# Biliary lipid secretion in hypercholesterolemia[1]

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**Abstract** A report on the effects of primary bile acid ingestion alone or in combination with plant sterols on serum cholesterol levels, biliary lipid secretion, and bile acid metabolism. Biliary bile acid and cholesterol secretion were measured in four patients with type IIa hypercholesterolemia before and after randomized treatment periods. During these periods either a bile acid mixture (cholic–chenodeoxycholic 2:1, a proportion similar to that endogenously synthesized in health), at a level of 20 mg/kg, or the same mixture plus sitosterols, 200 mg/kg, was fed. Serum cholesterol and the cholesterol saturation of fasting-state bile was also measured. Pretreatment biliary lipid secretion was within normal limits. Bile acid kinetic measurements were also recorded before treatment and showed that cholic acid synthesis was disproportionately decreased relative to that of chenodeoxycholic acid, a finding previously reported by others. Administration of the bile acid mixture increased biliary bile acid secretion in 3 of 4 patients, but did not influence biliary cholesterol secretion. The combination of sitosterol–bile acid, however, caused a relative decrease in cholesterol secretion in bile, and fasting-state bile became unsaturated in all patients. No change in fecal neutral sterol excretion occurred during the β-sitosterol–bile acid regimen, suggesting that simultaneous bile acid feeding blocks the compensatory increase in cholesterol synthesis known to be induced by β-sitosterol feeding in hypercholesterolemic patients. Serum cholesterol levels also fell modestly during the sitosterol–bile acid regimen, the decrease averaging 15%. We conclude that the abnormally low rate of bile acid synthesis in patients with type IIa hyperlipoproteinemia does not influence biliary lipid secretion; that increasing the input of the two primary bile acids into the enterohepatic circulation does not increase biliary cholesterol secretion or lower serum cholesterol levels in such patients; and that the usual increase in cholesterol synthesis induced by β-sitosterol feeding does not occur if bile acids are administered simultaneously.

**Supplementary key words** cholesterol metabolism · β-sitosterols · hyperlipoproteinemia

Patients with familial hypercholesterolemia (type IIa hyperlipoproteinemia) have increased serum cholesterol, largely as low density lipoprotein, by definition (2), and increased total body cholesterol, based on isotope dilution studies (3, 4). Langer, Strober, and Levy (5) showed that the fractional turnover of the apoproteins of low density lipoproteins was decreased, suggesting an impairment in apoprotein disposal. Subsequently, important studies by Brown and Goldstein (6–9) with cultured fibroblasts provided additional support for the hypothesis that apoprotein catabolism is defective in such patients.

In addition to this defect in apoprotein catabolism, such patients also have an abnormality in the conversion of cholesterol to bile acids (10–13). In healthy individuals, about twice as much cholic acid as chenodeoxycholic acid is synthesized, but in type IIa patients, less cholic than chenodeoxycholic acid is synthesized (14). Such patients also tend to have exchangeable bile acid pools that are smaller than those of healthy subjects (12).

Little information is available on biliary bile acid and cholesterol secretion in such patients. Biliary lipid secretion is important in overall cholesterol metabolism, since it provides the only route for cholesterol elimination, and indeed, the majority of the cholesterol entering the intestine is from biliary secretion (15–17).

We thought it worthwhile to define biliary lipid secretion in type IIa patients and reasoned further that we could define the significance of the impaired bile acid synthesis in such patients by exogenously correcting the defect. This could be achieved by administering primary bile acids in a proportion similar to that synthesized by the liver in health and in an amount exceeding the normal synthesis rate. We further speculated that such correction of bile acid metabolism might lead to increased biliary cholesterol secretion whose subsequent reabsorption could be prevented by simultaneous oral administration of plant sterols (cf. 18, 19). Were this sequence of events to occur, patients so treated would develop net negative cholesterol balances which could have therapeutic value.

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We report here the application of a previously reported (15–17) perfusion method for quantitation of biliary lipid secretion to patients with type IIa hyperlipidemia before treatment and after the feeding of a mixture of primary bile acid or of this bile acid mixture together with plant sterols. We also report the effect of these therapeutic regimens on serum cholesterol levels, fasting-state bile cholesterol saturation, and biliary bile acid composition, as well as sterol balance. The results suggest that the defects in bile acid synthesis in such patients are of minor importance and are in agreement with the emerging view that this genetic disease is caused by a defect in apoprotein metabolism.

While this work was in progress, studies were reported from our Swedish colleagues in which cholic (20) or chenodeoxycholic (21) acid was fed to patients with familial hypercholesterolemia. These workers found that cholic acid feeding caused a small (≃10%) decrease in serum cholesterol levels but a marked increase in fecal neutral sterols. Chenodeoxycholic acid, on the other hand, caused no change in serum cholesterol levels and probably no change in fecal neutral sterol excretion. Neither biliary lipid secretion nor the cholesterol saturation of fasting-state bile was measured.

## METHODS

### Patient material

Patients selected for this study had elevated serum cholesterol levels, normal serum triglyceride levels, and increased low density lipoproteins by serum lipoprotein electrophoresis; these abnormalities persisted after the initiation of a low-cholesterol (<300 mg/day) and moderate-fat (<60 g/day) diet (**Table 1**). All patients had at least one blood relative with hypercholesterolemia and fulfilled the criteria for the heterozygous form of primary hypercholesterolemia (2). All patients had normal oral cholecystograms, with the exception of one patient who participated in the outpatient study; this patient had radiolucent gallstones well-visualized in the gallbladder. Patients received no other drugs during our study except bile acids or sitosterol plus bile acids. Prior to the study, patients were receiving a variety of hypocholesterolemic agents, but all were discontinued at least one month prior to the study. Patients were instructed on a conventional low cholesterol diet (cholesterol <100 mg/day) at least one month prior to initiation of the study and remained on this diet throughout the entire study.

### Therapeutic regimens

*Bile acids*. Two therapeutic regimens were studied. In the first, termed bile acids, patients received a mixture of the primary bile acids, cholic acid and chenodeoxycholic acid. The bile acids were given as a mixture, cholic acid–chenodeoxycholic acid 2:1, a ratio similar to that synthesized by the liver in healthy individuals (14). Primary bile acids were obtained in pure form from Weddel Pharmaceuticals, London, and packaged in 250-mg gelatin capsules by Rowell Laboratories, Baudette, MN. Bile acids were ingested at a dosage of 20–30 mg/kg body weight, which is 5–10 times the endogenous synthesis rate.

*Sitosterol–bile acids*. This therapeutic regimen featured the addition of plant sterols to the primary bile

TABLE 1. Pertinent clinical features and treatment regimen of patients with type IIa hyperlipoproteinemia

| | | | | | Dosage[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Primary Bile Acids | | | Plant Sterols | | |
| Patient | Age | Sex | Weight | Height | Amount (mg) | mg/kg | μmol/kg | Amount (g) | mg/kg | μmol/kg |
| | *yr* | | *kg*[a] | *cm* | | | | | | |
| AN | 63 | F | 58.0 | 152.4 | 1200 | 20.7 | 51.8 | 12.0 | 206.9 | 53.5 |
| DH | 51 | M | 85.0 | 175.9 | 1920 | 22.6 | 56.5 | 18.0 | 211.8 | 54.7 |
| TL | 24 | M | 86.4 | 182.9 | 1200 | 13.9 | 34.8 | 18.0 | 208.3 | 53.8 |
| MH | 61 | F | 61.0 | 157.5 | 1200 | 19.7 | 49.2 | 15.0 | 245.9 | 63.5 |
| BV | 34 | M | 65.9 | 167.6 | 1200 | 18.2 | 45.5 | | | |
| SV | 60 | F | 55.5 | 154.9 | 1200 | 21.6 | 54.0 | 15.0 | 270.3 | 69.8 |
| JK | 37 | F | 66.8 | 170.2 | 1440 | 21.6 | 54.0 | 15.0 | 224.6 | 58.0 |
| PB | 34 | M | 82.7 | 177.8 | 1440 | 17.4 | 43.5 | 18.0 | 217.7 | 56.2 |
| M ± SE | 45.5 ± 5.3 | | 70.2 ± 4.5 | 167.4 ± 4.0 | 1350 ± 90 | 19.5 ± 1.0 | 48.7 ± 2.5 | 15.9 ± 0.9 | 226.5 ± 8.9 | 58.5 ± 2.3 |

[a] Entry weight. There were no significant weight changes during this study.
[b] Primary bile acids were administered as capsules containing two parts cholic and one part chenodeoxycholic acid. Plant sterols were administered as a liquid suspension (Cytellin, Eli Lilly and Company).

acid mixture. Plant sterols were administered as a suspension (Cytellin, Eli Lilly, Indianapolis, IN). The major constituent of Cytellin is β-sitosterol, 200 mg/ml. The plant sterols were fed at a dosage of 200–300 mg/kg body weight, a dosage similar to that used for a number of metabolic studies in man.

### Experimental design

*Outpatient study*. An outpatient study was designed to assess the influence of the two therapeutic regimens on serum lipid levels. Serum cholesterol and triglyceride levels were measured before treatment and at monthly intervals while patients ingested either bile acids or sitosterol–bile acids for periods of 8–25 weeks; the order of the regimens was randomized. The length of the periods was set at 8 weeks or greater, because this period of time has been shown to be sufficient for the bile acid pool to return to its pretreatment size after bile acid feeding (22); longer periods were used early in the study to prevent the missing of a delayed response.

*Inpatient study*. An inpatient study was designed to assess the influence of the two therapeutic regimens on a variety of aspects of bile acid and cholesterol metabolism. These studies, carried out in patients 1–4 (Table 1), featured measurements of bile acid and cholesterol metabolism before treatment and at the end of each randomized 8-week treatment period. Each metabolic study period lasted 1 week. The protocol was approved by the Mayo Clinic Human Studies Committee on July 10, 1974, and all subjects gave informed consent.

Patients were admitted to the Clinical Research Center. During the week prior to admission and during the period of hospitalization, each patient received chromium sesquioxide (500 mg p.o. three times a day) (23) and Cytellin (5 ml/day) (24, 25) as fecal flow markers; the Cytellin was not administered during the sitosterol–bile acid periods. Patients were fed a repeating diet with caloric fat, protein, and carbohydrate composition similar to that ingested by the patient at home, on the basis of a dietary history. Thus, admission to the Clinical Research Center was associated with no change in diet, and the patients were assumed to be in a steady state with respect to cholesterol and bile acid metabolism.

On the day of admission, after an overnight fast, patients received radioactive bile acids intravenously for measurement of bile acid kinetics. The following day a triple lumen tube was passed with its proximal aspiration site 5 cm distal to the ampulla of Vater, as visualized with fluoroscopy. Gallbladder bile was collected after gallbladder stimulation by cholecystokinin (75 Ivy dog units, Karolinska Institute, Stockholm, Sweden) at 24, 33, 48, 57, 72, and 81 hr after isotope administration. A 2-ml aliquot of bile was retained for analysis, and the remainder was returned to the duodenum. Each bile sample was diluted 1:9 with isopropanol, and stored at 5°C until analyzed.

Stool collections were obtained daily and those pooled during the first 4 days of hospitalization were analyzed individually for acidic and neutral fecal steroids.

Biliary lipid secretion over a 24-hr period, which included the ingestion of three liquid meals and an overnight fast, was measured hourly by a duodenal perfusion technique described previously in detail (15–17). In this technique, a recovery marker as well as radioactive lecithin and cholesterol are infused into the proximal portion of the duodenum. Intestinal content is sampled 20 cm distally and pooled hourly. Three equicaloric liquid meals were ingested at 0800, 1300, and 1800 hours. The meals were adjusted to 2 calories/ml with fat in the form of distilled butter oil containing 0.02% sterol by weight; the protein of the meal was a dried, nonfat, skimmed milk powder containing 0.01% sterol and 0.2% phospholipid by weight.

All unconjugated bile acids ingested by patients during this 24-hr period were extracted from the duodenal aspirates and quantitated by gas–liquid chromatography in a manner previously described (16). These unconjugated bile acids were subtracted from the total bile acid concentration so that reported values reflect only conjugated (of hepatobiliary origin) bile acid output.

### Bile acid kinetics by isotope dilution

The pool size and synthesis rate of primary bile acids were determined by isotope dilution (14). [11,12-$^{3}$H]Chenodeoxycholic acid was synthesized as described (26), and [24-$^{14}$C]cholic acid was purchased from New England Nuclear Corporation, Boston, MA. Both labeled bile acids had a radiopurity of 98% by zonal scanning. For isotope dilution studies, 30 μCi of the [$^{3}$H]chenodeoxycholic acid and 10 μCi of the [24-$^{14}$C]cholic acid were used.

We have recently found that 10–20% of the label of [11,12-$^{3}$H]chenodeoxycholic acid may be lost after its intravenous injection, possibly because some tritium is not located at the 11,12 positions (27). The lability of the tritium label produces an overestimate (averaging 13%) of the true pool size and synthesis rates, and we have accordingly corrected our figures for this error.

A 5-ml aliquot of the bile–isopropanol (1:10) solution was saponified in methanol–sodium hydroxide, extracted in acidified diethyl ether, evaporated to dryness, and redissolved in 3 ml of chloroform–

methanol 1:1 (v/v). One-third of this solution was used to determine biliary bile acid composition by gas–liquid chromatography as previously described (28), and the remaining two-thirds was evaporated to approximately 0.1 ml in volume for separation by thin-layer chromatography using iso-octane–isopropanol–acetic acid 6:2:0.5 (v/v/v) as the developing solvent (29) and 20 × 20 cm plates coated with 0.5 mm silica gel H. Standards of cholic, chenodeoxycholic, and deoxycholic acids were chromatographed and the locations of these bile acids in the individual bile samples were determined using an iodine chamber. The trihydroxy and dihydroxy zones were scraped from the plate and eluted twice with methanol; the solution was evaporated to dryness, and the residue was redissolved in 2 ml of 0.05 N NaOH. Aliquots (0.5 ml) of the NaOH solutions were analyzed for radioactivity by liquid scintillation spectroscopy with external standardization to correct for quenching. The mass of individual bile acids eluted from the chromatogram was determined with an automated modification of the steroid dehydrogenase method of Iwata and Yamasaki (30), using the 3-steroid dehydrogenase enzyme for cholic and total dihydroxy bile acid masses and the 7-steroid dehydrogenase enzyme (31) (Worthington Biochemicals, Freehold, NJ) for chenodeoxycholic acid mass. In validation experiments, we found optimal reaction conditions to be the same for both enzymes. The derived specific activity figures permitted calculations of chenodeoxycholic and cholic acid pool sizes, turnover rates, and synthesis rate. Bile acid kinetics were also determined during the bile acid and sitosterol–bile acid periods, but the data are not included because, in some subjects, implausibly high values were obtained for pool size and input (the sum of newly absorbed and endogenously synthesized bile acid, cf. 32).

### Biliary bile acid composition

Following deconjugation, methylation, and acetylation as previously described (28), methyl ester acetates were separated by gas–liquid chromatography at 265°C on a polysulfone column (1% Poly-S-179 on 100/120 mesh GasChrom Q, purchased from Applied Science Laboratories, Inc., State College, PA). The mean biliary bile acid composition was determined from the three morning bile samples.

### Biliary lipid composition of fasting gallbladder bile

Fasting morning bile samples in isopropanol solution (bile–isopropanol 1:10, v/v) were analyzed for phospholipid, total bile acid, and cholesterol concentrations. Phospholipid was measured as lipid-soluble phosphorus by the spectrophotometric method of Fiske and Subbarow (33). Total bile acids were measured by an automated steroid dehydrogenase enzyme method. Cholesterol concentration was determined by gas–liquid chromatography using cholesteryl isopropyl ether as an internal standard on a QF-1 column (4 ft, GCQ 100/200). The cholesterol was extracted from bile as described by Abell et al. (34).

### Fecal neutral and acidic sterols

Fecal neutral sterols were measured by a modified balance method of Miettinen, Ahrens, and Grundy (35) using $\beta$-sitosterol as an internal standard to correct for cholesterol losses (24, 25). Daily fecal collections were homogenized, sterols were extracted with petroleum ether after saponification, and individual sterols were separated by thin-layer chromatography prior to quantitative analysis by gas–liquid chromatography.

Fecal bile acids were determined by gas–liquid chromatography as described (36). After the neutral sterols had been extracted from the fecal homogenates, the bile acids were measured quantitatively by gas–liquid chromatography using hyocholic acid as an internal standard. Dietary cholesterol was calculated from the dietary history using published figures and subtracted from the fecal neutral sterol output.

### Calculation and expression of results

Biliary lipid composition was calculated and expressed as mole fractions of bile acid, lecithin, and cholesterol. Cholesterol saturation was then calculated from polynomial equations developed in our laboratories (37) based on the cholesterol solubility line proposed by Hegardt and Dam (38) and Holzbach et al. (39). All data are expressed as $\mu$mol/kg actual body weight.

## RESULTS

### Serum cholesterol levels

Serum cholesterol levels fell by an average of 15% during sitosterol–bile acid treatment. The decrease occurred within 8 weeks of initiating treatment, and no further decline occurred with prolonged treatment (**Fig. 1**). Bile acids alone did not influence serum cholesterol levels and neither regimen influenced serum triglyceride levels (data not presented).

### Biliary lipid secretion

*Biliary bile acid secretion* (**Table 2**). Biliary bile acid secretion before treatment averaged 0.5 mmol/kg-day, a figure quite similar to that found by us for non-

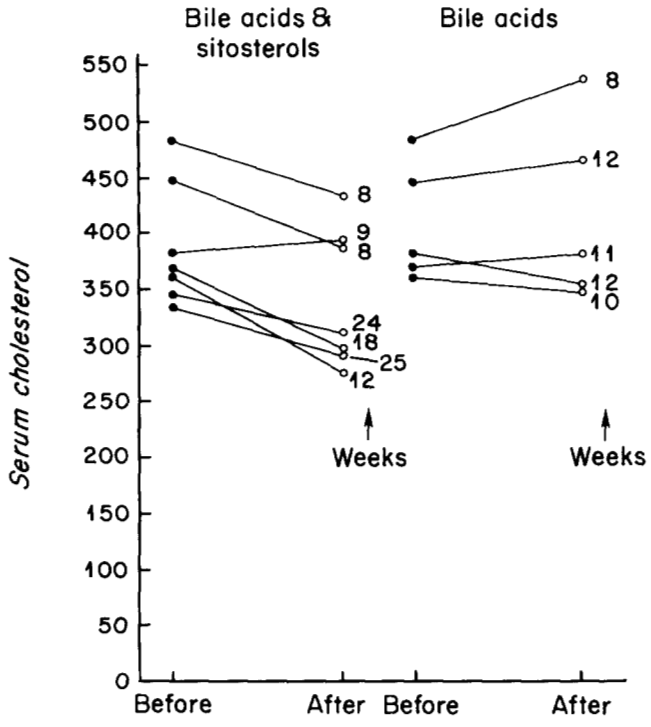


**Fig. 1.** Effect of treatment regimens on serum cholesterol levels in patients with type IIa familial hyperlipoproteinemia. The serum level at the end of the treatment period is plotted, as all post-treatment levels were quite stable. The number of weeks of treatment is indicated. The difference is statistically significant ($P = 0.02$) by the Wilcoxon signed rank sum test.

obese, middle-aged, apparently healthy volunteers (16), although somewhat lower than that reported by us for vigorous, healthy male medical students (17). With bile acid feeding, bile acid secretion increased markedly in three of the four individuals. When sitosterol was added to bile acids, bile acid secretion increased slightly more in all four individuals. Thus, bile acid feeding with or without sitosterol caused a marked increase in bile acid secretion in three of the four hypercholesterolemic patients.

*Biliary cholesterol secretion* (**Table 3**). Biliary cholesterol secretion averaged 0.046 mmol/kg-day and was probably within normal limits, i.e., values were similar to those previously reported by us for healthy nonobese, middle-aged men and women. Bile acid feeding had no effect on biliary lipid secretion. The addition of sitosterol to bile acids appeared to cause decreased cholesterol secretion in three of four patients and, during the sitosterol–bile acid regimen, all four patients had values below their pretreatment values. The ratio of cholesterol secretion to bile acid secretion was also calculated for the three periods. This fell in three of four individuals during the bile acid regimen and in all four individuals during the sitosterol–bile acid regimen. During the latter regimen, less than half as much cholesterol was secreted for a given amount of bile acid secreted.

TABLE 2. Biliary bile acid secretion (μmol/kg-day)[a] in four patients with type IIa hyperlipidemia before and during treatment with bile acid or sitosterol–bile acid regimens

| Patient | Regimen | | |
|---|---|---|---|
| | Pretreatment | Bile Acid | Sitosterol–Bile Acid |
| AN | 554 | 990 | 1223 |
| DH | 600 | 496 | 660 |
| TL | 424 | 763 | 925 |
| MH | 606 | 760 | 830 |
| M ± SE | 546 ± 42 | 752 ± 101 | 910 ± 118 |

[a] Using this methodology, biliary bile acid secretion expressed as μmol/kg-day, in six young healthy male volunteers was 627 ± 26 (M ± SE) (17) and in seven apparently healthy middle-aged men and women was 405 ± 47 (16).

*Fasting-state bile saturation* (**Table 4**). Bile saturation, determined on the first 3 days of the 5-day period that was used for isotope dilution studies, was unchanged by bile acid treatment but fell consistently during the sitosterol–bile acid regimen.

## Cholesterol metabolism

Fecal neutral sterols showed little change during treatment with bile acids or sitosterol–bile acids (**Table 5**). Values for all periods were within the range reported for patients with familial hypercholesterolemia. The majority (68%) of cholesterol in these

TABLE 3. Biliary cholesterol (Chol) secretion and ratio of cholesterol to bile acid (BA) in biliary lipid secretion[a] in four patients with type IIa familial hyperlipoproteinemia before and during treatment with bile acid and sitosterol–bile acid regimens

| Patient | Biliary Cholesterol Secretion (μmol/kg-day) | | | Ratio of Chol Secretion to BA Secretion | | |
|---|---|---|---|---|---|---|
| | Pretreatment | Bile Acid | Sitosterol–Bile Acid | Pretreatment | Bile Acid | Sitosterol–Bile Acid |
| AN | 40.9 | 66.4 | 39.0 | 0.074 | 0.067 | 0.032 |
| DH | 51.3 | 45.9 | 31.4 | 0.077 | 0.093 | 0.047 |
| TL | 37.0 | 27.9 | 34.6 | 0.087 | 0.037 | 0.037 |
| MH | 56.2 | 39.4 | 38.0 | 0.093 | 0.052 | 0.046 |
| M ± SE | 46.4 ± 4.5 | 44.9 ± 8.1 | 35.8 ± 1.7 | 0.083 ± 0.04 | 0.062 ± 0.01 | 0.041 ± 0.04 |

[a] Using this methodology, biliary cholesterol secretion, expressed as μmol/kg-day, in six young healthy male volunteers was (M ± SE) 39.8 ± 2.2 (17) and in seven apparently healthy middle-aged men and women with normal cholecystograms was 48.2 ± 7.2 (16).

TABLE 4. Fasting-state bile saturation,[a] %, in four patients with type IIa familial hyperlipoproteinemia before and during treatment with bile acid or sitosterol–bile acid regimens

| Patient | Day | Pretreatment | Bile Acid | Sitosterol–Bile Acid |
|---|---|---|---|---|
| AN | 1 | 91 | 73 | 68 |
| | 2 | 95 | 90 | 75 |
| | 3 | 100 | 96 | 80 |
| DH | 1 | 128 | 98 | 92 |
| | 2 | 182 | 119 | 83 |
| | 3 | 181 | 143 | 96 |
| TL | 1 | 75 | 77 | 67 |
| | 2 | 93 | 76 | 76 |
| | 3 | 102 | 89 | 71 |
| MH | 1 | 102 | 100 | 80 |
| | 2 | 96 | 149 | 94 |
| | 3 | 115 | 171 | 89 |
| M ± SE | | 113 ± 10 | 107 ± 9 | 81 ± 3 |

[a] Saturation was calculated using polynomial equations developed by us (32) to describe the solubility limits of cholesterol in a model system simulating bile as described by Hegardt and Dam (35).

individuals was lost as neutral rather than acidic sterols, in agreement with many previous reports (cf. 11–13).

### Bile acid metabolism

Biliary bile acid composition was unremarkable before and during treatment (**Table 6**). By isotope dilution, pretreatment chenodeoxycholic acid synthesis was at the upper limits of normal (cf. 32, 40), whereas cholic acid synthesis was within normal limits, so that the proportion of primary bile acid synthesis as cholic acid, 54 ± 2% (M ± SE), was lower than that generally reported for healthy individuals (**Table 7**). The estimate of bile acid synthesis by the isotope dilution method gave a figure greater than that obtained by measuring fecal bile acids by gas–liquid chromatography, as reported by some workers (41, 42) but not others (43).

During bile acid feeding, there was a marked increase in fecal bile acid output, representing the bile acid fed as well as continuing endogenous synthesis (Table 5).

## DISCUSSION

### Lack of therapeutic effect of sitosterol–bile acid

These results indicate that the combination of sitosterol–bile acid achieves no greater lowering of serum cholesterol levels in patients with familial hypercholesterolemia than that reported to be induced by sitosterol feeding alone (44, 45). The assumptions we made to rationalize this therapeutic modality were proved to be wrong by two observations reported here. First, biliary cholesterol secretion was not decreased in type IIa patients; and second, biliary cholesterol secretion was not increased by bile acid feeding.

### Therapeutic effect of sitosterol–bile acid on fasting-state bile saturation

Our results showed that sitosterol–bile acid caused a consistent decrease in the cholesterol saturation of fast-

TABLE 5. Daily fecal neutral and acidic sterols[a]

| Patient | Parameter | Pretreatment | Bile Acid | Sitosterol–Bile Acid |
|---|---|---|---|---|
| AN | Neutral sterols (mg/day) | 668.7 | 567.8 | 413.0 |
| | Neutral sterols (μmol/kg-day) | 29.8 | 25.3 | 18.4 |
| | Acidic sterols (mg/day) | 341.2 | 3021 | 1208 |
| | Acidic sterols (μmol/kg-day) | 14.7 | 130.2 | 52.1 |
| DH | Neutral sterols (mg/day) | 636.0 | 696.1 | 739.0 |
| | Neutral sterols (μmol/kg-day) | 19.3 | 21.2 | 22.5 |
| | Acidic sterols (mg/day) | 281.3 | 2240 | 2000 |
| | Acidic sterols (μmol/kg-day) | 8.3 | 65.9 | 58.8 |
| TL | Neutral sterols (mg/day) | 408.4 | 536.2 | 511.2 |
| | Neutral sterols (μmol/kg-day) | 12.2 | 16.0 | 15.3 |
| | Acidic sterols (mg/day) | 162.1 | 1521 | 979 |
| | Acidic sterols (μmol/kg-day) | 4.7 | 44.0 | 28.3 |
| MH | Neutral sterols (mg/day) | 440.8 | 448.2 | 456.7 |
| | Neutral sterols (μmol/kg-day) | 18.7 | 19.0 | 19.3 |
| | Acidic sterols (mg/day) | 236 | 1122 | 1419 |
| | Acidic sterols (μmol/kg-day) | 9.7 | 46.0 | 58.2 |
| M ± SE | Neutral sterols (mg/day) | 538.5 ± 66.4 | 562.1 ± 51.3 | 530 ± 72.5 |
| | Neutral sterols (μmol/kg-day) | 20.0 ± 3.6 | 20.4 ± 2.0 | 18.9 ± 1.5 |
| | Acidic sterols (mg/day) | 255.2 ± 37.8 | 1976 ± 418 | 1402 ± 219 |
| | Acidic sterols (μmol/kg-day) | 9.5 ± 2.1 | 61.5 ± 26.3 | 49.4 ± 7.2 |

[a] Dietary cholesterol has been subtracted from the total fecal neutral sterols.

ing-state bile in type IIa patients. We initially attributed this effect to the sitosterol alone since *a*) sitosterol alone has been reported to decrease cholesterol saturation of fasting-state gallbladder bile in healthy subjects (46), and *b*) neither cholic acid in gallstone patients (18) nor deoxycholic acid feeding in healthy volunteers (17) alters fasting-state gallbladder saturation. Indeed, we have recently tested the effect of sitosterol on bile saturation in gallstone patients and confirmed a real, but quite modest, desaturating effect (47). Whether the considerable change in fasting-state bile saturation observed here applies only to hypercholesterolemic patients or was caused by the combination of sitosterol and bile acid, or both, is not known.

The mechanism of this effect is obscure. Bile acid secretion was considerably increased in three of the four patients. The only proposed mechanism of action of β-sitosterol is to decrease intestinal cholesterol absorption, and, in man, there is not considered to be any simple relationship between cholesterol intake and fasting-state gallbladder bile saturation (48). Nonetheless, large amounts of dietary cholesterol may increase biliary cholesterol output in man (49) and hamster (50); in the prairie dog, a high cholesterol diet causes supersaturated bile (51).

We also observed no increase in fecal neutral sterol excretion when the sitosterol–bile acid regimen was compared to the bile acid regimen in contrast to the marked increase in sterol excretion observed by Grundy and Davignon (18) who administered β-sitosterol to hypercholesterolemic patients. The only reasonable explanation for these divergent results, assuming that steady-state conditions were present and that any increase could have been detected by our methodology, is that expanding the enterohepatic circulation by bile acid feeding abolished the compensatory increase in cholesterol synthesis that is induced by the feeding of β-sitosterol alone in hypercholesterolemic patients. A similar suppression by administered bile acids of the increased cholesterol synthesis induced by β-sitosterol has been described for the rat by Shefer et al. (52).

TABLE 6. Effect of treatment on biliary bile acid composition[a]

| Acid | Regimen (M ± SE) | | |
|---|---|---|---|
| | Pretreatment | Bile Acid | Sitosterol–Bile Acid |
| Cholic | 28.8 ± 2.2 | 32.9 ± 0.5 | 33.7 ± 3.1 |
| Chenodeoxycholic | 38.1 ± 2.4 | 47.4 ± 5.4 | 43.4 ± 1.6 |
| Deoxycholic | 30.1 ± 2.9 | 16.4 ± 5.5 | 19.8 ± 2.6 |
| Lithocholic | 1.4 ± 0.1 | 0.2 ± 0.1 | 0.03 ± 0.03 |
| Other | 2.6 ± 1.6 | 3.2 ± 0.3 | 3.4 ± 0.6 |

[a] Differences are not statistically significant.

## Lack of importance of altered bile acid metabolism in the pathogenesis of type IIa hypercholesterolemia

Although our results confirm numerous reports showing impaired cholic acid synthesis in type IIa hypercholesterolemia, these results, together with those of others in which cholic acid or chenodeoxycholic acid were fed, indicate that expanding the enterohepatic circulation of bile acids or changing its bile acid composition does not materially influence serum cholesterol levels in this genetic disease. Also, attempts to increase biliary cholesterol secretion

TABLE 7. Bile acid kinetics during the pretreatment period in four patients with type IIa familial hyperlipoproteinemia

| Patient | Pool, μmol/kg | Pool, mg | FTR, day$^{-1}$ | Syn., μmol/kg-day | Syn., mg/day |
|---|---|---|---|---|---|
| Cholic acid | | | | | |
| AN | 15.5 | 367 | 0.57 | 8.8 | 210 |
| DH | 36.3 | 1261 | 0.48 | 17.6 | 610 |
| TL | 28.5 | 1009 | 0.14 | 3.9 | 137 |
| MH | 18.5 | 462 | 0.60 | 11.0 | 279 |
| M ± SE | 24.7 ± 4.8 | 775 ± 215 | 0.45 ± 0.11 | 10.3 ± 2.8 | 309 ± 104 |
| Chenodeoxycholic acid | | | | | |
| AN | 23.8 | 544 | 0.30 | 7.1 | 163 |
| DH | 40.4 | 1348 | 0.28 | 11.2 | 376 |
| TL | 37.6 | 1275 | 0.08 | 3.0 | 99 |
| MH | 21.6 | 517 | 0.37 | 7.9 | 189 |
| M ± SE | 30.8 ± 4.8 | 921 ± 226 | 0.26 ± 0.06 | 7.3 ± 1.7 | 207 ± 60 |
| Total bile acids | Pool, μmol/kg | Pool, mg | BA Secretion, μmol/kg-day | BA Pool Recyc. F, no./d | BA Synthesis μmol/kg-day |
| AN | 73.8 | 1715 | 553.8 | 7.50 | 15.9 |
| DH | 119.7 | 4072 | 599.7 | 5.01 | 28.8 |
| TL | 88.5 | 3061 | 424.0 | 4.79 | 6.9 |
| MH | 60.1 | 1467 | 605.5 | 10.07 | 18.9 |
| M ± SE | 85.5 ± 12.8 | 2579 ± 609 | 545.8 ± 42.2 | 6.84 ± 1.24 | 17.6 ± 4.5 |

by cholic acid feeding in a patient with a biliary fistula failed, because synthesis kept pace with the induced increase in biliary cholesterol secretion (53).

Decreasing cholesterol absorption by sitosterol feeding causes only a 15–20% fall in serum cholesterol levels in hypercholesterolemic patients (44, 45). Further, the combination of decreasing cholesterol absorption and interrupting the enterohepatic circulation of bile acids (with an associated increase in cholesterol and bile acid synthesis) by cholestyramine feeding or ileal bypass causes only a slightly greater decrease in serum cholesterol (54). At present, the most successful therapeutic approach is the combination of cholestyramine and nicotinic acid, which not only decreases cholesterol and bile acid absorption, but is thought to inhibit lipoprotein release (55). Thus, this disease in which elevated serum cholesterol is considered to result from impaired lipoprotein catabolism is most effectively treated at present by measures that are aimed at altering not only cholesterol metabolism but also lipoprotein apoprotein metabolism.

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