Role of cholesterol synthesis in regulation of bile acid synthesis and biliary cholesterol secretion in humans[1]

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Abstract We used lovastatin, a specific inhibitor of HMG-CoA reductase, to study the role of cholesterol synthesis in regulation of both bile acid synthesis, measured by release of $^{14}CO_2$ from [26-^{14}C]cholesterol, and biliary cholesterol secretion, measured by standard marked perfusion techniques, in humans. Six volunteers were studied in each of four periods: *a*) control; *b*) 6–10 hours after a single 40 mg oral dose of lovastatin to study acute effects; *c*) after 5–6 weeks of lovastatin 40 mg orally twice a day to study steady-state effects; and *d*) 24 h after cessation of chronic lovastatin. Mean bile acid synthesis fell to 69% of control ($P < 0.01$) after single-dose lovastatin and remained at 83% of control after 5–6 weeks on lovastatin ($P < 0.05$). After withdrawal of lovastatin, mean bile acid synthesis was 88% of control (NS). Mean biliary cholesterol secretion did not change after single-dose lovastatin (103% of control), but fell to 81% of control during chronic lovastatin treatment ($P < 0.05$). After withdrawal of lovastatin, mean cholesterol secretion remained at 80% of control ($P < 0.05$). These data suggest that in humans cholesterol synthesis is an immediate regulator of bile acid synthesis. Cholesterol synthesis also regulates biliary cholesterol secretion, but the effect is not immediate and therefore may be indirect. —**Mitchell, J. C., B. G. Stone, G. M. Logan, and W. C. Duane.** Role of cholesterol synthesis in regulation of bile acid synthesis and biliary cholesterol secretion in humans. *J. Lipid Res.* 1991. **32:** 1143–1149.

Supplementary key words liver • hyperlipidemia • cholelithiasis • HMG-CoA reductase inhibitors • lecithin

Cholesterol homeostasis is the net result of all inputs and outputs. Input of cholesterol occurs either from endogenous synthesis or absorption of dietary cholesterol. Elimination of cholesterol is largely a result of two processes: direct secretion of cholesterol into bile followed by fecal excretion or conversion of cholesterol to bile acids. It has been hypothesized that the rate of cholesterol synthesis may regulate either cholesterol secretion into bile and/or bile acid synthesis (1, 2). There is evidence for and against both components of this hypothesis.

Studies of biliary cholesterol secretion in the rat have shown that sudden reduction of cholesterol synthesis lowers secretion (3). However, prolonged alteration of cholesterol synthesis induced by maneuvers such as fasting, cholesterol feeding, and cholestyramine administration have failed to alter biliary cholesterol secretion (4) except in rats simultaneously treated with pregnenolone-16α-carbonitrile (5). In humans, both obesity and administration of chenodeoxycholic acid result in parallel changes in cholesterol synthesis and biliary cholesterol secretion (1, 6, 7). However, the cause and effect relationship of these changes has not been established.

Recent studies of bile acid synthesis in bile fistula rats have shown that reduction in cholesterol synthesis lowers bile acid synthesis and activity of the rate-limiting enzyme, 7α-hydroxylase (8–11). Two recent studies in humans have reported lower mean bile acid synthesis and activity of 7α-hydroxylase after reduction of cholesterol synthesis, but in both the changes did not reach statistical significance (12, 13). Moreover, in both studies changes were assessed after prolonged reduction in cholesterol synthesis precluding any conclusion about direct, short-term regulation.

In the present study we have attempted to better define the role of cholesterol synthesis in regulation of both biliary cholesterol secretion and bile acid synthesis. To do this we have measured changes after administration of lovastatin, a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the synthesis of cholesterol. This approach has the advantage of reducing cholesterol synthesis directly. Moreover, because cholesterol synthesis is quickly lowered by lovastatin, it is possible to assess effects of both a sudden change in cholesterol synthesis and a prolonged alteration of cholesterol synthesis.

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; HDL, high density lipoproteins; LDL, low density lipoproteins; C.I., confidence interval.

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METHODS

Six male volunteers were studied in four separate time periods: *a*) control; *b*) 6–10 h after a single 40 mg oral dose of lovastatin to study acute effects; *c*) after 5–6 weeks of lovastatin 40 mg orally twice a day to study steady-state effects, and *d*) 24 h after cessation of chronic lovastatin treatment to assess possible withdrawal effects. All volunteers had participated in a larger study of effects of steady-state lovastatin on biliary lipid metabolism (12). Thus, some data reported here for the steady-state period have also been reported elsewhere (12). They are included here for comparison to the other periods and are specifically designated in the figures.

The volunteers ranged in age from 51 to 68 years, in percent ideal body weight from 98 to 132%, and in serum cholesterol from 140 to 210 mg/dl. Two of the volunteers had hypertriglyceridemia (412 and 478 mg/dl) without hypercholesterolemia. They were included because of the difficulty in recruiting volunteers for these rigorous studies, but their results did not appear to differ from those of the other four subjects. Each subject provided a detailed dietary history and was then instructed to continue his customary diet throughout the study.

Informed consent was obtained from all volunteers prior to study. All study protocols were approved by the Subcommittee on the Use of Human Subjects in Research of the Minneapolis VA Medical Center.

Serum total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides were measured by the clinical laboratory of the Minneapolis VA Medical Center. Low density lipoprotein (LDL) cholesterol was calculated from the above measurements by a standard formula (14).

Cholesterol synthesis was assessed by measuring incorporation of [^{3}H]acetate into sterols in each subject's mononuclear leukocytes as previously described by us and others (15, 16).

Bile acid synthesis was measured as release of $^{14}CO_2$ from administered [26-^{14}C]cholesterol (New England Nuclear, Boston, MA) as described previously (17). Synthesis was calculated by dividing output of $^{14}CO_2$ by the specific activity of cholesterol in stimulated bile. This specific activity was initially chosen for calculation because the precursor pools of cholesterol for bile acid synthesis and cholesterol secretion appear to be in near equilibrium (18). This precaution later proved unnecessary, however, because cholesterol specific activities in serum and bile eventually proved nearly identical (see below).

Specific activities of both biliary and serum free cholesterol were measured by gas–liquid chromatography and liquid scintillation counting (12, 19). For analysis of serum samples, free cholesterol was first isolated from a petroleum ether extract by thin-layer chromatography using a solvent system of ethyl ether–heptane 55:45. Biliary cholesterol for measurement of specific activity was obtained during measurement of stimulated secretion rates.

Biliary secretion rates of cholesterol, lecithin, and bile acid were measured by the method of Grundy and Metzger (20) as described previously (12). Values reported are the means of eight 1-h determinations. Of particular interest with respect to the studies of single-dose lovastatin, there was no consistent difference between lipid secretion values for the first versus the eighth hour of sampling.

For ease of comparison between periods and individuals, values for all measurements are expressed as percent of each individual's control period value. Statistical analysis was performed by analysis of variance and confidence interval (C.I.) testing.

RESULTS

Total serum cholesterol (**Fig. 1**), which averaged 170 mg/dl in the control period, averaged 95% of control after a single acute dose of lovastatin, a change that was not statistically significant (95% C.I. 88–102). After 5–6 weeks of lovastatin treatment in these six subjects, total cholesterol was significantly lowered to an average of 64% of control ($P < 0.01$, 99% C.I. 54–74). Finally 24 h after withdrawal of chronic lovastatin, total serum cholesterol averaged 66% of control, which continued to be significantly different from control ($P < 0.01$, 99% C.I. 56–76).

Average LDL cholesterol in the control period was 84 mg/dl and changes paralleled those of total cholesterol: after acute lovastatin, 102% of control (N.S., 95% C.I. 87–117); after 5–6 weeks on lovastatin, 59% of control ($P < 0.01$, 99% C.I. 39–80); and after withdrawal of chronic lovastatin, 51% of control ($P < 0.01$, 99% C.I. 30–72).

HDL cholesterol levels averaged 30 mg/dl in the control period and averaged 105% of control after a single dose of lovastatin (N.S., 95% C.I. 97–113). After 5–6 weeks of lovastatin, average HDL cholesterol had increased slightly but significantly to 115% of control ($P < 0.01$, 99% C.I. 104–126). Similarly, after withdrawal of chronic lovastatin, HDL cholesterol averaged 114% of control which was significantly greater than control ($P < 0.01$, 99% C.I. 103–125).

Sterol production by mononuclear leukocytes (**Fig. 2**) averaged 1.20 pmol/10^6 cells/h in the control period. After acute lovastatin administration, this production rate fell to an average of 69% of control ($P = 0.05$, 95% C.I. 37–100). After chronic lovastatin treatment in these six subjects, sterol production averaged 62% of control ($P < 0.05$, 95% C.I. 30–93). Finally, after withdrawal of chronic lovastatin, leukocyte sterol production was not

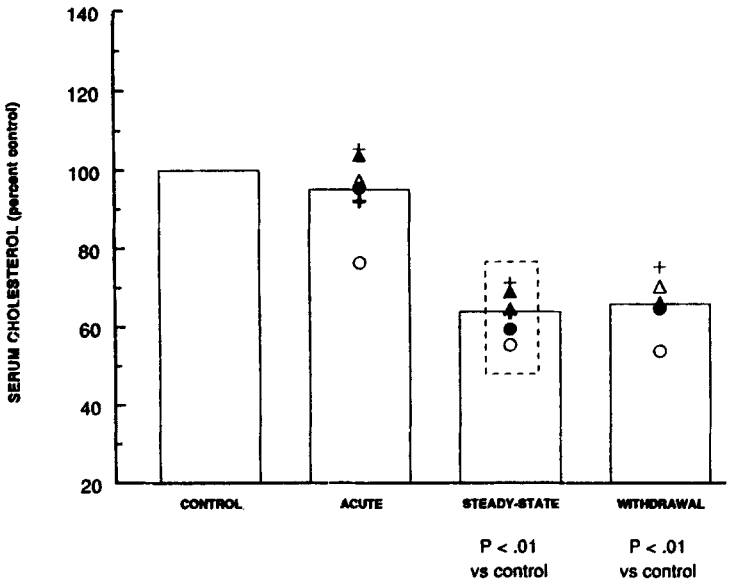

Fig. 1. Total serum cholesterol levels. In this and subsequent figures the data for acute, steady-state, and withdrawal periods are expressed as percent of each individual's control period value, and each individual is distinguished by a unique symbol. Data enclosed in broken lines have been published previously (12) and are included here for comparison. A single dose of lovastatin (acute period) did not appreciably alter serum cholesterol. In the steady-state period after 5–6 weeks of lovastatin, serum cholesterol was significantly reduced ($P < 0.01$). Twenty-four hours after withdrawal of lovastatin, serum cholesterol had not changed appreciably from the steady state level and remained significantly different from the control period ($P < 0.01$).

significantly different from control averaging 88% of control (95% C.I. 56–120).

Biliary cholesterol secretion (**Fig. 3**), which averaged 152 μmol/h in the control period, was unchanged after a single dose of lovastatin (mean 103% of control, 95% C.I. 85–120). Cholesterol secretion fell significantly to an average of 81% of control after 5–6 weeks of lovastatin treatment in these six subjects ($P < 0.05$, 95% C.I. 64–98). After withdrawal of chronic lovastatin, cholesterol secretion remained significantly different from control with values very similar to those in the chronic lovastatin period (mean 80% of control, $P < 0.05$, 95% C.I. 63–97).

Bile acid secretion into bile averaged 1154 μmol/h in the control period. Mean bile acid secretion was 99% of control after a single dose of lovastatin, 96% of control after 5–6 weeks of lovastatin (individual data points previously published (12)), and 99% of control 24 h after cessation of chronic lovastatin. None of these changes were statistically significant.

Biliary lecithin secretion averaged 441 μmol/h in the control period. Mean lecithin secretion was 106% of control after a single dose of lovastatin, 96% of control after 5–6 weeks of lovastatin (individual data points previously published (12)), and 98% of control 24 h after cessation of chronic lovastatin. Again, none of these changes were statistically significant.

Bile acid synthesis rate (**Fig. 4**) averaged 1.01 μmol/min in the control period. After a single dose of lovastatin, synthesis fell to an average of 69% of control ($P < 0.01$,

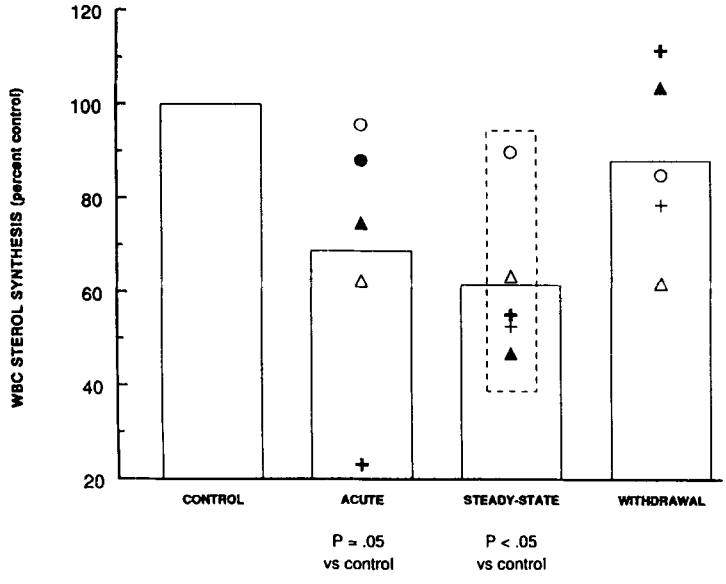

Fig. 2. Sterol production from acetate by mononuclear leukocytes. A single acute dose of lovastatin significantly lowered sterol production by these leukocytes ($P = 0.05$). Production remained significantly different from control after 5–6 weeks of lovastatin ($P < 0.05$). After withdrawal of chronic lovastatin, sterol production was indistinguishable from that in the control period. Data enclosed in broken lines have been published previously (12).

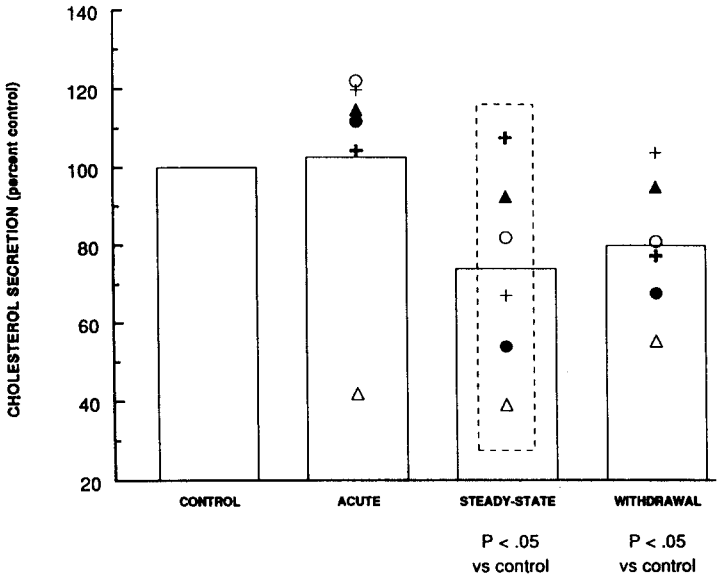

Fig. 3. Biliary cholesterol secretion. A single dose of lovastatin did not significantly alter secretion. After 5–6 weeks of lovastatin, secretion was significantly lower than in the control period ($P < 0.05$). After withdrawal of lovastatin, cholesterol secretion was similar to that prior to withdrawal (steady-state) and remained significantly lower than in the control period ($P < 0.05$). Data enclosed in broken lines have been published previously (12).

99% C.I. 50–87%). After long-term treatment with lovastatin, synthesis averaged 83% of control ($P < 0.05$, 95% C.I. 70–96). After withdrawal of chronic lovastatin synthesis averaged 88% of control N.S. 95% C.I. 75–102).

Specific activity of biliary cholesterol averaged 96% (95% C.I. 88–104) of specific activity of serum unesterified cholesterol. Each individual measurement of these two specific activities is plotted in **Fig. 5**. While there may have been a slight tendency for the serum free cholesterol specific activity to be higher than the bile cholesterol specific activity, the points clustered very close to the line of identity. Moreover, there did not appear to be a tendency for these two specific activities to differ more in any particular period. For the four periods: control, chronic lovastatin, acute lovastatin, and withdrawal, the bile cholesterol specific activity as percent of the serum free cholesterol specific activity averaged 93 (95% C.I. 86–99), 90 (95% C.I. 71–109), 98 (95% C.I. 82–113), and 103 (95% C.I. 72–134), respectively.

DISCUSSION

The present study provides new evidence in human subjects for regulatory effects of cholesterol synthesis on both cholesterol secretion into bile and the rate of bile acid synthesis. We used the inhibitor, lovastatin, to directly and primarily alter cholesterol synthesis, both acutely

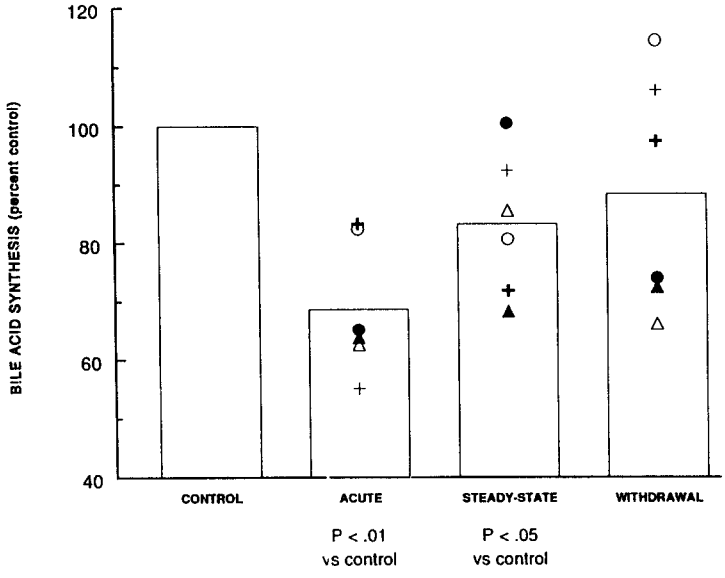

Fig. 4. Bile acid synthesis rate. A single dose of lovastatin induced a reduction in bile acid synthesis in all six subjects ($P < 0.01$). In the steady-state period bile acid synthesis had returned somewhat toward the baseline value, but remained significantly lower than in the control period ($P < 0.05$). After withdrawal of lovastatin, mean synthesis increased somewhat, but was not significantly different from either control or steady-state values.

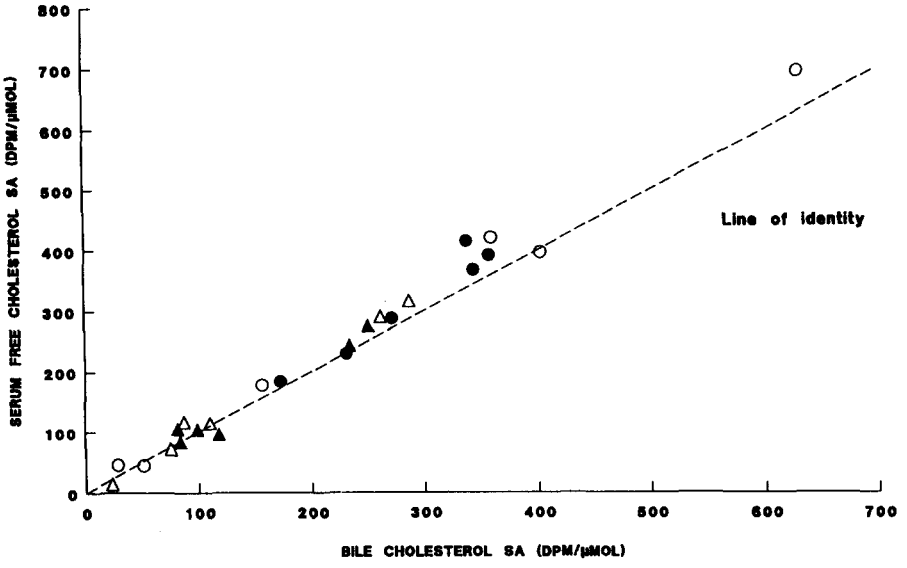

Fig. 5. Relationship of specific activity (SA) of cholesterol in bile compared to that of unesterified serum cholesterol. Closed circles correspond to control values; open circles to steady-state lovastatin values; closed triangles to values after a single dose of lovastatin; and open triangles to values after withdrawal of lovastatin. In general, serum values tended to be slightly higher than bile values, but the difference was small in all four periods, suggesting little preferential secretion of newly synthesized cholesterol.

and in the long-term. Changes in cholesterol synthesis were monitored by measuring sterol production in mononuclear leukocytes, a parameter that has been shown to correlate with other measures of cholesterol synthesis in rats and humans (16, 21–23). We also have recently summarized the evidence that lovastatin lowers cholesterol synthesis (12). Since that writing two other papers have been published, one showing lower cholesterol synthesis estimated by serum lathosterol levels in subjects on pravastatin (13) and the other showing no change in cholesterol synthesis on lovastatin measured by isotope dilution (24). The latter study used a lower dose of lovastatin than used in the present study. The bulk of the evidence, therefore, continues to suggest that at least relatively high dose lovastatin causes a persistent reduction in cholesterol synthesis in humans.

In response to acute lowering of cholesterol synthesis by a single dose of lovastatin, biliary cholesterol secretion remained at virtually identical levels to those in the control period. In contrast, as we have shown previously in ten subjects including the six studied here (12), 5–6 weeks of lovastatin administration significantly lowered biliary cholesterol secretion. These results are the opposite of those reported in the rat where lovastatin acutely lowered cholesterol secretion in two studies (3, 11) and multiple long-term alterations in cholesterol synthesis failed to alter cholesterol secretion in another study (4). These discrepancies may represent either species differences or differences in study design.

We also performed measurements in a withdrawal period after cessation of lovastatin. In the rat such a withdrawal causes a striking rebound in cholesterol synthesis (25, 26). In these human subjects, however, no such rebound occurred, perhaps again because of the more limited ability of humans to synthesize compensatory reductase protein (13, 15). Instead, mean sterol production by mononuclear leukocytes in the withdrawal period was intermediate between the chronic lovastatin and control periods, being significantly different from neither. Consistent with the lack of change in cholesterol secretion after short-term reduction in cholesterol synthesis, cholesterol secretion during the withdrawal period was nearly identical to that in the chronic lovastatin period.

One way cholesterol synthesis might affect cholesterol secretion is by altering the preferential secretion of newly synthesized cholesterol. However, in our subjects the specific activity of biliary cholesterol was, if anything, only slightly less than that of serum free cholesterol and this relationship was not significantly altered by either acute or long-term lovastatin (Fig. 5). Thus, unlike human subjects with an interrupted enterohepatic circulation (18), in these normal subjects there appeared to be little if any preferential secretion of newly synthesized cholesterol.

Perhaps the most striking result of the present study was the consistent reduction in bile acid synthesis after a single dose of lovastatin (Fig. 4). Synthesis was reduced in all six subjects and the magnitude of reduction was reasonably consistent. Could this change in synthesis

measured by the $^{14}CO_2$ method reflect a sudden alteration of the specific activity of the cholesterol substrate pool used for production of bile acid rather than a true change in synthesis? This seems an extremely unlikely possibility for two reasons. First, the specific activity of biliary cholesterol, which is usually in near equilibrium with the cholesterol pool used for bile acid synthesis (18), did not suddenly deviate from the serum free cholesterol specific activity following acute lovastatin (Fig. 5). Second, if anything one would expect acute lovastatin to suddenly increase cholesterol specific activity in this substrate pool. In that case we would have used a falsely low specific activity (the serum or bile value) to calculate a falsely high value for bile acid synthesis. We conclude that the rapid change in bile acid synthesis with a single dose of lovastatin was real and provides evidence that cholesterol synthesis is a significant regulator of bile acid synthesis in normal human subjects.

After 5–6 weeks of lovastatin, mean bile acid synthesis measured by release of $^{14}CO_2$ from [26-^{14}C]cholesterol remained significantly lower than control. These results are consistent with work done in rats where treatment with lovastatin has been shown to lower activity of 7α-hydroxylase (9–11). In human subjects Grundy and Bilheimer (27) found significantly lower levels of acidic sterol output in two of five patients treated with lovastatin. Also, a recent study of patients undergoing cholecystectomy reported a lower mean level of 7α-hydroxylase activity in those treated with pravastatin, but the results were not statistically significant (13). Finally, in ten human subjects, six of whom make up the cohort of the present study, we have previously reported a lower mean bile acid synthesis measured by isotope dilution during treatment with lovastatin, although the changes were not quite statistically significant (12). That we were able to show a significant reduction in synthesis in the present study for six of these ten subjects may reflect the simplicity of the $^{14}CO_2$ method compared to the isotope dilution method. There is no possiblity of selection bias, because the two studies were conducted simultaneously including as many subjects as would consent in the present study. Judging from the results in Fig. 4 it appears that long-term lovastatin does lower bile acid synthesis, although there may be so much individual variation in this response that it is difficult to demonstrate statistically.

The rapid response of bile acid synthesis to a single dose of lovastatin (Fig. 4) suggests that regulation of bile acid synthesis by cholesterol synthesis is relatively direct, but the mechanism of this regulation remains uncertain. Addition of mevinolinic acid to rat liver microsomes has been shown not to lower activity of 7α-hydroxylase (9) suggesting that this enzyme is not inhibited by inhibitors of HMG-CoA reductase. The reduction in bile acid synthesis and activity of 7α-hydroxylase in rats treated with both lovastatin and AY9944, which blocks conversion of 7-dehydrocholesterol to cholesterol, suggests that cholesterol synthesis may in some way directly regulate activity of this enzyme (9–11). Whether this is true in humans remains to be determined since the one existing study of this enzyme in subjects taking a reductase inhibitor was inconclusive (13). Alternatively, reduction in cholesterol synthesis could act by reducing cholesterol substrate available for conversion to bile acid. However, in both rat and human it appears that activity of 7α-hydroxylase is usually not substrate-limited (28, 29).

In summary, our data provide evidence that cholesterol synthesis is an immediate regulator of bile acid synthesis in humans, presumably serving to partially offset acute alterations in cholesterol input. Cholesterol synthesis also appears to regulate biliary cholesterol secretion, but the effect is not immediate and therefore may be indirect. Changes in both cholesterol secretion and bile acid synthesis tend to offset long-term changes in cholesterol synthesis.

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