Dietary Protein Effects on Gallstone Formation

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Semipurified lithogenic diets for hamsters contain casein as the protein source. Since substitution of soy protein isolate for casein reduces serum cholesterol concentrations in several species, we studied replacement of casein by soy protein for effects on gallstone formation. Feeding soy protein consistently resulted in a significantly reduced incidence of gallstones. Switching to a soy-based diet after induction of gallstones resulted in dissolution of a significant percentage of the stones. Partial substitution of soy for casein gave results intermediate between 100% casein and 100% soy. The lysine/arginine ratio of the proteins may be responsible for the observed differences in cholelithiasis. The reduction in lithogenicity associated with feeding soy protein appears to be mediated primarily through decreased secretion of cholesterol into bile.

Factors that have been associated with occurrence of cholesterol gallstones in human populations include obesity, gender and aging. It has been estimated that surgical and medical treatment for gall bladder disease accounts for more than 10% of total medical costs in the United States. Animal models for cholesterol cholelithiasis include the prairie dog and Syrian hamster. We have used the hamster to study dietary modification of gallstone formation. This paper summarizes our work to date on the effects of altering dietary protein while maintaining other components of the diet.

The diet which is used for induction of gallstones in hamsters was introduced by Dam and Christensen (1). It contains 74.3% sucrose, 20% casein, 5% mineral mix, 0.5% vitamin mix and 0.2% choline chloride. Dam and his coworkers fed numerous variants of this basic lithogenic diet in which fats were added or different types of carbohydrate were substituted for the sugar (2). However, variation of the protein source was not systematically investigated for effects on induction of gallstones. In light of the documented hypocholesterolemic effect of substitution of vegetable protein for animal protein in the diets of humans (3-6), rabbits (7,8) and swine (9), we decided to examine the effects of replacing casein in the standard lithogenic diet with soy protein isolate. Initially, we carried out four consecutive studies in which 25 to 30 hamsters per group were fed soy or casein for different lengths of time (Table 1). In each instance, feeding soy protein resulted in significantly fewer gallstones than did the standard casein-based diet ($p<0.001$) (10). Incidence of cholelithiasis in animals fed soy protein was approximately one-quarter that of those fed casein.

The effect of feeding soy protein on existing gallstones was also studied (10) (Table 2). Seventy-five hamsters were fed the casein-based lithogenic diet for 40 days. At that time one-third of the animals were examined for gallstones and a 50% incidence was observed. The remaining 50 animals were randomized into two groups that were either continued on the casein diet or transferred to the soy protein diet. After an additional 34 days, the incidence of gallstones in the hamsters fed casein was slightly increased to 58%, whereas gallstones were observed in only 32% of the animals fed soy protein. This suggests dissolution of

TABLE 1

Gallstone Formation in Hamsters: Substitution of Soy Protein for Casein in a Lithogenic Diet[a]

Duration (days)	Protein	Incidence of gallstones (%)
45	Casein	56
	Soy isolate	13
45	Casein	62
	Soy isolate	15
70	Casein	64
	Soy isolate	17
100	Casein	48
	Soy isolate	11

[a]Diet formula: 74.3% sucrose; 20% protein; 5% minerals; 1% vitamins; 0.2% choline chloride.

TABLE 2

Effect of Soy Protein of Existing Gallstones in Hamsters[a]

Protein	Duration (days)	Incidence of gallstones (%)
Casein	40	50
Casein	74	58
Casein	40	
Soy isolate	34	32

[a]See Table 1 footnote for dietary composition. Adapted from Reference 10.

TABLE 3

Gallstone Incidence in Hamsters Fed Varied Proportions of Casein and Soy Protein[a]

Protein (%)	Incidence of gallstones (%)
Casein (100)	44
Casein (75) Soy isolate (25)	38
Casein (50) Soy isolate (50)	23
Casein (25) Soy isolate (75)	15
Soy isolate (100)	12

[a]See Table 1 footnote for dietary composition.

TABLE 4

Bile Constituents (mM/dl) and Lithogenic Index of Hamsters Fed Varying Proportions of Casein and Soy Protein[a]

Protein	Cholesterol	Phospholipid	Bile acids	Lithogenic index
Casein	1.15 ± 0.54	2.51 ± 0.11	14.56 ± 0.83	0.66 ± 0.29
Casein (75%) Soy (25%)	0.94 ± 0.27	2.21 ± 0.28	18.62 ± 1.42	0.52 ± 0.14
Casein (50%) Soy (50%)	0.56 ± 0.19	2.42 ± 0.33	18.18 ± 1.96	0.34 ± 0.11
Casein (25%) Soy (75%)	0.35 ± 0.08	2.05 ± 0.21	20.16 ± 2.39	0.20 ± 0.04
Soy	0.27 ± 0.03	1.66 ± 0.06	17.33 ± 1.33	0.18 ± 0.01

[a]See Table 1 footnote for dietary composition.

gallstones in one-third of the animals fed soy protein.

We (11) and others (12) had shown previously that dilution of dietary animal protein with vegetable protein reduced serum cholesterol levels and degree of aortic atherosclerosis in experimental animals. In those studies, equal parts of animal and vegetable proteins yielded results comparable to 100% vegetable protein. In light of those findings we investigated the dilution of casein in the lithogenic diet by addition of graded amounts of soy protein isolate. Five dietary treatment groups were: (A) 100% casein; (B) 75% casein, 25% soy; (C) 50% casein, 50% soy; (D) 25% casein, 75% soy, and (E) 100% soy protein. Dilution of casein with soy protein resulted in a dose-dependent decrease in gallstone formation (Table 3). The correlation coefficient for gallstone incidence versus soy protein concentration was -0.977 ($P<0.005$). In this study we also obtained bile at the time of necropsy by direct aspiration with a μl syringe. Cholesterol (13), phospholipid (14) and bile acid (15) concentrations in the bile were determined and the lithogenic index calculated using the polynomial equation of Thomas and Hofmann (16). Although the presence of gallstones decreased the volume of bile obtained (which would tend to decrease lithogenic factors in pooled bile samples) from those animals with large and/or multiple gallstones, there were significant negative correlations of cholesterol ($r = -0.979$, $p< 0.005$), phospholipid ($r = -0.873$, $p<0.025$), and the lithogenic index ($r = -0.976$, $p<0.005$) with the percentage of soy protein in the diet (Table 4). Cholesterol concentration in the bile was most amenable to alteration by changes in dietary protein with a four-fold range in the sterol. Graded decreases in the lithogenic index paralleled decreased cholesterol concentration in the bile as soy protein replaced casein in the diet.

In an attempt to elucidate the mechanism of hypocholesterolemia associated with consumption of vegetable proteins, we have investigated the influence of the lysine/arginine ratio of dietary proteins on lipid metabolism. The lysine/arginine ratio of casein is approximately 2.0 and that of soy protein isolate is about 1.0. We have shown that the alteration of the lysine/arginine ratio of these two proteins by the addition of lysine to soy or the addition of arginine to casein to approximate the lysine/arginine ratio of the other protein alters the serum cholesterol response of rabbits and the amount of atherosclerosis they develop (17,18). An examination of three animal proteins with varying lysine/arginine ratios (but similar absolute lysine contents) for atherogenicity resulted in a statistically significant positive correlation between aortic atherosclerosis and lysine/arginine ratio (19).

TABLE 5

Influence of Lysine/Arginine Ratio of Dietary Protein on Gallstones[a]

Protein	Incidence of gallstones (%)
Casein	26
Soy isolate	8
Casein plus arginine	18
Soy plus lysine	36

[a]See Table 1 footnote for dietary composition.

Because our previous studies implicated the lysine/arginine ratio of dietary proteins as a significant mediator of serum cholesterol and aortic atherosclerosis, we investigated whether the lysine/arginine ratio of dietary protein was also a determinant of bile cholesterol saturation as reflected by gallstone incidence (20). To this end, we fed groups of 25 hamsters the standard lithogenic diet containing casein, a soy protein diet, 19.0% casein plus 1.0% arginine, or 18.8% soy protein plus 1.2% lysine. As usual, feeding soy protein resulted in significantly fewer gallstones than feeding casein. Addition of arginine to casein had a slight protective effect against lithogenesis, while addition of lysine to soy protein had a significant enhancing effect on formation of gallstones (Table 5).

A recent report on gallstones in vegetarian women as compared with meat eaters suggests some factor in the diet of vegetarians protects against gallstone formation (21). The incidence of gallstones in 632 female nonvegetarians was 25%, while in 130 vegetarians the in-

cidence of stones was 12% ($p<0.01$). While vegetarians may be characterized by a number of dietary differences that may impinge upon development of gallstones, this study does support the hypothesis that vegetable protein may decrease risk of cholelithiasis in humans.

Our series of studies in hamsters demonstrates reproducibly that the type of protein influences cholesterol saturation of bile and the incidence of cholelithiasis. Soy protein feeding results in significantly fewer gallstones than does casein feeding. Mixing the two proteins results in decreased gallstone formation as the proportion of soy protein increases. This effect appears to be mediated primarily via decreased cholesterol concentration in bile.

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