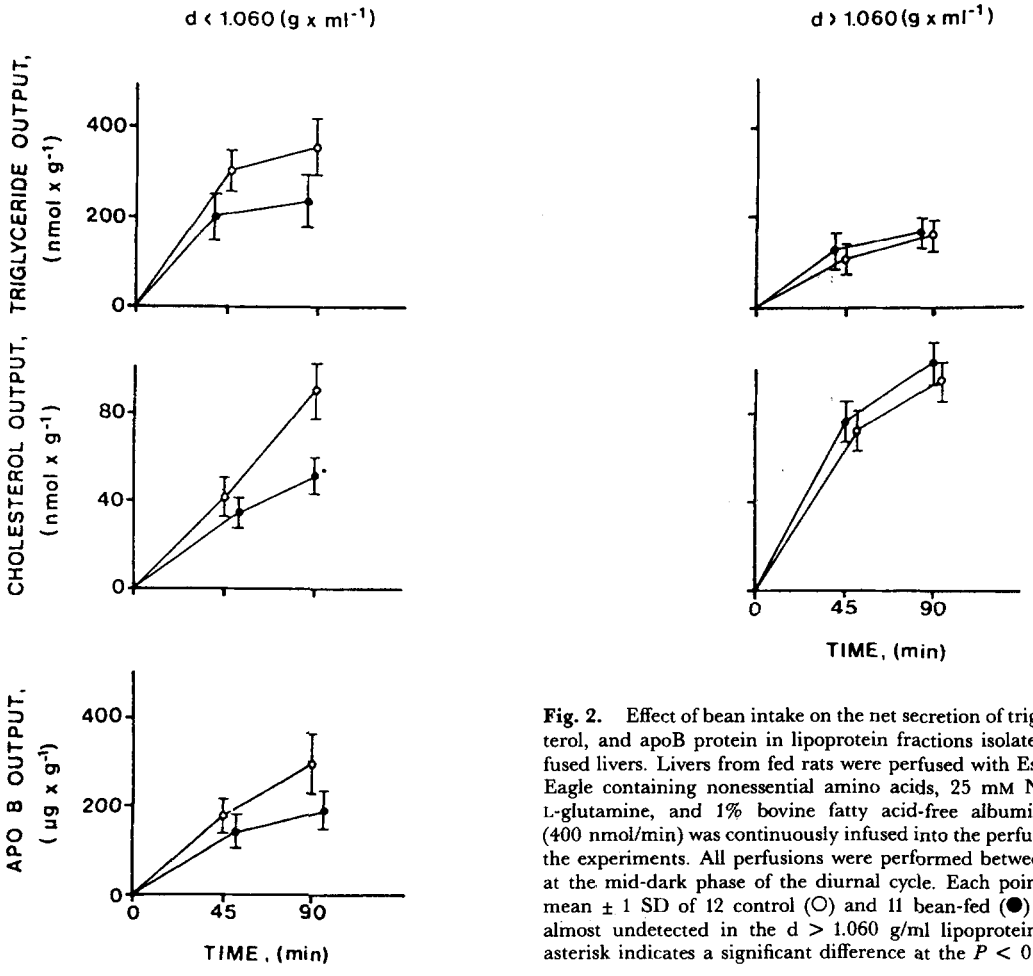

Fig. 2. Effect of bean intake on the net secretion of triglyceride, cholesterol, and apoB protein in lipoprotein fractions isolated from the perfused livers. Livers from fed rats were perfused with Essential Medium Eagle containing nonessential amino acids, 25 mM $NaHCO_3$, 2 mM L-glutamine, and 1% bovine fatty acid-free albumin. Taurocholate (400 nmol/min) was continuously infused into the perfusates throughout the experiments. All perfusions were performed between 9 and 11 AM, at the mid-dark phase of the diurnal cycle. Each point represents the mean ± 1 SD of 12 control (○) and 11 bean-fed (●) rats. ApoB was almost undetected in the d > 1.060 g/ml lipoprotein fractions. The asterisk indicates a significant difference at the $P < 0.05$ level.

induced by a bean diet is related to both an increment of the clearance of lipoproteins containing apoB/E and a lower rate of lipoprotein cholesterol secretion by the liver into the sinusoids. It was also found that peripheral tissues (i.e., kidney, spleen) as well as the liver of bean-fed rats had a higher rate of cholesterol synthesis as compared to the control animals. This observation presumably reflected a relative depletion of tissue cholesterol that could not be compensated for by the induced uptake of lipoprotein cholesterol, at least in the liver. It is interesting to note that soy feeding (another legume) increased the catabolism of LDL in human leucocytes in vitro, suggesting a derepression of the activity of the apoB/E receptor (19). It is very likely that the apoB/E receptor activity was induced in the liver of bean-fed rats, as well as in peripheral tissues, presumably as a consequence of the marked stimulation of biliary cholesterol output (1) and the significantly higher rate of bile salt synthesis demonstrated in this study.

It has been previously demonstrated that heterologous h-LDL could be cleared from the circulation through the apoB/E receptor in the rat (20), although at a rate lower than that obtained with homologous LDL (21, 22), indicating a lower affinity of the heterologous lipoproteins for the apoB/E receptor of the rat. It has also been shown that induced apoB/E receptor activity by estrogens in the rat could be detected with heterologous h-LDL (23). We took advantage of this observation to study the effect of a bean diet on apoB/E receptor activity by using h-LDL in the whole animal and in the isolated perfused liver. We also used smaller amounts of r-LDL in a limited number of perfused livers to confirm the results obtained with the h-LDL turnover studies. The liver is the most important site in the body for the uptake of LDL through the apoB/E receptor (24, 25) and the only organ for the excretion of cholesterol (biliary cholesterol and bile salts) from the body (26). A major fraction of serum cholesterol is transported in HDL in rats. These lipoprotein particles may contain apoE and, therefore, share with LDL a high affinity for the apoB/E receptor. Although total HDL concentration remained in the normal range in bean-fed rats (1), the clearance of HDL containing apoE could also increase through the apoB/E receptor in the bean-fed animals. It has been previously shown that HDL turnover is increased in diosgenin-fed rats, which have high rates of biliary cholesterol output (27). The reciprocal relationship between serum and biliary cholesterol found in the bean-fed rat is consistent with the existence of a common precursor pool of free cholesterol for both secretory pathways, very low density lipoproteins and biliary vesicles, as suggested by previous studies from this laboratory (12). This functional interrelationship found in the rat may also be present in humans and might explain, in part, the reported negative correlation between gallstone disease and serum cholesterol (28).

We also found a 100% increment in bile salt synthesis in bean-fed rats. As these animals had normal rates of biliary bile salt output (and presumably a normal bile salt pool size), it is likely that the increment in bile salt synthesis was paralleled by an increment in their fecal excretion. Although this parameter was not measured in the present study, it has been previously found that soy feeding increased fecal steroid excretion (29), blocking both the enterohepatic circulation of bile salts and the intestinal absorption of cholesterol. A number of studies have shown that newly synthesized bile salts preferentially originate from newly synthesized hepatic cholesterol (30, 31) and that biliary cholesterol preferentially originates from a different preformed metabolic pool of free cholesterol mainly fed through the hepatic uptake of serum lipoproteins (26). This metabolic compartmentalization of hepatocyte cholesterol is also supported by other studies from this laboratory (12). The expanded pool of newly synthesized free cholesterol in the microsomes could drive bile salt synthesis. This new metabolic setting in the hepatocyte would imply an overflow of metabolically active free cholesterol in the endoplasmic reticulum, which would drive the 7α-hydroxylase reaction by increasing the availability of substrate for bile salt synthesis. Another mechanism may be related to a direct derepression of the negative feedback mechanism of the 7α-hydroxylase reaction secondary to the interruption of the enterohepatic circulation of bile salts. Recent studies from this laboratory have shown that the nonsoluble component of bean fiber significantly decreased biliary bile salt output, suggesting a partial depletion of the bile salt pool (5). This situation would be compensated with more newly synthesized bile salts, as occurs with ileal resection or cholestyramine feeding (32). It is interesting to note that the hypocholesterolemia induced by feeding a bean diet was associated with the starch component of beans, which also reproduced the increment of biliary cholesterol output but not the increment of bile salt synthesis (5). These observations are important in that they suggest that conditions associated with a depletion of metabolically active hepatic free cholesterol through the biliary cholesterol secretory pathway may also contribute to derepress LDL-receptor activity at the sinusoidal membrane. We have recently found that sapogenins of beans may be the most potent stimulatory factor of the bile salt-related cosecretory mechanism of biliary cholesterol output found in the bean-fed rat (5). It has been previously shown that sapogenins, such as diosgenin, may be absorbed from the intestine and that the subcutaneous administration of plant steroids may induce biliary cholesterol secretion (33).

The hepatic production of apoB-containing lipoprotein particles is regulated by several mechanisms, including substrate availability and apoB synthesis (34). In the present studies, the sinusoidal secretion of apoB and tri-

glycerides in the d < 1.060 g/ml lipoprotein remained in the normal range in the isolated perfused liver of bean-fed rats. This observation suggested that the complex multistep mechanism of lipoprotein assembly, intracellular transport, and sinusoidal exocytosis remained normal in the bean-fed animal. It is very likely that the lower rate of sinusoidal lipoprotein cholesterol output found in the d < 1.060 g/ml fractions of perfusates of perfused livers from bean-fed rats was the consequence of a decreased availability of free cholesterol in the endoplasmic reticulum. This metabolic situation would primarily depend on the stimulation of biliary cholesterol output, as previously found in another model of biliary cholesterol hypersecretion in our laboratory (12).

It is not possible to identify a single metabolic event responsible for the hypocholesterolemia induced by beans. It is more likely that different components (i.e., starch, fiber) of the bean diet may alter differents steps in the homeostatic regulatory mechanisms of cholesterol metabolism that finally determine the derepression of the apoB/E receptor activity in the whole animal and specifically in the liver. This new metabolic setting in the hepatocyte is related to the increased canalicular secretion of both biliary cholesterol and newly synthesized bile salt molecules found in the present study and presumably to a higher elimination of fecal sterols, as occurs with soy and saponin feeding (35, 36).

The authors thank Dr. Roger Davis (University of Colorado) for the generous gift of the monoclonal antibody DB-11 anti-apoB for the quantification of apoB. The authors also wish to acknowledge Profs. Geltrude Mingrone and Aldo Greco (Universita Cattolica del Sacro Cuore, Rome, Italy) for their comments and fruitful discussions. They also acknowledge Miriem Aguad for typing the manuscript. This study was partially supported by the Direzzione Generale Cooperazione allo svilupo, Ministereo degli Affari Esteri de Italia; Istituto per la Cooperazione Universitaria (ICU); and by Grant Fondecyt 0833 from the Fondo Nacional de Desarrollo Científico y Tecnológico, Chile.

Manuscript received 8 September 1992 and in revised form 3 December 1992.

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