Effects of lovastatin and chenodiol on bile acid synthesis, bile lipid composition, and biliary lipid secretion in healthy human subjects

David S. Hanson and William C. Duane[1]

Department of Medicine, Veterans Affairs Medical Center, and University of Minnesota, Minneapolis, MN 55417

Abstract To assess the relationship between cholesterol synthesis and feedback inhibition of bile acid synthesis, we studied seven normal human subjects taking three different doses of chenodiol, 0, 5, and 15 mg/kg per day: once while taking no lovastatin and again while taking lovastatin 80 mg/day. Lovastatin and both doses of chenodiol significantly lowered bile acid synthesis measured by the $^{14}CO_2$ method, but there was no significant interaction between the perturbations. Both also lowered cholesterol saturation index of gallbladder bile without appreciable interaction, and the combination was distinctly more effective than either medication alone. Lovastatin and low-dose chenodiol both lowered biliary cholesterol secretion without affecting bile acid secretion. Increasing the dose of chenodiol did not further lower cholesterol secretion, but did further reduce saturation index because of an increase in secretion of bile acid and phospholipid. These studies indicate that there is no interaction between cholesterol synthesis and feedback return of bile acid in the enterohepatic circulation with respect to either bile acid synthesis or biliary lipid secretion; that the combination of chenodiol and lovastatin is better than either alone for improving biliary cholesterol saturation; and that the mechanism by which chenodiol lowers cholesterol saturation is dose-dependent.—**Hanson, D. S., and W. C. Duane.** Effects of lovastatin and chenodiol on bile acid synthesis, bile lipid composition, and biliary lipid secretion in healthy human subjects. *J. Lipid Res.* 1994. **35:** 1462–1468.

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Lovastatin and other inhibitors of HMG-CoA reductase lower both serum and biliary cholesterol (1–3). They also constitute a powerful new tool for advancing our understanding of sterol homeostasis in human subjects. Studying human subjects treated with HMG-CoA reductase inhibitors, we and others have conclusively demonstrated that cholesterol synthesis is an important regulator of biliary cholesterol secretion (3–6). Defining the role of cholesterol synthesis in regulation of bile acid synthesis in humans has proven more difficult.

Using output of $^{14}CO_2$ from [26-^{14}C]cholesterol, we have shown that lovastatin lowers bile acid synthesis, both acutely (4) and in the steady-state (3). Vanhanen, Kesaniemi, and Miettinen (7) have also demonstrated reduction in bile acid synthesis by fecal acidic sterol output in subjects taking pravastatin. However, we have not been able to confirm reduced fecal acidic sterol output during lovastatin treatment (5). Moreover, using Lindstedt isotope dilution kinetics, we have also found no significant change in bile acid synthesis on long-term lovastatin (3).

We reasoned that part of this difficulty might stem from lovastatin inducing changes in bile acid synthesis that were relatively small and therefore difficult to measure. We thought it possible that lovastatin might have a greater effect under conditions where feedback inhibition of synthesis was enhanced by administration of a bile acid such as chenodiol. Conversely, it seemed possible that reduction in cholesterol synthesis by lovastatin might enhance sensitivity of bile acid synthesis to feedback inhibition by chenodiol. We therefore designed a study to test the effects of lovastatin on bile acid synthesis while varying feedback inhibition. Seven human subjects were studied on and off lovastatin at three different doses (0, 5, and 15 mg/kg per day) of chenodiol, a bile acid known to inhibit bile acid synthesis (8). Hepatic secretion of bile acid was measured in each of these six periods to provide an estimate of bile acid flux through the liver.

In addition to addressing questions about bile acid synthesis, this design provided the opportunity to test the combined effect of lovastatin and chenodiol on cholesterol saturation of bile. Combining lovastatin with ursodiol is known to lower saturation index more than either drug alone (9). However, chenodiol and ursodiol act by different mechanisms (8), and the combination of lovastatin

[1]To whom correspondence should be addressed at: GI Section (111D), Veterans Affairs Medical Center, 1 Veterans Drive, Minneapolis, MN 55417.

with chenodiol has never been evaluated with respect to cholesterol saturation of bile. Moreover, by measuring biliary lipid secretion in each period, we were able to determine to what extent any combined improvement was a result of decreased cholesterol secretion versus increased bile acid and phospholipid secretion.

METHODS

The protocol for this study was approved by committees overseeing use of human subjects in research at both the Minneapolis VA Medical Center and the University of Minnesota. Informed consent was obtained from all volunteers prior to enrolling in the study. Seven male volunteers ranging in age from 48 to 73 years participated in this investigation. None had undergone cholecystectomy and all were free of major illness by previously published criteria (10). Each subject also underwent ultrasonography to document absence of gallstones. Dietary histories were obtained at the beginning of the study, and each participant was asked not to alter his dietary habits while enrolled. While it would have been preferable to complete all studies on a metabolic ward with strict diet control, that constraint for such a long study would have made recruitment of volunteers very difficult and greatly increased the cost of the study. Moreover, our recent studies show that quadrupling dietary cholesterol increases bile acid synthesis by only about 15% and has no discernible effect on biliary cholesterol secretion (5).

The protocol was designed so that each subject served as his own control. As shown in **Figure 1,** it consisted of two separate, randomly ordered 5-week periods: one during which the subject took lovastatin, 40 mg b.i.d., and one during which the subject took no lovastatin. The two periods were separated by a 2-week respite. After the first 2 weeks of each of these two periods, during which the subject took no chenodiol, samples of breath and bile were obtained. Each subject was then randomly assigned to take either 5 mg/kg per day or 15 mg/kg per day of chenodiol in three divided doses for the next 10 days, after which breath and bile samples were obtained. Finally, the alternate dose of chenodiol was given for the last 10 days, followed by breath and bile sampling. A 10-day interval was chosen for two reasons. First, it was short enough to maintain [26-^{14}C]cholesterol at levels sufficient to measure output of $^{14}CO_2$ without administering excessive isotope (see below). Second, it was long enough so that biliary levels of chenodeoxycholic acid, which has a half-life of about 2 days (8, 10), would be expected to approach equilibration.

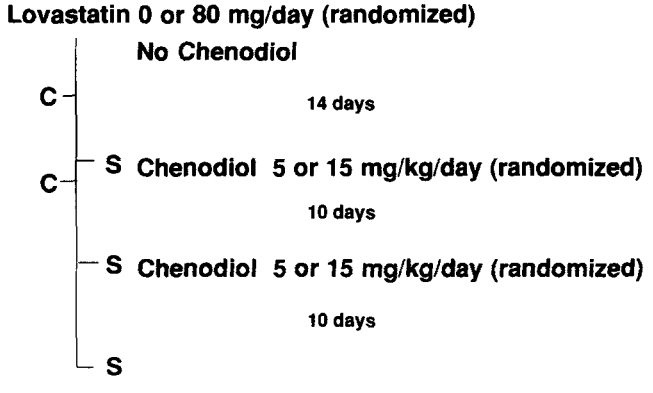

Fig. 1. Schematization of the 5-week study protocol. Each subject was studied in two such 5-week periods, one with and one without lovastatin by random assignment. The two 5-week periods were separated by a 2-week respite. For the first 14 days of each period the subject took no chenodiol. This was followed by chenodiol, either 5 or 15 mg/kg per day, for 10 days, and then by the alternate dose of chenodiol for the next 10 days, again by random assignment. 'C' indicates times when each subject was given 25 μCi [26-^{14}C]cholesterol. 'S' indicates times when the subject provided samples of breath and bile. Subjects 1, 4, and 6 received lovastatin during the first 5 weeks. The other subjects received lovastatin during the second 5 weeks.

Fifty μCi [26-^{14}C]cholesterol (New England Nuclear, Boston, MA) was orally administered during each 5-week cycle. This permitted measurement of bile acid synthesis by the previously described $^{14}CO_2$ technique (11). Synthesis was calculated by dividing $^{14}CO_2$ output on the breath by the specific activity of free cholesterol determined from a serum sample obtained at the time of breath sampling, done in duplicate, as previously described (11).

Gallbladder bile samples were obtained via peroral duodenal tube using cholecystokinin octapeptide to stimulate gallbladder contraction as previously described (3). Samples were analyzed for bilirubin, cholesterol, phospholipid, and total and individual bile salts as described in previous publications (3-5, 12). Cholesterol saturation index was calculated according to formulas of Carey and Small (13) assuming a total solid concentration of 10 g/dl.

Biliary secretion rates were determined from the analysis of gallbladder bile and the endogenous production rate of carbon monoxide (CO), determined by breath analysis as previously described (12). This method utilizes the fact that CO production rate reflects both bilirubin production and secretion. Biliary secretion for any bile constituent can then be determined from the ratio of constituent/bilirubin measured in gallbladder bile multiplied by the endogenous CO production rate. We have shown that this method accurately reflects cholesterol secretion compared to standard marker perfusion techniques and that it is also more reproducible than marker perfusion (12). The method consistently provides an estimate of bile acid and phospholipid secretion that is about 25% lower than those measured by marker perfusion (12). However, in the present study, where effects were assessed comparing each subject to himself, that should not have represented a significant disadvantage.

Statistical analysis was performed using SAS software

TABLE 1. Bile acid synthesis

	No Lovastatin			Lovastatin		
	Chenodiol (mg/kg/day)			Chenodiol (mg/kg/day)		
Subject	0	5	15	0	5	15
	μmol/day			*μmol/day*		
1	1515	1141	463	1422	997	497
2	2069	1795	1856	1759	1256	1058
3	1576	703	424	721	989	231
4	588	490	414	626	604	401
5	489	279	222	202	272	172
6	607	383	320	639	366	315
7	1407	658	219	1003	468	261
Mean	1179	779	560	910	707	419

(SAS Institute, Carey, NC) on a Northgate 486 personal computer. Analysis of variance was used to test the null hypothesis for an effect of lovastatin, an overall effect of chenodeoxycholic acid, and an interaction between these two medications. We also used contrast analysis to compare periods of differing chenodiol doses (14).

The term "interaction" is used here in a precise statistical sense (14). Thus, if there is no interaction between lovastatin and chenodiol with respect to (for example) bile acid synthesis, then lovastatin affects bile acid synthesis the same with and without chenodiol treatment and chenodiol affects synthesis the same with and without lovastatin treatment. Absence of interaction does not preclude an additive effect of the two medications. On the contrary, if each medication by itself has an effect and the effects are in the same direction, absence of interaction implies that the combination has a greater effect than either medication alone.

RESULTS

Quantitative values are provided in **Tables 1-6,** and results of all statistical testing are provided in **Table 7.**

Lovastatin induced a small, but significant (P = 0.036), reduction in bile acid synthesis (Tables 1 and 7). Chenodiol also lowered bile acid synthesis overall (P = 0.0001), and this effect was significant for each incremental increase in dose (Tables 1 and 7). There was no significant interaction (P = 0.530) between lovastatin and chenodiol (Table 7).

Cholesterol saturation index was reduced by lovastatin (P = 0.0001, Tables 2 and 7). Chenodiol also lowered saturation index (overall P = 0.0001) and there was a significant incremental reduction with each increase in dose of chenodeoxycholic acid (Tables 2 and 7). There was no significant interaction (P = 0.248) between lovastatin and chenodiol with respect to saturation index (Table 7).

Biliary secretion of cholesterol was significantly reduced by lovastatin (P = 0.0001, Tables 3 and 7). There was a significant overall effect of chenodiol on cholesterol secretion (P = 0.032); however, increasing the dose of chenodiol from 5 to 15 mg/kg per day did not further decrease cholesterol secretion (P = 0.533, Tables 3 and 7). There was no significant interaction between lovastatin and chenodiol with respect biliary cholesterol secretion (P = 0.871, Table 7).

Secretion of phospholipid into bile was not significantly affected by lovastatin (= 0.680), but was significantly in-

TABLE 2. Cholesterol saturation index

	No Lovastatin			Lovastatin		
	Chenodiol (mg/kg/day)			Chenodiol (mg/kg/day)		
Subject	0	5	15	0	5	15
1	1.07	0.87	0.74	0.95	0.62	0.46
2	1.79	1.77	1.17	1.14	0.99	0.87
3	1.20	1.02	0.74	0.90	0.79	0.65
4	1.68	1.37	1.21	0.97	1.03	0.86
5	0.95	0.74	0.66	0.60	0.62	0.54
6	0.76	0.60	0.65	0.82	0.56	0.56
7	0.87	0.70	0.62	0.74	0.61	0.56
Mean	1.19	1.01	0.83	0.87	0.74	0.64

TABLE 3. Biliary secretion of cholesterol

	No Lovastatin			Lovastatin		
	Chenodiol (mg/kg/day)			Chenodiol (mg/kg/day)		
Subject	0	5	15	0	5	15
	μmol/h			*μmol/h*		
1	90	65	67	67	46	35
2	144	124	150	149	122	121
3	120	92	76	74	67	66
4	78	67	80	38	37	22
5	28	28	34	22	26	24
6	70	74	63	66	45	58
7	85	84	99	74	66	89
Mean	88	76	81	70	59	59

TABLE 4. Biliary secretion of lecithin

	No Lovastatin			Lovastatin		
	Chenodiol (mg/kg/day)			Chenodiol (mg/kg/day)		
Subject	0	5	15	0	5	15
	μmol/h			*μmol/h*		
1	252	223	278	201	218	228
2	223	204	385	410	382	431
3	311	283	322	255	265	312
4	140	154	200	120	101	43
5	86	115	154	109	128	134
6	293	392	297	241	255	331
7	297	373	500	312	338	529
Mean	229	249	305	235	241	287

TABLE 5. Biliary secretion of bile acid

	No Lovastatin			Lovastatin		
	Chenodiol (mg/kg/day)			Chenodiol (mg/kg/day)		
Subject	0	5	15	0	5	15
	μmol/h			*μmol/h*		
1	858	812	938	865	907	920
2	871	631	1290	1177	1205	1363
3	906	824	1051	805	845	1075
4	396	408	625	403	479	761
5	340	429	603	414	514	546
6	902	1236	1057	865	824	1057
7	991	1192	1689	991	1139	1480
Mean	752	790	1036	789	845	1029

TABLE 6. Biliary secretion of chenodeoxycholic acid

	No Lovastatin			Lovastatin		
	Chenodiol (mg/kg/day)			Chenodiol (mg/kg/day)		
Subject	0	5	15	0	5	15
	μmol/h			*μmol/h*		
1	274	486	737	240	549	770
2	175	230	943	207	485	1005
3	323	490	884	309	529	936
4	97	259	449	140	311	559
5	158	338	513	331	418	482
6	357	899	722	363	588	808
7	348	729	1568	303	708	1256
Mean	247	490	831	270	513	831

creased by chenodiol overall (P = 0.006, Tables 4 and 7). The increase in phospholipid secretion occurred predominantly when the dose of chenodiol was increased from 5 to 15 mg/kg per day (P = 0.013, Tables 4 and 7). Increasing chenodiol from 0 to 5 mg/kg per day did not significantly alter phospholipid secretion (P = 0.500, Tables 4 and 7). There was no significant interaction between lovastatin and chenodiol (P = 0.803) with respect to phospholipid secretion.

Bile acid secretion changed in a manner similar to phospholipid secretion showing no significant lovastatin effect (P = 0.479) and a significant overall chenodiol effect (P = 0.0001, Tables 5 and 7). Here, too, increasing chenodiol from 0 to 5 mg/kg per day had no significant effect (P = 0.333) while increasing from 5 to 15 mg/kg per day significantly increased bile acid secretion (P = 0.0002, Tables 4 and 7). For this variable as well there was no significant interaction between lovastatin and chenodiol (P = 0.806, Table 7).

Finally, biliary secretion of chenodeoxycholic acid was independent of lovastatin (P = 0.777), but showed a highly significant overall chenodiol effect (P = 0.0001, Tables 6 and 7). Each increase in chenodiol dose resulted in a significant increase in secretion of chenodeoxycholic acid (Tables 6 and 7). There was no significant interaction between lovastatin and chenodiol with respect to secretion of chenodeoxycholic acid (P = 0.981, Table 7).

DISCUSSION

We have shown that the dose of lovastatin used in the present study inhibits cholesterol synthesis in human subjects, both acutely (15) and over the longterm (5). Moreover, addition of this agent to microsomes does not affect activity of 7α-hydroxylase (16). Lovastatin, therefore, provides an ideal means for testing the effects of cholesterol synthesis on bile acid synthesis. Acute studies in both animal models and human subjects have shown that lovastatin does rapidly lower bile acid synthesis (4, 16, 17). Potential mechanisms for this effect have been discussed elsewhere (4, 16–18). In the long-term steady-state situation, lovastatin has lowered bile acid synthesis by the $^{14}CO_2$ technique (4), but not by isotope dilution (3).

TABLE 7. Results of statistical analysis

	Analysis of Variance for Main Effects[a]			Contrast Analysis of Chenodiol Doses[b]		
Variable	Lovast	Chenod	Interac	0 vs 5	0 vs 15	5 vs 15
	P-value			*P*-value		
Bile acid synthesis	0.0356	0.0001	0.5300	0.0022	0.0001	0.0081
Saturation index	0.0001	0.0001	0.2478	0.0005	0.0001	0.0010
Cholesterol secretion	0.0001	0.0323	0.8708	0.0125	0.0513	0.5226
Lecithin secretion	0.6800	0.0062	0.8031	0.5004	0.0025	0.0130
Bile acid secretion	0.4794	0.0001	0.8045	0.3328	0.0001	0.0002
Cheno secretion[c]	0.7769	0.0001	0.9808	0.0011	0.0001	0.0001

[a]Lovast, lovastatin; Chenod, chenodiol; Interac, interaction between lovastatin and chenodiol.
[b]Comparison of chenodiol at doses of 0, 5, and 15 mg/kg per day.
[c]Secretion of chenodeoxycholic acid.

Determined by fecal acidic sterol output, some studies have shown a reduction in bile acid synthesis on HMG-CoA reductase inhibitors while others have not (5, 7).

In the present study, bile acid synthesis measured by the $^{14}CO_2$ technique was significantly lowered by lovastatin (Tables 1 and 7). As expected, chenodiol also lowered bile acid synthesis, but there was no significant interaction between lovastatin and chenodiol (Tables 1 and 7). This means that the effect of lovastatin on bile acid synthesis was the same regardless of changing levels of feedback inhibition with chenodiol. It also means that feedback inhibition by chenodiol was unaffected by lovastatin, indicating that reduced cholesterol synthesis did not alter sensitivity of the feedback inhibition mechanism. These negative findings are perhaps especially remarkable in view of the fact that feedback inhibition of bile acid synthesis by chenodiol may also be accompanied by lower activity of HMG-CoA reductase and/or altered cholesterol absorption (8). It is also conceivable that chenodiol might affect absorption or pharmacokinetics of lovastatin, although there is no evidence for that effect. In any case, manipulation of feedback inhibition and its attendant secondary effects did not prove to be a useful strategy for magnifying effects of lovastatin on bile acid synthesis.

Could the $^{14}CO_2$ method provide a systematic underestimation of bile acid synthesis during lovastatin treatment? This seems an extremely unlikely explanation. Only two measurements enter into this determination: output of $^{14}CO_2$ on the breath and specific activity of serum free cholesterol. There is no reason to suspect that lovastatin would falsely lower output of $^{14}CO_2$. A falsely low rate of synthesis would be measured by this method if the serum free cholesterol specific activity were higher than that of the actual precursor pool, as would be the case if newly synthesized cholesterol were preferentially used for bile acid synthesis. However, lovastatin would, if anything, reduce preferential use of newly synthesized cholesterol and tend to raise, not lower, apparent bile acid synthesis.

On the other hand, inherent methodologic variability is probably lower for the relatively simple $^{14}CO_2$ method than for either fecal acidic sterol output or isotope dilution, both of which require complex analytical procedures and sample averaging over prolonged time intervals. It is possible, therefore, that our studies with these more complex methods were subject to type II statistical error (19). This possibility is supported by the fact that in the study of Vanhanen et al. (7) pravastatin did significantly lower fecal acidic sterol output, and in another of our studies (3) mean synthesis by isotope dilution during lovastatin treatment was lower, though the difference was not quite statistically significant. Finally, we have also demonstrated a statistically significant reduction in bile acid pool size by the one-sample method (3) suggesting that lovastatin lowers bile acid synthesis. Taking an overview of all available data, we are forced to conclude that in the steady-state lovastatin may slightly lower bile acid synthesis, but if so, the change is small enough and inconsistent enough to be very difficult to document.

During administration of high dose chenodiol the decrease in bile acid synthesis was associated with a significant increase in bile acid secretion (Tables 5 and 7), which is equivalent to flux through the liver. However, bile acid synthesis was also significantly inhibited by low-dose chenodiol despite the fact that bile acid secretion was not significantly increased (Tables 5 and 7). This is presumably explained by the significant increase in secretion of chenodeoxycholic acid during treatment with low-dose chenodiol (Tables 6 and 7). In human subjects this finding lends support to the observation that more hydrophobic bile acids, such as chenodeoxycholic acid, are better inhibitors of bile acid synthesis than more hydrophilic bile acids, such as cholic acid (8, 20).

The present study shows for the first time that there is no interaction between lovastatin and chenodiol with respect to cholesterol saturation index. Thus chenodiol had the same effect on saturation index regardless of lovastatin treatment and lovastatin had the same effect regardless of chenodiol treatment (Tables 2 and 7). It is noteworthy, however, that at each dose of chenodiol, addition of lovastatin resulted in a further lowering of saturation index, and the combination of high-dose chenodiol with lovastatin lowered mean saturation index by a remarkable 50% (Table 2).

Both lovastatin (3–6) and chenodiol (21, 22) are known to lower biliary cholesterol secretion (3–6). Interestingly, in the present study nearly all the reduction in cholesterol secretion induced by chenodiol occurred at a dose of 5 mg/kg per day with no further reduction as the dose was tripled (Table 3). This dose-plateau effect has not been appreciated previously, perhaps because the arduous marker dilution methods limited study of dose-response with respect to cholesterol secretion. It was possible to delineate this relationship in the present study because our new facilitated method for measuring cholesterol secretion much more easily permits multiple studies in an individual subject and has less inherent variability than standard marker dilution techniques (12).

It is most noteworthy that despite the plateau of cholesterol secretion, cholesterol saturation index was further reduced when chenodiol was increased from 5 to 15 mg/kg per day (Tables 2 and 7). That was because secretion of both bile acid and phospholipid increased between these two doses (Tables 4, 5, and 7), an effect that also has not been previously appreciated. Indeed, it is generally stated that chenodiol lowers cholesterol saturation without increasing secretion of bile acid (8, 23). The present study strongly argues against that assertion. Thus, for every one of the fourteen comparisons, going from 0 to 15 mg/kg per day of chenodiol increased bile acid secretion (Table 5).

Close examination of the literature shows that a similar increase is discernible in existing data. We could find a total of 31 subjects for whom bile acid secretion had been measured in a control period and again on chenodiol at a dose of at least 12.5 mg/kg per day (22–25). As percent of control, mean bile acid secretion for these 31 subjects on chenodiol was 119% with a 95% confidence interval of 100–138%. Thus, although previously not clearly appreciated, the decrease in saturation index on high dose chenodiol occurs in large part because of an increase in bile acid and phospholipid secretion.

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