Effect of Type of Dietary Fat, Cholesterol and Chenodeoxycholic Acid on Gallstone Formation, Bile Acid Kinetics and Plasma Lipids in Squirrel Monkeys

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ABSTRACT To explore the effect of type of dietary fat, cholesterol and chenodeoxycholic acid on gallstone formation, bile formation, bile composition, bile acid kinetics and plasma lipids in squirrel monkeys, 39 monkeys were studied using seven different diets. Safflower oil, a highly unsaturated fat, added to a diet with cholesterol resulted in as least as high an incidence of cholesterol gallstones as butter added to the same diet. On the other hand, diets with high levels of saturated or unsaturated fat without cholesterol did not result in gallstone formation. Dietary chenodeoxycholic acid (0.1%) did not reduce the incidence of cholesterol gallstones, although the proportion of bile acids as chenodeoxycholic acid increased. Gallbladder bile from monkeys fed semipurified diets with cholesterol had a significantly higher lithogenic index than the comparable groups without cholesterol. Pool sizes of bile acids in all semipurified diet groups were reduced and the lithogenic indices were increased compared with the group fed a commercial feed. Dietary chenodeoxycholic acid caused a decrease in plasma cholesterol in butter groups and an increase in triglyceride concentrations in safflower groups. Diet influences bile composition and bile acid kinetics, as well as the incidence of gallstones, in squirrel monkeys. J. Nutr. 106: 1123–1134, 1976.

INDEXING KEY WORDS gallstones • bile kinetics • dietary lipid • chenodeoxycholic acid • squirrel monkey

In patients with cholelithiasis, the hepatic synthesis and biliary secretion of cholesterol appear to increase whereas the synthesis and pool size of bile acids decrease (1–6) with proportionally less chenodeoxycholic acid in the bile than in healthy subjects (7). Many patients with cholesterol gallstones are now being treated with chenodeoxycholic acid. Since bile promptly returns to the supersaturated state and gallstones recur after chenodeoxycholic acid therapy is discontinued, continuous or intermittent prophylactic therapy is necessary. No significant hepatic dysfunction in man has been associated with chenodeoxycholic acid treatment (8–10), but some animal studies have raised

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the possibility of hepatotoxicity (11-14). Squirrel monkeys fed certain semipurified diets are susceptible to the formation of cholesterol gallstones (15-17). Although not all of the nutritional factors that affect gallstone formation in squirrel monkeys have been established, the level of dietary cholesterol and the level and type of fat are probably important. These studies were designed to explore the effects of type of dietary fat, cholesterol and chenodeoxycholic acid on gallstone formation, bile acid kinetics and plasma lipids in squirrel monkeys.

MATERIAL AND METHODS

Experimental animals and diets. Thirty-nine (19 male and 20 female) sexually mature squirrel monkeys (Saimiri sciureus), weighing from 550 to 1,200 g, were divided into seven groups and fed different diets for 24 months. The mean weight at the beginning of the experiments (mean ± se) was 913.7 ± 30.0 g for males and 696.5 ± 15.3 g for females. The mean weights of the seven groups were similar at the beginning and at the end of the experiments. The basal semipurified diet is described in table 1 and the distribution of monkeys into different diet groups and the incidence of gallstones after 1 year are shown in table 2.

**TABLE 1**

Composition of the basal semipurified diet

<table>
<thead>
<tr>
<th>%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>21.25</td>
</tr>
<tr>
<td>Sucrose</td>
<td>38.46</td>
</tr>
<tr>
<td>Fat</td>
<td>20.19</td>
</tr>
<tr>
<td>Salt mix</td>
<td>3.40</td>
</tr>
<tr>
<td>Synthetic fiber</td>
<td>15.00</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.70</td>
</tr>
</tbody>
</table>

1. Hepsted IV Salt Mix (%): CaCO3, 29.9740; CaHPO4·2H2O, 7.4950; CuSO4·5H2O, 0.0290; FeC6H12O7·H2O, 2.7470; MgSO4·7H2O, 10.1910; MnSO4·4H2O, 0.4990; KI, 0.0799; KH2PO4, 32.2220; NaCl, 16.7550; ZnCl2, 0.0249 (56). 2. Alphacel (ICN Pharmaceutical Inc., Cleveland, Ohio.) 3. The vitamin mix contained per kilogram of diet: 12.5 mg retinyl acetate, 100 mg α-tocopherol, 500 mg ascorbic acid, 1 g inositol, 5 g choline, 40 mg menaquinone, 49 mg niacin, 10 mg riboflavin, 10 mg thiamin, 10 mg pyridoxine, 20 mg calcium pantothenate, 0.2 mg biotin, 1 mg folic acid, 0.02 mg vitamin B12, 0.05 mg crystalline cholecalciferol and 10.24 g dextrose.

Procedure for determining bile acid kinetic factors. The purity of [24-14C] Cholic acid and [24-14C] chenodeoxycholic acid was evaluated in the thin-layer chromatographic system of Mitropoulos and Myant (18). In some cases, the bile acids were further purified with thin-layer chromatography. At 1500 hours, the monkeys fed diets for 16 to 24 months were injected intravenously with 1.5 μCi each of C-labeled cholic and chenodeoxycholic acids which had been neutralized with dilute NaOH and made isotonic with saline. The animals were given access to their diet until 2300 hours. During digestion, the labeled compounds were well mixed with the bile acid pool so that sampling of the bile acid pool some time later (greater than 6 hours) gave a valid point on the specific activity decay curve (19).

At 0900 hours the next day, 18 hours after isotope injection and 10 hours after their last meal, surgery was performed to obtain a small sample (about 50 μl) of gallbladder bile. The samples (termed α samples) were obtained through a fine needle inserted into the gallbladder. The openings were closed with fine ligatures and the sample sites were thoroughly wiped with sponges to ensure recovery of all radioactivity. The monkeys were again given access to their food by 1600 hours. A second sample of bile was obtained by emptying the gallbladder 1 week later (β sample).

Analysis of bile. Measured aliquots of gallbladder bile which were obtained after overnight fasting at cholecystotomy 1 year or more after the inception of the special diets were transferred to cholecystotomy 1 year or more after the inception of the special diets were transferred to cholecystotomy 1 year or more after the inception of the special diets were transferred to 12 ml of chloroform-methanol (2:1) and stored at 2°C for analysis.

We used the method of Bartlett (20) to measure phospholipids on lipid fractions from gallbladder bile and thin-layer chromatography (16) to measure cholesterol and different bile acids of the gallbladder.

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4. Mallinckrodt Chemical Works, St. Louis, Missouri 63147.
TABLE 2

Description of diets and frequency of cholesterol gallstones in squirrel monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet code</th>
<th>Diet</th>
<th>Gallstones at 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Bu + Chl</td>
<td>Semipurified diet with butter 19.5% (40.9% of energy), corn oil 0.7% (1.43% of energy), and 0.38% cholesterol (0.9 mg/kcal)</td>
<td>5/6 (3/3F)</td>
</tr>
<tr>
<td>B</td>
<td>Bu</td>
<td>Same as A but without cholesterol</td>
<td>0/6</td>
</tr>
<tr>
<td>C</td>
<td>Bu + Chl + CDCA</td>
<td>Same as A but with CDCA 0.1%</td>
<td>4/6 (1/3F)</td>
</tr>
<tr>
<td>D</td>
<td>Sn + Chl</td>
<td>Semipurified diet with safflower oil 20.2% (42.4% of energy) and cholesterol 0.38% (0.9 mg/kcal)</td>
<td>5/6 (3/3F)</td>
</tr>
<tr>
<td>E</td>
<td>Sn</td>
<td>Same as D but without cholesterol</td>
<td>0/6</td>
</tr>
<tr>
<td>F</td>
<td>Sn + Chl + CDCA</td>
<td>Same as D but with CDCA 0.1%</td>
<td>6/6 (3/3F)</td>
</tr>
<tr>
<td>G</td>
<td>Stock</td>
<td>Commercial feed + vitamin supplements</td>
<td>0/3</td>
</tr>
</tbody>
</table>

1 All groups except G consisted of three males and three females. Group G consisted of one male and two females. 2 Numbers in column = animals with gallstones/total animals. 3 Numbers in parentheses = females with gallstones/total females. 4 CDCA = chenodeoxycholic acid, kindly supplied by Dr. Peter Ziegler of Canada Packers. We could not detect lithocholic acid in thin-layer chromatographic plates that were run with high levels of chenodeoxycholic acid. 5 Purina monkey chow, Ralston Purina Co., St. Louis, Missouri. 6 The mean weights of gallstones in afflicted animals were 76.6, 39.5, 76.6, and 102.2 mg for groups A, C, D, and F. 7 The same vitamin supplement that was in the semipurified diets (Footnote to Table 1), but at one half the final concentration (w/w), was added.

Bile. Free bile acids and cholesterol were isolated after alkaline hydrolysis, acidification, and extraction with diethyl ether. Silica gel G thin-layer plates had 15 lanes, 10 for biological samples and 5 for standards—0.5, 1, 2, 3, and 4 μg each of cholic, deoxycholic, chenodeoxycholic, and lithocholic acids and cholesterol. After development in the system of Mitropoulos and Myant (18), spots were developed with phosphomolybdic acid and quantified with a thin-layer scanner on the basis of peak heights. The precision (coefficient of variation) for 20 duplicates was 5.1%. After α and β samples of gallbladder bile were analyzed for cholesterol, phospholipids, and the different bile salts, radioactivity measurements on the bile acids were made by scraping the appropriate areas into scintillation vials and adding scintillation fluid. Samples were counted on a liquid scintillation spectrometer and corrected by a channels ratio method. Recovery was greater than 97%.

Calculation of pool sizes and biological half-lives of bile acids. In a preliminary experiment, the sampling procedures were repeated three times, 4, 8, and 13 days after the first bile sampling, on two squirrel monkeys fed the commercial diet. One monkey died a few days after the fourth operation, but the other gave a linear biological decay curve of specific activity versus time on logarithmic-arithmetic paper for both bile acids (fig. 1). Since bile salt metabolism apparently followed simplified first-order kinetics (see also 21), we fixed on a 2-sample procedure for most of our kinetic studies. The second or β sample

![Graph showing specific activity decay curves for chenodeoxycholic acid.](https://academic.oup.com/jn/article-abstract/106/8/1123/4768873/112.-(

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8 Farrand VIS-UV.  
9 Toluene with OmniFluor + BBS-3 (10%).  
10 Model 3003, Packard.
was obtained 7 days after the first (7 days + 18 hours after isotope injection).

The pool size of each bile acid was determined from the specific activity of the zero time intercept of the line joining the $\alpha$ and $\beta$ values for gallbladder bile according to the formula:

$$\text{pool size} = \frac{\text{total radioactivity of bile acid injected}}{\text{specific activity at 0 time intercept}}.$$

The radioactivity lost by the $\alpha$ sample collection of gallbladder bile was less than 5% and was ignored when half-life was determined from the decay curve.

The fractional turnover rate in days$^{-1}$ was

$$\text{the natural log 2 \over \text{half-life (days)}}.$$

The daily replacement rate (synthesis + absorbed dietary bile acid) of each bile acid was equal to the pool size $\times$ the fractional turnover rate. The pool size and the daily replacement rate were then expressed on the basis of kg of body weight.

**Liver biopsies.** One year after the inception of the special diets, we opened but did not remove the gallbladder (cholecystotomy) of squirrel monkeys to determine the extent of gallstone formation. We took approximately 200 mg pieces of liver from the median parts of the central lobes and put them immediately into Hollander-Bouin solution. After overnight fixation, these samples were washed, embedded in paraffin, cut, and stained with hematoxylin-eosin and PAS.$^{11}$

**Blood samples.** Several times during the experiments, the following determinations were performed on blood samples: total cholesterol concentration, hemoglobin, red blood cell count, hematocrit, serum alkaline phosphatase, serum glutamic pyruvic transaminase, total protein, albumin/globulin ratios, triglycerides, and lipoproteins by agarose electrophoresis.

Calculations of SEM and the use of Student’s $t$ test to determine the statistical differences between means were performed according to procedures outlined by Snedecor and Cochran (22).

RESULTS

**Effect of diet and chenodeoxycholic acid on the composition of gallbladder bile and the incidence of stones.** The incidence of cholesterol gallstones for squirrel monkeys fed different diets is shown in table 2. As in studies by Melchior et al.$^{5,6}$ highly unsaturated fat added to the cholesterol diet (groups D and F) resulted in at least as high an incidence of gallstones as butter added to the same diet (A and C). The incidence of gallstones in monkeys of group A (fed diets with butter plus cholesterol) in this experiment was high compared to our overall experience with monkeys fed that diet for 1 year or longer (53.6%; 30 of 56). On the other hand, diets with high levels of saturated or unsaturated fat without cholesterol did not result in gallstone formation (B and E). Neither the three monkeys fed the commercial monkey diet (G) during this experiment nor 37 fed previously had gallstones. Dietary chenodeoxycholic acid (0.1%) did not reduce the incidence of gallstones (C and F).

Of the monkeys fed diets containing cholesterol (A, C, D, and F), 15 of the 15 that had their gallstones removed as completely as possible during the first cholecystotomy after consuming the same diet for 1 year showed a recurrence of stones during a second cholecystotomy whereas two others that were originally free of stones remained so. No obvious sex differences in susceptibility to gallstones were observed in any diet group.

The relative molar composition of three constituents in gallbladder bile (total bile acids, cholesterol, and phospholipid, where the total of the three constituents is equal to 100%) are shown in table 3. The mean molar percentages of cholesterol were highest in the groups fed the cholesterol diets (A, C, D, and F) and lowest in those fed diets free of cholesterol (B, E, and G), especially in group C. Although there were only three monkeys fed the modified commercial diet (G) in this experiment, the mean molar percentage of biliary cholesterol was consistently low in the past (1.99% = mean for 25 monkeys). We have expressed the relative saturation or lithogenic index of gallbladder bile by the

$^{11}$ Periodic acid Schiff.
### TABLE 3
The concentration of total lipids, relative concentrations of different constituents and the lithogenic indices of gallbladder bile from squirrel monkeys of 7 groups

<table>
<thead>
<tr>
<th>Group diet code</th>
<th>A Bu+Chl 6</th>
<th>B Bu 6</th>
<th>Bu+Chl+CDCA 5</th>
<th>C Sa+Chl 6</th>
<th>D Sa+Chl 6</th>
<th>E Sa+Chl+CDCA 6</th>
<th>F Stock 3</th>
<th>G Stock 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids, (w/v %)</td>
<td>8.90 ± 2.34*</td>
<td>10.05 ± 1.15a</td>
<td>11.73 ± 2.82a</td>
<td>6.39 ± 0.93a</td>
<td>11.06 ± 1.02b</td>
<td>8.82 ± 1.41*</td>
<td>6.97 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, (molar %)</td>
<td>6.5 ± 0.9a</td>
<td>3.3 ± 0.5a</td>
<td>5.0 ± 0.4a</td>
<td>9.2 ± 1.4a</td>
<td>3.8 ± 0.4d</td>
<td>10.3 ± 2.7a</td>
<td>1.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>with cholesterol gallstones</td>
<td>6.8 ± 1.0d</td>
<td>5.3 ± 0.6a</td>
<td>10.2 ± 1.2a</td>
<td>10.3 ± 2.7a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile acids, (molar %)</td>
<td>70.4 ± 2.6a</td>
<td>72.7 ± 1.8a</td>
<td>73.6 ± 1.1a</td>
<td>67.5 ± 2.6a</td>
<td>73.8 ± 2.4a</td>
<td>65.5 ± 1.5a</td>
<td>78.2 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Lecithin, (molar %)</td>
<td>23.1 ± 2.2d</td>
<td>24.0 ± 1.4a</td>
<td>21.4 ± 1.0a</td>
<td>22.4 ± 1.4a</td>
<td>24.2 ± 1.5a</td>
<td>20.0 ± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithogenic index</td>
<td>66.5 ± 8.3a</td>
<td>32.9 ± 5.7a</td>
<td>50.5 ± 3.5a</td>
<td>94.8 ± 14.5a</td>
<td>39.3 ± 4.2b</td>
<td>103.1 ± 27.7a</td>
<td>19.5 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Admirand-Small (25), (lipids 10%)</td>
<td>69.6 ± 9.5a</td>
<td>53.6 ± 5.4a</td>
<td>105.0 ± 12.6a</td>
<td>103.1 ± 27.7a</td>
<td>146.2 ± 44.1a</td>
<td>33.5 ± 10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with cholesterol gallstones</td>
<td>97.9 ± 13.1a</td>
<td>47.4 ± 7.9a</td>
<td>77.6 ± 5.0a</td>
<td>100.0 ± 6.9a</td>
<td>146.2 ± 44.1a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hegardt-Dam (26)-Holzbach (27), (lipids 10%)</td>
<td>103.4 ± 13.2a</td>
<td>83.8 ± 7.0a</td>
<td>159.5 ± 23.8a</td>
<td>146.2 ± 44.1a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with cholesterol gallstones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Total for Cholesterol + Lecithin + Bile acids = 100. * not significant; * P < 0.05; * P < 0.02; * P < 0.01; * P < 0.001; all values are means ±SE. The letters indicating the significance of statistical tests are for paired comparisons of A vs. B, B vs. G, C vs. A, D vs. E, E vs. G, and F vs. D. The superscript letter indicating the order of statistical significance is placed by the mean for the first group designated in each pairing. The lithogenic index is defined as the ratio of cholesterol (CH) actually present to the maximal amount that would be soluble at the phospholipid-bile acid (PL/BA) ratio of the sample. When multiplied by 100, this index is identical to the percent saturation. The calculation of lithogenic index is simplified (24) by plotting maximal cholesterol solubility on rectangular coordinates, with molar percent cholesterol (100 × CH/[CH + PL + BA]) as the ordinate and the molar ratio of phospholipid to phospholipid plus bile acid as the abscissa. The resulting graph is very similar to the triangular coordinate plot (25).
method of Metzger et al. (23) as modified by Thomas and Hofmann (24).

Among the seven groups, the mean concentrations of total lipid varied from a low of 6.39% (w/v) in group D to a high of 11.73% in group C. According to the criteria of Admirand and Small (25), the mean lithogenic index was much less than 100 for all groups except D and F. On the other hand, all of the groups fed the cholesterol diets except group C had a mean lithogenic index of about 100 or more if judged by the criteria of Hegardt and Dam (26) and Holzbach et al. (27). Monkeys fed the semipurified diets with cholesterol (A and D) had significantly higher lithogenic indices ($P < 0.01$) than the comparable groups (B and E) fed the semipurified diets without cholesterol.

The effects of dietary chenodeoxycholic acid, cholesterol and type of fat on the bile acid composition of gallbladder bile after 1 year are shown on table 4. In groups C and F which were fed chenodeoxycholic acid, chenodeoxycholic acid increased over 200%, but lithocholic acid remained less than 1% of the total (we did not measure sulfated forms). Dietary chenodeoxycholic acid caused a corresponding significant decrease in the cholic and deoxycholic acid concentrations, expressed as percentages of total bile acids.

**Turnover of primary bile acids in different diet groups.** Table 5 shows the pool sizes of cholic, chenodeoxycholic and total primary bile acids for the seven groups of squirrel monkeys calculated by the two-point method.

Group G, which in this study and previous ones (16) had always been free of gallstones, had the largest pool of cholic acid ($75.86 \pm 18.22$ mg/kg which is significantly greater than the mean of all other groups) and a chenodeoxycholic acid pool of $25.54 \pm 1.34$ mg/kg. In a previous study (28), the pool sizes of cholic and chenodeoxycholic acids for five squirrel monkeys fed the diet containing the same commercial feed were $86.0 \pm 12.3$ and $29.6 \pm 6.5$ mg/kg respectively.

Although the mean chenodeoxycholic acid pools of groups C and F (fed chenodeoxycholic acid) were three times greater than those of groups A and D (the comparable groups without chenodeoxycholic acid), the cholic acid pools were reduced about 50% and the total pool of primary bile acids in the chenodeoxycholic acid feeding groups were approximately 150% of those for control groups; all of the differences were not significant because of the large intragroup variability and small sample numbers. Cholesterol did not have a significant effect on pool size (A vs. B and D vs. E). The cholic acid and total pool sizes of the semipurified diet groups without cholesterol (B and E) were significantly lower than those of the commercial diet group (G).

**Half-lives and daily replacement rates of primary bile acids.** Except for the reduced half-lives of both bile acids as a result of the addition of cholesterol to the diets with butter (A vs. B), there were no statistically significant effects on half-lives (table 6). Except in group G, the half-lives of chenodeoxycholic acid were longer than those of cholic acid.

The daily synthesis of cholic and chenodeoxycholic acid is also shown in table 6. It is impossible to determine the rate of endogenous chenodeoxycholic acid synthesis in groups C and F fed diets with chenodeoxycholic acid. We have used the term *daily replacement rate* to express the product of the fractional turnover rate and pool size which is equal to the daily synthesis plus the exogenous bile acid absorbed into the body pool. Exogenous bile acids were only a factor for groups C and F.

Dietary cholesterol significantly increased the daily replacement of cholic, chenodeoxycholic and total bile acids in monkeys fed the butter diet. Although dietary chenodeoxycholic acid caused an increase in the replacement of chenodeoxycholic acid, it did not alter the total bile acid replacement.

**Plasma cholesterol and triglyceride levels.** The effect of cholesterol, fat and chenodeoxycholic acid feeding on the concentrations of plasma cholesterol and triglyceride is shown in table 7. Dietary chenodeoxycholic acid resulted in significantly decreased plasma cholesterol levels in the monkeys fed the butter and cholesterol diets (C vs. A) and significantly increased triglyceride levels in monkeys fed the safflower oil and cholesterol diets (F vs. D).
## TABLE 4

Effect of chenodeoxycholic acid feeding on bile acid composition of gallbladder bile in seven groups of squirrel monkeys after 1 year

<table>
<thead>
<tr>
<th>Group diet code</th>
<th>A Bu+Chl</th>
<th>B Bu</th>
<th>C Bu±Chl+CDCA</th>
<th>D Sa+Chl</th>
<th>E Sa</th>
<th>F Sa±Chl+CDCA</th>
<th>G Stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Bu+Chl 6</td>
<td>51.65±3.94*</td>
<td>48.35±6.34*</td>
<td>12.80±2.20*</td>
<td>47.13±6.31*</td>
<td>41.58±2.43*</td>
<td>7.26±3.75*</td>
<td>65.81±3.64</td>
</tr>
<tr>
<td>B Bu 6</td>
<td>34.70±3.04*</td>
<td>43.90±6.76*</td>
<td>86.20±2.69*</td>
<td>40.22±4.77*</td>
<td>44.57±2.77b</td>
<td>92.10±3.75c</td>
<td>26.98±2.29</td>
</tr>
<tr>
<td>C Bu±Chl+CDCA 5</td>
<td>13.59±3.29*</td>
<td>8.06±2.28*</td>
<td>0.91±0.72e</td>
<td>15.18±3.84a</td>
<td>13.85±2.15a</td>
<td>0.65±0.65</td>
<td>1.21±4.29</td>
</tr>
</tbody>
</table>

* Not significant; † P < 0.01; ‡ P < 0.001; all values are means ± se. The letters indicating the significance of statistical tests are for paired comparisons of A vs. B, B vs. G, C vs. A, D vs. E, E vs. G, and F vs. D. The superscript letter indicating the order of statistical significance is placed by the mean for the first group designated in each pairing.

## TABLE 5

Effects of chenodeoxycholic acid, cholesterol, and type of fat (saflower oil or butter) on the bile acid pool size in squirrel monkeys

<table>
<thead>
<tr>
<th>Group diet code</th>
<th>A Bu+Chl 6</th>
<th>B Bu 6</th>
<th>C Bu+Chl+CDCA 3</th>
<th>D Sa+Chl 5</th>
<th>E Sa 5</th>
<th>F Sa±Chl+CDCA 4</th>
<th>G Stock 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholic acid, (mg/kg) with cholesterol gallstones</td>
<td>26.7±6.3a</td>
<td>28.0±2.4c</td>
<td>12.3±5.0a</td>
<td>21.2±5.3a</td>
<td>23.1±3.5a</td>
<td>13.0±3.4a</td>
<td>75.9±18.2</td>
</tr>
<tr>
<td>Chenodeoxycholic acid, (mg/kg) with cholesterol gallstones</td>
<td>21.2±4.0a</td>
<td>15.8±2.3†</td>
<td>53.7±21.2a</td>
<td>16.7±4.3*</td>
<td>17.8±3.0a</td>
<td>49.9±20.9a</td>
<td>25.5±1.3</td>
</tr>
<tr>
<td>Total pool size of primary bile acid, (mg/kg) with cholesterol gallstones</td>
<td>14.4±2.9a</td>
<td>53.7±21.2a</td>
<td>13.9±4.3a</td>
<td>49.9±20.9a</td>
<td>10.4±3.4a</td>
<td>75.9±18.2</td>
<td></td>
</tr>
</tbody>
</table>

* Not significant; † P < 0.05; ‡ P < 0.01; all values are means ± se. The letters indicating the significance of statistical tests are for paired comparisons of A vs. B, B vs. G, C vs. A, D vs. E, E vs. G, and F vs. D. The superscript letter indicating the order of statistical significance is placed by the mean for the first group designated in each pairing.
TABLE 6
Effect of chenodeoxycholic acid and fat on half-lives and daily replacement ratea of primary bile acids

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>diet code</td>
<td>Bu+Chl</td>
<td>Bu</td>
<td>Bu+Chl+CDCA</td>
<td>Sa+Chl</td>
<td>Sa</td>
<td>Sa+Chl+CDCA</td>
<td>Stock</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Half-lives, (days):
- cholic acid: 1.4±0.1a, 3.0±0.5a, 1.3±0.2a, 2.3±0.7a, 3.9±0.8a, 1.7±0.4a, 5.8±2.0a
- chenodeoxycholic acid: 1.8±0.2a, 3.9±0.8a, 1.8±0.3a, 4.1±1.1a, 4.5±1.1a, 3.0±1.6a, 3.1±0.5a

Daily replacement rate, (mg/kg/day):
- cholic acid: 14.0±3.0a, 7.0±0.8a, 6.3±2.3a, 8.4±3.3a, 5.1±1.6a, 6.3±2.6a, 9.8±1.1a
- chenodeoxycholic acid: 6.5±1.1a, 3.1±0.4a, 20.5±5.5a, 3.9±1.7a, 3.0±0.6a, 15.2±4.3a, 6.0±1.0a
- total: 20.5±3.9a, 10.0±1.2b, 26.8±7.7a, 12.3±4.9a, 8.1±2.1a, 21.5±6.0a, 15.8±2.0a

a The replacement rate, which is the product of the fractional turnover rate and pool size is equal to the daily synthesis rate plus the exogenous bile acids absorbed into the body pool. Exogenous bile acids are a factor only for Groups C and F. * Not significant; \(P < 0.05\); \(P < 0.02\); all values are means ± se. The letters indicating the significance of statistical tests are for paired comparisons of A vs. B, B vs. G, C vs. A, D vs. E, E vs. G, and F vs. D. The superscript letter indicating the order of statistical significance is placed by the mean for the first group designated in each pairing.

TABLE 7
Effect of chenodeoxycholic acid and fat on plasma cholesterol and triglycerides in squirrel monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>diet code</td>
<td>Bu+Chl</td>
<td>Bu</td>
<td>Bu+Chl+CDCA</td>
<td>Sa+Chl</td>
<td>Sa</td>
<td>Sa+Chl+CDCA</td>
<td>Stock</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Total cholesterola (mg/dL): 420±26a, 237±13b, 286±30d, 242±16e, 163±10a, 211±17a, 181±22a
Triglycerideb (mg/dL): 19± 4, 32±10a, 3± 1b, 9± 1c, 10± 2e, 40±12c

a The plasma total cholesterol value is the mean of four bleedings during the 2 years of feeding. b The plasma triglyceride value was obtained at the end of 2 years of feeding. * Not significant; \(P < 0.05\); \(P < 0.02\); \(P < 0.01\); \(P < 0.001\). The letters indicating significance of statistical tests are for paired comparisons of A vs. B, B vs. G, C vs. A, D vs. E, E vs. G, and F vs. D. The superscript letter indicating the order of statistical significance is placed by the mean for the first group designated in each pairing. — The triglyceride value of Group B was not available.
Dietary cholesterol increased the plasma cholesterol regardless of the type of fat, and under all conditions, diets with safflower oil were associated with lower cholesterol and triglyceride levels than those with butter.

**Effect of CDCA feeding on liver histology.** No evidence of hyperplasia could be found in any part of the biliary tree of control monkeys or in those on chenodeoxycholic acid (CDCA) for 1 year, despite an occasional parasite in the bile ducts of these feral primates. Focal inflammatory cell infiltration in hepatic acini and cellular infiltration in Glisson’s triad were seen in several samples from each group, but these findings did not differ significantly in either frequency or severity in different groups.

**Liver function tests.** By human standards, squirrel monkeys have high and very variable serum alkaline phosphatase and glutamic pyruvic transaminase activities which make it extremely difficult to evaluate the toxicological effects of the dietary treatments. There were, however, no significant differences between the squirrel monkeys that had received chenodeoxycholic acid (groups C and F) and those that did not (groups A and D).

**DISCUSSION**

Whereas dietary cholesterol is the most lithogenic of the factors tested so far in squirrel monkeys, it is not an absolute requirement for cholesterol gallstone formation. For example, some animals fed diets containing 15% of energy as corn oil and no cholesterol sometimes formed stones (16). In spite of the high incidence of gallstones in squirrel monkeys fed cholesterol and safflower oil, a highly unsaturated fat, and in men fed diets rich in safflower oil (29), no cholesterol gallstones formed in monkeys fed diets containing 42% of energy as butter or safflower oil unless cholesterol was included in the diet. The high levels of dietary fat contribute to the lithogenicity of diets containing cholesterol since the commercial feed supplemented with 25% (w/w) butter plus cholesterol was associated with gallstones (28). On the other hand, the feeding of semipurified diets with the same amount of cholesterol but only 2% safflower oil did not cause cholesterol gallstones. Increased cholesterol intake led to increased concentrations of cholesterol in gallbladder bile. We also showed that dietary cholesterol increased the absolute level of cholesterol secretion in hepatic bile (28). Although substantial quantities of cholesterol are absorbed by both squirrel monkeys (30-32) and man (33), diets with high levels of cholesterol contribute a smaller share to the exchangeable cholesterol pool in man than in squirrel monkey (31). Dietary cholesterol caused a drastic reduction in the hepatic synthesis of cholesterol in squirrel monkeys (34) and is undoubtedly the source of much of the biliary cholesterol and bile acids.

The oral administration of chenodeoxycholic acid to human patients caused the dissolution of cholesterol gallstones in functioning gallbladders (9, 10, 35), but how it did so is not known. Chenodeoxycholic acid treatment in fasting subjects makes gallbladder bile less saturated with cholesterol (7), and bile acid feeding usually increases the total bile acid pool size (36). Chenodeoxycholic acid probably acts by decreasing the hepatic synthesis and biliary secretion of cholesterol (37).

In squirrel monkeys, chenodeoxycholic acid had little effect on the lithogenic index of gallbladder bile and did not reduce the incidence of cholesterol gallstones. The high level of dietary cholesterol (0.9 mg/kcal) used in our squirrel monkeys to induce a high incidence of gallstones is much higher than that in the diets of most human subjects treated with chenodeoxycholic acid to dissolve cholesterol gallstones. We estimate that the squirrel monkeys in our studies ingested 62 mg of chenodeoxycholic acid/kg body weight/day (0.1% w/w of the diet). The dosage used in man ranged from 250 mg to 4.5 g/day or 3.6 to 64 mg/kg body weight daily (9, 10, 36). This represents 0.1 to 1.8 mg/kcal for man compared with the 0.24 mg/kcal used in these experiments on squirrel monkeys. In general, the higher the dose of chenodeoxycholic acid used in man, the more the saturation of bile with cholesterol is reduced, but 14 to 15 mg/kg/day seems to be an effective dose (39). Chenodeoxycholic acid dissolves only cholesterol-rich gallstones in man, but the

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12 Unpublished data.
gallstones of squirrel monkeys are almost pure cholesterol (15). Depending on the dose, chenodeoxycholic acid treatment raised the percentage of chenodeoxycholic acid in bile acids to 76% (40) or over 90% (36) in man. Dietary chenodeoxycholic acid inhibited to a greater extent the endogenous synthesis of cholic acid than of chenodeoxycholic acid (41). In our experiments, chenodeoxycholic acid feeding increased the percentage of chenodeoxycholic acid to over 85% of the total bile acids.

The failure of dietary chenodeoxycholic acid to protect against cholesterol gallstone formation in squirrel monkeys has its parallel in the studies of Dam in hamsters (42-44). Perhaps the effects of chenodeoxycholic acid on gallstones in different species are related to quantitative differences in the effects on HMG CoA reductase and 7α-hydroxylase, the main regulatory enzymes for cholesterol and bile acid synthesis by the liver (2, 3, 35, 45-48). It will be important to estimate both control enzymes in squirrel monkeys fed various diets, including chenodeoxycholate. We conjecture that although cholesterol feeding resulted in increased hepatic 7α-hydroxylase activity and decreased HMG CoA reductase activity in squirrel monkeys, high concentrations of dietary cholesterol overwhelmed these control mechanisms and caused an increase in the levels of hepatic and biliary cholesterol. Plasma cholesterol concentrations increased slightly (46) or remained unchanged (9, 39) and plasma triglycerides tended to decrease (10, 39, 46) or remain unchanged (49) during chenodeoxycholate treatment in man. But in the squirrel monkey, plasma cholesterol tended to decrease and plasma triglyceride to increase during chenodeoxycholate feeding and depending on the type of dietary fat. Bile acid feeding (20 mg/kg body weight/day) caused no significant change in the levels of serum triglyceride or serum cholesterol in the rhesus monkey (50).

Although it was not the major focus of this study, the commercial diet had remarkable effects on bile composition, bile acid kinetics and gallstone formation in squirrel monkeys. There was protection against gallstones and expansion of the bile acid pool in squirrel monkeys (16, 28) and increased bile acid production and pool size in rats (51-53) fed commercial feeds based on mixtures of grains. The binding of bile salts by specific plant fiber (54, 55) seems the best explanation of these effects of the unrefined diets. Although deoxycholic acid is readily formed by the intestinal flora of monkeys fed the unrefined diets, it is not reabsorbed (16).

Diet influences bile composition and bile acid kinetics, as well as the incidence of gallstones, in squirrel monkeys. Nevertheless, the apparent differences between the effects of chenodeoxycholic acid on cholelithiasis in man and squirrel monkeys indicate caution in translating findings in one species directly to the other.

ACKNOWLEDGMENTS

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