# Cholesterol metabolism in primary biliary cirrhosis during simvastatin and UDCA administration

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**Abstract** Little is known about the effects of cholesterol-lowering agents in hypercholesterolemic patients with primary biliary cirrhosis (PBC). The aim of this study was to compare the changes induced by simvastatin and ursodeoxycholic acid (UDCA) on cholesterol metabolism in patients with PBC and preserved liver function. Six patients with PBC were administered simvastatin (40 mg/day) for 30 days and, after a washout period of 30 days, ursodeoxycholic acid (600 mg/day) for 30 days. Serum levels of lathosterol, campesterol, 7α-hydroxycholesterol, and 27-hydroxycholesterol were measured by gas chromatography-mass spectrometry. During simvastatin administration, reduction of cholesterol levels (34% in 30 days) was paralleled by the decrease of lathosterol (55%), whereas concentrations of campesterol and of the two hydroxysterols were not substantially modified. During ursodeoxycholic acid administration, a trend toward a decrease of serum cholesterol concentrations was observed after only one year of treatment, and these changes were paralleled by the decrease of campesterol serum levels. Both simvastatin and UDCA were well tolerated, and a reduction of serum liver enzyme levels occurred with the latter. Simvastatin proved to be safe and effective in reducing serum cholesterol levels in patients with PBC by an inhibitory effect on cholesterol synthesis occurring within 24 h.—Del Puppo, M., M. Galli Kienle, A. Crosignani, M. L. Petroni, B. Amati, M. Zuin, and M. Podda. **Cholesterol metabolism in primary biliary cirrhosis during simvastatin and UDCA administration.** *J. Lipid Res.* 2001. **42:** 437–441.

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Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease commonly associated with marked alterations of the enterohepatic circulation of various compounds and, especially, lipid moieties (1, 2). Several studies have demonstrated that cholesterol metabolism is markedly deranged in PBC patients (3–5), leading to hypercholesterolemia that is severe in some cases (2, 6). Both synthesis and biliary elimination of cholesterol are impaired (4, 5), with concomitant reduction of serum levels of lathosterol and increased serum concentrations of dietary sterols,

such as campesterol (4, 5). In addition, reduced cholesterol absorption may occur in patients with the latest stage of the disease (5), and data from a recent study suggest that both the feedback regulation of retained bile acids on cholesterol 7α-hydroxylase and the cholesterol 27-hydroxylase scavenger effect on excess serum cholesterol are deficient in these patients (7).

The question of the long-term clinical consequences of hypercholesterolemia in PBC patients is still open, because the available data do not come from prospective cohort studies (2, 6, 8). It is probable that at least a subset of PBC patients, selected on the basis of the degree of elevation of serum cholesterol and with relatively low high density lipoprotein (HDL) cholesterol serum levels, will benefit from administration of inhibitors of HMGCoA reductase, especially if additional risk factors for cardiovascular disease coexist. However, caution in the use of cholesterol-lowering drugs for PBC patients is suggested by the observation in the past of detrimental effects during clofibrate administrations with paradoxical elevation of cholesterol (9, 10). In contrast, several occasional observations have indicated that cholestasis may improve during simvastatin (11) and pravastatin (12) administration. In view of the profound alteration of cholesterol metabolism occurring in PBC, pilot studies aimed at evaluating whether the metabolic effects of inhibitors of HMGCoA reductase in patients with PBC are similar to those observed in hypercholesterolemic patients with normal liver function are needed.

On the other hand, ursodeoxycholic acid (UDCA), a well-established therapy for PBC (13, 14), may exert significant changes on cholesterol metabolism in these patients and, ultimately, significantly reduce the risk associated with hypercholesterolemia (15, 16). Therefore, a comparison of the extent and type of changes in cholesterol me-

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tabolism induced by UDCA with those observed during the administration of inhibitors of HMGCoA reductase may provide information on the best way to treat hypercholesterolemia in PBC patients.

For this purpose, we have designed a pilot study in which six patients with hypercholesterolemia and well-compensated PBC have been enrolled. The effects on parameters related to cholesterol synthesis, absorption, elimination, and catabolism have been evaluated in details, and the kinetics of these changes are reported.

## MATERIALS AND METHODS

### Patients

Six patients with PBC and hypercholesterolemia (serum cholesterol >240 mg/dl) were enrolled in the study. In addition, to study the short-term effects of simvastatin administration on cholesterol metabolism, a control group of six PBC patients receiving no treatment was included. Characteristics of all patients are reported in **Table 1**. Diagnosis of PBC was made according to the usual biochemical, serological, clinical, and histological criteria (1). Only patients with elevated serum concentrations of liver enzymes related to cholestasis, e.g., alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT), with well-compensated liver disease and normal bilirubin concentrations, were enrolled. Ultrasound examination of the upper abdomen had been carried out within the previous 3 months, to exclude the presence of liver tumors or extrahepatic biliary obstruction. None of the patients had been treated with UDCA in the previous 3 months.

The protocol of the study was approved by the bioethics committee of San Paolo Hospital, and patients gave their consent after being carefully informed about the aims and the procedures of the study.

### Study design

At the beginning of the study, all patients were put on a standardized 1,800-kcal hypolipidemic diet regimen for at least 4 weeks before blood collection. Blood samples used for the evaluation of sterol serum levels were always collected after an overnight fast. Automated routine methods were used to measure serum liver enzyme and plasma lipid concentrations.

After baseline evaluation, the six PBC patients were administered 40 mg of simvastatin orally, once daily, in the morning, for a period of 30 days. To monitor the early changes induced by the drug on cholesterol metabolism, simvastatin was administered to each patient at 8:00 AM. Venous blood was collected 2, 4, 6, 8, and 24 h after the first dose. Treatment continued for 30 days; blood was collected after 10 days and at the end of the treatment. Venous blood was also collected from the six control patients at 8:00 AM and after 2, 4, 6, 8, and 24 h. After centrifugation, butylated hydroxytoluene (BHT) was added to avoid oxidation at a final concentration of 50 μg/ml serum. Serum samples were then frozen immediately at −20°C.

After a washout period of 30 days, the six patients were put on UDCA treatment, 600 mg daily, administered in two divided doses. Blood collection for the study of cholesterol metabolism was scheduled after 1 month of treatment. In view of the significant improvement in survival induced by UDCA in patients with PBC (13), bile acid treatment was scheduled to be continued indefinitely. Therefore, for three patients, data are also available after 12 months of UDCA administration.

### Chemicals

All solvents were obtained from Merck (Darmstadt, Germany) and were of analytical grade. Deuterated lathosterol, used as internal standard, was synthesized as described previously (17); 5α-cholestane and 19-hydroxycholesterol, also used as internal standards, and BHT were purchased from Sigma Chemical Co. (St. Louis, MO). All internal standards were dissolved in ethyl acetate containing BHT (50 μg/ml). Silica cartridge columns (Supelco LC-Si) and trimethylsilylimidazole were obtained from Supelco, Inc. (Bellefonte, PA).

### Lathosterol and plant sterol serum concentrations

Deuterated lathosterol (1 μg) and 5α-cholestane (1 μg) were added to 0.1 ml serum samples as internal standards for the measurement of lathosterol and dietary sterols, respectively. After alkaline hydrolysis with 1 ml 1N NaOH in 90% ethanol at 60°C for 90 min under nitrogen, samples were extracted, transformed into trimethylsilylimidazole (TMS) derivatives, and analyzed as described previously (7).

### Hydroxysterol serum concentrations

After addition of 250 ng of 19-hydroxycholesterol as internal standard to 0.2 ml serum, samples were hydrolyzed and extracted as described (7). The organic phase was evaporated to dryness under a stream of nitrogen and purified by solid phase extraction. The fraction containing the hydroxysterols was dried and treated with trimethylsilylimidazole:piperidine (1:1). (TMSIM-PIP).

### Gas chromatography-mass spectrometry analysis

Analysis was carried out under previously described conditions, monitoring ions at m/z 372 for detection of cholestane, m/z 382 for campesterol, m/z 255 and 259 for lathosterol and deuterated lathosterol, respectively, m/z 353 for 19-hydroxycholesterol, and m/z 456 for 7α-hydroxycholesterol and 27-hydroxycholesterol. Calibration curves were prepared, spiking serum with fixed amounts of each internal standard and increasing amounts of the above-mentioned sterols, and were treated and analyzed as the samples. Concentrations were calculated on the basis of the slope of the standard curve and on the peak area ratio (sterol-internal standard) found in the sample.

### Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). The significance of the differences was evaluated by Duncan's new multiple range test and by paired $t$-test for comparisons within groups.

TABLE 1. Clinical characteristics of patients with primary biliary cirrhosis (PBC) enrolled in the study

| | Gender | Age | Bilirubin | Albumin | Total cholesterol | HDL cholesterol |
|---|---|---|---|---|---|---|
| | F/M | yrs | mg/dl | g/dl | mg/dl | mg/dl |
| Treated patients (n = 6) | 5/1 | 63 (56–72) | 0.6 (0.5–0.8) | 4.7 (4.3–4.9) | 312 (247–454) | 82 (52–149) |
| Controls (n = 6) | 5/1 | 60 (50–73) | 0.7 (0.4–1.0) | 4.5 (4.2–4.9) | 300 (246–371) | 55 (33–79) |

Values are expressed as means (range). HDL, high density lipoprotein.

TABLE 2. Changes in serum liver enzyme concentrations induced by simvastatin and ursodeoxycholic acid (UDCA)
administration to six patients with primary biliary cirrhosis

| | | Simvastatin | | | UDCA | |
| | Baseline | 10 days | 30 days | Washout | 30 days | 12 months[a] |
|---|---|---|---|---|---|---|
| ALT | 87 (22–214) | 83 (31–211) | 91 (23–225) | 83 (23–186) | 52 (22–110) | 70 (48–103) |
| AST | 70 (24–164) | 70 (24–135) | 69 (21–153) | 65 (20–150) | 46 (19–77) | 55 (33–68) |
| GGT | 602 (83–1,439) | 581 (66–1,410) | 581 (59–1,300) | 596 (79–1,302) | 325 (69–695) | 537 (89–863) |
| ALP | 806 (307–1,790) | 807 (279–1,781) | 890 (255–1,800) | 769 (274–1,726) | 527 (349–937) | 612 (366–915) |

Values are means (range) for the six tested patients. Concentrations of both serum liver enzymes are expressed as IU/l. Normal values: ALT < 50; AST < 45; GGT < 32; ALP < 279. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase.

[a] Values for 12-month treatment with UDCA are available only for three patients.

## RESULTS

### Safety and compliance

Both simvastatin and UDCA were well tolerated, and no patient complained of adverse effects during treatment. Compliance was assessed by counting unused capsules and was judged to be good, as all patients took more than 95% of the dispensed dose. Serum concentrations of liver enzymes related to cytolysis and cholestasis before and during treatment are reported in **Table 2**. No substantial changes were observed at any time during simvastatin administration, whereas a reduction occurred as expected during UDCA administration.

### Changes induced by simvastatin on cholesterol metabolism

**Figure 1** shows the acute changes in serum levels of cholesterol and lathosterol induced by the first administration of simvastatin; in the control group receiving no



*LATHOSTEROL*

*CHOLESTEROL*

*$P$ < 0.01 versus controls.

**Fig. 1** Changes during day of serum concentrations of cholesterol and lathosterol after the first administration of 40 mg of simvastatin to primary biliary cirrhosis (PBC) patients. In a control group of PBC patients administered no treatment, blood was sampled on the same schedule. Data are expressed as percentages of basal values.
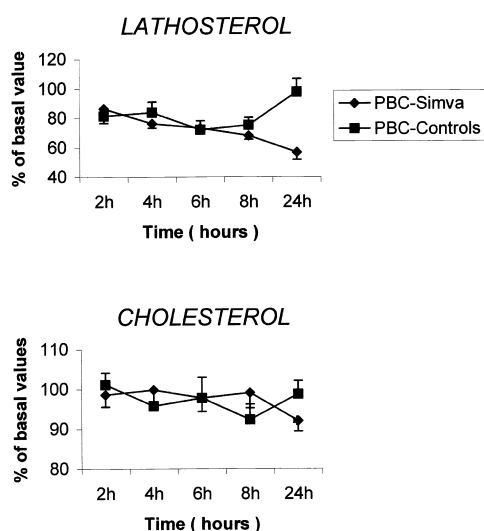
treatment, blood was taken according to the same schedule. In both groups, lathosterol decreased during the day (from 8:00 AM to 4:00 PM). In treated patients, a further reduction was observed after 24 h; these values returned to baseline in the control group. Therefore, a significant difference between the two groups was observed after only 24 hours. During the first day of treatment, campesterol, 7α-hydroxycholesterol, and 27-hydroxycholesterol serum levels did not change significantly in either group.

In the days following treatment, serum cholesterol concentrations progressively decreased to about 66% of basal values at 30 days, whereas lathosterol showed a parallel but more marked decrease to 45% of initial levels (**Table 3**). A substantial but not significant decrease in serum levels of 7α-hydroxycholesterol was found, but no relevant changes occurred in 27-hydroxycholesterol levels. (Table 3).

### Changes induced by UDCA on cholesterol metabolism

One month after UDCA treatment, only 7α-hydroxycholesterol serum levels showed a marked decrease (Table 3).

In three patients, after long-term UDCA administration (12 months), we found a slight reduction of serum cholesterol (316 ± 16 vs. 355 ± 31 mg/dl, or 11%), whereas serum concentrations of campesterol decreased in all patients (from 333 ± 147 to 186 ± 50 μg /100 mg cholesterol). In the same patients, serum levels of 27-hydroxycholesterol paralleled cholesterol concentrations (from 27 ± 5 to 23 ± 1 μg/dl)

## DISCUSSION

The results of our pilot study indicate that simvastatin has a marked effect on cholesterol metabolism in patients with PBC and hypercholesterolemia. In the present study, parameters of cholesterol synthesis, elimination, and catabolism (3–5, 18) have been studied before and during the administration of inhibitors of HMGCoA reductase in patients with PBC. These effects were compared with those seen during administration of UDCA. No data on lipoprotein composition are available, as these measurements were not part of the study design.

The reduction of serum cholesterol levels occurred within 24 h after simvastatin administration. Because a correlation between serum lathosterol concentrations and

TABLE 3. Effects of simvastatin or UDCA administration to patients with PBC
on parameters related to cholesterol metabolism[a]

| Sterol | Baseline | Simvastatin 10 days | Simvastatin 30 days | Washout | UDCA 30 days |
|---|---|---|---|---|---|
| Cholesterol (mg/dl) | 295 ± 39 | 229 ± 31[b] | 195 ± 15[b] | 292 ± 33 | 312 ± 37 |
| Lathosterol (μg/100 mg chol.) | 100 ± 23 | 41 ± 11[c] | 45 ± 14[c] | 96 ± 25 | 89 ± 24 |
| Campesterol (μg/100 mg chol.) | 201 ± 98 | 253 ± 109 | 236 ± 76 | 226 ± 82 | 232 ± 49 |
| 7α-hydroxycholesterol (μg/dl) | 7.9 ± 1.1 | 6.2 ± 0.6 | 5.5 ± 0.7 | 10.6 ± 2.9 | 6.4 ± 0.5 |
| 27-hydroxycholesterol (μg/dl) | 21.4 ± 1.7 | 19.5 ± 2.5 | 18.1 ± 2.9 | 26.5 ± 4.1 | 25.7 ± 2.8 |

[a] Values are mean ± SEM of the tested patients.
[b] $P < 0.05$ versus baseline and washout (paired $t$-test).
[c] $P < 0.01$ versus baseline and washout (paired $t$-test).

HMGCoA reductase activity in the liver of non-PBC patients has been described (19), the concomitant decrease of lathosterol in our patients is in keeping with a direct inhibitory effect of simvastatin on this enzyme similar to that observed in patients with hypercholesterolemia and normal liver function (20). The paradoxical effect reported with clofibrate in PBC patients (9, 10) was obviously related to the different mechanisms of action.

In gallstone patients with normal liver function, plant sterols were shown to increase significantly during long-term simvastatin administration, and this effect was attributed to decreased biliary sterol elimination (21). In contrast, campesterol serum levels did not change in our PBC patients, suggesting that neither sterol absorption nor biliary elimination was affected by short-term administration of the drug. Both 7α-hydroxycholesterol and 27-hydroxycholesterol are parameters of cholesterol catabolism (18, 22, 23), which takes place in the liver by the activity of the cholesterol 7α-hydroxylase (18, 22) or in extrahepatic tissues by the activity of the sterol 27-hydroxylase (23–26). In our patients, serum levels of 27-hydroxycholesterol in basal conditions were lower than expected; this confirms previous observations of our group (7). During short-term simvastatin administration, no significant changes in parameters of cholesterol catabolism were observed, and the nonsignificant reduction of 7α-hydroxycholesterol may be due to a reduced availability of cholesterol.

Simvastatin was well tolerated, and no impairment of the biochemical expression of liver disease was observed in any of the patients. The previously described improvement of the cholestatic profile (11, 12) was not seen in our study, but that may have been due to the limited number of patients and the short duration of treatment.

Serum concentrations of cholesterol slightly decreased during UDCA administration, as previously reported (15), but the decrease was less than that induced by simvastatin administration. In contrast to findings in patients with normal liver function (27), no effects on cholesterol synthesis were observed during both short- and long-term administration of UDCA, as indicated by the unmodified lathosterol concentrations. In gallstone patients with normal liver function, plant sterols were shown to increase during UDCA administration, which was attributed to decreased cholesterol biliary elimination (27). The substantial decrease of campesterol serum levels after long-term UDCA administration is consistent with sterol malabsorption induced by UDCA, as observed in gallstone patients (21), but in cholestatic patients, the improvement of biliary secretion (27) may also play a role. Also, the trend toward a reduction of 7α-hydroxycholesterol that has been observed during UDCA administration may be explained by a reduced availability of cholesterol owing to reduced intestinal absorption (21) or increased biliary elimination (28).

In summary, in patients with PBC, simvastatin reduces cholesterol serum levels to an extent similar to that observed in hypercholesterolemic patients with normal liver function (20). In contrast, UDCA has only limited effects on cholesterol serum levels and only after long-term administration. To evaluate the effects induced on cholesterol metabolism by inhibitors of HMGCoA reductase and whether they may be additive to those induced by UDCA, as already reported in patients with normal liver function (29), future studies should include larger numbers of patients and go on for longer periods of time.

## REFERENCES

1. Neuberger, J. 1997. Primary biliary cirrhosis. *Lancet.* **350:** 875–879.
2. Crippin, J. S., K. D. Lindor, R. Jorgensen, B. A. Kottke, J. M. Harrison, P. A. Murtaugh, and E. R. Dickson. 1992. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? *Hepatology.* **15:** 858–862.
3. Miettinen, T. A. 1972. Lipid absorption, bile acids and cholesterol metabolism in patients with chronic liver disease. *Gut.* **13:** 682–689.
4. Nikkilä, K., and T. A. Miettinen. 1988. Serum cholesterol precursors, cholestanol, and plant sterols in primary biliary cirrhosis. *Scand. J. Gastroenterol.* **23:** 967–972.
5. Nikkila, K., K. Hockerstedt, and T. A. Miettinen. 1991. High cholestanol and low campesterol-to-sitosterol ratio in serum of patients with primary biliary cirrhosis before liver transplantation. *Hepatology.* **13:** 663–669.
6. Propst, A., T. Propst, M. Lechleitner, F. Hoppichler, H. Kathrein, W. Vogel, G. Judmaier, E. Knapp, and H. Braunsteiner. 1993. Hypercholesterolemia in primary biliary cirrhosis is no risk factor for atherosclerosis. *Dig. Dis. Sci.* **38:** 379–380.
7. Del Puppo, M., M. Galli Kienle, M. L. Petroni, A. Crosignani, and

M. Podda. 1998. Serum 27-hydroxycholesterol in patients with primary biliary cirrhosis suggests alteration of cholesterol catabolism to bile acids via the acidic pathway. *J. Lipid Res.* **39:** 2477–2482.

8. Van Dam, G. M., and C. H. Gips. 1997. Primary biliary cirrhosis in the Netherlands. An analysis of associated diseases, cardiovascular risk, and malignancies on the basis of mortality figures. *Scand. J. Gastroenterol.* **32:** 77–83.

9. Summerfield, J. A., E. Elias, and S. Sherlock. 1975. Effects of clofibrate in primary biliary cirrhosis hypercholesterolemia and gallstones. *Gastroenterology.* **69:** 998–1000.

10. Schaffner, F. 1969. Paradoxical elevation of serum cholesterol by clofibrate in patients with primary biliary cirrhosis. *Gastroenterol.* **57:** 253–255.

11. Kamisako, T., and Y. Adachi. 1995. Marked improvement in cholestasis and hypercholesterolemia with simvastatin in a patient with primary biliary cirrhosis. *Amer. J. Gastroenterol.* **90:** 1187–1188.

12. Kurihara, T., M. Akimoto, K. Abe, H. Ishiguro, A. Niimi, A. Maeda, M. Shigemoto, K. Yamashita, I. Yokoyama, Y. Suzuki, and H. Adachi. 1993. Experimental use of pravastatin in patients with primary biliary cirrhosis associated with hypercholesterolemia. *Clin. Ther.* **15:** 890–898.

13. Poupon, R. E., K. D. Lindor, K. Cauch-Dudek, E. R. Dickson, R. Poupon, and E. J. Heathcote. 1997. Combined analysis of randomised controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. *Gastroenterol.* **113:** 884–890.

14. Rubin, R. A., T. E. Kowalsky, M. Khandelwal, and P. F. Malet. 1994. Ursodiol for hepatobiliary disorders. *Ann. Inter. Med.* **121:** 207–218.

15. Poupon, R. E., K. Ouguerram, Y. Chrétien, C. Verneau, E. Eschwège, T. Magot, and R. Poupon. 1993. Cholesterol lowering effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. *Hepatology.* **17:** 577–582.

16. Miettinen, T. A., M. Farkkila, M. Vuoristo, A. L. Karvonen, R. Leino, J. Lehtola, C. Friman, K. Seppala, and J. Tuominen. 1995. Serum cholestanol, cholesterol precursors, and plant sterols during placebo-controlled treatment of primary biliary cirrhosis with ursodeoxycholic acid or colchicine. *Hepatology.* **21:** 1261–1268.

17. Galli Kienle, M., M. Anastasia, G. Cighetti, G. Galli, and A. Fiecchi. 1980. Studies on the 14α-demethylation mechanism in cholesterol biosynthesis. *Eur. J. Biochem.* **110:** 93–105.

18. Hahn, C., C. Reichel, and K. von Bergmann. 1995. Serum concentration of 7α-hydroxycholesterol as an indicator of bile acid synthesis in humans. *J. Lipid Res.* **36:** 2059–2066.

19. Bjorkem, I., T. Miettinen, E. Reihner, S. Ewerth, B. Angelin, and K. Einarsson. 1987. Correlation between serum levels of some cholesterol precursors and activity of HMGCoA reductase in human liver. *J. Lipid Res.* **28:** 1137–1143.

20. Feillet, C., M. Farnier, L. H. Monnier, C. Percheron, C. Colette, B. Descomps, and A. Crastes De Paulet. 1995. Comparative effects of simvastatin and pravastatin on cholesterol synthesis in patients with primary hypercholesterolemia. *Atherosclerosis.* **118:** 251–258.

21. Miettinen, T. E., T. Kiviluoto, M. Taavitsainen, M. Vuoristo, and T. A. Miettinen. 1998. Cholesterol metabolism and serum and biliary noncholesterol sterols in gallstone patients during simvastatin and ursodeoxycholic acid treatments. *Hepatology.* **27:** 649–655.

22. Oda, H., H. Yamashita, K. Kosahara, S. Kuroki, and F. Nakayama. 1990. Esterified and total 7α-hydroxycholesterol in human serum as an indicator for hepatic bile acid synthesis. *J. Lipid Res.* **31:** 2209–2218.

23. Axelson, M., and J. Sjovall. 1990. Potential bile acid precursors in plasma: possible indicators of biosynthetic pathways to cholic and chenodeoxycholic acids in man. *J. Steroid Biochem.* **36:** 631–640.

24. Axelson, M., and O. Larsson. 1995. Low-density lipoprotein (LDL) cholesterol is converted to 27-hydroxycholesterol in human fibroblast. Evidence that 27-hydroxycholesterol can be an important intracellular mediator between LDL and the suppression of cholesterol production. *J. Biol. Chem.* **270:** 15102–15110.

25. Babiker, A., O. Andersson, E. Lund, R. J. Xiu, S. Deeb, A. Reshef, E. Leitersdorf, U. Diczfalusy, and I. Bjorkhem. 1997. Elimination of cholesterol in macrophages and endothelial cells by the sterol 27-hydroxylase mechanism. Comparison with high density lipoprotein-mediated reverse cholesterol transport. *J. Biol. Chem.* **272:** 26253–26261.

26. Princen, H. M. G., P. Meijer, B. G. Wolthers, R. J. Vonk, and F. Kuipers. 1991. Cyclosporin A blocks bile acid synthesis in cultured hepatocytes by specific inhibition of chenodeoxycholic acid synthesis. *Biochem. J.* **275:** 501–505.

27. Miettinen, T. E., S. Tarpila, and H. Gylling. 1997. The effects of ursodeoxycholic acid on serum and biliary noncholesterol sterols in patients with gallstones. *Hepatology.* **25:** 514–518.

28. Jazrawi, R. P., J. S. de Caestecker, P. M. Goggin, A. J. Britten, A. E. A. Joseph, J. D. Maxwell, and T. C. Northfield. 1994. Kinetics of hepatic bile acid handling in cholestatic liver disease: effect of ursodeoxycholic acid. *Gastroenterol.* **106:** 134–142.

29. Lanzarotto, F., B. Panarotto, R. Sorbara, M. Panteghini, F. Pagani, S. Sosta, and A. Lanzini. 1999. Effect of long term simvastatin administration as an adjunct to ursodeoxycholic acid: evidence for a synergistic effect on biliary bile acid composition but not on serum lipids in humans. *Gut.* **44:** 552–556.