Dietary Protein Effects on Cholelithiasis in Hamsters: Interaction With Amino Acids and Bile Acids

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The objective of this experiment was to study the effects of dietary cottonseed protein and casein on plasma and biliary lipids, plasma amino acids and gallstones in hamsters. Thirty-four male hamsters (60 ± 5 g) were fed either the lithogenic "Dam Diet" (containing 20% casein, 74.3% sucrose and 5.7% vitamin-mineral mix) or a similar diet that contained 20% cottonseed protein for 30 days. Both diets contained protein as a protein isolate. The concentration of alpha-aminobutyric acid was significantly elevated in the casein-fed group. Significant differences in the total plasma cholesterol or lipoprotein cholesterol concentrations were not observed between the two dietary groups.

A significant elevation in the absolute concentration of biliary cholesterol was observed in the casein-fed hamsters. Cottonseed protein-fed animals exhibited a significantly elevated concentration of bile acids. The ratio of glycochenodeoxycholic:glycocholic acid was significantly higher in the cottonseed protein-fed group. This study reports that an elevated concentration of biliary cholesterol with a concomitant decrease in bile acid concentration yields a condition favorable to gallstone formation. It is proposed that cottonseed protein may have a specific effect on the bile acid pool by increasing the ratio of glycochenodeoxycholic acid:glycocholic acid which, in turn, prevents formation of cholesterol gallstones.

A variety of dietary factors have been implicated in development of gallstone disease. Several studies have reported a reduction in incidence of cholesterol gallstones in hamsters fed dietary plant proteins as compared with animal proteins (1-4). It has been proposed that the lysine/arginine ratio of these proteins may influence their lithogenicity via its effect on cholesterol metabolism (2,5,6). Although dietary proteins do not appear to directly affect serum cholesterol and lipoprotein cholesterol concentrations in hamsters (2,7), these proteins do affect concentrations of the three primary biliary lipid constituents (bile acids, cholesterol and phospholipids) (2-5).

It is generally accepted that the abnormality which leads to formation of lithogenic bile involves an insufficient concentration of bile acid and/or phospholipid to prevent biliary cholesterol from precipitating out of solution. Several studies using human subjects (8-17) have shown that the bile acid/phospholipid/cholesterol ratio can be altered by feeding chenodeoxycholic acid and thereby dissolving cholesterol gallstones. Ursodeoxycholic acid consumption has also been shown to be effective in dissolving cholesterol gallstones in humans (18) and hamsters (19). In prairie dogs, the incidence of gallstones can be diminished by feeding hyodeoxycholic acid (20).

Since past human and animal studies have shown that the bile acid pool can be altered by feeding specific bile acids and thereby preventing/dissolving gallstones, it is the intent of the present study to determine whether dietary proteins alter the bile acid pool and thereby diminish gallstone formation in hamsters. The specific objectives of this study were to study effects of dietary animal (casein) and plant (cottonseed) protein on serum amino acids, serum lipids (cholesterol and lipoproteins), and biliary lipids (cholesterol, phospholipids and individual bile acids) in the hamster.

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TABLE 1

Effect of Dietary Protein on Plasma Amino Acid Concentrations (μmol/l) in Hamsters[a]

Amino acid	Group 1 casein (N = 10)	Group 2 cottonseed (N = 13)	Significance
Phosphoserine	6.0 ± 2.0	7.3 ± 2.5	NS
Taurine	377.7 ± 23.4	347.7 ± 20.2	NS
Hydroxyproline	10.4 ± 2.9	18.8 ± 2.5	$p \leq 0.05$
Aspartic acid	26.6 ± 1.9	31.7 ± 2.0	NS
Threonine	225.6 ± 9.4	231.6 ± 10.8	NS
Serine	282.8 ± 17.1	313.3 ± 16.4	NS
Asparagine	62.1 ± 10.8	53.6 ± 6.7	NS
Glutamic acid	309.6 ± 16.9	343.8 ± 22.0	NS
Proline	191.1 ± 20.7	185.9 ± 22.8	NS
Glutamic acid	65.4 ± 7.6	89.0 ± 12.8	NS
Citrulline	33.7 ± 3.9	43.7 ± 3.6	NS
Glycine	312.3 ± 23.3	377.1 ± 22.9	NS
Alanine	567.3 ± 26.0	653.8 ± 30.9	$p \leq 0.05$
α-Aminobutyric acid	71.7 ± 5.9	19.9 ± 5.7	$p \leq 0.05$
Valine	389.2 ± 28.6	333.2 ± 22.2	NS
Cystine	21.7 ± 5.3	12.8 ± 3.4	NS
Methionine	63.9 ± 2.9	62.0 ± 3.4	NS
Isoleucine	143.4 ± 7.6	132.0 ± 8.1	NS
Leucine	241.4 ± 13.9	207.2 ± 12.2	NS
Tyrosine	80.6 ± 1.6	80.7 ± 2.9	NS
Phenylalanine	67.3 ± 3.2	72.0 ± 4.7	NS
Ornithine	167.7 ± 18.7	207.3 ± 23.4	NS
Ammonia	371.9 ± 45.7	302.4 ± 53.2	
Lysine	350.3 ± 13.7	356.3 ± 18.6	NS
Histidine	96.2 ± 4.1	102.4 ± 6.3	NS
Tryptophan	289.1 ± 61.9	281.7 ± 33.1	NS
Arginine	134.3 ± 16.7	115.6 ± 16.1	NS

[a]Values are means ± SE.

EXPERIMENTAL PROCEDURES

Description and care of the animals. Thirty four male Golden Syrian hamsters (Engle Laboratory, Farmersberg, Indiana) weighing 60 ± 5 g were used. Animals were housed individually in wire cages (9.5″ × 7″ × 7″) in a well-ventilated room which was illuminated from 7 a.m. to 7 p.m. Upon arrival, animals were weighed and then fed equilibration diets (Purina 5001) for seven days. Body weights were recorded every two days for the duration of

TABLE 2

Effect of Dietary Protein on the Concentration of Total Plasma Cholesterol as Well as Cholesterol Fractions and Subfractions in Hamsters[a,b]

Group	Protein source	Total serum cholesterol (mg/dl)	Total HDL-cholesterol (mg/dl)	HDL_2-cholesterol (md/dl)	HDL_2-cholesterol (mg/dl)	VLDL-LDL cholesterol (mg/dl)
1	Casein	157.9 ± 12.2[1] (N = 12)	104.5 ± 4.9[1] (N = 11)	90.5 ± 4.9[1] (N = 12)	17.6 ± 4.5[1] (N = 11)	45.5 ± 9.1[1] (N = 11)
2	Cottonseed	154.4 ± 8.4[1] (N = 12)	109.4 ± 7.5[1] (N = 12)	80.8 ± 6.0[1] (N = 12)	28.6 ± 5.0[1] (N = 12)	49.2 ± 8.4[1] (N = 12)

[a]Values are means ± SE.

[b]Means followed by different superscripts within a column are significantly different ($p \leq 0.05$).

the study. Following the equilibration period, hamsters were randomly assigned to one of two experimental groups and were fed pelleted experimental diets containing 20% casein or cottonseed protein (2) for 30 days. Diets and water were provided ad libitum throughout the experiment.

Collection and preparation of samples for analysis. Animals were fasted for 12–16 hr, anesthetized with ether and killed between 8 and 10:30 a.m. Blood was removed by heart puncture using 3-ml syringes and placed in heparinized test tubes. Plasma was separated by centrifugation (4,000 rpm) and frozen for later analysis. Gallbladder bile was aspirated using a 50-µl Hamilton syringe and was then frozen for later analysis.

Analytical techniques. Total plasma cholesterol and lipoprotein cholesterol fractions were analyzed using the enzymatic procedures of Allain et al. (21) and Warnick et al. (22), respectively. Analysis of plasma amino acids was performed using a Beckman Model 121-M Amino Acid Analyzer (23). The three primary constituents of gallbladder bile (total bile acids, phospholipids and cholesterol) were quantitatively analyzed using the methods of Turley and Dietschy (24), Trudinger (25), and Reyes and Kern (26), respectively. Individual bile acids were analyzed via high pressure liquid chromatography (HPLC) using a Beckman #112 high pressure liquid chromatograph according to the method of Kamada et al. (27).

Statistical analysis. Comparison of casein versus cottonseed protein-fed groups was done using two-sample independent t-tests. Pearson correlation coefficients were calculated to measure the strength of the relationship between selected variables (28).

RESULTS

Effect of dietary proteins on weight gain. A steady increase in weight was observed in both the casein and cottonseed protein-fed hamsters throughout the entire experimental period. Cottonseed protein-fed animals exhibited a significantly greater weight at time of sacrifice than the casein-fed animals, as has been reported in other studies (2-3).

Effect of dietary proteins on plasma amino acids and lipids. Plasma amino acid concentrations in the casein and cottonseed protein-fed hamsters are shown in Table 1. No significant differences in plasma lysine/arginine (L/A) ratios were observed in comparing the casein- and cottonseed protein-fed hamsters, although the plasma (L/A) ratio of the cottonseed protein-fed animals was higher (3.1) than that of the casein group (2.6). Significant differences were observed in three of the 28 amino acid concentrations determined. The cottonseed protein-fed group exhibited a significantly higher concentration of hydroxyproline and alanine, while the casein-fed group exhibited a significantly higher concentration of alpha-aminobutyric acid ($p \leq 0.05$).

No significant differences were observed between the casein and cottonseed protein-fed hamsters in concentrations of total serum cholesterol, total HDL-cholesterol, or HDL_2- or HDL_3-cholesterol (Table 2).

Effect of dietary protein on biliary lipids. Absolute and relative concentrations of the three primary biliary constituents (total bile acids, phospholipid and cholesterol) are shown in Table 3. A significant elevation in the absolute concentration of biliary cholesterol was observed in the casein-fed hamsters ($p \leq 0.05$). Cottonseed protein-fed

TABLE 3

Effect of Dietary Protein on Biliary Fluid Composition of Hamsters[a,b]

Group	Protein source	Absolute concentration (µmol/ml)			Relative concentration (mol %)		
		Bile acid	Phospholipid	Cholesterol	Bile acid	Phospholipid	Cholesterol
1	Casein	131.20 ± 28.05[1] (N = 6)	17.21 ± 2.83[1] (N = 9)	1.40 ± 0.13[1] (N = 8)	85.65 ± 6.4[1] (N = 5)	12.53 ± 5.3[1] (N = 5)	1.82 ± 1.0[1] (N = 5)
2	Cottonseed	198.46 ± 17.20[2] (N = 14)	13.51 ± 1.14[1] (N = 16)	0.90 ± 0.13[2] (N = 17)	93.10 ± 0.5[1] (N = 13)	6.46 ± 0.5[1] (N = 13)	0.45 ± 0.1[1] (N = 13)

[a]Values are means ± SE.

[b]Means followed by different superscripts within a column are significantly different ($p \leq 0.05$).

animals exhibited a significantly elevated concentration of total bile acids ($p \leq 0.05$). Concentrations of biliary phospholipid were not significantly different between the two groups. Significant differences in relative concentrations (molar %) of total bile acids, phospholipids or biliary cholesterol were not observed between the two groups (Table 3); however, cholesterol values were substantially increased and bile acids were decreased in casein-fed animals.

The ratios of eight individual bile acids to glycocholic acid were computed and are shown in Table 4. Glycocholic acid was chosen as the bile acid to which comparisons could be made because it was the only major bile acid present in every sample. Of the eight bile acid:glycocholic acid ratios compared, only the glycochenodeoxycholic acid:glycocholic acid ratio was significantly different between the casein and cottonseed protein-fed groups of hamsters. The animals fed cottonseed protein exhibited a significantly higher glycochenodeoxycholic acid:glycocholic acid ratio than the casein-fed hamsters ($p \leq 0.01$).

Effect of dietary protein on cholelithiasis. Eight of 10 animals (80%) in the casein-fed group exhibited gallstone formation. Only 2 of 17 hamsters (11.7%) fed cottonseed protein diets exhibited gallstone formation.

TABLE 4

Effect of Dietary Proteins (casein and cottonseed) on the Ratios of Individual Bile Acids to Gylcocholic Acid[a]

Bile acid	Casein diet	Cottonseed diet	Significance
Glycocholic acid	1.0	1.0	NS
Glycoursodeoxycholic acid	0.6 ± 0.3 (N = 7)	0.6 ± 0.3 (N = 14)	NS
Glycochenodeoxycholic acid	0.4 ± 0.1 (N = 7)	1.1 ± 0.1 (N = 15)	$p \leq 0.05$
Glycodeoxycholic acid	0.4 ± 0.2 (N = 7)	0.4 ± 0.1 (N = 14)	NS
Glycolithic acid	0.3 ± 0.1 (N = 7)	0.1 ± 0.1 (N = 15)	NS
Ursodeoxycholic acid	0.4 ± 0.4 (N = 7)	0.2 ± 0.2 (N = 15)	NS
Cholic acid	0.9 ± 0.7 (N = 7)	0.9 ± 0.4 (N = 5)	NS
Chenodeoxycholic acid	2.0 ± 1.0 (N = 7)	0..6 ± 0.5 (N = 15)	NS
Deoxycholic acid	0.9 ± 0.4 (N = 7)	0.5 ± 0.4 (N = 15)	NS

[a]Values are means ± SE.

[b]Means followed by different superscripts within a column are significantly different ($p \leq 0.05$).

DISCUSSION

Effect of dietary proteins on serum amino acids and lipids. Presently, there is no clear explanation as to how dietary proteins alter the mechanism of gallstone formation. Results from recent studies are equivocal as to possible roles that either total plasma cholesterol or the various lipoprotein fractions and subfractions may play in cholelithiasis. It has been proposed that the L/A ratio of dietary proteins may affect cholesterol metabolism and thereby influence lithogenicity (2,5,6). The present research does not reflect a direct relationship between L/A ratios in the diet and plasma L/A ratios.

Recent human (29) and animal (2,3,30) studies have shown little or no relationship between plasma concentrations of total cholesterol and biliary cholesterol. High density lipoprotein cholesterol has been associated with biliary cholesterol saturation in healthy, non-cholelithic women (31) and with incidence of gallstone disease in women (32). Other human (29) and animal studies (2,3,30) have shown no relationships between concentrations of various lipoproteins and biliary cholesterol saturation in cholelithic and non-cholelithic patients and animals. Results of the present study suggest that there is no direct relationship between concentrations of total plasma cholesterol or its various lipoprotein fractions and subfractions and biliary cholesterol in hamsters fed lithogenic diets.

Effect of dietary proteins on biliary lipids. Studies involving humans (33,34) and experimental animals (19,35,36) have shown relationships between biliary cholesterol, bile acid and phospholipid levels, and formation of cholesterol gallstones. A good deal of work has indicated that dietary proteins have at least some effect on gallstone formation by altering lipid ratios in biliary fluid (2-6,30). Studies investigating effects of dietary proteins in hamsters (1-6,30) have shown that dietary casein consistently is associated with an increase in biliary cholesterol, a decrease in biliary bile acids and an increase in gallstone formation. In contrast, plant proteins such as soy and cottonseed have the opposite effect. In the present study, the same patterns were shown to occur.

In order to develop a better understanding of the interaction between bile acids and gallstone formation, individual bile acids from animals within each dietary group were analyzed. This was particularly important, because bile acids such as ursodeoxycholic, chenodeoxycholic and hyodeoxycholic acid have been shown to have the ability to dissolve gallstones when administered in the diet of humans (10-18). The present study seems to indicate that dietary proteins may have an effect on gallstone formation by altering concentrations of specific bile acids in hamsters.

Animal studies by Pearlman et al. (37), using hamsters as the model for cholelithiasis, prevented gallstone formation by adding chenodeoxycholic and ursodeoxycholic acid to a gallstone-inducing diet. Singhal et al. (38) reported that addition of individual bile acids to the diet tends to shift bile acid composition in the direction of the dietary bile acid. It is possible that dietary cottonseed protein altered the bile acid pool by increasing the relative amount of glycochenodeoxycholic acid present, which in turn created conditions not favoring cholelithiasis.

Effect of dietary proteins on cholelithiasis. The specific mechanism by which cottonseed protein affects the bile acid pool and cholelithiasis is unknown at this time. The significantly elevated ratio of glycochenodeoxycholic:glycocholic acid in the cottonseed protein-fed group may reflect a greater concentration of plasma glycine. Concentration of plasma glycine in the cottonseed protein-fed hamsters was greater than in the casein-fed animals, although not significantly. It is proposed that this

increase of glycine in the blood may increase the glycine conjugated bile acids, thereby increasing the amount of cholesterol held in solution in the bile.

Gallstones form when the concentration of bile acids and/or phospholipid is insufficient to prevent cholesterol from precipitating out of solution. This and other studies (2,3,30) have shown that an elevated concentration of biliary cholesterol with a concomitant decrease in the concentration of bile acids yields a condition favorable to gallstone formation. It is proposed that cottonseed protein may have a specific effect on the bile acid pool by increasing the ratio of glycochenodeoxycholic acid:glycocholic acid, which in turn prevents the formation of cholesterol gallstones.

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