Influence of Newly Synthesized Cholesterol on Bile Acid Synthesis During Chronic Inhibition of Bile Acid Absorption

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The effects of newly synthesized cholesterol availability on bile acid synthesis are largely unknown, particularly in humans. The present study was aimed to study the changes induced on bile acid synthesis by simvastatin, a competitive inhibitor of hydroxymethyl glutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis, during pharmacologic interruption of the enterohepatic circulation. Six patients with primary hypercholesterolemia were studied in basal conditions, after treatment with the bile acid binding resin cholestyramine alone (8-16 g/d for 6-8 weeks) and subsequently in combination with simvastatin (40 mg/d for 6-8 weeks). Cholesterol 7α-hydroxylation rate, a measure of total bile acid synthesis, was assayed *in vivo* by tritium release analysis. Serum lathosterol levels were assayed by gas chromatography–mass spectrometry as a measure of cholesterol synthesis. Serum total and low-density lipoprotein-cholesterol were reduced significantly after cholestyramine (by 26% and 30%, respectively) and during combined treatment (by 47% and 55%). 7α-Hydroxylation rates increased nearly 4-fold with cholestyramine alone; addition of simvastatin induced a significant decrease of hydroxylation rates (cholestyramine alone, 1,591 ± 183 mg/d; plus simvastatin, 1,098 ± 232 mg/d; mean ± SEM; $P < .05$). Hydroxylation rates significantly correlated with serum lathosterol/cholesterol ratio ($r = 0.79$, $P < .05$). In conclusion, in conditions of chronic stimulation bile acid synthesis may be affected by changes in newly synthesized cholesterol availability. The finding might relate to the degree of substrate saturation of microsomal cholesterol 7α-hydroxylase; alternatively, newly synthesized cholesterol might induce a stimulatory effect on cholesterol 7α-hydroxylase transcription. (HEPATOLOGY 2003;38:939-946.)

Bile acid production is a major mechanism whereby cholesterol is eliminated from the organism and, therefore, represents a crucial event in the maintenance of cholesterol homeostasis.[1-3] Two metabolic pathways leading to primary bile acid synthesis have been described: the classical or *neutral* pathway, where the first step is hydroxylation at the 7α position, takes place entirely in the liver and is likely to be the most relevant one, in quantitative terms, in normal conditions. On the other hand, the first step of the alternative, or *acidic* pathway, *i.e.*, hydroxylation at the 27 position of the side chain, may take place in liver and extrahepatic tissues as well.[4,5]

Hydrophobic bile acids returning to the liver via the enterohepatic circulation have long been shown to exert feedback inhibition on bile acid synthesis and on the rate-limiting enzyme of the classical metabolic pathway, cholesterol 7α-hydroxylase (CYP7A1, EC 1.14.13.17), both in experimental models and in humans.[1-3,6,7] A body of evidence in very recent years has improved our knowledge greatly on the molecular regulation of cholesterol 7α-hydroxylase gene transcription and has highlighted the role of nuclear receptors on cholesterol metabolism.[8-11] This impressive work has provided a sound basis for the feedback inhibition exerted by bile acids with hydrophobic structure.

Abbreviations: HMG-CoA, hydroxymethyl glutaryl-CoA; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

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Table 1. Relevant Clinical Data of Patients Investigated

Patient	Age	Sex	BMI kg/m²	Cholesterol	HDL Cholesterol	Triglyceride	Diagnosis
				mg/dL			
1	46	M	23.8	337	49	101	FH
2	60	M	33.2	504	40	250	FCH + type II DM
3	62	F	23.8	335	46	243	FCH
4	64	F	29.1	368	69	118	FH
5	66	F	24.3	380	64	108	FH
6	69	F	25.6	450	63	131	FH

Abbreviations: FH, familial hypercholesterolemia; FCH, familial combined hyperlipidemia; DM, diabetes mellitus.

The effects induced on bile acid synthesis by the availability of free cholesterol are much less defined even if an induction of its degradation to bile acid is certainly plausible from a finalistic point of view. Work in cell cultures[12] and in animal models[13] indeed seems to support such hypothesis. Direct experimental evidence in humans is rather scarce, mainly because of the difficulty of modulating cholesterol availability for bile acid production by dietary changes. Evidence coming either from anecdotal reports[14] or controlled studies[15] seems to confirm a stimulatory effect induced by cholesterol on bile acid production.

Modifications of the availability of newly synthesized cholesterol theoretically can be accomplished more easily by use of statins, competitive inhibitors of hydroxymethyl glutaryl-CoA (HMG-CoA) reductase, the limiting enzyme of cholesterol synthesis.[16] Nonetheless, evidence in humans is again inconclusive because the results of studies on the effects of statins on bile acid synthesis have proven conflicting and largely influenced by the experimental setting: chronic treatment with HMG-CoA reductase inhibitors does not seem to affect bile acid synthesis or cholesterol 7α-hydroxylase activity,[7,17] whereas bolus administration of a statin to patients with external biliary drainage significantly impairs bile acid output and synthesis.[18] This of course represents an extreme and unphysiologic condition, both in terms of drug administration and (mostly) because of the surgical manipulation.

This study was designed to investigate the effect of a widely prescribed HMG-CoA reductase inhibitor, simvastatin,[19] on bile acid synthesis *in vivo* in a more physiologic situation and in patients with intact biliary tract; pharmacologic interruption of the enterohepatic circulation was achieved by treatment with the resin cholestyramine, which is well known to inhibit bile acid absorption and stimulate bile acid production. The correlation between the effects on bile acid synthesis and changes in serum levels of lathosterol, a precursor of cholesterol synthesis, also were addressed to clarify the relationship between the two biosynthetic pathways.

Patients and Methods

Patients and Study Design. We investigated six patients with primary hypercholesterolemia, isolated or associated with hypertriglyceridemia, and a clinical diagnosis of either familial hypercholesterolemia or familial combined hyperlipidemia according to currently accepted criteria.[20] They were seen as outpatients in the Lipid Clinic of the University Hospital of Modena and were on a hypocholesterolemic diet adequate to keep their weight constant. Body weight did not change throughout the study.

Table 1 shows the clinical data of studied subjects in the absence of drug treatment. Intestinal, liver, and thyroid dysfunction as well as alcohol abuse were ruled out on the basis of clinical and biochemical evaluation. Patient 2 was obese and had type II diabetes mellitus; he had received a fixed dose of metformin for many years, and this was maintained throughout the study.

Patients gave their informed consent to the protocol of the study, which was conducted according to the Declaration of Helsinki and approved by the local ethical committee. The design of the study is summarized in Fig. 1. Patients were investigated in basal, untreated conditions. Then chronic pharmacologic interruption of the enterohepatic circulation was accomplished by treatment with cholestyramine (Questran; Bristol-Myers Squibb Italia, Rome, Italy) at the highest tolerated dose (8-16 g/d in 2 or 3 daily doses before meals); after 6 to 8 weeks of treatment, the patients were restudied. Finally, the last evaluation was performed after an additional 6 to 8 weeks when the same dosage of cholestyramine was superimposed to a standard dose of simvastatin (Sinvacor; Merck, Sharp and Dohme Italia, Rome, Italy [40 mg as a single bedtime dose]). Patient compliance was assessed by interview and by drug preparation count.

Methods. Routine laboratory tests were performed by automated analysis. Cholesterol and triglyceride concentrations were determined by enzymatic assay. Cholesterol

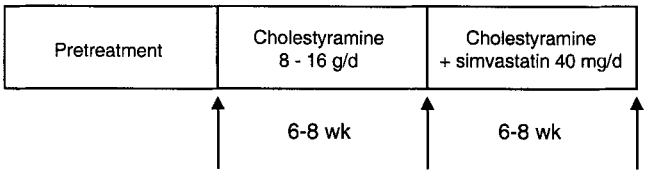

Fig. 1. Schematic of study design. ↑, blood sampling for serum lipid and lathosterol levels and assay of cholesterol 7α-hydroxylation rates *in vivo*.

concentrations also were determined after sequential ultracentrifugation of serum in the different density fractions: < 1.006 g/mL, mainly composed of very-low-density lipoprotein; 1.006 to 1.063, mainly composed of low-density lipoprotein (LDL); and > 1.063 g/mL, represented by high-density lipoprotein (HDL).

Cholesterol 7α-hydroxylation rates *in vivo* were determined by tritium release assay as previously described.[7,21] Briefly, trace amounts (200-300 μCi) of [7α-^{3}H]cholesterol (specific activity, 3-10 mCi/mmol) were injected intravenously in the morning after an overnight fast. Blood and urine samples were taken at fixed intervals for 5 to 6 days after tracer infusion. The amount of tritium released is proportional to the 7α-hydroxylation reactions and can be measured by determination of body water radioactivity. The rate of 7α-hydroxylation can be quantified as the ratio between the enrichment over time in body water tritium, assayed by liquid scintillation counting of distilled urine samples, and the mean specific radioactivity (radioactivity/mass ratio) of serum cholesterol, assayed in the same time interval (usually 60 to 72 hours after tracer infusion). Hydroxylation rates were expressed as the amount of cholesterol undergoing 7α-hydroxylation per day (mg/d). Because this assay quantifies total tritium release, regardless of the pathway involved, calculated hydroxylation rates should reflect total bile acid synthesis, deriving from both classical and alternative pathways.

Serum levels of lathosterol were assayed as described.[22] Deuterated lathosterol (1 μg) as internal standard was added to 0.2 mL serum samples as solution in ethyl acetate. Alkaline hydrolysis was carried out with 1 mL 1N NaOH in 90% ethanol at 60°C for 90 minutes under nitrogen; after addition of saline (1 mL), sterols were extracted with 2 mL of petroleum ether and taken to dryness under a stream of nitrogen. Sterols were then converted into trimethylsilylethers with 20 μL of trimethylsilylimidazole:piperidine (1:1) for 10 minutes at room temperature. Gas chromatography–mass spectrometry analysis of samples was carried out in the selected ion monitoring mode, recording ions at m/z 255 and m/z 259 for the detection of lathosterol and deuterated lathosterol, respectively. Calibration curves were prepared, spiking 0.2 mL serum with fixed amounts of internal standard (1 μg) and increasing amounts of lathosterol (0-1,000 μg/dL) 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 for the tested ions (lathosterol/deuterated lathosterol) found in the sample of total lathosterol. Results were expressed as the serum lathosterol to cholesterol ratio, which is regarded by most authors as the most reliable marker of whole body and hepatic cholesterol production.[23,24]

Statistical Evaluation. When appropriate, data were expressed as the mean ± SEM and the significance of differences was evaluated according to Student's *t* test for paired data. Linear regression analysis was performed by the least squares methods. Statistical analysis was conducted with the aid of the SPSS/PC statistical package (SPSS Italia, Bologna, Italy). Significance was accepted at the $P < .05$ level.

Results

Figure 2 shows the effects of treatment with cholestyramine with or without simvastatin on cholesterol concentration in total serum and in the 1.006-1.063 g/dL density fraction; the latter is mainly composed of LDL and therefore is regarded as LDL even if a component of intermediate density lipoprotein might be present as well. As expected, circulating cholesterol levels significantly decreased during cholestyramine and further decreased when simvastatin was superimposed (mean values of total cholesterol ± SEM: pretreatment, 407 ± 33 mg/dL; cholestyramine alone, 300 ± 18 mg/dL; cholestyramine plus simvastatin, 216 ± 15 mg/dL; values for LDL-cholesterol: pretreatment, 296 ± 27 mg/dL; cholestyramine alone, 206 ± 23 mg/dL; cholestyramine plus simvastatin, 134 ± 19 mg/dL). The data support proper drug efficacy and are indirectly consistent with good patient compliance.

Drug treatment induced a much lesser effect on serum HDL cholesterol and triglyceride levels, as shown in Fig. 3. No significant changes were observed in HDL cholesterol concentration, whereas triglyceride levels tended to increase with cholestyramine alone and were decreased significantly, when compared with treatment with resin alone, during combined drug administration. No significant changes were detected on serum very-low-density lipoprotein–cholesterol levels (data not shown).

The effects on cholesterol 7α-hydroxylation rates *in vivo* are shown in Fig. 4. After cholestyramine alone hydroxylation rates increased nearly 4-fold, consistently with previous evidence from this laboratory.[7] When simvastatin was added to the fixed cholestyramine regimen,

hydroxylation rates significantly decreased (cholestyramine alone, 1,591 ± 183 mg of cholesterol/day; cholestyramine plus simvastatin, 1,098 ± 232 mg/d; $P < .05$) consistently with relatively reduced availability of newly synthesized cholesterol for bile acid synthesis. Compatibly with the limited number of observations, variable responses were observed among patients in the sense that a more pronounced effect of cholestyramine treatment appeared to associate with higher bile acid synthesis rates also after adding simvastatin. However, hydroxylation rates during combined treatment remained significantly higher compared with pretreatment values.

To better relate the alterations of bile acid production to the changes induced on cholesterol synthesis, serum levels of total lathosterol were determined. Figure 5 shows the changes induced by drug treatment on the lathosterol-to-cholesterol ratio, which is considered the most reliable marker of cholesterol synthesis. Lathosterol levels markedly increased during cholestyramine treatment (2.48 ± 0.40 μg/mg of cholesterol compared with pretreatment, 0.73 ± 0.11 μg/mg; $P < .05$) and decreased towards pretreatment values when simvastatin was added (1.17 ± 0.30 μg/mg; $P < .05$ vs. cholestyramine, P = n.s. vs. pretreatment).

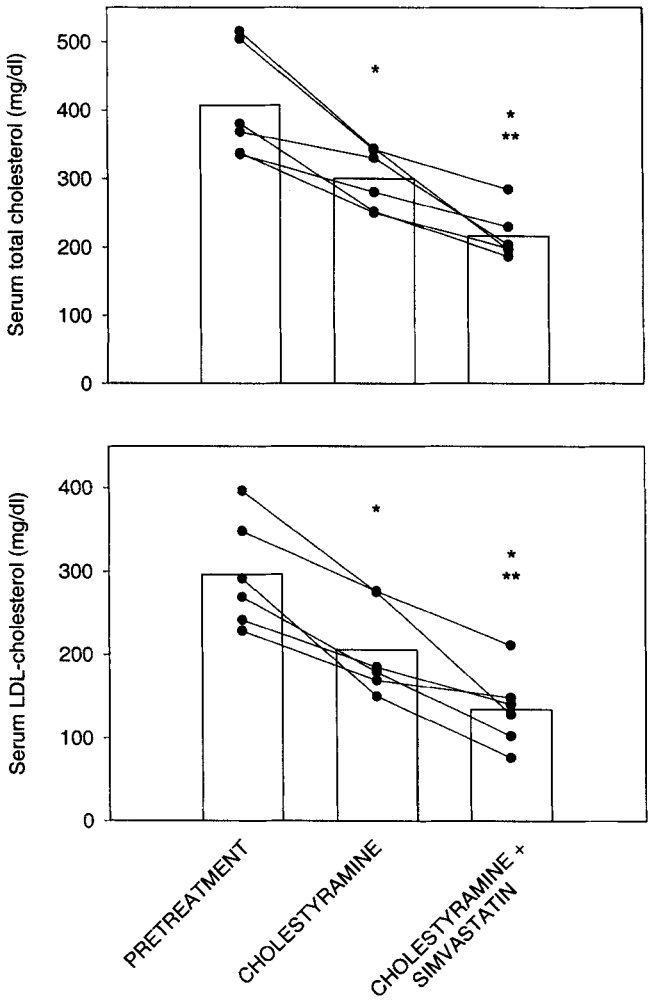

Fig. 2. Concentrations of cholesterol in total serum (**upper panel**) and in the LDL fraction (**lower panel**) in the different phases of the study. Bars indicate mean values. *$P < .05$ versus pretreatment and **$P < .05$ versus cholestyramine alone. Student's t test for paired data.

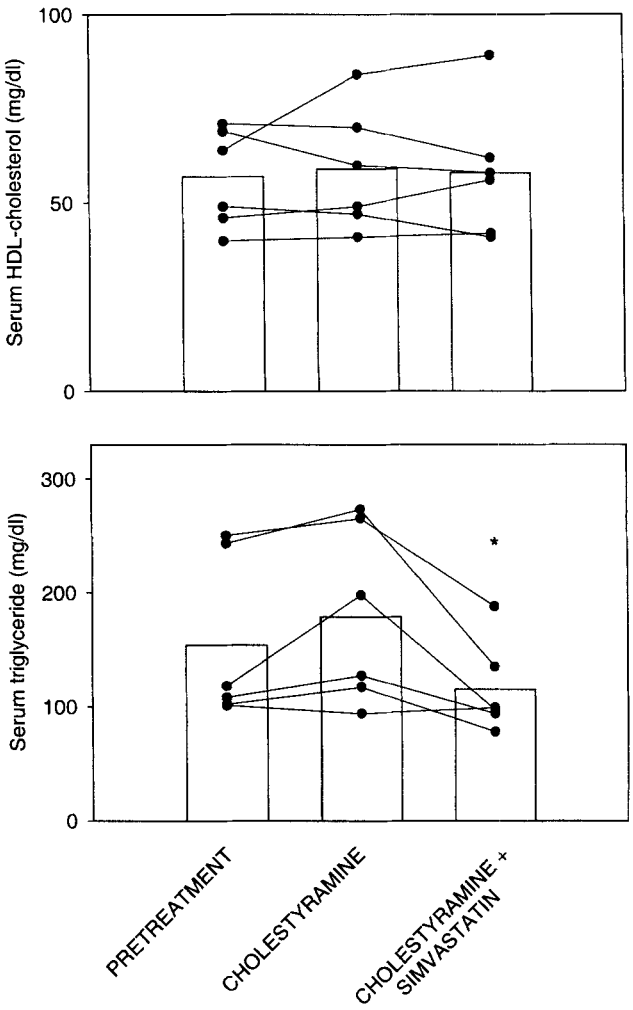

Fig. 3. Concentrations of HDL cholesterol (**upper panel**) and total serum triglycerides (**lower panel**) in the different phases of the study. Bars indicate mean values. *$P < .05$ versus cholestyramine alone, Student's t test for paired data.

When the values of lathosterol-to-cholesterol ratio were plotted against cholesterol 7α-hydroxylation rates *in vivo* during all treatment phases, a highly significant correlation was observed, as shown in Fig. 6 ($r = 0.79$; $P < .05$); again this suggests that cholesterol synthesis may be a limiting factor for bile acid synthesis in the experimental conditions studied. A significant correlation still was present ($r = 0.72$; $P < .05$) when considering only the data during cholestyramine treatment, with and without simvastatin. No significant correlation between cholesterol and bile acid synthesis was observed, compatibly with the small number of observations, in pretreatment

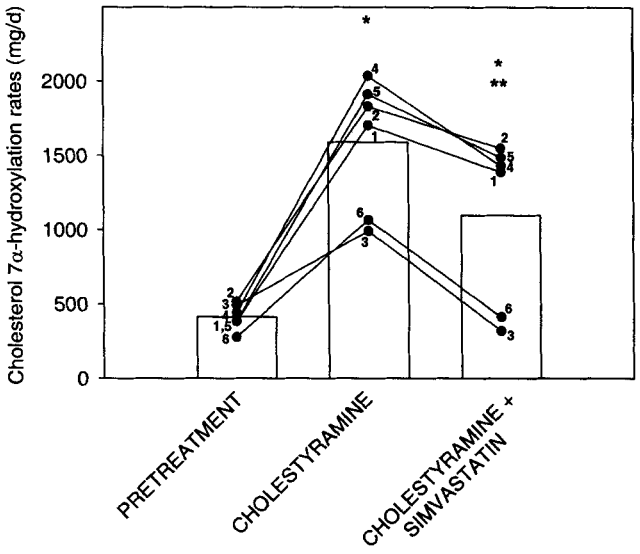

Fig. 4. Rates of cholesterol 7α-hydroxylation in the different phases of the study. Bars indicate mean values. Data points for individual patients (numbered as described in Table 1) are shown. *$P < .05$ versus pretreatment. **$P < .05$ versus cholestyramine alone, Student's t test for paired data.

conditions. The findings were substantially similar when considering absolute serum lathosterol levels (data not shown).

Discussion

Despite the recent acquisitions on the molecular regulation of cholesterol 7α-hydroxylase transcription, the regulatory effects of cholesterol availability on bile acid synthesis are largely unknown. Human studies in particular are relatively scarce, mainly because of the difficulty of designing physiologic and reproducible experimental conditions; moreover, the results obtained often have been inconsistent because of the different experimental settings and different techniques used to quantify bile acid synthesis.

The present study was aimed to assess the effect of inhibition of HMG-CoA reductase, achieved by statin administration, in a context of chronically interrupted recirculation of bile acids, obtained by treatment with cholestyramine. Data obtained with cholestyramine alone as a model of pure derepression of bile acid synthesis were compared with those obtained by combined treatment. A technique validated in this laboratory was used to investigate bile acid synthesis *in vivo*, and serum levels of lathosterol were used to assess changes of cholesterol synthesis.

The effects on serum lipid concentrations were predictable largely on the basis of the pharmacologic effects of the drugs used. Total and LDL cholesterol levels were decreased significantly during cholestyramine alone and even more so during combined drug treatment. The data confirm adequate drug efficacy and are indirectly consistent with proper compliance. No effects were observed on HDL cholesterol levels; the slight increase of serum triglyceride with cholestyramine alone is in line with previous observations linking bile acid synthesis with serum triglyceride and, presumably, hepatic lipoprotein and/or triglyceride production in experimental models and in humans.[20,25,26] Likewise, the decrease observed with combined treatment is consistent with the well-known hypotriglyceridemic action of statins.[19]

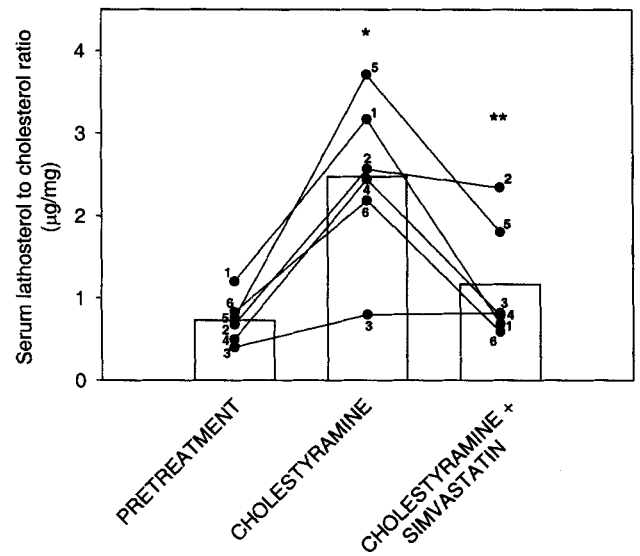

Fig. 5. Serum lathosterol to cholesterol ratio in the different phases of the study. Bars indicate mean values. Data points for individual patients (numbered as described in Table 1) are shown. *$P < .05$ versus pretreatment; **$P < .05$ versus cholestyramine alone, Student's t test for paired data.

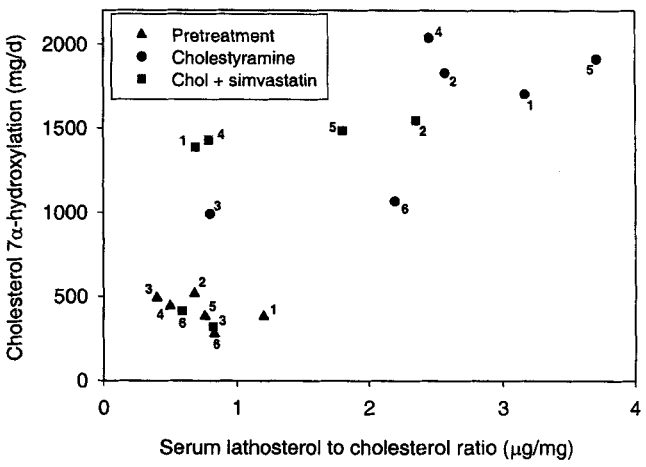

Fig. 6. Correlation between serum lathosterol to cholesterol ratio and cholesterol 7α-hydroxylation rates in the different phases of the study in all patients, numbered as shown in Table 1; $r = 0.79$; $P < .05$ by linear regression analysis.

Bile acid synthesis was increased markedly during cholestyramine in complete agreement with our previous findings.[7] Interestingly, supplementation with simvastatin significantly reduced 7α-hydroxylation rates when compared with treatment with cholestyramine alone. Compatibly with variable individual responses, the finding suggests that in stimulated conditions bile acid production may be affected by concurrent pharmacologic inhibition of HMG-CoA reductase. This is in agreement with previous evidence in bile fistula animals[27] and in humans in whom bile acid synthesis was stimulated by either partial[28] or total[18] biliary diversion after cholecystectomy. Such experimental conditions were quite different from the present one: statin was administered acutely as a single high dose to patients recovering from surgical manipulation of the biliary tract. Similar findings were observed after chronic statin administration in patients with ileal bypass.[29,30]

The present study was designed instead to provide a completely physiologic condition: chronic administration of a common dose of simvastatin in patients with intact biliary and gastrointestinal anatomy, with cholestyramine as a pharmacologic tool to induce interruption of the enterohepatic circulation. The inhibitory effect on bile acid synthesis is in agreement with a previous study in which serum levels of 7α-hydroxy-4-cholesten-3-one were assayed as a marker of bile acid production.[30] Our *in vivo* isotope release technique brings the theoretical advantage to give an absolute estimate of bile acid synthesis rates, which can be used in terms of cholesterol balance evaluation. This technique relies on the assumption that plasma cholesterol rapidly equilibrates with the hepatic microsomal compartment; during cholesterol-lowering treatment the theoretical possibility exists that such equilibration may be affected. On the other hand, we believe that the chronic conditions of the present study should allow complete equilibration within the rapidly exchanging cholesterol pool as previously discussed in detail.[7,31]

Such inhibitory effect of HMG-CoA reductase inhibitors is not evident in nonstimulated conditions: indeed, almost all studies with chronic statin monotherapy failed to show any effect on bile acid synthesis, measured with different techniques, or on hepatic cholesterol 7α-hydroxylase activity.[6,7,15,30,32,33] Few reports showed reduced rates of bile acid synthesis during chronic treatment with statins.[29,34] Differences in methodology or in study design may account for such discrepancy; however, it is interesting to note that in one of these studies[34] bile acid synthesis rates tended to increase toward normal values with longer-term drug treatment.

Taken together, these pieces of evidence generally support the view that during chronic treatment with statins, if bile acid synthesis is not up-regulated, compensatory mechanisms may take place in the liver (*i.e.*, increased lipoprotein uptake, increased synthesis of HMG-CoA reductase protein) to restore a sufficient amount of free cholesterol for adequate bile acid production.

To better define the relationship between bile acid and cholesterol synthesis, we also investigated the changes induced on serum lathosterol, a metabolic precursor of cholesterol. Even if such an approach does not allow determination of cholesterol synthesis in quantitative terms, circulating lathosterol, in particular the serum lathosterol-to-cholesterol ratio, generally is considered to be a reliable marker of cholesterol synthesis and to correlate well with hepatic HMG-CoA reductase activity.[23,24] This parameter previously was used to assess changes in cholesterol synthesis during treatment with HMG-CoA reductase inhibitors.[35]

Changes in serum lathosterol-to-cholesterol ratio essentially mirrored those regarding bile acid synthesis rates, namely, a sharp increase with cholestyramine alone and a significant decrement on addition of simvastatin. The two parameters of cholesterol and bile acid synthesis strictly were correlated, particularly in stimulated conditions, even if individual variability in the response to drug treatment was observed. With the limitations attributable to the limited number of observations, no correlation was present in pretreatment conditions. We have not assayed such a relationship directly in patients receiving chronic treatment with statin alone, but published data suggest that no correlation is likely to take place.[30]

Interestingly, the lathosterol-to-cholesterol ratio during combined treatment was lowered to levels quite similar to those of pretreatment, suggesting near-normalization of cholesterol synthesis rates after adding simvastatin, whereas 7α-hydroxylation rates during combined treatment still remained significantly higher than in untreated condition. This supports the view that, regardless of the effects induced by HMG-CoA reductase inhibition, cholesterol used for bile acid production still derives to a large extent from preformed sources. On the other hand, when intestinal cholesterol absorption is inhibited by nearly 50%, as recently shown with ezetimibe, bile acid synthesis is only marginally affected, probably because in this particular model (in the absence of statin treatment), increased cholesterol synthesis can compensate for reduced availability of chylomicron cholesterol.[36]

These findings altogether suggest that both preformed and newly synthesized cholesterol may represent critical sources for bile acid synthesis; in particular, partial inhibition of cholesterol synthesis can lead to variable and

patient-dependent responses of the contribution of each cholesterol source to bile acid production according to the different pathophysiologic conditions, with an inhibitory effect on bile acid synthesis showing up in a stimulated situation.

Such relative inhibition should not bear any adverse effect on hepatic cholesterol homeostasis, considering the underlying stimulation of bile acid synthesis; this is at variance with previous evidence with other hypolipidemic agents, *i.e.*, fibric acid derivatives, which are well known to increase biliary cholesterol output in association with reduced bile acid synthesis and hepatic cholesterol 7α-hydroxylase activity during chronic treatment.[20,37]

Indeed, long-term treatment with statins was described as reducing biliary cholesterol secretion significantly.[15,32-34] Because biliary cholesterol represents a major component of cholesterol in the intestinal lumen, this might affect the delivery of chylomicron cholesterol to the liver as well. This raises the possibility that when simvastatin is given in combination with a bile acid sequestrant, there is increased use of LDL cholesterol as a substrate for bile acid production.

The mechanisms by which statin treatment can affect bile acid production during interruption of the enterohepatic circulation are not completely clear even if a number of speculations can be made. First of all, microsomal cholesterol 7α-hydroxylase was reported to be fully saturated with cholesterol in normal conditions, whereas the degree of saturation with cholesterol markedly decreases during interruption of the enterohepatic circulation[38]; this might explain why bile acid synthesis may become relatively dependent on the availability of newly synthesized cholesterol during treatment with cholestyramine, but not in basal conditions. Another hypothesis, which is not exclusive of the former one, involves a role for nuclear receptors as sensors of intracellular cholesterol availability. As shown in animal models, but not in humans, increased levels of cholesterol can increase hepatic expression of cholesterol 7α-hydroxylase via activation of liver X receptor α by hydroxylated cholesterol derivatives (oxysterols).[39] The physiologic relevance of this pathway in humans is highly uncertain; however, previous evidence from this group has shown increased *in vitro* expression and activity of cholesterol 7α-hydroxylase in patients with obstructive cholestasis in association with increased microsomal cholesterol content, despite reduced *in vivo* bile acid synthesis rates.[31] Nuclear receptors or coactivators of cholesterol 7α-hydroxylase transcription other than liver X receptors certainly can be involved.[10,11,40] Again, such *feedforward* stimulation exerted by cholesterol (or the inhibition attributable to deprivation of newly synthesized cholesterol as in this case) might be particularly evident in conditions of maximally stimulated cholesterol degradation.

In conclusion, this report brings new evidence concerning the role of newly synthesized cholesterol in regulating bile acid synthesis in physiologically stimulated conditions; further insight on the role of nuclear receptors and/or transcription factors involved in hepatic and extrahepatic cholesterol homeostasis in humans, presently underway in this laboratory, may help to clarify some still unresolved issues regarding cholesterol metabolism and its perturbations.

Acknowledgment: This article is dedicated to the memory of our friend and colleague Prof. Giovanni Galli.

References

1. Princen HMG, Post SM, Twisk J. Regulation of bile acid biosynthesis. Curr Pharm Des 1997;3:59-84.
2. Chiang JYL. Regulation of bile acid synthesis. Front Biosci 1998;3:D176-D193.
3. Vlahcevic ZR, Pandak WM, Stravitz RT. Regulation of bile acid biosynthesis. Gastroenterol Clin North Am 1999;28:1-25.
4. Björkhem I. Mechanism of degradation of the steroid side chain in the formation of bile acids. J Lipid Res 1992;33:455-472.
5. Javitt NB. Bile acid synthesis from cholesterol:regulatory and auxiliary pathways. FASEB J 1994;8:1308-1311.
6. Reihnér E, Björkhem I, Angelin B, Ewerth S, Einarsson K. Bile acid synthesis in humans: regulation of hepatic microsomal cholesterol 7α-hydroxylase activity. Gastroenterology 1989;97:1498-1505.
7. Bertolotti M, Abate N, Loria P, Dilengite M, Carubbi F, Pinetti A, Digrisolo A, et al. Regulation of bile acid synthesis in humans: effect of treatment with bile acids, cholestyramine or simvastatin on cholesterol 7α-hydroxylation rates *in vivo*. HEPATOLOGY 1991;14:830-837.
8. Repa JJ, Mangelsdorf DJ. Nuclear receptor regulation of cholesterol and bile acid metabolism. Curr Opin Biotechnol 1999;10:557-563.
9. Russell DW. Nuclear orphan receptors control cholesterol catabolism. Cell 1999;97:539-542.
10. Chiang JYL. Bile acid regulation of gene expression: roles of nuclear hormone receptors. Endocr Rev 2002;23:443-463.
11. Goodwin B, Kliewer SA. Nuclear receptors. I. Nuclear receptors and bile acid homeostasis. Am J Physiol Gastrointest Liver Physiol 2002;282: G926-G931.
12. Davis RA, Hyde PM, Kuan J-C W, Malone-McNeal M, Archambault-Schexnayder J. Bile acid secretion by cultured rat hepatocytes. Regulation by cholesterol availability. J Biol Chem 1983;258:3661-3667.
13. Björkhem I, Eggertsen G, Andersson U. On the mechanism of stimulation of cholesterol 7 alpha-hydroxylase by dietary cholesterol. Biochim Biophys Acta 1991;1085:329-335.
14. Kern F Jr. Normal plasma cholesterol in an 88-year-old man who eats 25 eggs a day. Mechanisms of adaptation. N Engl J Med 1991;324:896-899.
15. Duane WC. Effects of lovastatin and dietary cholesterol on sterol homeostasis in healthy human subjects. J Clin Invest 1993;92:911-918.
16. Grundy SM. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. N Engl J Med 1988;319:24-33.
17. Reihnér E, Rudling M, Ståhlberg D, Berglund L, Ewerth S, Björkhem I, Einarsson K, et al. Influence of pravastatin, a specific inhibitor of HMG-CoA reductase, on hepatic metabolism of cholesterol. N Engl J Med 1990; 323:224-228.
18. Loria P, Bertolotti M, Cassinadri MT, Dilengite MA, Bozzoli M, Carubbi F, Concari M, et al. Short-term effects of simvastatin on bile acid synthesis and bile lipid secretion in human subjects. HEPATOLOGY 1994;19:882-888.

19. Plosker GL, McTavish D. Simvastatin. A reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolemia. Drugs 1995;50:334-363.
20. Bertolotti M, Concari M, Loria P, Abate N, Pinetti A, Guicciardi ME, Carulli N. Effects of different phenotypes of hyperlipoproteinemia and of treatment with fibric acid derivatives on the rates of cholesterol 7α-hydroxylation in humans. Arterioscler Thromb Vasc Biol 1995;15:1064-1069.
21. Bertolotti M, Carulli N, Menozzi D, Zironi F, Digrisolo A, Pinetti A, Baldini MG. *In vivo* evaluation of cholesterol 7α-hydroxylation in humans: effect of disease and drug treatment. J Lipid Res 1986;27:1278-1286.
22. Del Puppo M, Galli Kienle M, Petroni ML, Crosignani A, Podda M. Serum 27-hydroxycholesterol in patients with primary biliary cirrhosis suggests alteration of cholesterol catabolism to bile acids via the acidic pathway. J Lipid Res 1998;39:2477-2482.
23. Björkhem I, Miettinen T, Reihnér E, Ewerth S, Angelin B, Einarsson K. Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver. J Lipid Res 1987;28:1137-1143.
24. Kempen HJ, Glatz JF, Gevers Leuven JA, van der Voort HA, Katan MB. Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. J Lipid Res 1988;29:1149-1155.
25. Angelin B, Hershon KS, Brunzell JD. Bile acid metabolism in hereditary forms of hypertriglyceridemia: evidence for an increased synthesis rate in monogenic familial hypertriglyceridemia. Proc Natl Acad Sci U S A 1987; 84:5434-5438.
26. Davis RA, Hui TY. Atherosclerosis is a liver disease of the heart. Arterioscler Thromb Vasc Biol 2001;21:887-898.
27. Pandak WM, Heuman DM, Hylemon PB, Vlahcevic ZR. Regulation of bile acid synthesis. IV. Interrelationship between cholesterol and bile acid biosynthesis pathways. J Lipid Res 1990;31:79-90.
28. Muraca M, Baggio G, Miconi L, Vilei MT, Martini S, Gabelli C, Belluco C, et al. Acute effects of HMG-CoA reductase inhibitors on biliary lipids in patients with interrupted enterohepatic circulation. Eur J Clin Invest 1991;21:204-208.
29. Vanhanen H, Kesäniemi YA, Miettinen T. Pravastatin lowers serum cholesterol, cholesterol-precursor sterols, fecal steroids, and cholesterol absorption in man. Metabolism 1992;41:588-595.
30. Naoumova RP, O'Neill FH, Dunn S, Neuwirth CKY, Taylor GW, Axelson M, Thompson GR. Effect of inhibiting HMG-CoA reductase on 7α-hydroxy-4-cholesten-3-one, a marker of bile acid synthesis: contrasting findings in patients with and without prior up-regulation of the latter pathway. Eur J Clin Invest 1999;29:404-412.
31. Bertolotti M, Carulli L, Concari M, Martella P, Loria P, Tagliafico E, Ferrari S, et al. Suppression of bile acid synthesis, but not of hepatic cholesterol 7α-hydroxylase expression, by obstructive cholestasis in humans. HEPATOLOGY 2001;34:234-242.
32. Mazzella G, Parini P, Festi D, Bazzoli F, Aldini R, Roda A, Tonelli D, et al. Effect of simvastatin, ursodeoxycholic acid and simvastatin plus ursodeoxycholic acid on biliary lipid secretion and cholic acid kinetics in nonfamilial hypercholesterolemia. HEPATOLOGY 1992;15:1072-1078.
33. Duane WC. Effects of lovastatin and dietary cholesterol on bile acid kinetics and bile lipid composition in healthy male subjects. J Lipid Res 1994; 35:501-509.
34. Mitchell JC, Stone BG, Logan GM, Duane WC. Role of cholesterol synthesis in regulation of bile acid synthesis and biliary cholesterol secretion in humans. J Lipid Res 1991;32:1143-1149.
35. Pfohl M, Naoumova RP, Kim K-D, Thompson GR. Use of cholesterol precursors to assess changes in cholesterol synthesis under non–steady-state conditions. Eur J Clin Invest 1998;28:491-496.
36. Sudhop T, Lütjohann D, Kodal A, Igel M, Tribble DL, Shah S, Perevozskaya I, et al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. Circulation 2002;106:1943-1948.
37. Ståhlberg D, Reihnér E, Rudling M, Berglund L, Einarsson K, Angelin B. Influence of bezafibrate on hepatic cholesterol metabolism in gallstone patients: reduced activity of cholesterol 7 alpha-hydroxylase. HEPATOLOGY 1995;21:1025-1030.
38. Einarsson K, Reihnér E, Björkhem I. On the saturation of the cholesterol 7α-hydroxylase in human liver microsomes. J Lipid Res 1989;30:1477-1481.
39. Peet DJ, Turley SD, Ma WM, Janowski BA, Lobaccaro J-MA, Hammer RE, Mangelsdorf DJ. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRα. Cell 1998;93:693-704.
40. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331-340.