Effects of legume consumption on serum cholesterol, biliary lipids, and sterol metabolism in humans

William C. Duane

Department of Medicine, Veterans Affairs Medical Center and University of Minnesota, 1 Veterans Drive, Minneapolis, MN 55417

Abstract Legume consumption appears to lower serum cholesterol and to increase cholesterol saturation of bile, but the mechanisms of these effects have not been established. We studied nine human subjects on a metabolic ward during two randomly ordered 6–7 week periods: one during consumption of a control diet and the other during consumption of the same diet with 120 gm mixed legumes substituted for foods having equivalent calories, fat, protein, and carbohydrate. Mean serum LDL cholesterol was significantly lower during legume consumption (126 vs. 138 mg/dl, $P = 0.039$). Legume consumption significantly increased mean cholesterol saturation index of gallbladder bile from 1.07 to 1.26 ($P = 0.016$), largely because of an increase in hepatic secretion of cholesterol from a mean of 90.2 μmol/h to 100.8 μmol/h ($P = 0.042$). Fecal neutral sterol output was unaffected by legumes, but fecal acidic sterols increased from a mean of 861 to 1202 μmol/day ($P = 0.002$) during legume consumption. Mean sterol balance became significantly more negative during legume consumption (−2140 vs. −2700 μmol/day, $P = 0.037$) indicating an increase in cholesterol synthesis. Mean fractional absorption of bile acid was lower during legume consumption than (0.947 vs. 0.960, $P = 0.003$). These data suggest that legume consumption lowers LDL cholesterol by partially interrupting the enterohepatic circulation of bile acids and increases cholesterol saturation of bile by increasing hepatic secretion of cholesterol.—**Duane, W. C.** Effects of legume consumption on serum cholesterol, biliary lipids, and sterol metabolism in humans. *J. Lipid Res.* 1997. **38:** 1120–1128.

Supplementary key words bile acids and salts • cholelithiasis • atherosclerosis • sapogenins • triglycerides

Elevation of serum low density lipoprotein (LDL) cholesterol is a major risk factor for cardiovascular disease. Recent studies have conclusively shown that lowering LDL cholesterol diminishes both cardiovascular and overall mortality with every 1% reduction in LDL cholesterol resulting in approximately a 1% reduction in cardiovascular mortality (1, 2). This has led to more aggressive treatment of hypercholesterolemia as well as a renewed focus on modifications of life-style and diet which might promote reduction in LDL cholesterol even in subjects with relatively normal cholesterol levels.

One dietary constituent with a propensity to lower LDL cholesterol is legumes. Soybean products are known to have hypocholesterolemic effects as documented in a recent meta-analysis (3). In addition, Nervi et al. (4) found that a diet rich in mixed legumes, not including soybeans, lowered serum LDL cholesterol in Chilean men. That observation was all the more interesting because accompanying the reduction in serum cholesterol by mixed legumes was an increase in cholesterol saturation of bile.

Supersaturation of bile with cholesterol predisposes to development of cholesterol gallstones (5). Interestingly, Pima and Papago Native Americans of the Southwest, like Chileans, consume diets rich in mixed legumes (4, 6) suggesting that the legume component of the diet might be in part responsible for the increased incidence of gallstones and the decreased risk of coronary artery disease found in these populations (7, 8).

There are several potential mechanisms by which dietary legumes might affect sterol metabolism. In general, cholesterol supersaturation of bile can occur either because of increased biliary secretion of cholesterol or decreased secretion of bile acid and/or phospholipid, lipid constituents of bile that solubilize cholesterol (5). Studies in animal models have suggested that the mechanism by which legumes increase cholesterol saturation of bile is by increasing biliary se-

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; CO, carbon monoxide; HMG-CoA, hydroxymethylglutaryl coenzyme A.

cretion of cholesterol (9, 10), perhaps through effects of diosgenin, one of several sapogenins liberated from legumes during digestion (11). Diosgenin is known to stimulate cholesterol secretion in the rat (12, 13). A primary increase in biliary cholesterol secretion could conceivably deplete hepatocellular cholesterol levels thus stimulating LDL receptors and lowering serum LDL cholesterol. However, there are few data available defining the effect of legumes on biliary lipid secretion in human subjects.

Perhaps a more likely mechanism by which legumes lower serum LDL cholesterol would be reduction in intestinal re-uptake of bile acids, which would increase bile acid synthesis and lower LDL cholesterol by a mechanism similar to that of cholestyramine. It is know that legumes increase bile acid synthesis and output of fecal acidic sterols in the rat (10, 14). There is also one study in human subjects showing that consumption of soybean protein increased output of fecal acidic sterols (15). However, another study reported a reduction of fecal acidic sterol output with increased consumption of mixed legumes. In both studies, time on diet ranged between 1 and 3 weeks, an interval reported to be too short for reproducible measurement of fecal sterol outputs (16).

To further evaluate the effects of mixed legumes on sterol metabolism we studied not only serum cholesterol and biliary cholesterol saturation, but also biliary lipid secretion and fecal sterol balance in human subjects living on a metabolic ward, alternately consuming a control diet and the same diet supplemented with legumes.

METHODS

We studied nine male subjects ranging in age from 41 to 78 years (mean 58) and ranging in body mass index from 22.1 to 38.9 (mean 28.0). All were without significant medical problems as judged by previously published criteria (17), and all were shown to be free of gallstones by ultrasonography. After a detailed explanation of study procedures, each gave his written consent to participate. The study protocol was approved by committees overseeing use of human subjects in research at both the Minneapolis VA Medical Center and the University of Minnesota.

All subjects lived on the metabolic ward during the entire study and ate only meals served by the metabolic kitchen. Meals consisted of regular food selected and weighed to provide constant daily amounts of fat (23%), carbohydrate (60%), protein (17%), calories, and cholesterol. The daily intake of cholesterol varied somewhat from subject to subject (range 192 to 456 mg/day, mean 350 mg/day), but for each subject cholesterol intake was held constant throughout the study. Subjects were weighed daily to assure that caloric intake was appropriate to maintain a steady-state. The menu was repeated on a weekly basis to provide overall consistency.

Each subject was studied in two randomly ordered 6–7 week periods. During one period (control) each subject ate the above diet specifically constructed to contain no legumes. During the other period (legume) each subject consumed the same diet except 120 grams (dry weight) of mixed legumes were substituted so as to maintain calories, cholesterol, fat, carbohydrate, and protein constant. Fiber content of the legume diet averaged 24.1 gms/day and of the control diet averaged 19.1 gms/day. Fatty acid composition of the diet was not determined. The legume component of the diet consisted of 60% beans (including red, navy, and lima beans), 27% peas, and 13% lentils.

Serum lipids including cholesterol, triglyceride, and high density lipoprotein (HDL) cholesterol were measured weekly by the clinical laboratory of the Minneapolis Veterans Affairs Medical Center. For all subjects except #7 and #8, LDL cholesterol levels were calculated from the serum triglyceride level by standard methods assuming a ratio of triglyceride/cholesterol in VLDL of 5.0. Because subjects #7 and #8 had elevated serum triglycerides, their serum VLDL was separated by ultracentrifugation, and the ratio of cholesterol/triglyceride was determined using enzymatic methods (18). The three serum lipid levels from the final 2 weeks of each study period were used for statistical comparison.

For the last 20 days of each period subjects ingested 200 mg chromic oxide three times each day as a nonabsorbable marker. For the last 10 days of each period, stool was quantitatively collected. Collections for each of the five 2-day intervals were homogenized with an equal volume of water. Aliquots of these homogenates were analyzed for neutral and acidic sterols as previously reported (19). Daily acidic sterol output (A) was calculated by multiplying daily chromium intake times the mean concentration ratio of acidic sterol/chromium in stool. Daily neutral stool output (N) was calculated analogously from mean concentration ratio of neutral sterol/chromium in stool. Cholesterol balance was calculated from the formula: Balance = D − A − N, where D = daily intake of cholesterol from the diet.

For the 4 final days of each period, gallbladder bile was collected via duodenal tube using intravenous cholecystokinin octapeptide (Kinevac, Squibb & Sons, Inc., Princeton, NJ) to stimulate gallbladder contraction.

Each bile sample was analyzed for cholesterol, phospholipid, total bile acid, bile acid composition, and bilirubin as described in previous publications (17, 20, 21). Also, on at least two separate occasions, subjects underwent measurement of output of carbon monoxide (CO) on breath to estimate bilirubin production as previously described (21). These measurements permitted calculation of cholesterol saturation index by the equations of Carey and Small (5) and secretion of lipids into bile by a method we have recently described (21). This latter method utilizes the fact that CO production rate reflects both bilirubin production and secretion. Biliary secretion for any bile constituent can then be determined from the ratio of constituent/bilirubin measured in gallbladder bile multiplied by the endogenous CO production rate, which for the subjects in the present study averaged 21 μmol/h with a range of 15–28 μmol/h. We have shown that this method accurately reflects cholesterol secretion compared to standard marker perfusion techniques, and that it is also more reproducible than marker perfusion (21). We used this method in part because of this reproducibility and in part because it is much easier for the study subjects than marker perfusion measurements. The method consistently provides an estimate of bile acid and lecithin secretion that is about 25% lower than those measured by marker perfusion. However, as all subjects in the present study were studied by the same method and each subject was compared to himself in control and legume periods, the comparison should be valid and informative.

Also, from these measurements we calculated fractional absorption of bile acid by the formula: $1 - [A/(O \times 24)]$ where again A = daily fecal output of acidic sterols and O = hourly secretion of bile acid into bile. It should be noted that because our method of measuring bile acid secretion yields a consistently lower value than marker perfusion, the above estimates of absorption will be somewhat lower than those published by others. Because the same method was used in both dietary periods, the comparison should provide valid estimates of relative differences.

Statistical testing was by paired *t*-test.

RESULTS

Individual and mean values for serum total cholesterol and LDL cholesterol levels are shown in **Fig. 1.** Mean serum total cholesterol was lower during legume consumption than in the control period (194 vs. 204 mg/dl), although this change was of borderline statistical significance ($P = 0.061$). Mean serum LDL cholesterol was significantly lower during legume consumption compared to control (126 vs. 138 mg/dl, $P = 0.039$). **Table 1** provides values for HDL cholesterol and triglyceride levels, neither of which were significantly affected by legume consumption. Mean HDL cholesterol during legume consumption was 31 mg/dl compared to 32 mg/dl during control ($P = 0.171$). Mean VLDL cholesterol during legume consumption was 36 mg/dl compared to 34 mg/dl during control ($P = 0.246$). Mean triglyceride level during legume con-

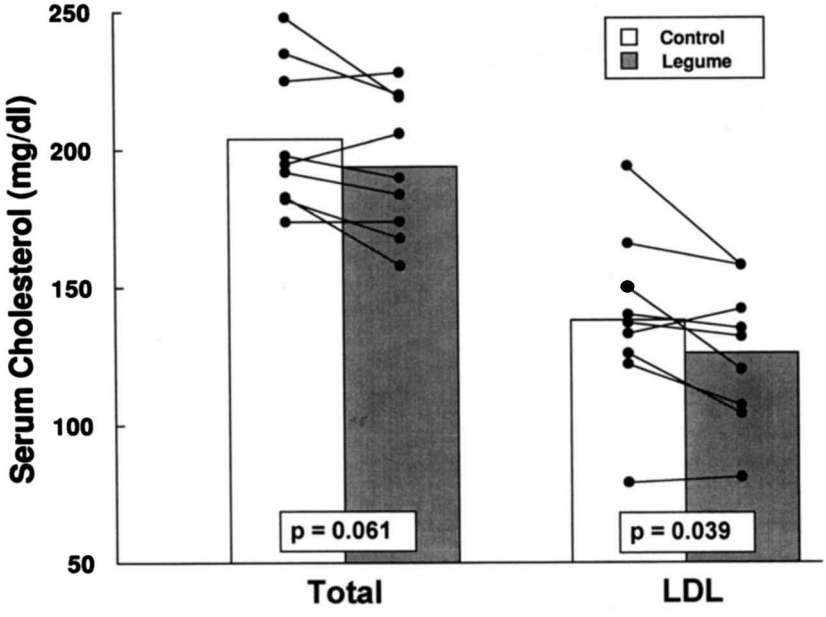

Fig. 1. Individual and mean values for serum total and LDL cholesterol levels after 6–7 weeks on the control diet (white bars) and 6–7 weeks on the legume diet (gray bars). On the legume diet mean LDL cholesterol was significantly lower than on the control diet ($P = 0.039$). Mean total cholesterol was also lower during legume consumption, but the results were of borderline statistical significance ($P = 0.061$).

TABLE 1. Serum triglyceride, HDL cholesterol, and VLDL cholesterol

Subject	Triglyceride		HDL Cholesterol		VLDL Cholesterol	
	Control	Legume	Control	Legume	Control	Legume
			mg/dl			
1	108	96	40	32	22	19
2	108	154	32	30	22	31
3	130	156	33	33	26	31
4	120	162	32	32	24	32
5	147	151	33	34	29	30
6	111	104	36	34	22	21
7	329	309	29	31	64	61
8	294	340	29	28	57	65
9	204	182	29	26	41	36
Mean	172	184	32	31	34	36
P value	0.263		0.171		0.246	

sumption was 184 mg/dl compared to 172 during control ($P = 0.263$).

Mean and individual values for cholesterol saturation index of gallbladder bile are shown in **Fig. 2.** Legume consumption significantly increased mean saturation index from 1.07 in the control period to 1.26 during legume consumption ($P = 0.016$). Examination of the data for lipid composition of gallbladder bile (**Table 2**) used to calculate saturation index showed that mean molar percent phospholipid was significantly lower during the legume period (16.8 vs. 18.5, $P = 0.030$). Mean molar percent cholesterol was higher during legume consumption (7.44 vs. 6.80), although the difference was not quite statistically significant ($P = 0.083$). Mean molar percent bile acid was not significantly altered by the legume diet (75.8 vs. 74.7, $P = 0.184$). It should be noted that because these three molar percent values are interdependent, the observed reduction in molar percent phospholipid does not necessarily imply a reduction in phospholipid secretion (see below).

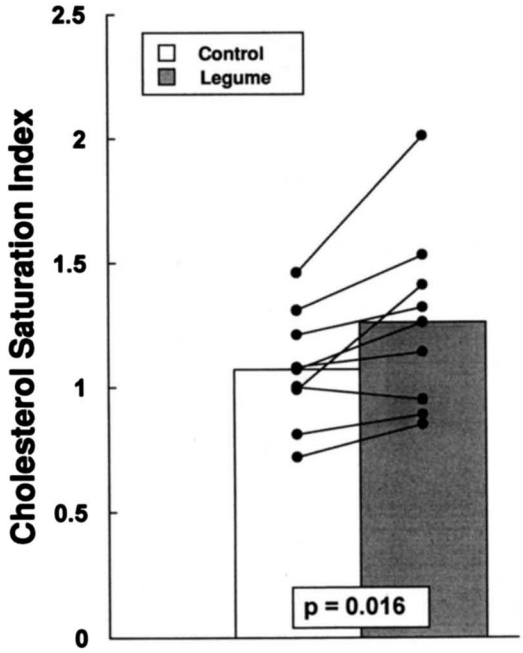

Fig. 2. Individual and mean values for cholesterol saturation index of gallbladder bile at the end of the control period (white bars) and legume period (gray bars). Legume consumption significantly increased cholesterol saturation index ($P = 0.016$).

Legume consumption did not appreciably alter the composition of individual bile acids in gallbladder bile. For legume versus control periods, the mean percentage of total bile acid was 37.2% versus 38.0% for cholic acid, 36.1% versus 36.1% for chenodeoxycholic acid, 22.8% versus 21.6% for deoxycholic acid, 2.9% versus 3.3% for ursodeoxycholic acid, and 1.0% versus 1.0% for lithocholic acid.

Values for secretion rates of biliary lipids (**Table 3**) showed a significant effect of legume consumption only on secretion of cholesterol, which averaged 100.8 μmol/h during legume consumption and 90.2 μmol/h during the control period ($P = 0.042$). Secretion of phospholipid during legume consumption averaged 242 μmol/h compared to 261 μmol/h during control ($P = 0.369$) while secretion of bile acid averaged 1090 μmol/h during the legume period and 1025 μmol/h during control ($P = 0.310$). As noted above, these values for bile acid and phospholipid secretion determined by the CO method are probably 20–30% lower than would be obtained had secretion been measured by marker perfusion (21). Even cholesterol secretion may be 10% lower than if measured by marker perfusion (21).

Individual and mean values for output of fecal sterols and sterol balance are shown in **Fig. 3.** There was a consistent and highly significant increase in output of fecal acidic sterols during consumption of legumes com-

TABLE 2. Biliary lipid composition

Subject	Bile Acid		Phospholipid		Cholesterol	
	Control	Legume	Control	Legume	Control	Legume
	molar percent					
1	75.7	74.8	16.5	16.2	7.80	9.08
2	74.0	74.6	18.2	17.2	7.83	8.18
3	71.9	77.9	20.7	15.1	7.41	7.01
4	78.1	77.8	15.8	15.7	6.14	6.50
5	76.5	76.5	17.4	17.8	6.12	5.68
6	75.7	76.2	19.6	18.4	4.73	5.36
7	73.6	75.6	17.5	13.7	8.83	10.63
8	73.8	72.6	19.6	18.4	6.61	8.95
9	72.6	76.1	21.7	18.3	5.71	5.60
Mean	74.7	75.8	18.5	16.8	6.80	7.44
P value	0.184		0.030		0.083	

pared to control (1202 vs. 861 μmol/day, $P = 0.002$). Output of neutral sterols was not significantly different during the legume period compared to control period (2400 vs. 2170 μmol/day, $P = 0.255$). Mean cholesterol balance was significantly more negative during legume consumption compared to control (−2700 vs. −2140 μmol/day, $P = 0.037$).

Fractional absorption of bile acid was consistently and significantly reduced during legume consumption compared to control (0.947 vs. 0.960, $P = 0.003$). The converse, fraction of bile acid unabsorbed, is shown in **Fig. 4** and increased from a mean control value of 0.040 to a mean of 0.053 during legume consumption ($P = 0.003$). Here too it should be noted that use of the CO method for measuring bile acid secretion provides a value 20–30% lower than values obtained by marker perfusion (21). Consequently these values for bile acid absorption are presumably proportionately lower (and the values for bile acid unabsorbed proportionately higher) than if bile acid secretion had been measured by marker perfusion.

DISCUSSION

The present study constitutes a particularly rigorous assessment of effects of dietary legumes on sterol metabolism. All subjects lived on a metabolic ward throughout the study and ate only food prepared for them by a metabolic kitchen. As a result the only substantial difference between the two study periods was the presence of legumes in place of an equivalent amount of nonlegumes in the diet. The study periods were long enough to permit full achievement of a steady-state. The two study periods were randomly assigned to eliminate potential effects of diet sequence. Each subject was studied in both periods permitting the use of paired

TABLE 3. Secretion rates of biliary lipids

Subject	Bile Acid		Phospholipid		Cholesterol	
	Control	Legume	Control	Legume	Control	Legume
	μmol/h					
1	789	589	172	128	81.3	71.8
2	810	959	190	217	76.6	101.4
3	1612	1755	462	339	165.9	156.0
4	499	651	100	132	39.0	54.2
5	1132	1488	253	332	89.0	109.8
6	1224	1262	309	303	73.7	88.1
7	636	653	157	122	72.9	84.8
8	1446	1239	382	312	128.8	152.6
9	1078	1213	321	291	84.7	88.7
Mean	1025	1090	261	242	90.2	100.8
P value	0.310		0.369		0.042	

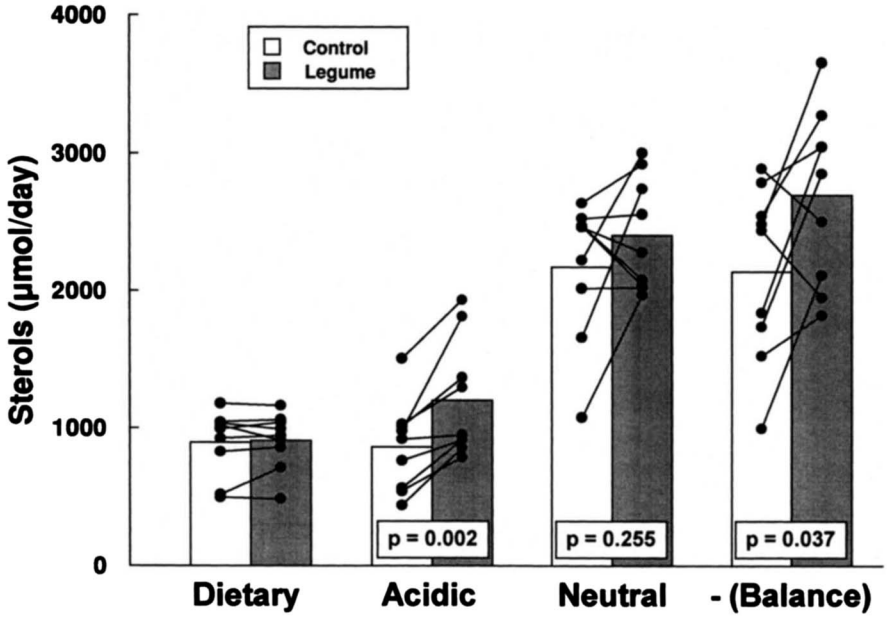

Fig. 3. Individual and mean values for dietary cholesterol, fecal output of acidic and neutral sterols, and negative cholesterol balance (equivalent to cholesterol synthesis), during the last 10 days of the control period (white bars) and the legume period (gray bars). Mean acidic sterol output was significantly higher during legume consumption ($P = 0.002$), while neutral sterol output was not significantly affected by legumes. Cholesterol balance was significantly more negative during legume consumption ($P = 0.037$).

statistical analysis and eliminating the effects of individual variation. As a result of this rigorous study design, differences between measurements in the two periods can be attributed to legume consumption with a reasonably high degree of confidence.

Legume consumption in our subjects lowered total serum cholesterol by an average of 5%, a change that was of borderline statistical significance ($P = 0.061$, Fig. 1). Legume consumption also lowered LDL cholesterol by an average of 9% ($P = 0.039$, Fig. 1). Serum HDL cholesterol, VLDL cholesterol, and serum triglyceride were all unaffected by legume consumption (Table 1). These results are similar to those reported by Nervi et al. (4), although those authors observed somewhat larger reductions in both total and LDL cholesterol (12% and 15%, respectively). Anderson et al. (22) reported even larger reductions in total and LDL cholesterol (19% and 24%, respectively), as well as a significant reduction in HDL cholesterol. However, they exclusively studied hypercholesterolemic subjects, and their subjects lost substantial amounts of weight during the 21-day legume diet period (22). Both these factors could have contributed to more pronounced reductions of serum cholesterol induced by legumes in their study compared to our own. Consumption of soybean protein is also known to lower total and LDL cholesterol, but unlike

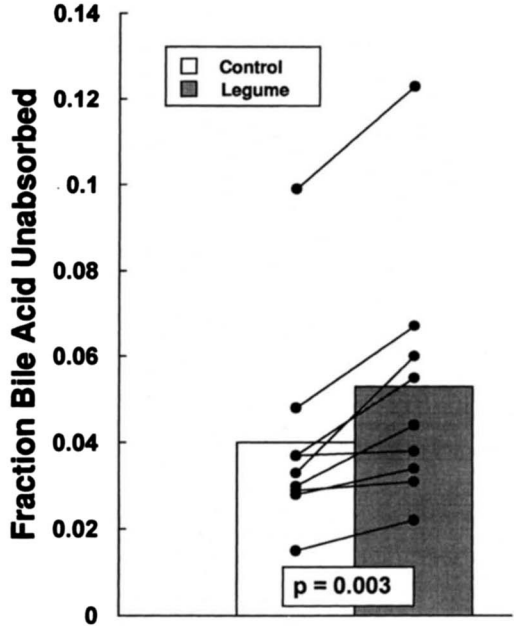

Fig. 4. Individual and mean values for fractional absorption of bile acid during control (white bars) and legume (gray bars) periods. Consumption of legumes significantly reduced bile acid absorption ($P = 0.003$).

mixed legumes, soy protein apparently also lowers serum triglyceride level (3).

The only previous study of effects of mixed legumes on biliary lipids in human subjects was that of Nervi et al. (4) in which measurements were limited to serum lipids and biliary lipid composition. Although that was a carefully controlled study using paired statistical design, the subjects were not studied on a metabolic ward. Moreover, the study periods were somewhat shorter than in the present study (30–35 days vs. 36–42 days) and all subjects were studied first in the control period followed by the legume period, rather than randomly varying the order of periods. Nevertheless, the findings of that study agree well with our own. Like Nervi et al. (4) we found that legume consumption increased cholesterol saturation index of gallbladder bile, although the magnitude of this increase was somewhat less in our study, 18% versus a 54% increase in the study of Nervi et al. In both studies the change in saturation index was, in part, the result of a significant decrease in molar percent phospholipid (Table 2). In both studies molar percent cholesterol increased during legume consumption, but the change was not quite statistically significant. Nervi et al. (4) observed a significant increase in molar percent bile acid during legume consumption. Our subjects also showed this effect, but the change was not statistically significant.

It is reassuring that the pattern of change in biliary lipid composition was similar in our subjects compared to those of Nervi et al. (4). Nevertheless, these lipid composition profiles, which consist of three interdependent percentages, do not indicate the change in lipid secretion that caused the change in composition. Our measurements of lipid secretion revealed that the major effect of legume consumption on biliary lipids was to increase secretion of cholesterol into bile (Table 3). Secretion of phospholipid trended downward and secretion of bile acid trended upward, but neither change even approached statistical significance. To our knowledge, these are the first reported measurements of biliary lipid secretion during legume consumption in humans. In the rat, bean consumption has been reported to increase output of cholesterol into bile (9, 10); however, this animal model may not be comparable to humans because output of phospholipid also increased during legume consumption in rats while phospholipid secretion in our subjects was unchanged, or may even have been slightly lower, during legume consumption.

To investigate potential mechanisms by which legume consumption might affect serum cholesterol levels and biliary secretion of cholesterol, we measured steady-state outputs of neutral and acidic sterols in stool. Legume consumption did not significantly increase fecal output of neutral sterols (Fig. 3). This finding is in agreement with the data of Anderson et al. (22) who also found that beans did not appreciably increase output of fecal neutral sterols.

On the other hand, legume consumption did significantly increase output of acidic sterols (Fig. 3). Because our subjects were in a steady-state, this finding indicates that legumes increased synthesis of bile acids. These data differ from those of Anderson et al. (22) who reported no change in acidic sterol output on a bean diet. This discrepancy could conceivably have resulted from differences in composition of study diets. Another difference in the two studies was that Anderson et al. (22) had a control period of only 1 week and a bean diet period of only 3 weeks. Moreover, many of their subjects lost weight during this 3-week period indicating lack of a steady-state. In contrast, our study periods were 6–7 weeks and none of our subjects lost weight.

Fractional absorption of bile acid, calculated from bile acid secretion and acidic sterol output, was significantly lowered by consumption of legumes (Fig. 4). This reduction of bile acid absorption likely was the reason that bile acid synthesis increased during legume consumption, just as administration of bile acid binding resins stimulate bile acid synthesis through partial interruption of negative feedback control (23–25). In conjunction with the stimulation of bile acid synthesis, legume consumption also resulted in significantly more negative cholesterol balance (Fig. 3) indicating increased cholesterol synthesis. Presumably this represents an attempt by the liver to compensate for increased conversion of cholesterol to bile acid. During treatment with bile acid binding resins the liver attempts to maintain cholesterol homeostasis, both by increasing its biosynthesis and by stimulation of LDL receptors (24, 25), which results in lowered LDL cholesterol levels. Although we did not measure LDL receptors, it is likely that the reduction of LDL cholesterol during legume consumption occurred by the same mechanism operative during treatment with bile acid binding resins, namely stimulation of LDL receptors ultimately induced by increased loss of bile acid from the enterohepatic circulation.

The mechanism by which legume consumption increased secretion of cholesterol into bile is less clear. We have previously shown that primary changes in cholesterol synthesis, induced by lovastatin, are accompanied by corresponding changes in biliary cholesterol secretion (19, 20). That does not necessarily imply, however, that increased cholesterol synthesis occurring as a compensatory response to bile acid loss would increase biliary cholesterol secretion. Indeed, treatment with bile acid binding resins has little or no affect on biliary cholesterol secretion and saturation of choles-

terol in gallbladder bile (24, 26). It seems likely, therefore, that some component of legumes, independent of their capacity to reduce bile acid absorption, increased biliary cholesterol secretion.

A potential candidate for this component is sapogenins, particularly diosgenin. Legumes contain appreciable quantities of saponins which can be hydrolyzed by intestinal bacteria to sapogenins, one of which is diosgenin (11–13). Diosgenin has been shown in animal models to greatly increase secretion of cholesterol into bile (12, 13, 27). This effect has been noted as a result of both oral and intraperitoneal administration suggesting it is a direct effect on the liver, rather than an effect in the intestinal lumen (13). Diosgenin has also been reported to increase activity of HMG-CoA reductase (27), but whether this is a cause or an effect of increased biliary secretion of cholesterol is unclear. In either case, it is possible that part of the increase in cholesterol synthesis noted in our subjects during legume consumption resulted from diosgenin generated from the saponin component of legumes.

In summary, dietary legume consumption lowers LDL cholesterol, but increases output of cholesterol into bile, an effect that could predispose to gallstone formation. Reduced bile acid absorption is apparently the major reason for the hypocholesterolemic effect of legumes. Increased hepatic secretion of cholesterol appears to be responsible for increased cholesterol saturation of bile during legume consumption, but defining the mechanism by which legumes increase cholesterol secretion requires further investigation. Judging from animal studies it would seem reasonable to begin this investigation by assessing, to the extent possible, effects of diosgenin and perhaps other sapogenins on cholesterol secretion and metabolism in human subjects.

I wish to acknowledge the dedicated organizational and technical work of Ms. Cathy Bagne, Margaret Jordan, and Linda Hanson, without which these studies would not have been possible. Ms. Heidi Hoover and the Dietetic Service of the Minneapolis VA Medical Center provided invaluable assistance in designing and preparing dietary regimens. Financial support was provided by grants from the Department of Veterans Affairs and the National Institutes of Health (R01-DK42433).

Manuscript received 17 December 1996 and in revised form 25 February 1997.

REFERENCES

1. Shepherd, J., S. M. Cobbe, I. Ford, C. G. Isles, A. R. Lorimer, P. W. Macfarlane, J. H. Mckillop, and C. J. Packard. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N. Engl. J. Med.* **333:** 1301–1307.
2. Scandinavian Simvastatin Survival Study Group. 1994. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet.* **344:** 1383–1389.
3. Anderson, J. W., B. M. Johnstone, and M. E. Cook-Newell. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.* **333:** 276–282.
4. Nervi, F., C. Covarrubias, P. Bravo, N. Velasco, N. Ulloa, F. Cruz, M. Fava, C. Severin, R. Del Pozo, C. Antezana, V. Valdivieso, and A. Arteaga. 1989. Influence of legume intake on biliary lipids and cholesterol saturation in young Chilean men: identification of a dietary risk factor for cholesterol gallstone formation in a highly prevalent area. *Gastroenterology.* **96:** 825–830.
5. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
6. Reid, J. M., S. D. Fullmer, K. D. Pettigrew, T. A. Burch, P. H. Bennett, M. Miller, and G. D. Whedon. 1971. Nutrient intake of Pima Indian women: relationships to diabetes mellitus and gallbladder disease. *Am. J. Clin. Nutr.* **24:** 1281–1289.
7. Sampliner, R. E., P. H. Bennett, L. J. Comess, F. A. Rose, and T. A. Burch. 1970. Gallbladder disease in Pima Indians: demonstration of high prevalence and early onset by cholecystography. *N. Engl. J. Med.* **283:** 1358–1364.
8. Covarrubias, C., V. Valdivieso, and F. Nervi. 1984. Epidemiology of gallstone disease in Chile. *In* Epidemiology and Prevention of Gallstone Disease. L. Capocaccia, G. Ricci, M. Angelico, and A.F. Attili, editors. MTP Press, Lancaster. 26–30.
9. Rigotti, A., P. Marzolo, N. Ulloa, O. Gonzalez, and F. Nervi. 1989. Effect of bean intake on biliary lipid secretion and on hepatic cholesterol metabolism in the rat. *J. Lipid Res.* **30:** 1041–1048.
10. Marzolo, M. P., L. Amigo, and F. Nervi. 1993. 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.* **34:** 807–814.
11. Fenwick, D. E., and D. Oakenfull. 1983. Saponin content of food plants and some prepared foods. *J. Sci. Food Agric.* **34:** 186–191.
12. Thewles, A., R. A. Parslow, and R. Coleman. 1993. Effect of diosgenin on biliary cholesterol transport in the rat. *Biochem. J.* **291:** 793–798.
13. Ulloa, N., and F. Nervi. 1985. Mechanism and kinetic characteristics of the uncoupling by plant steroids of biliary cholesterol from bile salt output. *Biochim. Biophys. Acta.* **837:** 181–189.
14. Santier, C., C. Doucet, C. Flament, and D. Lemonier. 1979. Effect of soy protein and saponins on serum, tissue and fecal steroids in rats. *Atherosclerosis.* **34:** 233–241.
15. Fumagalli, R., L. Soleri, R. Farina, R. Musanti, O. Mantero, G. Noseda, E. Gatti, and C. R. Sirtori. 1982. Fecal cholesterol excretion studies in type II hypercholesterolemic patients treated with soybean diet. *Atherosclerosis.* **43:** 341–353.
16. Lin, D. S., and W. E. Connor. 1980. The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. *J. Lipid Res.* **21:** 1042–1052.
17. Duane, W. C. 1978. Simulation of the defect of bile acid

metabolism associated with cholesterol cholelithiasis by sorbitol ingestion in man. *J. Lab. Clin. Med.* **91:** 969–978.
18. McGowan, M. W., J. D. Artiss, D. R. Strandbergh, and B. Zak. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29:** 538–542.
19. Duane, W. C. 1993. Effects of lovastatin and dietary cholesterol on sterol homeostasis in healthy human subjects. *J. Clin. Invest.* **92:** 911–918.
20. Mitchell, J. C., G. M. Logan, B. G. Stone, and W. C. Duane. 1991. Effects of lovastatin on biliary lipid secretion and bile acid metabolism in humans. *J. Lipid Res.* **32:** 71–78.
21. Duane, W. C., M. D. Levitt, and M. K. Elson. 1993. Facilitated method for measurement of biliary secretion rates in healthy humans. *J. Lipid Res.* **34:** 859–863.
22. Anderson, J. W., L. Story, B. Sieling, W. L. Chen, M. S. Petro, and J. Story. 1984. Hypocholesterolemic effects of oat-bran or bean intake for hypercholesterolemic men. *Am. J. Clin. Nutr.* **40:** 1146–1155.
23. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78:** 94–121.
24. Reihner, E., B. Angelin, M. Rudling, S. Ewerth, I. Björkhem, and K. Einarsson. 1990. Regulation of hepatic cholesterol metabolism in humans: stimulatory effects of cholestyramine on HMG-CoA reductase activity and low density lipoprotein receptor expression in gallstone patients. *J. Lipid Res.* **31:** 2219–2226.
25. Packard, C. J., and J. Shepherd. 1982. The hepatobiliary axis and lipoprotein metabolism effects of bile acid sequestrants and ileal bypass surgery. *J. Lipid Res.* **23:** 1081–1098.
26. Carrella, M., S. Ericsson, C. Del Piano, B. Angelin, and K. Einarsson. 1991. Effect of cholestyramine treatment on biliary lipid secretion rates in normolipidaemic men. *J. Intern. Med.* **229:** 241–246.
27. Cayen, M. N., and D. Dvornik. 1979. Effect of diosgenin on lipid metabolism in rats. *J. Lipid Res.* **20:** 162–174.