Serum plant sterols and biliary cholesterol secretion in humans: studies with ursodeoxycholic acid

Bernhard Lindenthal,* Thomas Sudhop,* Peter Schiedermaier,† Mohamed Agnan,* Tilman Sauerbruch,† and Klaus von Bergmann[1,*]

Department of Clinical Pharmacology* and Department of Internal Medicine,† University of Bonn, Germany

Abstract **Ratios of cholestanol, campesterol, and sitosterol to cholesterol in serum are known to reflect cholesterol absorption efficiency. Here, a possible link between these ratios and biliary secretion rates of cholesterol was investigated. Biliary lipid secretion rates and serum sterols were determined in 13 patients with gallstones. Seven were treated with ursodeoxycholic acid (UDCA) (1,000 mg/d). Serum cholesterol and non-cholesterol sterols were also measured in a cross over study in 20 healthy volunteers, who received either placebo or UDCA (750 mg/d). Biliary cholesterol secretion was significantly lower, whereas the non-cholesterol sterols and their ratio to cholesterol were higher in patients with gallstones treated with UDCA. A highly significant negative linear correlation between the ratios of non-cholesterol sterols to cholesterol and biliary cholesterol secretion was observed. In volunteers, administration of UDCA for 4 weeks was followed by a significant increase in non-cholesterol sterols and their ratios. Even 4 weeks after discontinuing UDCA administration, campesterol and sitosterol were still significantly higher than pretreatment levels, which was also true for the campesterol-cholesterol ratio after 8 weeks. The results suggest that the ratios of cholestanol, campesterol, and sitosterol to cholesterol can be used as indicators of changes in biliary cholesterol secretion rates.**—Lindenthal, B., T. Sudhop, P. Schiedermaier, M. Agnan, T. Sauerbruch, and K. von Bergmann. **Serum plant sterols and biliary cholesterol secretion in man: studies with ursodeoxycholic acid.** ***J. Lipid Res.*** **2002.** 43: **1072–1077.**

Supplementary key words cholestanol • sitosterol • campesterol • non-cholesterol sterols

Cholestanol and the two plant sterols campesterol and sitosterol are present in low concentrations in human blood (1, 2), but their concentrations are 400- to 1,000-fold lower than cholesterol (1). The concentrations in serum are the results of synthesis (for cholestanol only), absorption efficiency (campesterol and sitosterol), lipoprotein metabolism, and rate of hepatic excretion into bile (3). They are absorbed from the intestine to a much lesser extent than cholesterol (4, 5). Cholestanol is synthesized in the liver from cholesterol (6), and is almost absent from the diet. Whereas sitosterol is not metabolized to a significant extent in humans (7), a conversion of cholestanol to bile acids has been reported (6). The ratios of cholestanol, campesterol, and sitosterol to cholesterol in serum have been shown to correlate positively with the cholesterol absorption efficiency (2, 8), and have therefore been regarded as markers for changes in the absorption of cholesterol (8–10).

Recently it has been reported that treatment with ursodeoxycholic acid (UDCA), a bile acid known to reduce the hepatic secretion of cholesterol (11–14), increases serum concentrations of plant sterols and cholestanol in patients with primary biliary cirrhosis (15) and patients with radiolucent gallstones (16, 17). Considering that cholesterol absorption is not affected or even reduced by UDCA (13, 14, 18–21), we thought that these results were not consistent with the observation that the ratio of plant sterols to cholesterol are only markers of cholesterol absorption efficiency. The purpose of the present study was to examine whether the ratios of non-cholesterol sterols to cholesterol in serum also reflect biliary secretion of cholesterol and for how long the non-cholesterol sterols remain elevated after discontinuing UDCA administration.

Thus, we conducted two studies. In study I we measured serum cholestanol and plant sterols concentrations and their ratio to cholesterol as well as biliary cholesterol secretion in patients with gallstones. Seven of these patients were treated with UDCA, and six were not. In study II, serum concentrations of the non-cholesterol sterols were measured before, during, and after UDCA administration in healthy volunteers in a randomized, placebo controlled cross-over study.

Manuscript received 21 December 2001 and in revised form 9 April 2002.
DOI 10.1194/jlr.M100438-JLR200

Abbreviations: UDCA, ursodeoxycholic acid.

[1] To whom correspondence should be addressed.
e-mail: vonbergmann@uni-bonn.de

PATIENTS AND METHODS

Study I

Biliary lipid secretion rates were measured in 13 patients with radiolucent gallstones. Seven patients were treated with UDCA (1,000 mg/day) for 4 weeks and six patients were not treated. Measurements of biliary lipid secretion were performed by the method of Grundy and Metzger (22), as described previously (12, 23). Briefly, on the evening before the study the patients were admitted to the metabolic ward of the Department of Clinical Pharmacology and swallowed a triple-lumen tube. The next morning the tube was positioned by X-ray guidance in the duodenum with the two most proximal outlets opposite to the ampulla of Vater and the third 10 cm distally. A liquid formula diet (Nutrison, R. Braun, Melsungen, Germany) was infused constantly into the most proximal outlet (1.42 kcal/kg × h). The liquid formula contained sitosterol (7 mg/100 ml), which was used as a marker to calculate biliary secretion rates. When liquid formula infusion was started, patients on therapy swallowed two capsules of UDCA (500 mg). After allowing 4 hours for gallbladder contraction and stabilization of hepatic lipid secretion, hourly samples were collected for the following 6 h from the second proximal and distal outlets as described previously (12, 23). Fasting blood samples were obtained after an overnight fast and serum samples were stored at −20°C for analysis of cholesterol and non-cholesterol sterols. Characteristics of the patients are given in **Table 1**.

Study II

Serum samples of 20 healthy, non-smoking volunteers (5 female, 15 male, aged 19–38) had been stored at –20°C from a previous study, in which the effect of UDCA on splanchnic and systemic hemodynamics and gallbladder motility had been investigated (24). All individuals had been randomly assigned to either group I or group II. Group I had received a placebo first for 4 weeks followed by a washout period of 4 weeks, and thereafter UDCA (250 mg tid) for 4 weeks. Group II had received UDCA first for 4 weeks, and after the washout period, placebo for 4 weeks. Before and at the end of each treatment period, fasting blood samples had been obtained after an overnight fast. None of the subjects had a history of gastrointestinal diseases, diabetes, or renal impairment. None of the volunteers were taking any kind of drugs, including oral contraceptives. One volunteer from group I dropped out before administration of UDCA for personal reasons. Serum samples were analyzed for cholesterol and non-cholesterol sterols.

Both study protocols were in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the ethical committee of the University of Bonn. Written informed consent was obtained from each subject.

Analytical methods

Biliary lipids were measured after extraction (25) and bile acids, phospholipids, and cholesterol were measured as described previously (12). Serum cholesterol, cholestanol, campesterol, and sitosterol were analyzed by gas-liquid chromatography (GLC) (4). Briefly, 5α-cholestane (50 μg) was added to 0.1 ml serum as internal standard. After alkaline hydrolysis, extraction, and derivatisation to the trimethylsilyl ethers, the sterols were quantified by GLC.

TABLE 1. Characteristics of patients with radiolucent gallstones

Treatment	Age	Weight	BMI[a]
	years	*kg*	*kg/m²*
Control (n = 6)			
(four women, two men)	38 ± 12	72 ± 7	23.4 ± 0.9
UDCA (n = 7)			
(three women, four men)	43 ± 17	72 ± 5	23.9 ± 0.4

Values are mean ± SD.
[a] BMI, body mass index [weight (kg) / height² (m)].

Statistical analysis

Data are expressed as mean ± SD to show variation within a group. The changes in sterol concentration within the groups treated with UDCA or placebo were analyzed with the Student's *t*-test for dependent samples. The subgroup analysis in patients with gallstones was performed with a Student's *t*-test for independent samples. *P* of less than 0.05 was considered statistically significant. Correlation analysis was calculated as Pearson's product moment using Fisher's test for significance. All other calculations were performed with the statistical software SPSS™ 9.0 package (SPSS Inc., Chicago, IL).

RESULTS

Study I

Biliary lipid secretion rates of cholesterol, phospholipids, and bile acids in patients with radiolucent gallstones with and without UDCA treatment are summarized in **Table 2**. As expected, cholesterol output was significantly lower (−28%, $P < 0.05$) in patients treated with UDCA than in patients without treatment, whereas total bile acid and phospholipid secretions did not differ. The concentrations of cholesterol and the non-cholesterol sterols together with their ratio to cholesterol are given in **Table 3**. In patients treated with UDCA, serum concentrations of cholesterol were 11% lower than in the other group, but this difference did not reach statistical significance. The levels of cholestanol, campesterol, and sitosterol were 57%, 40%, and 53% higher in patients treated with UDCA. This was only significant for sitosterol ($P < 0.05$). However, the ratios of cholestanol, campesterol, and sitosterol to cholesterol were significantly higher than in patients not treated with UDCA (71%, 69%, and 76%). Combining the results of all patients, a strong negative linear correlation between the ratios of cholestanol, campesterol, and sitosterol to cholesterol in serum and biliary cholesterol secretion was observed (**Fig. 1**). Even after biliary cholesterol secretions were adjusted for body weight, this correlation remained highly significant for all ratios ($P < 0.002$ or less; data not shown).

Study II

In both groups of volunteers, administration of UDCA had no effect on total cholesterol concentrations in serum (**Table 4**). In group I, all sterol concentrations also remained remarkably constant during the 8 weeks without UDCA acid (4 weeks placebo and 4 weeks washout period), indicating metabolic steady state conditions. Cholestanol increased significantly during UDCA treatment in both groups compared with pretreatment levels. The increase during treatment with UDCA in group I and II averaged 16% and 20%, respectively. In group II, cho-

TABLE 2. Biliary lipid secretion in patients with radiolucent gallstones with and without treatment with UDCA

Group	Cholesterol	Phospholipids	Bile Acids
		mg/h	
Control (n = 6)	60 ± 14	363 ± 112	1170 ± 487
UDCA (n = 7)	43 ± 13[a]	391 ± 115	1169 ± 381

Values are mean ± SD.
[a] Significantly different from control ($P < 0.05$).

lestanol decreased to pretreatment levels 4 weeks after discontinuing UDCA and remained constant thereafter (Table 4). Four weeks of UDCA administration also significantly increased the ratio of cholestanol to cholesterol in group I and II by 13% and 24%, respectively (**Table 5**). Campesterol and sitosterol showed an even more pronounced increase during UDCA treatment, and the concentration of campesterol rose by 42% in group I and 55% in group II. Sitosterol increased by 61% in both groups. Four weeks after UDCA was discontinued in group II, campesterol and sitosterol levels were still significantly higher than pretreatment levels (14% and 21%) (Table 4); even after 8 weeks, the concentrations of campesterol and sitosterol were still 8% and 10% higher but not of statistical significance ($P = 0.07$ for campesterol and $P = 0.08$ for sitosterol). Also, the ratios of campesterol to cholesterol and sitosterol to cholesterol increased significantly after 4 weeks of administration of UDCA by 38% and 67%, respectively (Table 5). Even after 8 weeks, the ratio of both plant sterols to cholesterol did not return to pretreatment values. The ratio of campesterol to cholesterol and sitosterol to cholesterol were still 8% ($P < 0.05$) and 10% ($P = 0.117$) higher compared with pretreatment levels.

The ratios of noncholesterol sterols to cholesterol in the six patients with radiolucent gallstones were lower compared with the values in the healthy volunteers during the control period and even after UDCA administration. These results might indicate that patients have higher secretion rates of biliary cholesterol than controls.

TABLE 3. Serum sterols and their ratios to cholesterol in serum

	Control (n = 6)	UDCA (n = 7)
Cholesterol (mg/dl)	196 ± 40	175 ± 57
Cholestanol (mg/dl)	0.282 ± 0.065	0.441 ± 0.207
Campesterol (mg/dl)	0.422 ± 0.150	0.594 ± 0.218
Sitosterol (mg/dl)	0.165 ± 0.034	0.259 ± 0.091[a]
Cholestanol/cholesterol (μg/mg)	1.477 ± 0.381	2.520 ± 0.805[a]
Campesterol/cholesterol (μg/mg)	2.155 ± 0.615	3.631 ± 1.337[a]
Sitosterol/cholesterol (μg/mg)	0.908 ± 0.332	1.587 ± 0.531[a]

Values are mean ± SD. Serum sterols and their ratios to cholesterol in serum in patients with radiolucent gallstones with and without treatment with ursodeoxycholic acid (UDCA 1000 mg/day).
[a] Significantly different from control ($P < 0.05$).

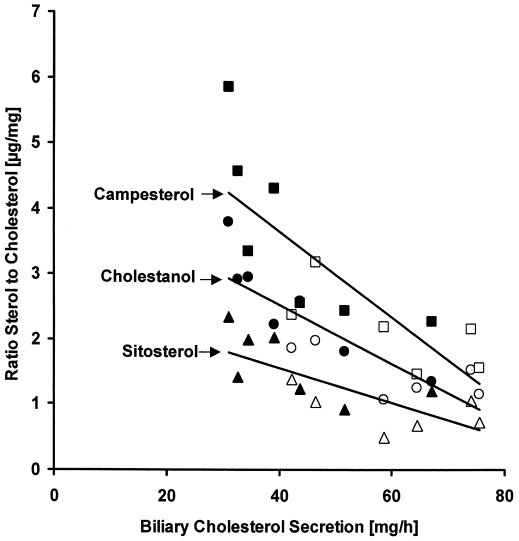

Fig. 1. Linear regression analysis between biliary cholesterol secretion and ratio of campesterol ($r = -0.806$, $P < 0.001$), cholestanol ($r = -0.863$, $P < 0.001$), and sitosterol (-0.752, $P < 0.005$) to cholesterol in patients with radiolucent gallstones, with (closed symbols) and without (open symbols) ursodeoxycholic acid treatment.

DISCUSSION

The present study was carried out to evaluate the effect of UDCA administration on serum concentrations of cholestanol, campesterol, and sitosterol in patients with gallstones and normolipemic, healthy volunteers, and to elucidate a possible link between serum concentrations of these sterols and biliary secretion of cholesterol. After UDCA treatment, the concentrations of these sterols, as well as their ratios to cholesterol, increased significantly. The results are in agreement with those recently reported in patients with gallstones (16, 17) and primary biliary cirrhosis (15). Miettinen et al. (15–17) found a negative relationship between the change of the ratio of campesterol to cholesterol in serum before and during UDCA therapy and the change in cholesterol saturation in gallbladder bile. From the results, the authors suggest that under these circumstances levels of plant sterols might be affected by their biliary elimination and therefore reflect changes in biliary cholesterol secretion. However, measurements of biliary cholesterol secretion were not performed. Therefore, a direct relationship between serum concentrations of these sterols and biliary cholesterol secretion could not be proven.

Administration of UDCA is known to reduce hepatic secretion of cholesterol into bile (11–14). Some, but not all, studies have shown that UDCA also reduces intestinal cholesterol absorption efficiency (13, 14, 18–21), which one would also expect to cause a reduction of the ratio of plant sterols to cholesterol in serum. Therefore, the findings of the present study in patients with radiolucent gallstones and normolipemic volunteers cannot be related to reduced intestinal absorption of cholesterol and plant sterols by UDCA. The reduction in biliary cholesterol secre-

TABLE 4. Serum cholesterol, cholestanol, campesterol, and sitosterol in normolipemic volunteers before and after administration of ursodeoxycholic acid (250 mg tid) for 4 weeks in Group I and II

	Week 0	Week 4	Week 8	Week 12
		sterols (mg/dl)		
Cholesterol				
Group I	186 ± 19	183 ± 34	178 ± 45	180 ± 28
Group II	186 ± 24	181 ± 31	184 ± 24	188 ± 32
Cholestanol				
Group I	0.547 ± 0.054	0.543 ± 0.041	0.543 ± 0.055	0.628 ± 0.041[a]
Group II	0.594 ± 0.097	0.710 ± 0.104[b]	0.617 ± 0.084	0.605 ± 0.085
Campesterol				
Group I	0.388 ± 0.090	0.426 ± 0.118	0.433 ± 0.165	0.613 ± 0.185[a]
Group II	0.490 ± 0.102	0.761 ± 0.134[b]	0.558 ± 0.140[c]	0.530 ± 0.117[d]
Sitosterol				
Group I	0.249 ± 0.061	0.261 ± 0.068	0.268 ± 0.090	0.431 ± 0.120[a]
Group II	0.281 ± 0.051	0.453 ± 0.663[b]	0.339 ± 0.082[c]	0.310 ± 0.050

Values are mean ± SD. Group I received placebo (week 0 to 4) and after a washout period ursodeoxycholic acid (week 8 to 12), Group II received UDCA (week 0 to 4) and after a washout period placebo (week 8 to 12).

[a] Significantly different from Week 0, 4, and 8 ($P < 0.005$).

[b] Significantly different from Week 0, 8, and 12 ($P < 0.005$).

[c] Significantly different from Week 0 ($P < 0.05$), [d]($P = 0.073$).

tion has been attributed to the lower micellar capacity of UDCA to dissolve cholesterol in bile acid-lecithin mixtures (26, 27). Indeed, as we have previously shown, UDCA has no influence on the mobilization of hepatic cholesterol into bile in patients with gallstones (12). Therefore, it is likely that UDCA also reduces biliary output of plant sterols and cholestanol. Since UDCA has no effects on lipoprotein metabolism (16, 17, 28), retained plant sterols in the liver probably lead to higher absolute serum concentrations and their ratios to cholesterol. This led us to consider whether the ratio of cholestanol and plant sterols to cholesterol might be markers of cholesterol secretion into bile. In support of this hypothesis, we showed a close and highly significant negative linear relationship between the ratio of the non-cholesterol sterols to cholesterol with biliary cholesterol secretion in patients with radiolucent gallstones (Fig. 1). This is in line with the results of Miettinen et al. (17). These authors confirmed that UDCA decreases biliary cholesterol secretion by 26%, from 16.4 to 12.1 mg/kg × h^{-1}. However, the ratio of campesterol and sitosterol to cholesterol in bile did not change, indicating that hepatic secretion of campesterol and sitosterol were also reduced, whereas the ratios in serum increased. From the present results, it might also be considered that UDCA affects the expression of the ATP-binding-cassette transporters ABCG5 and/or ABCG8. These sterol transporters have recently been identified in the rare inherited disease of phytosterolemia (29, 30). In this disease, one of these two transporters is defective, leading to hyperabsorption and diminished biliary secretion of cholesterol and plant sterols. However, more specific investigations are necessary to prove this speculation.

Furthermore, the ratios of noncholesterol sterols to cholesterol in the six patients with radiolucent gallstones were lower compared with the values in the healthy volunteers during the control period and even after UDCA administration. These results might indicate that patients with cholesterol gallstones have higher secretion rates of biliary cholesterol than controls.

It is notable that in healthy volunteers cholestanol had returned to pretreatment levels 4 weeks after UDCA administration was stopped, whereas campesterol and sito-

TABLE 5. Ratio of serum cholestanol, campesterol, and sitosterol to cholesterol in normolipemic volunteers before and after administration of UDCA (250 mg tid) for 4 weeks in group I and II

	Week 0	Week 4	Week 8	Week 12
		μg/mg		
Cholestanol/cholesterol				
Group I	2.981 ± 0.393	3.020 ± 0.568	3.140 ± 0.600	3.540 ± 0.443[a]
Group II	3.193 ± 0.367	3.950 ± 0.397[b]	3.374 ± 0.374	3.262 ± 0.518
Campesterol/cholesterol				
Group I	2.092 ± 0.425	2.428 ± 0.856	2.497 ± 0.881	3.442 ± 1.111[a]
Group II	2.658 ± 0.611	4.256 ± 0.812[b]	3.066 ± 0.756[c]	2.855 ± 0.663[c]
Sitosterol/cholesterol				
Group I	1.361 ± 0.215	1.489 ± 0.419	1.586 ± 0.441	2.418 ± 0.717[a]
Group II	1.539 ± 0.324	2.573 ± 0.592[b]	1.871 ± 0.499[c]	1.686 ± 0.374

Values are mean ± SD. Group I received placebo (week 0 to 4) and after a washout period UDCA (week 8 to 12), Group II received UDCA (week 0 to 4) and after a washout period placebo (week 8 to 12).

[a] Significantly different from Week 0, 4, and 8 ($P < 0.005$).

[b] Significantly different from Week 0, 8, and 12 ($P < 0.005$).

[c] Significantly different from Week 0 ($P < 0.05$).

sterol were still significantly elevated at this time. The half-life of UDCA is short, and it is eliminated from the body in only 1 week (31, 32). Thus, biliary cholesterol secretion returned to pretreatment rates. Therefore, the continuing higher ratio of campesterol and sitosterol to cholesterol must be due to other mechanisms. One explanation may be the half-life of these sterols. The terminal half-life of sitosterol after intravenous injection in five normal subjects was 15.8 ± 2.4 (SD) days (1). This half-life could indeed explain that sitosterol and the ratio of sitosterol to cholesterol is still elevated 56 days after discontinuing UDCA (3.5 half-lives). Since the structure of campesterol (24-methyl-Δ5-cholesten-3β-ol) is more closely related to cholesterol than sitosterol (24-ethyl-Δ5-cholesten-3β-ol), the half-life of campesterol would be between that of sitosterol and cholesterol. This would explain why the ratio of campesterol to cholesterol is still significantly higher at the end of the study. From the present study, the half-life of cholestanol (5α-cholestan-3β-ol) must be markedly shorter than that of the two plant sterols. Indeed, earlier studies by Salen and Grundy (33) revealed a terminal half-life of 7.0 ± 2.8 (SD) days in five subjects. For this reason, cholestanol might be considered to be the better marker of changes in biliary cholesterol secretion as demonstrated in the present study. On the other hand, cholestanol is an endogenous product of cholesterol, and other mechanisms might influence its metabolism and serum concentration.

From our results, it can be concluded that the ratios of the sterols to cholesterol are not always accurate markers for intestinal cholesterol absorption efficiency, but can under special conditions be indicators for changes of biliary cholesterol secretion rates. However, when non-cholesterol sterols are used as indicators for these purposes, it is essential that metabolic steady state conditions have been stable for at least several weeks.

The authors thank Heike Prange, Katja Wilmersdorf, and Silvia Winnen for excellent technical assistance. This study was supported by a research grant from the Bundesministerium für Bildung, Forschung, Wissenschaft und Technologie (01EC9402). M.A. received a grant from the Government of Libya.

REFERENCES

1. Salen, G., E. H. Ahrens, Jr., and S. M. Grundy. 1970. Metabolism of beta-sitosterol in man. *J. Clin. Invest.* **49:** 952–967.
2. Gylling, H., and T. A. Miettinen. 1988. Serum noncholesterol sterols related to cholesterol metabolism in familial hypercholesterolemia. *Clin. Chim. Acta.* **178:** 41–49.
3. Weihrauch, J., and J. Gardner. 1978. Sterol content of foods of plant origin. *J. Am. Diet. Assoc.* **73:** 39–46.
4. Heinemann, T., G. Axtmann, and K. von Bergmann. 1993. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur. J. Clin. Invest.* **23:** 827–831.
5. Lütjohann, D., I. Bjorkhem, U. F. Beil, and K. von Bergmann. 1995. Sterol absorption and sterol balance in phytosterolemia evaluated by deuterium-labeled sterols: effect of sitostanol treatment. *J. Lipid Res.* **36:** 1763–1773.
6. Björkhem, I., and K. Boberg. 1995. Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. *In* The Metabolic and Molecular Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill Book, New York. 2073–2099.
7. Boberg, K. M., K. Einarsson, and I. Bjorkhem. 1990. Apparent lack of conversion of sitosterol into C24-bile acids in humans. *J. Lipid Res.* **31:** 1083–1088.
8. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesäniemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **131:** 20–31.
9. Miettinen, T. A., and H. Vanhanen. 1994. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atherosclerosis.* **105:** 217–226.
10. Tilvis, R. S., and T. A. Miettinen. 1986. Serum plant sterols and their relation to cholesterol absorption. *Am. J. Clin. Nutr.* **43:** 92–97.
11. Nilsell, K., B. Angelin, B. Leijd, and K. Einarsson. 1983. Comparative effects of ursodeoxycholic acid and chenodeoxycholic acid on bile acid kinetics and biliary lipid secretion in humans. Evidence for different modes of action on bile acid synthesis. *Gastroenterology.* **85:** 1248–1256.
12. von Bergmann, K., M. Epple-Gutsfeld, and O. Leiss. 1984. Differences in the effects of chenodeoxycholic and ursodeoxycholic acid on biliary lipid secretion and bile acid synthesis in patients with gallstones. *Gastroenterology.* **87:** 136–143.
13. Leiss, O., K. von Bergmann, U. Streicher, and H. Strotkoetter. 1984. Effect of three different dihydroxy bile acids on intestinal cholesterol absorption in normal volunteers. *Gastroenterology.* **87:** 144–149.
14. Hardison, W. G., and S. M. Grundy. 1984. Effect of ursodeoxycholate and its taurine conjugate on bile acid synthesis and cholesterol absorption. *Gastroenterology.* **87:** 130–135.
15. 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.
16. 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.
17. Miettinen, T. E., T. Kiviluoto, M. Taavitsainen, M. Vuoristo, and T. A. Miettinen. 1997. Cholesterol metabolism and serum and biliary noncholesterol sterols in gallstone patients during simvastatin and ursodeoxycholic acid treatments. *Hepatology.* **27:** 649–655.
18. Ponz de Leon, M., N. Carulli, P. Loria, R. Iori, and F. Zironi. 1980. Cholesterol absorption during bile acid feeding. Effect of ursodeoxycholic acid (UDCA) administration. *Gastroenterology.* **78:** 214–219.
19. LaRusso, N. F., and J. L. Thistle. 1983. Effect of litholytic bile acids on cholesterol absorption in gallstone patients. *Gastroenterology.* **84:** 265–271.
20. Salvioli, G., R. Lugli, and J. M. Pradelli. 1985. Cholesterol absorption and sterol balance in normal subjects receiving dietary fiber or ursodeoxycholic acid. *Dig. Dis. Sci.* **30:** 301–307.
21. von Bergmann, K., and D. Lütjohann. 1990. Effect of ursodeoxycholic acid on cholesterol absorption and faecal excretion or neutral sterols and bile acids in patients with primary biliary cirrhosis. *In* Bile Acids as Therapeutic Agents from Basic Science to Clinical Practice. G. Paumgartner, A. Stiehl, and W. Gerok, editors. Kluwer Academic Publishers, London. 257–262.
22. Grundy, S. M., and A. L. Metzger. 1972. A physiological method for estimation of hepatic secretion of biliary lipids in man. *Gastroenterology.* **62:** 1200–1217.
23. Leiss, O., K. Meyer-Krahmer, and K. von Bergmann. 1986. Biliary lipid secretion in patients with heterozygous familial hypercholesterolemia and combined hyperlipidemia. Influence of bezafibrate and fenofibrate. *J. Lipid Res.* **27:** 213–223.
24. Schiedermaier, P., S. Hansen, D. Asdonk, K. Brensing, and T. Sauerbruch. 2000. Effects of ursodeoxycholic acid on splanchnic and systemic hemodynamics: a double-blind, cross-over, placebo-controlled study in healthy volunteers. *Digestion.* **61:** 107–112.
25. Grundy, S. M., S. P. Lan, and J. Lachin. 1984. The effects of chenodiol on biliary lipids and their association with gallstone dissolution in the National Cooperative Gallstone Study (NCGS). *J. Clin. Invest.* **73:** 1156–1166.
26. Carey, M. C., J. C. Montet, M. C. Phillips, M. J. Armstrong, and N. A. Mazer. 1981. Thermodynamic and molecular basis for dissimilar

cholesterol-solubilizing capacities by micellar solutions of bile salts: cases of sodium chenodeoxycholate and sodium ursodeoxycholate and their glycine and taurine conjugates. *Biochemistry.* **20:** 3637–3648.
27. Igimi, H., and M. C. Carey. 1981. Cholesterol gallstone dissolution in bile: dissolution kinetics of crystalline (anhydrate and monohydrate) cholesterol with chenodeoxycholate, ursodeoxycholate, and their glycine and taurine conjugates. *J. Lipid Res.* **22:** 254–270.
28. Erlinger, S., A. Le Go, J. M. Husson, and J. Fevery. 1984. Franco-Belgian cooperative study of ursodeoxycholic acid in the medical dissolution of gallstones: a double-blind, randomized, dose-response study, and comparison with chenodeoxycholic acid. *Hepatology.* **4:** 308–314.
29. Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science.* **290:** 1771–1775.
30. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, A. K. Srivastava, G. Salen, M. Dean, and S. B. Patel. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat. Genet.* **27:** 79–83.
31. Fedorowski, T., G. Salen, A. Calallilo, G. S. Tint, E. H. Mosbach, and J. C. Hall. 1977. Metabolism of ursodeoxycholic acid in man. *Gastroenterology.* **73:** 1131–1137.
32. Hofmann, A. F. 1994. Pharmacology of ursodeoxycholic acid, an enterohepatic drug. *Scand. J. Gastroenterol.* **204 (Suppl.):** 1–15.
33. Salen, G., and S. M. Grundy. 1973. The metabolism of cholestanol, cholesterol, and bile acids in cerebrotendinous xanthomatosis. *J. Clin. Invest.* **52:** 2822–2835.