Effects of Soybean Protein and Very Low Dietary Cholesterol on Serum Lipids, Biliary Lipids, and Fecal Sterols in Humans

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Soy-base texturized vegetable protein (TVP; Archer Daniels Midland, Decatur, IL) has been used to decrease serum cholesterol and as a substitute for animal protein to achieve very low levels of dietary cholesterol. The effect of very low dietary cholesterol and of TVP on biliary lipids and fecal sterols is unclear. The study objective was to determine the effects of very low intake of dietary cholesterol, as well as TVP itself, on serum lipids, biliary lipids, and fecal sterols. We studied eight normal subjects living on a metabolic ward during three randomly ordered 6- to 7-week periods: (1) standard cholesterol diet (190 to 550 mg/d), (2) TVP–low-cholesterol diet (17 to 30 mg/d), and (3) TVP–standard cholesterol diet. By analysis of covariance (ANCOVA), reducing dietary cholesterol to these very low levels significantly decreased serum low-density lipoprotein (LDL) cholesterol ($P = .048$) but did not affect high-density lipoprotein (HDL) cholesterol or triglyceride. TVP resulted in a borderline significant reduction in LDL cholesterol ($P = .058$) with a highly significant reduction in HDL cholesterol ($P = .004$) and an increase in serum triglyceride ($P = .010$). During TVP ingestion, there was a highly significant increase in the output of fecal neutral sterols ($P = .005$) and a tendency for a higher output of fecal acidic sterols ($P = .100$). Fecal sterol balance was significantly more negative (indicating increased cholesterol synthesis) during TVP ingestion ($P = .016$). Neither TVP nor the very–low-cholesterol diet appreciably affected the gallbladder bile molar percent cholesterol or saturation index. The data are consistent with the hypothesis that to the extent TVP decreases serum LDL cholesterol (an effect of borderline significance in this study), the effect occurs via a reduction in the absorption of cholesterol and perhaps bile acid. However, the potential benefit of decreasing LDL cholesterol in this way seems to be at least partially offset by a concomitant reduction in HDL cholesterol and an increase in serum triglycerides.

AS PART OF A STRATEGY to control serum cholesterol, many people in Western society strive to decrease the dietary intake of fat and cholesterol. Although a modest reduction of cholesterol intake can be accomplished without major dietary modification, achieving a very low level of cholesterol intake usually requires limiting the intake of animal protein and fat. A common substitute for animal protein is texturized vegetable protein (TVP) produced from soybean flour.

Interestingly, soybean vegetable protein itself seems to decrease serum cholesterol, particularly in hypercholesterolemic subjects.[1-3] However, although there is general agreement that soy protein decreases low-density lipoprotein (LDL) cholesterol,[1] there is conflicting evidence regarding its effect on high-density lipoprotein (HDL) cholesterol and triglyceride.[1-3] Moreover, the mechanism by which soy protein alters serum lipids is unclear. Data from animal models suggest that soy protein increases the output of neutral and acidic sterols in the feces.[4-6] However, the only study of this question in humans did not confirm increased fecal sterol output.[7]

The effects of dietary modifications on biliary cholesterol are also of interest because of the known role of biliary cholesterol saturation in the pathogenesis of cholelithiasis.[8] Two early studies suggested that curtailing the intake of dietary cholesterol decreased biliary cholesterol,[9,10] but subsequent studies failed to demonstrate an effect of cholesterol consumption on biliary cholesterol.[11-15] However, none of these prior studies investigated the changes in biliary cholesterol following a reduction of cholesterol consumption to extremely low levels. Moreover, the effect of soy protein on biliary cholesterol in humans has not been systematically studied.

To help answer some of these questions, we sought to assess the effects both of very low dietary cholesterol and of TVP on serum lipids, biliary cholesterol, and fecal sterols. This was accomplished by studying subjects in three separate periods to enable separation of the effects of very low dietary cholesterol from the effects of TVP itself.

SUBJECTS AND METHODS

We studied eight men aged 43 to 80 years with a body mass index of 19.9 to 31.4 kg/m^2 (Table 1). Subjects were not selected for any particular characteristic other than normal serum lipids, good general health, and willingness to participate in these long and difficult studies. Females were not specifically excluded, but represented a fairly small percentage of the veteran population from which the study participants emerged. All subjects were without significant medical problems as judged by previously published criteria,[16] and all were shown to be free of gallstones by ultrasonography. Following a detailed explanation of the study procedures, each subject provided written consent to participate. The study protocol was approved by committees overseeing the 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 typical American foods including a variety of meats, dairy products, breads, fruits, and vegetables. These were selected and weighed to provide constant daily amounts of fat, carbohydrate, and protein. Dietary fat constituted 17% to 22% of caloric intake, and protein constituted 15% to 18% of calories. Subjects were weighed daily to ensure that caloric intake was appropriate to maintain a steady state. As a consequence of this monitoring, none of the subjects had a weight change of more than 2 lbs throughout the study. The menu was repeated on a weekly basis to provide overall consistency.

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Table 1. Age, Weight, Body Mass Index, Initial Serum Lipids, and Dietary Cholesterol Intake for Each Subject

Subject No	Age (yr)	Weight (kg)	BMI (kg/m²)	Total Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)	HDL Cholesterol (mg/dL)	Triglycerides (mg/dL)	Dietary Cholesterol (mg/d)		
								Standard Cholesterol	TVP-Cholesterol	TVP-Low Cholesterol
1	80	72	19.9	183	126	38	97	192	505	20
2*	55	92	29.2	211	73	35	120	—	499	22
3	49	95	29.3	214	147	42	124	394	525	27
4	64	72	24.4	161	93	29	193	200	505	30
5	51	99	31.4	207	139	34	169	320	515	26
6	68	82	27.2	170	94	38	191	550	502	17
7	43	82	28.4	204	132	38	168	494	497	19
8	72	56	20.5	152	99	33	102	503	494	24

Abbreviation: BMI, body mass index.

*Subject no. 2 did not complete the standard cholesterol period.

Each subject was studied in three randomly ordered 6- to 7-week periods. During one period, designated the standard cholesterol period, each subject ate the above-mentioned diet designed to contain a moderate amount of cholesterol (192 to 550 mg/d). During a second period, we attempted to remove nearly all dietary cholesterol. This was accomplished in large part by substituting TVP (TVP, a texturized vegetable protein produced from soybeans; Archer Daniels Midland, Decatur, IL) for most animal protein. This period was designated TVP–low cholesterol. In the third period, designated the TVP-cholesterol period, the diet was the same as for the TVP–low cholesterol period except that eggs were isocalorically substituted for an equivalent amount of protein and fat to return dietary cholesterol levels to the moderate range. Except for subject no. 2, who did not complete the standard cholesterol period, all subjects were studied in each of the three periods (Table 1).

Serum lipids including cholesterol, triglyceride, and HDL cholesterol were measured weekly by the clinical laboratory of the Minneapolis VA Medical Center. LDL cholesterol was calculated from serum triglyceride by standard methods, assuming a ratio of triglyceride to cholesterol in very–low-density lipoprotein of 5.0.

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, the 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.[17] Daily acidic sterol output (A) was calculated by multiplying daily chromium intake by the mean concentration ratio of acidic sterol to chromium in the stool. Daily neutral sterol output (N) was calculated analogously from the mean concentration ratio of neutral sterol to chromium in the stool. Cholesterol balance was calculated from the formula, balance = D − A − N, where D is the daily intake of cholesterol from the diet.

For the 4 final days of each period, gallbladder bile was collected via a duodenal tube using intravenous cholecystokinin octapeptide (Kinevac; Squibb & Sons, Princeton, NJ) to stimulate gallbladder contraction. Each bile sample was analyzed for cholesterol, phospholipid, and bile acid as described in previous publications.[16,18] These measurements permitted calculation of the cholesterol saturation index by the equations of Carey and Small.[8]

Statistical testing was performed by SAS software (SAS Institute, Cary, NC) using analysis of covariance (ANCOVA) with a model that accounted for subject and TVP status as discrete variables and dietary cholesterol as a continuous variable. This permitted testing for the effects of both TVP and very low dietary cholesterol intake using each subject as his own control. In addition, it accounted for within-subject variation of dietary cholesterol in the standard cholesterol and TVP-cholesterol periods. Although in some subjects, this variation was larger than the ideal level (the largest being 192 to 505 mg/d; Table 1), judging from the results of this study (vide infra) and previous studies,[11,17] we would expect this degree of variation in dietary cholesterol to exert a minimal effect, if any, on biliary lipids, sterol balance, and even serum lipids. In any case, the statistical procedure used should have compensated for whatever minimal effect resulted from this variation.

RESULTS

Figure 1 provides individual and mean values for serum total cholesterol, LDL cholesterol, and triglyceride. Relative to the standard cholesterol diet, the TVP diet with moderate amounts of cholesterol had slightly lower mean values for both total cholesterol and LDL cholesterol. The TVP–low cholesterol diet showed further reductions in mean total and LDL cholesterol. By ANCOVA, there was a significant effect of dietary cholesterol on total cholesterol ($P = .051$), but no significant effect of TVP ($P = .215$). For LDL cholesterol, there was a significant effect of dietary cholesterol ($P = .048$) and a borderline effect of TVP ($P = .058$) (Table 2).

Mean serum triglyceride was higher in both TVP periods compared with the standard cholesterol period, but there was only a slight difference in mean values between the two TVP periods. By ANCOVA, there was a highly significant TVP effect on triglyceride ($P = .010$) but no significant effect of dietary cholesterol ($P = .645$) (Table 2).

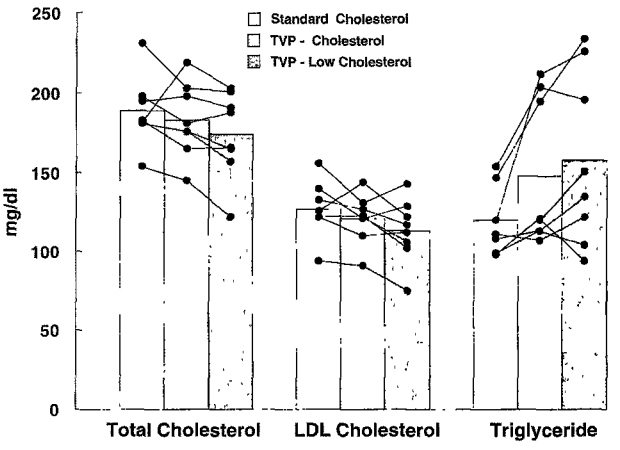

Fig 1. Individual and mean values for serum total cholesterol, LDL cholesterol, and triglyceride during 3 study periods. For statistical analysis of the data in all figures, see Table 2.

Table 2. Means Values for Each Dietary Period and *P* Values From ANCOVA Testing

Parameter	Standard Cholesterol	TVP-Cholesterol	TVP-Low Cholesterol	*P* Value: TVP Effect	*P* Value: Cholesterol Effect
Total cholesterol (mg/dL)	189	183	174	.215	.051
LDL cholesterol (mg/dL)	127	121	113	.058	048
HDL cholesterol (mg/dL)	37.6	32.0	30.4	.004	.128
Triglyceride (mg/dL)	120	148	158	.010	.645
LDL/HDL ratio	3.42	3.80	3.75	.124	.808
Acidic sterols (μmol/d)	1,040	1,320	1,260	.100	449
Neutral sterols (μmol/d)	2,440	3,210	1,930	.005	.0001
Sterol balance (μmol/d)	2,500	3,220	3,130	.016	.515
Bile salt (molar %)	77.1	75.9	77.3	242	.450
Phospholipid (molar %)	16.9	17.6	16.4	111	.178
Cholesterol (molar %)	5.96	6.46	6.28	.881	.613
Cholesterol saturation index	1.03	1.07	1.18	.363	.116

The mean HDL cholesterol level was slightly lower in the TVP–low cholesterol period versus TVP-cholesterol, but both mean values were appreciably lower than the mean in the standard cholesterol period. ANCOVA showed a highly significant TVP effect on HDL cholesterol ($P = .004$) without a significant dietary cholesterol effect ($P = .128$). The mean ratio of LDL/HDL cholesterol was similar in the two TVP periods, although both mean values were slightly higher than in the standard cholesterol period. For the LDL/HDL ratio, ANCOVA showed no significant TVP effect ($P = .124$) and no significant effect of dietary cholesterol ($P = .808$) (Fig 2 and Table 2).

Figure 3 provides individual and mean values for fecal sterols. For acidic sterol output, mean values tended to be higher during both TVP periods compared with the standard cholesterol period. By ANCOVA, the TVP effect on acidic sterol output did not reach statistical significance ($P = .100$). There was no significant dietary cholesterol effect on acidic sterols ($P = .449$) (Table 2).

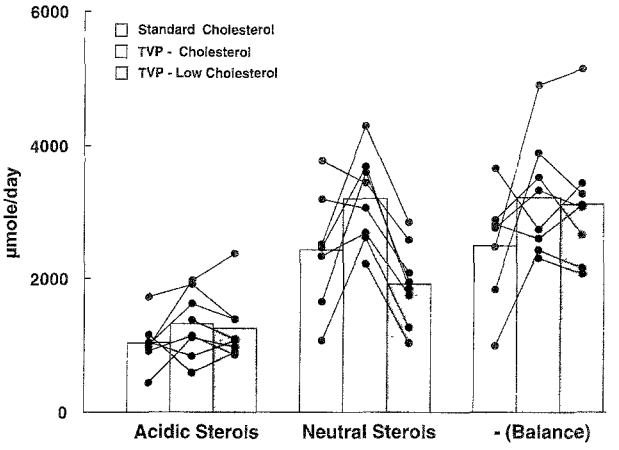

Fig 3. Individual and mean values for fecal output of acidic and neutral sterols and negative sterol balance during 3 study periods.

The mean output of neutral sterols was higher during the TVP-cholesterol period versus the standard cholesterol period (Fig 3). As expected, the mean neutral sterol output was strikingly lower during the TVP–low cholesterol period compared with the TVP-cholesterol period. For neutral sterols, ANCOVA showed highly significant effects of both TVP ($P = .005$) and dietary cholesterol ($P = .0001$).

Mean values for sterol balance were similar in the two TVP periods, but both were appreciably more negative compared with the standard cholesterol period (Fig 3). By ANCOVA, sterol balance showed a significant TVP effect ($P = .016$) but no significant dietary cholesterol effect ($P = .515$) (Table 2).

Figure 4 shows individual and mean values for the molar percent cholesterol and cholesterol saturation index of gallbladder bile. Although there were slight differences in mean values

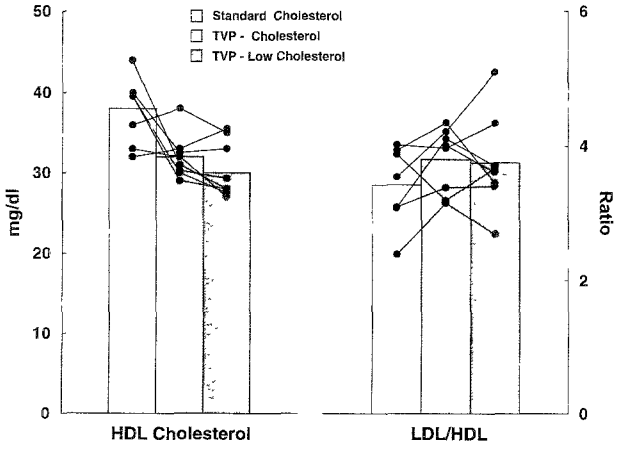

Fig 2. Individual and mean values for serum HDL cholesterol and the ratio of LDL/HDL cholesterol during 3 study periods.

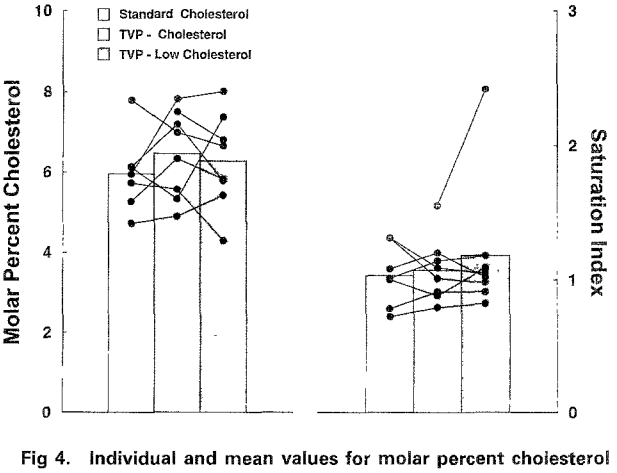

Fig 4. Individual and mean values for molar percent cholesterol and cholesterol saturation index of gallbladder bile during each of 3 study periods.

across the periods for both parameters, the changes were not statistically significant. For the molar percent cholesterol, ANCOVA showed no significant effect of either TVP ($P = .881$) or dietary cholesterol ($P = .613$). Also, for the cholesterol saturation index, there was no significant effect of TVP ($P = .363$) or dietary cholesterol ($P = .116$). Similarly, ANCOVA showed no TVP effect ($P = .242$) or dietary cholesterol effect ($P = .450$) for the molar percent bile salt and no TVP effect ($P = .111$) or dietary cholesterol effect ($P = .178$) for the molar percent phospholipid (Table 2).

DISCUSSION

As expected, decreasing dietary cholesterol to very low levels significantly decreased both total cholesterol and LDL cholesterol without appreciable changes in either HDL cholesterol or triglyceride (Figs 1 and 2 and Table 2). TVP also decreased LDL cholesterol, although the effect was of borderline statistical significance. There was a tendency for TVP to decrease total cholesterol, but the change did not reach statistical significance. It is likely that the effect of TVP on total and LDL cholesterol would be more pronounced if our subjects were hypercholesterolemic. A recent meta-analysis demonstrated that the effect of soy protein on serum cholesterol is a function of the subject's initial cholesterol.[1]

Of particular interest is the observation in our subjects that TVP decreased serum HDL cholesterol and increased serum triglyceride, both at reasonably high levels of statistical significance (Figs 1 and 2 and Table 2). Shorey et al,[2] in a study of 24 subjects, also found that soy supplementation decreased HDL cholesterol and increased triglyceride when compared with a control diet of mixed animal protein. However, in comparison to a casein protein diet, van Raaij et al[3] reported an actual increase in HDL cholesterol during soy supplementation. The meta-analysis by Anderson et al[1] reported that soy protein did not significantly affect HDL cholesterol and significantly decreased serum triglyceride. The discrepancy between studies with respect to the effects of soy on HDL cholesterol and triglyceride may be a result of differences in soy products, control diets, and study populations. In any case, our data clearly show the potential in normocholesterolemic subjects for at least one soy preparation to decrease HDL cholesterol and increase serum triglyceride. This effect may at least partially offset the beneficial effect of decreasing LDL cholesterol with TVP. Indeed, the LDL/HDL ratio tended to be slightly higher during the TVP periods, although by ANCOVA this effect was not statistically significant ($P = .124$).

In the present study, neither dietary cholesterol nor TVP had a significant effect on biliary lipid composition and cholesterol saturation. This finding is consistent with a study in monkeys showing that bile lipid composition was similar on casein versus soybean diets.[19] In humans, consumption of mixed legumes is known to increase the cholesterol content of bile,[20,21] but the effect of soy products on biliary lipids has not been systematically assessed. The lack of a TVP effect in our subjects suggests that, unlike mixed legumes, soybean products do not increase the biliary secretion of cholesterol and are therefore unlikely to predispose to formation of cholesterol gallstones.

We have previously shown that decreasing dietary cholesterol from 1,070 to 250 mg/d does not affect biliary lipid composition.[11] Four other studies of dietary cholesterol in this range also reported no significant effect on biliary lipid composition,[12-15] although two earlier studies reported increased biliary cholesterol content in response to increasing dietary cholesterol.[9,10] The present study achieved a more stringent reduction of dietary cholesterol than any of these previous studies, yet even these extreme reductions did not alter the biliary cholesterol content and saturation (Fig 4 and Table 2).

We measured the output of fecal sterols in an effort to elucidate the mechanisms by which dietary changes might affect serum and biliary lipids. These data showed a highly significant effect of TVP on neutral sterol output (Fig 3 and Table 2). In animal studies, soy products generally increase the output of neutral sterols.[4-6,22] The only previous study of soy-based diets and fecal sterol output in human subjects found no effect of soybean protein on the output of either neutral or acidic sterols.[7] However, the capacity to discern changes in fecal sterols in that study may have been compromised because the study periods were only 2 to 3 weeks in duration, which, at least in some subjects, is too short an interval for reproducible measurements of fecal sterol output.[23]

An increased output of neutral sterols must result either from an increased biliary secretion of cholesterol or a decreased intestinal absorption of cholesterol. Because our subjects demonstrated no appreciable TVP effect with respect to biliary cholesterol content (Fig 4 and Table 2), it is unlikely that TVP caused an increase in biliary cholesterol secretion. Thus, the increased output of neutral sterols during TVP ingestion most likely reflects a reduction of intestinal absorption of cholesterol. This conclusion is consistent with animal studies showing a reduction in cholesterol absorption induced by soy protein diets.[5,6] Certainly, decreased absorption of cholesterol would be a plausible explanation for the reduction of LDL cholesterol observed with TVP consumption. However, it would not explain the observed effect of TVP on HDL cholesterol and triglyceride, both of which are usually unaffected by isolated changes in cholesterol absorption.[24] Parenthetically, it is of interest that any affect of TVP on cholesterol absorption would not have been mediated by β-sitosterol, which is present at concentrations of less than 5 mg/100 g TVP, or by other phytosterols, which are present at even lower concentrations (Rasik Daftary, personal communication, July 1998).

Fecal acidic sterol output was not significantly affected by either TVP or the very low cholesterol diet (Fig 3 and Table 2), although there was a trend for increased acidic sterol output during TVP ingestion. Again, animal studies have generally found an increase in fecal acidic sterol output during soy protein ingestion,[4-6] but the results are neither as striking nor as consistent as for neutral sterol output.[22] The only other previous study of this question in human subjects found no effect of a soy diet on acidic sterol output.[7] If the increase in the mean output of acidic sterols on the TVP diet represents a true increase (Fig 3), it could help to explain not only the tendency for TVP to

decrease LDL cholesterol but also its tendency to increase serum triglyceride and decrease HDL cholesterol (Fig 1 and Table 2), both of which are known to accompany a reduction in bile acid absorption.[25-27] The absence of statistical significance precludes such a conclusion, but the trend suggests the need for further study.

Because fecal sterol balance is calculated by subtracting dietary cholesterol from total sterol output, the increase in both acidic and neutral sterols also translated into significantly more negative sterol balance on the TVP diet (Fig 3 and Table 2). Assuming that our subjects were in a steady state, which seems reasonable in view of the fairly long study periods on a metabolic ward and the stability of the subjects' body weight, this change in sterol balance indicates an increase in whole-body cholesterol synthesis during TVP ingestion. This increase in synthesis would be an expected compensatory response to a reduction in cholesterol absorption induced by TVP.

It is noteworthy that decreasing dietary cholesterol to very low levels did not appreciably decrease cholesterol synthesis (as determined by sterol balance) or increase acidic sterol output (Fig 3 and Table 2). In a previous study, we observed both compensatory responses when dietary cholesterol was decreased from 1,070 to 250 mg/d.[17] In that study, the changes may have been more easily detected because the absolute change in dietary cholesterol was about 800 mg/d, as opposed to only about 400 mg/d in the present study (Table 1). Moreover, if TVP decreased the absorption of cholesterol as appears to be the case, a given change in cholesterol intake would have even less effect on hepatic cholesterol metabolism than at higher rates of absorption.

In summary, our data in normocholesterolemic subjects demonstrate a borderline significant reduction of LDL cholesterol during ingestion of TVP, but with a concomitant reduction of HDL cholesterol and an increase of triglyceride. The mechanism of this change appears to be a reduction of cholesterol absorption. A reduction in the absorption of bile acid also may have contributed to these changes, but this possibility will require further confirmation. There appears to be no effect of TVP on biliary lipid composition.

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