Effects of lovastatin and dietary cholesterol on bile acid kinetics and bile lipid composition in healthy male subjects

William C. Duane

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

Abstract We measured bile acid kinetics and bile lipids in 12 human subjects on a metabolic ward in four randomly allocated, 6–7 week periods: *1)* lovastatin (40 mg b.i.d) + low cholesterol diet (mean 246 mg/day); *2)* lovastatin + high cholesterol diet (mean 1071 mg/day); *3)* low cholesterol diet alone; and *4)* high cholesterol diet alone. Lovastatin did not significantly alter fractional turnover, synthesis, absorption, enterohepatic cycling, or pool sizes of bile acid measured by the Lindstedt method. The high cholesterol diet increased fractional turnover and synthesis rate of cholic acid, but not chenodeoxycholic acid, without altering pool size of either bile acid. The high cholesterol diet decreased bile acid absorption, but only during lovastatin treatment, suggesting the possibility of a "cholestyramine-like" effect of dietary cholesterol, appreciable at least when biliary cholesterol secretion is reduced by lovastatin. As in previous studies, lovastatin markedly lowered saturation index of gallbladder bile. Increased cholesterol consumption did not significantly alter cholesterol saturation index, suggesting that dietary cholesterol may not be a major factor in cholesterol gallstone pathogenesis.—**Duane, W. C.** 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.

Supplementary key words cholesterol • bile acids and salts • atherosclerosis • hydroxymethylglutaryl-CoA reductases • cholelithiasis

Regulation of bile acid metabolism is accomplished in part via feedback inhibition of synthesis by bile acid returning to the liver (1). In addition, cholesterol input may regulate bile acid metabolism, but this potential regulatory mechanism remains incompletely characterized, especially in humans.

There are only two routes for cholesterol input: ingestion and biosynthesis. The role of cholesterol biosynthesis in regulation of bile acid metabolism has been most effectively studied using HMG-CoA reductase inhibitors such as lovastatin. In short-term animal studies these inhibitors have been shown to cause acute reduction in bile acid synthesis, presumably as a result of reduced cholesterol biosynthesis (2). In studies of normal human volunteers and monitoring conversion of [26-^{14}C]cholesterol to $^{14}CO_2$, we have also been able to show an acute reduction in bile acid synthesis after a single dose of lovastatin (3).

Steady-state effects are less clear. We have documented a reduction in bile acid pool size measured by the one-sample method after 4–6 weeks on lovastatin (4). This suggests a decrease in bile acid synthesis, and indeed we found such a decrease by measuring synthesis by the $^{14}CO_2$ method after 4 weeks on lovastatin (3). Others have also found a reduction in synthesis by fecal acidic sterol output measurements in human subjects on pravastatin (5). However, we have not been able to confirm reduction in fecal acidic sterol output in subjects taking lovastatin (6). Moreover, using a third measure of synthesis, isotope dilution, we have found equivocal changes after 4 weeks on lovastatin: a reduction in mean synthesis that did not quite reach statistical significance (4). Part of the reason for variability in that study could have been uncontrolled dietary cholesterol because dietary restrictions were not imposed.

The second source of cholesterol input, dietary consumption, may affect bile acid metabolism in two ways. First, in certain animal models, an increase in dietary cholesterol increases bile acid synthesis (7–9). This has also been observed in some human studies (10, 11), although not in others (12–14). Second, some animal studies have suggested that excess dietary cholesterol has the effect of holding bile acid in the intestinal lumen, thus decreasing bile acid absorption in a manner similar to cholestyramine (1). This effect, which could also account for an increase in bile acid synthesis, has received little direct study in human subjects.

Finally, in addition to possible effects on bile acid metabolism, cholesterol input can regulate biliary cholesterol secretion and hence cholesterol saturation of bile.

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl.

We and others have demonstrated that decreasing cholesterol synthesis with lovastatin and related drugs lowers cholesterol secretion and saturation index (4, 15–18). The effect of dietary cholesterol on cholesterol saturation of bile is less clear. Two studies have reported increased cholesterol saturation following an increase in dietary cholesterol (19, 20), but three others have found no change (14, 21, 22).

To better define the role of cholesterol input in regulation of bile acid metabolism and bile lipid composition, we studied 12 patients on a metabolic ward with careful dietary control. We perturbed both cholesterol consumption with dietary manipulation and cholesterol biosynthesis with lovastatin in a dose previously shown to lower whole body cholesterol synthesis in this group of subjects (6). All 12 subjects were studied in four randomly assigned 6-week periods to include all combinations of high and low dietary cholesterol with and without lovastatin. We used isotope dilution methods because they permit measurement of pool size and fractional turnover as well as bile acid synthesis, and because we hoped to resolve any ambiguity of our previous isotope dilution study (4).

METHODS

Subjects, diets, and lovastatin treatment protocols are described in detail as part of a recent study of serum lipids and fecal sterols (6). Briefly, 12 healthy male volunteers were studied. Two subjects (#2 and #7) had mild elevation of serum triglycerides and another two (#9 and #11) had mild elevation of serum cholesterol (6). All subjects gave written informed consent to participate. The study procedures were approved by committees overseeing use of human subjects in research at both the Minneapolis Veterans Administration Medical Center and the University of Minnesota.

All subjects ate only meals served by the metabolic kitchen of the Special Diagnostic and Treatment Unit of the Minneapolis VA Medical Center. All subjects except one (#8) resided in this metabolic unit throughout the study. They consumed either a low cholesterol diet (mean 246 mg/day) or a high cholesterol diet (mean 1071 mg/day) that was identical to the low cholesterol diet except for isocaloric daily substitution of five eggs. Meals consisted of regular food with a weekly menu rotation to provide consistency.

All subjects were studied in four separate 6- to 7-week periods. For each subject the chronological order of periods was random. The four periods were designated as follows: LN, low dietary cholesterol, no lovastatin; HN, high dietary cholesterol, no lovastatin; LL, low dietary cholesterol, lovastatin, 40 mg b.i.d.; HL, high dietary cholesterol, lovastatin, 40 mg b.i.d.

All stools were collected for the last 10 days of each study period for measurement of fecal sterols as previously described (6). During the last week of each period, subjects were given about 5 μCi each of [24-^{14}C]cholic acid and [24-^{14}C]chenodeoxycholic acid (New England Nuclear, Boston, MA) via duodenal tube. For the 4 subsequent days, gallbladder bile was collected via duodenal tube for measurement of Lindstedt bile acid kinetics (23), one-sample total bile acid pool size (24), and bile lipid composition, all as previously described (4). On the last day of each period, measurements of biliary lipid secretion were performed by marker perfusion as previously outlined (6) using the method originally described by Grundy and Metzger (25).

Fractional bile acid absorption was calculated from the formula: $1 - [A/(S \times 24)]$ where A = acidic sterol output in μmoles/day and S = rate of secretion of bile acid into bile in μmoles/h. Daily frequency of enterohepatic cycling was calculated from the total bile acid pool (P) determined by the Lindstedt method using the formula: $(S \times 24)/P$. Cholesterol saturation index was calculated using the equations of Carey and Small (26) as previously described (4).

Statistical testing was performed using SAS software (SAS Institute, Cary, NC) on a Northgate personal computer equipped with a 486DX microprocessor. All testing was by analysis of variance (ANOVA) with randomized block design followed by contrast analysis (27). This permitted use of the entire data set to test the null hypothesis for a lovastatin effect, a dietary cholesterol effect, and an interaction between dietary cholesterol and lovastatin. Contrast analysis was also used to compare treatment periods, but this less comprehensive statistic was used only secondarily. It should also be noted that we did not calculate standard deviations because that method of comparing groups eliminates subject identity from the analysis, reflecting instead subject-to-subject variation and potentially masking changes induced by the experimental perturbations. Alternatively, we have provided each individual measurement for all important parameters, both in this manuscript and in the manuscript describing serum and fecal sterol measurements in these subjects (6).

RESULTS

Fig. 1 summarizes average systemic cholesterol inputs, namely synthesized and absorbed dietary cholesterol, in the four study periods for all subjects. These values are taken from reference 6, where all individual values and statistical analysis of these values are reported.

Fractional turnover rates for cholic and chenodeoxycholic acids are given in **Table 1.** (Mean values and results of ANOVA testing are presented in Table 7.) Lovastatin administration did not significantly alter turnover

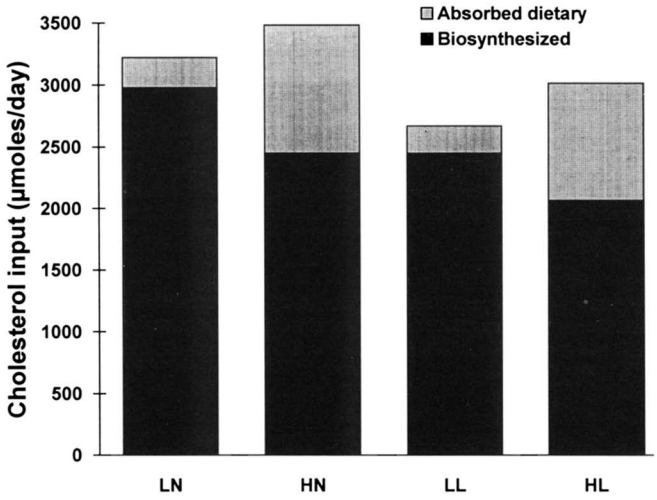

Fig. 1. Summary of average systemic cholesterol inputs. Values are taken from reference 6, which also presents individual values in tabular form. Total input is the sum of cholesterol synthesis (determined by sterol balance) and absorbed dietary cholesterol (determined by mass measurements in diet, bile, and stool). ANOVA showed a significant lovastatin effect for cholesterol synthesis and total input as well as a significant diet effect for synthesis, absorbed dietary cholesterol, and total systemic input of cholesterol (6).

of either bile acid. Increasing dietary cholesterol also did not change fractional turnover of chenodeoxycholic acid. However, cholesterol consumption did significantly increase fractional turnover of cholic acid (P = 0.025, Table 7). There was no significant interaction between dietary cholesterol and lovastatin with respect to fractional turnover rate.

Table 2 shows pool sizes of the two primary bile acids measured by the method of Lindstedt (23). There was no statistically significant effect of either lovastatin or increased dietary cholesterol on pool size of either primary bile acid (see Table 7).

Total bile acid pool determined by the Lindstedt method also was not significantly altered by either lovastatin or dietary cholesterol (**Table 3,** and Table 7). Mean total bile acid pool measured by the one-sample method was lower during lovastatin treatment, although the change was of borderline statistical significance (P = 0.073, see Tables 3 and 7). Dietary cholesterol did not significantly alter one-sample bile acid pool, and there was no significant interaction between dietary cholesterol and lovastatin with respect to one-sample pool size.

Lovastatin had no significant effect on synthesis of either primary bile acid (**Table 4** and Table 7). Consistent with the increased fractional turnover and unchanged pool size of cholic acid, synthesis rate of this bile acid was significantly increased by increasing dietary cholesterol (see Tables 4 and 7). Dietary cholesterol did not alter synthesis of chenodeoxycholic acid.

Lovastatin did not appreciably alter total bile acid synthesis measured by this method. Mean total bile acid synthesis was higher during increased cholesterol consumption, although the change was of borderline statistical significance (P = 0.054, **Table 5** and Table 7).

Overall, bile acid absorption was not significantly affected by either lovastatin or dietary cholesterol (see Tables 5 and 8). However, there was a borderline statistically significant interaction between lovastatin and dietary cholesterol with respect to bile acid absorption (P = 0.081, Table 8). Moreover, by contrast analysis comparing periods, absorption was significantly lower in period HL than in period LL (P = 0.028). Together these suggest that dietary cholesterol reduces bile acid absorption, but only during lovastatin treatment.

TABLE 1. Fractional turnover rates

Subject	Cholic Acid				Chenodeoxycholic Acid			
	LN	HN	LL	HL	LN	HN	LL	HL
	day^{-1}				*day*$^{-1}$			
1	0.218	0.198	0.154	0.362	0.286	0.157	0.131	0.274
2	0.449	0.441	0.568	0.539	0.286	0.299	0.423	0.367
3	0.240	0.360	0.261	0.396	0.070	0.282	0.247	0.252
4	0.366	0.432	0.227	0.502	0.292	0.275	0.231	0.374
5	1.176	1.337	1.070	1.321	0.663	0.419	0.639	0.651
6	0.399	0.785	0.474	0.743	0.326	0.428	0.306	0.426
7	0.599	0.934	0.504	0.488	0.479	0.578	0.330	0.388
8	0.797	0.682	0.820	0.706	0.558	0.488	0.533	0.569
9	0.638	0.370	0.322	0.407	0.482	0.345	0.341	0.357
10	0.419	0.528	0.623	0.514	0.189	0.400	0.645	0.450
11	0.232	0.472	0.172	0.219	0.335	0.422	0.174	0.200
12	0.797	0.690	0.858	0.902	0.436	0.424	0.392	0.562
Mean	0.527	0.602	0.504	0.592	0.367	0.376	0.366	0.406

LN, low dietary cholesterol, no lovastatin; HN, high dietary cholesterol, no lovastatin; LL, low dietary cholesterol, lovastatin, 40 mg b.i.d.; HL, high dietary cholesterol, lovastatin, 40 mg b.i.d.

TABLE 2. Primary bile acid pool sizes (Lindstedt)

Subject	Cholic Acid				Chenodeoxycholic Acid			
	LN	HN	LL	HL	LN	HN	LL	HL
	μmoles				*μmoles*			
1	2180	4930	2500	1820	2290	2670	3130	2250
2	1680	1300	1400	1510	1940	1370	1550	1750
3	2460	2100	2070	1290	3320	2090	1990	1660
4	2670	2500	3350	2360	2030	2260	1980	1720
5	600	740	520	530	600	970	540	520
6	2340	1730	3440	3050	1570	1700	2460	1760
7	1360	810	1390	1460	1330	920	1480	1120
8	1710	1580	1440	1310	1350	1200	1560	1130
9	1260	2470	1880	2070	1000	1760	1160	1310
10	1520	2010	1170	2020	2870	1390	710	1310
11	2160	1250	2850	2140	1890	1280	3240	1980
12	1280	2290	1950	1620	1330	2020	2060	1550
Mean	1770	1980	2000	1760	1790	1640	1820	1510

TABLE 3. Total bile acid pool size by Lindstedt and one-sample methods

Subject	Lindstedt Pool Size				One-Sample Pool Size			
	LN	HN	LL	HL	LN	HN	LL	HL
	μmoles				*μmoles*			
1	6060	10000	7160	5280	11700	8060	5930	6840
2	5780	4500	4380	4940	7050	5600	5430	6120
3	8270	7460	6170	4910	7510	9750	6910	5750
4	7510	6650	7130	5700	9050	7950	7860	6920
5	1930	2720	1820	2010	2770	3640	3070	3390
6	8190	7640	11400	10100		8680	12200	8120
7	4710	2880	6100	4960	6760	4670	5740	5620
8	4000	3740	3870	3330	5940	5230	4920	5190
9	2850	5190	3540	4420	3920	5010	4430	5350
10	4900	4620	2870	4830	4900	5530	5960	6030
11	4480	3390	6730	5810	5950	5720	3360	6120
12	3730	5700	5200	4180	6500	6180	6150	5560
Mean	5200	5370	5530	5040	6550	6330	5990	5920

TABLE 4. Individual bile acid synthesis rates

Subject	Cholic Acid				Chenodeoxycholic Acid			
	LN	HN	LL	HL	LN	HN	LL	HL
	μmole/day				*μmole/day*			
1	475	975	385	658	657	419	410	616
2	755	572	798	811	557	410	655	644
3	590	754	541	511	231	591	492	419
4	978	1080	762	1180	593	622	458	643
5	700	983	557	701	395	407	348	341
6	932	1360	1630	2260	512	729	754	750
7	814	760	702	711	637	530	487	436
8	1360	1080	1180	922	756	584	830	644
9	805	912	605	841	482	607	394	467
10	637	1060	727	1040	541	554	455	590
11	502	591	491	469	633	541	564	396
12	1020	1580	1680	1460	579	857	807	873
Mean	797	976	838	964	548	571	554	568

TABLE 5. Total bile acid synthesis rate and bile acid absorption

Subject	Synthesis				Bile Acid Absorption			
	LN	HN	LL	HL	LN	HN	LL	HL
	μmoles/day				*fraction*			
1	1130	1390	800	1270	0.983	0.990	0.981	0.970
2	1310	980	1450	1460	0.934	0.952	0.957	0.913
3	820	1350	1030	930	0.966	0.972	0.972	0.966
4	1570	1700	1220	1830	0.969	0.943	0.979	0.946
5	1090	1390	910	1040	0.978	0.952	0.972	0.975
6	1440	2090	2380	3010	0.962	0.959	0.926	0.936
7	1450	1290	1190	1150	0.972	0.965	0.974	0.967
8	2110	1660	2010	1570	0.960	0.962	0.971	0.937
9	1290	1520	1000	1310	0.961	0.973	0.975	0.972
10	1180	1610	1180	1630	0.962	0.931	0.941	0.947
11	1130	1130	1050	870	0.978	0.971	0.966	0.968
12	1600	2440	2480	2330	0.879	0.953	0.958	0.894
Mean	1340	1550	1390	1530	0.959	0.960	0.964	0.949

Frequency of enterohepatic cycling of the bile acid pool was not significantly affected by either lovastatin or increasing dietary cholesterol and there was no significant interaction for this variable (Table 8).

Both molar percent cholesterol in gallbladder bile and cholesterol saturation index were strikingly reduced by lovastatin ($P < 0.0001$, **Table 6** and Table 8). Increasing dietary cholesterol, however, had no significant effect on cholesterol content of bile or saturation index. There was no significant interaction between cholesterol consumption and lovastatin for these parameters.

Finally because two subjects had hypertriglyceridemia (#2 and #7) and two others had hypercholesterolemia (#9 and #11), the data were separately analyzed omitting each of these two pairs. In each case *P*-values were essentially identical to those for the group as a whole. Specifically no *P*-value <0.05 in the original analysis was >0.05 in the re-analysis and vice versa.

DISCUSSION

Bile acid metabolism: effects of lovastatin

Reducing cholesterol synthesis with lovastatin had no discernible effect on either fractional turnover or synthesis rate for both cholic acid and chenodeoxycholic acid (**Table 7**). Pool sizes of these two primary bile acids as well as total pool size determined by the method of Lindstedt (23) were also unaffected by lovastatin. There was, however, a reduction in mean one-sample pool size of bile acid on lovastatin (Tables 3 and 7). Although this change was of borderline statistical significance ($P = 0.073$), it is probably real because a similar (and statistically significant) reduction in one-sample pool size was also observed in our previous study (4).

There is no good physiological explanation for these discrepant results provided by Lindstedt kinetics versus the one-sample method. Total bile acid pool size could be

TABLE 6. Cholesterol content and saturation index of gallbladder bile

Subject	Cholesterol Content				Cholesterol Saturation Index			
	LN	HN	LL	HL	LN	HN	LL	HL
	molar percent							
1	7.61	9.31	8.10	6.14	1.14	1.36	1.20	0.84
2	11.00	10.20	3.76	7.56	1.54	1.68	0.69	1.19
3	6.47	11.90	6.34	6.88	0.88	1.82	0.87	1.12
4	5.59	6.00	4.59	4.68	0.86	0.93	0.69	0.68
5	9.63	11.70	7.02	5.81	1.27	1.74	1.04	0.96
6	11.40	11.20	7.25	7.09	1.53	1.57	1.04	1.11
7	9.02	8.14	7.26	6.69	1.31	1.24	1.02	1.08
8	6.02	6.32	5.76	6.64	1.07	0.91	0.82	0.90
9	8.17	7.57	4.25	5.14	1.21	1.22	0.66	0.81
10	7.94	7.43	5.65	5.32	1.23	1.20	0.90	0.84
11	8.57	8.37	4.85	5.38	1.45	1.32	0.78	0.91
12	7.19	5.44	6.48	5.05	1.16	0.80	1.14	0.77
Mean	8.21	8.63	5.94	6.03	1.22	1.32	0.91	0.93

TABLE 7. Summary of means and ANOVA testing for bile acid kinetics

Variable	Means				ANOVA effects		
	LN	HN	LL	HL	Diet	Lov	Int
						P-value	
Fractional turnover (day^{-1})							
Cholic	.527	.602	.504	.592	.025	.627	.861
Chenodeoxycholic	.367	.376	.366	.406	.395	.622	.602
Pool sizes (μmoles)							
Cholic	1770	1980	2000	1760	.938	.962	.211
Chenodeoxycholic	1790	1640	1820	1510	.102	.719	.578
Total (Lindstedt)	5200	5370	5530	5040	.662	.995	.359
Total (one-sample)	6550	6330	5990	5920	.385	.073	.511
Synthesis (μmole/day)							
Cholic	797	976	838	964	.025	.826	.689
Chenodeoxycholic	548	571	554	568	.563	.949	.882
Total	1340	1550	1390	1530	.054	.850	.721
Bile acid composition (percent)							
Cholic	33.5	33.5	32.7	31.4	.505	.174	.531
Chenodeoxycholic	37.0	34.6	38.1	35.8	.076	.363	.987
Deoxycholic	24.5	26.7	23.8	27.2	.065	.921	.704
Lithocholic	2.2	2.4	2.7	2.6	.681	.066	.476
Ursodeoxycholic	2.8	2.8	2.7	3.0	.539	.954	.462

Abbreviations: Diet, dietary cholesterol; Lov, lovastatin; Int, interaction between dietary cholesterol and lovastatin.

reduced without a change in fractional turnover or synthesis rates of primary bile acids only if pool sizes of secondary bile acid fell. That was not the case, however, because composition of the bile acid pool was not appreciably altered by lovastatin (Table 7). It therefore remains possible that the reduction in one-sample pool size by lovastatin results from a slight lowering of bile acid synthesis rate as suggested by measurements with the $^{14}CO_2$ method (3). Nevertheless, based on both our isotope dilution measurements (Table 7) and our fecal acidic sterol measurements (Table 7 and reference 6), we are forced to conclude that if lovastatin does lower bile acid synthesis, the effect is small enough and inconsistent enough to be very difficult to measure. Certainly reduction in bile acid synthesis is not a major mechanism by which humans compensate for decreased cholesterol synthesis during lovastatin treatment.

Bile acid metabolism: effects of dietary cholesterol

Increasing cholesterol consumption increased both fractional turnover and synthesis rate for cholic acid, but not for chenodeoxycholic acid (Table 7). Total bile acid synthesis also was increased (P = 0.054) consistent with the increased output of acidic sterols on the high cholesterol diet (Table 7 and reference 6).

It is noteworthy that bile acid synthesis exhibited little or no change in response to changes in cholesterol synthesis, but increased in response to increases in dietary cholesterol, even though part of the increased dietary input was offset by reduced cholesterol synthesis (Fig. 1). One explanation for this difference would be that the liver distinguishes between inputs of dietary versus synthetic cholesterol. That possibility is supported by recent sophisticated modeling of tracer kinetics in human subjects with bile fistulae (28). These studies suggested that the hepatic pool of cholesterol influencing bile acid synthesis is regulated not by cholesterol synthesis, but rather by free cholesterol coming from plasma lipoproteins, especially high density lipoprotein (HDL). Thus dietary cholesterol, which unlike synthesized cholesterol must pass through HDL and chylomicrons in transit to the liver, may act through this regulatory pool of hepatic cholesterol to stimulate bile acid synthesis.

A second possible explanation for the differing effects of dietary versus synthetic cholesterol inputs with respect to bile acid metabolism is that cholesterol in the gut lumen may exert a "cholestyramine-like" effect as reported in animal models (29). While the present study does not provide strong support for this possibility, significantly increased fractional turnover of cholic acid on the high cholesterol diet (Table 7) does favor it. In addition, while there was not a significant dietary cholesterol effect on overall bile acid absorption (Table 7), there was a significant reduction in bile acid absorption by cholesterol consumption when subjects were taking lovastatin (period HL versus LL). Moreover, ANOVA results suggested a possible interaction between dietary cholesterol and lovastatin (P = 0.081, Table 7). This suggests that dietary cholesterol had an appreciable effect on bile acid absorption only when lovastatin was present, possibly because

TABLE 8. Summary of means and ANOVA testing for enterohepatic cycling and bile lipids

Variable	Means				ANOVA effects		
	LN	HN	LL	HL	Diet	Lov	Int
						P-value	
Bile acid secretion (μmole/h)[a]	1400	1550	1400	1240	.974	.189	.174
Fecal acid sterols (μmole/day)[a]	1220	1330	1120	1390	.001	.743	.115
Fractional bile acid absorption	.959	.960	.964	.949	.155	.569	.081
Enterohepatic cycling (day^{-1})	7.81	7.47	7.52	6.89	.488	.534	.835
Gallbladder bile lipids							
Cholesterol (molar percent)	8.21	8.63	5.94	6.03	.514	.0001	.671
Lecithin (molar percent)	19.7	19.0	19.9	19.7	.451	.483	.654
Bile acid (molar percent)	72.1	72.4	74.2	74.2	.798	.014	.898
Cholesterol saturation index	1.22	1.32	0.91	0.93	.290	.0001	.580

Abbreviations: Diet, dietary cholesterol; Lov, lovastatin; Int, interaction between dietary cholesterol and lovastatin.
[a]Values taken from reference 6 and presented here because they were used in calculation of bile acid absorption and enterohepatic cycling.

lovastatin substantially lowered cholesterol secretion into bile (6) making dietary cholesterol a greater proportion of total cholesterol entering the intestinal lumen.

Regardless of how dietary cholesterol increased bile acid synthesis, it is not clear why cholic acid, but not chenodeoxycholic acid, was affected. A similar differential effect has been noted when bile acid synthesis is stimulated by cholestyramine administration to normal subjects (30). This has been attributed to preferential use of newly synthesized cholesterol for cholic acid synthesis, but that speculation has not been proven. Moreover, it is difficult to see how stimulation of bile acid synthesis either by increasing cholesterol substrate to 7α-hydroxylase or decreasing feedback inhibition of 7α-hydroxylase would favor production of cholic acid, which requires additional enzymatic steps, over chenodeoxycholic acid. Rather one would expect a disproportionate increase in cholic acid synthesis to require either a change in activity of 12α-hydroxylase or a change in relative use of an alternate pathway such as the 27-hydroxylation pathway, which is known to favor production of chenodeoxycholic acid (31). However, to date there is no evidence that dietary cholesterol affects either of these (31, 32). Finally, it is possible that previously noted imprecision in the Lindstedt isotope dilution method (24) may have prevented detection of changes in chenodeoxycholic acid metabolism induced by dietary cholesterol.

Gallbladder bile lipid composition

The striking reduction in cholesterol saturation of gallbladder bile by inhibitors of HMG-CoA reductase is well recognized (4, 15–18). The present study not only confirms that effect, but also shows that there was no significant interaction between dietary cholesterol and lovastatin with respect to cholesterol content of bile in these subject (**Table 8**). This demonstrates that the effect of lovastatin on biliary cholesterol content is not affected by changes in dietary cholesterol.

We also found no significant effect of dietary cholesterol itself on cholesterol saturation of bile (Tables 6 and 8). The effect of dietary cholesterol on cholesterol content of bile has been controversial. Lee et al. (19) reported that increasing dietary cholesterol from 500 to 750 or 1000 mg/day increased cholesterol saturation index in both normal subjects and cholesterol patients. Also, DenBesten, Connor, and Bell (20) found that adding 750 mg/day of cholesterol to a cholesterol-free diet increased biliary cholesterol content. However, three other studies (14, 21, 22) reported no change or a decrease in saturation index during increased cholesterol consumption by normal subjects. The present study, by virtue of its rigorous design and number of comparisons, lends strong support to these latter three studies. This finding is consistent with the lack of effect of cholesterol consumption on cholesterol secretion into bile demonstrated in these same subjects (6). This stability of biliary cholesterol presumably reflects the compensatory decrease in cholesterol synthesis and increase in bile acid synthesis that occur in response to cholesterol consumption (6).

Because the amount of cholesterol in the diets given our volunteers reasonably approximates the range of dietary cholesterol in Western society (33), these data suggest that dietary cholesterol may play little if any role in pathogenesis of cholesterol gallstone disease. That conclusion would be compatible with two recent large epidemiological studies, both of which found no relationship between dietary cholesterol and cholelithiasis (34, 35). Alternatively, it is possible that subjects predisposed to gallstones respond differently to dietary cholesterol than do normal subjects, although that was not the case in the study of Lee et al. (19). It is also possible that more stringent elimination of cholesterol from the diet could lower cholesterol saturation index compared to either of our diets as suggested by the study of DenBesten et al. (20). Both of these possibilities deserve further evaluation.

I wish to acknowledge the dedicated organizational and technical work of Ms. Cathy Pinther-Evans, Ann McHale, Margaret Jordan, and Linda Hartich, without which these studies would not have been possible. Financial support was provided by grants from the Department of Veterans Affairs and the National Institutes of Health (R01-DK42433).

Manuscript received 26 July 1993 and in revised form 4 November 1993.

REFERENCES

1. Vlahcevic, Z. R., D. M. Heuman, and P. B. Hylemon. 1991. Regulation of bile acid synthesis. *Hepatology.* **13:** 590–600.
2. Pandak, W. M., D. M. Heuman, P. B. Hylemon, and Z. R. Vlahcevic. 1990. Regulation of bile acid synthesis. IV. Interrelationship between cholesterol and bile acid biosynthesis pathways. *J. Lipid Res.* **31:** 79–90.
3. Mitchell, J. C., B. G. Stone, G. M. Logan, and W. C. Duane. 1991. Role of cholesterol synthesis regulation of bile acid synthesis and biliary cholesterol secretion in humans. *J. Lipid Res.* **32:** 1143–1149.
4. Mitchell, J. C., G. M. Logan, B. G. Stone, and W. C. Duane. 1991. Effects of lovastatin on biliary lipid secretion and bile acid metabolism in humans. *J. Lipid Res.* **32:** 71–78.
5. Vanhanen, H., Y. A. Kesaniemi, and T. A. Miettinen. 1992. Pravastatin lowers serum cholesterol, cholesterol-precursor sterols, fecal steroids, and cholesterol absorption in man. *Metabolism.* **41:** 588–595.
6. Duane, W. C. 1993. Effects of lovastatin and dietary cholesterol on sterol homeostasis in healthy human subjects. *J. Clin. Invest.* **92:** 911–918.
7. Gustafsson, B. E., B. Angelin, K. Einarsson, and J. Gustafsson. 1977. Effects of cholesterol feeding on synthesis and metabolism of cholesterol and bile acids in germfree rats. *J. Lipid Res.* **18:** 717–721.
8. Pertsemlidis, D., E. H. Kirchman, and E. H. Ahrens, Jr. 1973. Regulation of cholesterol metabolism in the dog. I. Effects of complete bile diversion and of cholesterol feeding on absorption, synthesis, accumulation, and excretion rates measured during life. *J. Clin. Invest.* **52:** 2353–2367.
9. Eggan, D. A. 1974. Cholesterol metabolism in rhesus monkey, squirrel monkey, and baboon. *J. Lipid Res.* **15:** 139–145.
10. Lin, D. S., and W. E. Connor. 1980. The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. *J. Lipid Res.* **21:** 1042–1052.
11. Gylling, H., and T. A. Miettinen. 1992. Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men with different apoE phenotypes. *J. Lipid Res.* **33:** 1361–1371.
12. Quintão, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res.* **12:** 233–247.
13. Nestel, P. J., and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. *Metabolism.* **25:** 1591–1599.
14. Andersén, E., and K. Hellström. 1979. The effect of cholesterol feeding on bile acid kinetics and biliary lipids in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res.* **20:** 1020–1027.
15. Freeman, M. L., W. F. Prigge, D. B. Hunninghake, W. C. Duane, and R. L. Gebhard. 1988. Intestinal HMG-CoA reductase activity is low in hypercholesterolemic patients and is further decreased with lovastatin therapy. *J. Lipid Res.* **29:** 839–845.
16. Duane, W. C., D. B. Hunninghake, M. L. Freeman, P. A. Pooler, L. A. Schlasner, and R. L. Gebhard. 1988. Simvastatin, a competitive inhibitor of HMG-CoA reductase, lowers cholesterol saturation index of gallbladder bile. *Hepatology.* **8:** 1147–1150.
17. Hoogerbrugge-van der Linden, N., F. W. M. de Rooy, H. Jansen, and M. van Blankenstein. 1990. Effect of pravastatin on biliary lipid composition and bile acid synthesis in familial hypercholesterolaemia. *Gut.* **31:** 348–350.
18. Mazzella, G., P. Parini, D. Festi, F. Bazzoli, R. Aldini, A. Roda, D. Tonelli, A. Cipolla, A. Salzetta, and E. Roda. 1992. Effect of simvastatin, ursodeoxycholic acid and simvastatin plus ursodeoxycholic acid on biliary lipid secretion and cholic acid kinetics in nonfamilial hypercholesterolemia. *Hepatology.* **15:** 1072–1078.
19. Lee, W. T., C. J. Gilmore, G. Bonorris, H. Cohen, J. W. Marks, M. Cho-Sue, M. S. Meiselman, and L. Schoenfield. 1985. Effect of dietary cholesterol on biliary lipids in patients with gallstones and normal subjects. *Am. J. Clin. Nutr.* **42:** 414–420.
20. DenBesten, L., W. E. Connor, and S. Bell. 1973. The effect of dietary cholesterol on the composition of human bile. *Surgery.* **73:** 266–273.
21. Everson, G. T., C. McKinley, and F. Kern, Jr. 1991. Mechanism of gallstone formation in women: effects of exogenous estrogen (Premarin) and dietary cholesterol on hepatic lipid metabolism. *J. Clin. Invest.* **87:** 237–246.
22. Dam, H., I. Prange, M. K. Jensen, H. E. Kallehauge, and H. J. Fenger. 1971. Studies on human bile. IV. Influence of ingestion of cholesterol in the form of eggs on the composition of bile in healthy subjects. *Z. Ernahrungswiss.* **10:** 178–187.
23. Lindstedt, S. 1957. The turnover of cholic acid in man: bile acids and steroids 51. *Acta Physiol. Scand.* **40:** 1–40.
24. Duane, W. C., R. D. Adler, L. J. Bennion, and R. L. Ginsberg. 1975. Determination of bile acid pool size in man: a simplified method with advantages of increased precision, shortened analysis time, and decreased isotope exposure. *J. Lipid Res.* **16:** 155–158.
25. 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.
26. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. *J. Clin. Invest.* **61:** 998–1026.
27. Snedecor, G. W., and W. G. Cochran. 1989. Statistical Methods. 8th ed. Iowa State University Press, Ames, IA. 217–236.
28. Schwartz, C. C., L. A. Zech, J. M. Vandenbroek, and P. S. Cooper. 1993. Cholesterol kinetics in subjects with bile fistula: positive relationship between size of the bile acid precursor pool and bile acid synthetic rate. *J. Clin. Invest.* **91:** 923–938.
29. Björkhem, I., G. Eggertsen, and U. Anderson. 1991. On the mechanism of stimulation of cholesterol 7α-hydroxylase by dietary cholesterol. *Biochim. Biophys. Acta.* **1085:** 329–335.
30. Einarsson, K., S. Ericsson, S. Reihnér, M. Rudling, D. Ståhlberg, and B. Angelin. 1991. Bile acid sequestrants: mechanisms of action on bile acid and cholesterol metabolism. *Eur. J. Clin. Pharmacol.* **40:** S53–S58.
31. Javitt, N. B. 1990. 26-Hydroxycholesterol: synthesis,

metabolism, and biologic activities. *J. Lipid Res.* **31:** 1527–1533.
32. Björkhem, I., M. Eriksson, and K. Einarsson. 1983. Evidence for a lack of regulatory importance of the 12α-hydroxylase in formation of bile acids in man: an in vivo study. *J. Lipid Res.* **24:** 1451–1456.
33. Keys, A., J. T. Anderson, and F. Grande. 1965. Serum cholesterol response to changes in the diet. II. The effect of cholesterol in the diet. *Metabolism.* **14:** 759–765.
34. Maclure, K. M., K. C. Hayes, G. A. Colditz, M. J. Stampfer, and W. C. Willett. 1990. Dietary predictors of symptom-associated gallstones in middle-aged women. *Am. J. Clin. Nutr.* **52:** 916–922.
35. Jørgensen, T., and L. M. Jørgensen. 1989. Gallstones and diet in a Danish population. *Scand. J. Gastroenterol.* **24:** 821–826.