Serum lathosterol levels in human subjects reflect changes in whole body cholesterol synthesis induced by lovastatin but not dietary cholesterol

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Abstract We measured serum lathosterol levels and whole body cholesterol synthesis by sterol balance 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. Whole body cholesterol synthesis was significantly reduced both by lovastatin ($P = 0.0004$) and by high dietary cholesterol ($P = 0.0005$). Serum total lathosterol (μg/dl) was reduced by lovastatin ($P < 0.0001$), but was not significantly altered (and actually tended to increase) during consumption of the high cholesterol diet, presumably because eggs contained appreciable lathosterol as demonstrated by direct analysis. Results were similar for total versus free lathosterol and for lathosterol expressed as μg/dl serum versus μg/100 mg cholesterol. We conclude that serum lathosterol does not reflect changes in cholesterol synthesis induced by dietary cholesterol. Studies using serum lathosterol as an indicator of cholesterol synthesis must be carefully controlled for dietary cholesterol.—**Duane, W. C.** Serum lathosterol levels in human subjects reflect changes in whole body cholesterol synthesis induced by lovastatin but not dietary cholesterol. *J. Lipid Res.* 1995. **36:** 343–348.

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Direct measurement of whole body cholesterol synthesis in human subjects is expensive, time-consuming, and difficult for both investigator and subject. Therefore, beginning with the pioneering work of Miettinen (1), investigators have pursued the possibility that serum levels of intermediates in the cholesterol synthetic pathway might reflect whole body cholesterol synthesis. The most successful of these efforts has been for lathosterol (5α-cholest-7-en-3β-ol), levels of which have been shown to correlate quite well with whole body cholesterol synthesis, especially during perturbations such as administration of HMG-CoA reductase inhibitors, cholestyramine and chenodeoxycholic acid (2–6).

One critical perturbation that has not been carefully examined in these studies is cholesterol consumption which, at least according to some studies (7–10), lowers cholesterol synthesis in humans and therefore should also lower serum lathosterol levels. We recently had the opportunity to measure serum lathosterol levels in 12 human subjects undergoing studies of sterol balance in response to dietary cholesterol manipulation and lovastatin administration. As expected, lovastatin lowered both lathosterol levels and cholesterol synthesis measured by sterol balance. However, to our surprise, increasing dietary cholesterol, which also reduced cholesterol synthesis, did not lower (and actually tended to increase) serum lathosterol. Because this observation has important implications for studies utilizing lathosterol levels to reflect cholesterol synthesis, we decided to formally report these results.

METHODS

Study procedures were approved by committees overseeing use of human subjects in research at both the Minneapolis VA Medical Center and the University of Minnesota. All subjects gave written informed consent to participate.

Subjects, diets, and lovastatin treatment protocols are described in detail as part of a recent study of serum lipids and fecal sterols (10). Briefly, 12 healthy male volunteers were studied. Two had mild elevation of serum triglycerides and another two had mild elevation of serum cholesterol (10). 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 resided on this metabolic unit throughout the study. They consumed either a low cholesterol diet (mean 246 mg/day) or a high cholesterol

Abbreviations: LDL, low density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

diet (mean 1071 mg/day) that was identical to the low cholesterol diet except for isocaloric daily substitution of five eggs. Both diets contained 30% fat, 54% carbohydrate, and 16% protein with calories adjusted to maintain constant weight. Meals consisted of regular food with a weekly menu rotation to provide consistency.

All subjects were studied in four separate, randomly ordered 6–7 week periods designed as follows: *a)* low dietary cholesterol, no lovastatin; *b)* high dietary cholesterol, no lovastatin; *c)* low dietary cholesterol, lovastatin 40 mg b.i.d.; *d)* high dietary cholesterol, lovastatin 40 mg b.i.d. Stools were collected for the last 10 days of each study period for determination of cholesterol synthesis by fecal sterol balance as previously described (10). Serum samples were drawn just before breakfast in the last week of each period. An aliquot was analyzed for total cholesterol, triglycerides, and high density lipoprotein cholesterol by the clinical laboratory of the Minneapolis VA Medical Center (11). Low density lipoprotein (LDL) cholesterol was calculated by standard formula (12). All these values for serum lipids have been reported previously (10). Another aliquot of serum was frozen for later analysis of lathosterol levels.

Reference lathosterol (5α-cholest-7-en-3β-ol) was purchased from Sigma Chemical (St. Louis, MO). Serum levels of free and esterified lathosterol were determined using a modification of the procedure described by Björkhem et al. (2). Like those authors, we began these analyses intending to quantitate squalene, lanosterol, and other methylsterols. Later we decided to focus exclusively on lathosterol, but steps required for separation of other sterols were retained to make all analyses uniform. Briefly, our procedure was to combine 1 cc of serum with a small amount of [4-^{14}C]cholesterol (New England Nuclear, Boston, MA) as a recovery standard and extracted with $CHCl_3$–MeOH 8:3. The chloroform extract was applied to a thin-layer chromatography plate of silica gel G and developed first in hexane–benzene 9:1, a step originally intended to separate squalene. After this first development, the plates were developed in heptane–ethyl ether 45:55, a step designed to separate esterified from free sterols and to separate free lathosterol and cholesterol from other free sterols. The ester band was subjected to saponification in ethanolic KOH for 20 min at 65°C and re-extracted with $CHCl_3$–MeOH. Resulting free sterols were separated on thin-layer plates using the heptane–ethyl ether system described above. For both the original separation (free) and that following saponification (esterified), sterols in the band containing lathosterol and cholesterol were converted to trimethylsilyl ethers using N,O-bis(trimethylsilyl)-acetamide (Sigma Chemical Company, St. Louis, MO) and combined with cholestane as an internal standard. Derivatized sterols were quantitated by capillary gas–liquid chromatography on a Hewlett-Packard 5890 chromatograph equipped with a 30 m × 0.25 mm capillary column of SAC-5 (Supelco, Bellefonte, PA) at 273°C. Helium was used as the carrier gas at a linear flow of 20 cm/sec and a split ratio of 90:1.

For analysis of lathosterol in eggs, a small amount of [4-^{14}C]cholesterol was added as a recovery standard to four separate eggs. Each egg was then homogenized and extracted with $CHCl_3$–MeOH 8:3. The extract was analyzed for lathosterol as described for serum samples above. Similarly, all the food contained in each of 2 separate days of the low cholesterol diet was homogenized with [4-^{14}C]cholesterol, extracted with $CHCl_3$–MeOH, and analyzed for lathosterol.

Statistical testing was performed using SAS software (SAS Institute, Cary, NC) on a Northgate personal computer equipped with a 486DX microprocessor. Testing was by analysis of variance (ANOVA) with randomized block design. 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.

RESULTS

As shown in **Figure 1,** mean total lathosterol levels, expressed as μg/dl of serum, were lower during administration of lovastatin for both low and high dietary cholesterol periods. ANOVA of these data indicated a highly significant lovastatin effect ($P < 0.0001$). Mean total lathosterol levels on the high cholesterol diet were actually higher than on the low cholesterol diet both on and off lovastatin (Fig. 1), although ANOVA indicated that there was not a statistically significant effect of dietary cholesterol ($P = 0.175$).

This same pattern (lower mean levels on lovastatin with significant lovastatin effects; higher mean levels for high versus low dietary cholesterol but without a significant dietary effect) was observed for free and esterified lathosterol expressed as μg/dl of serum as well as for free, esterified, and total lathosterol expressed as μg/100 mg cholesterol (**Table 1**).

For none of these variations of lathosterol was there any statistically significant interaction between lovastatin and dietary cholesterol (Table 1). This indicates that for serum lathosterol levels the effect of lovastatin was the same regardless of dietary cholesterol status and the effect of dietary cholesterol was the same regardless of lovastatin status.

Results of cholesterol synthesis measured by sterol balance on these subjects have been published previously (10), but are provided here (**Fig. 2,** Table 1) to permit easy comparison with changes in lathosterol levels. Mean cholesterol synthesis was lower during lovastatin treatment, during both low and high dietary cholesterol intake. Analysis of these data by ANOVA indicated a highly significant lovastatin effect ($P = 0.0004$). Mean

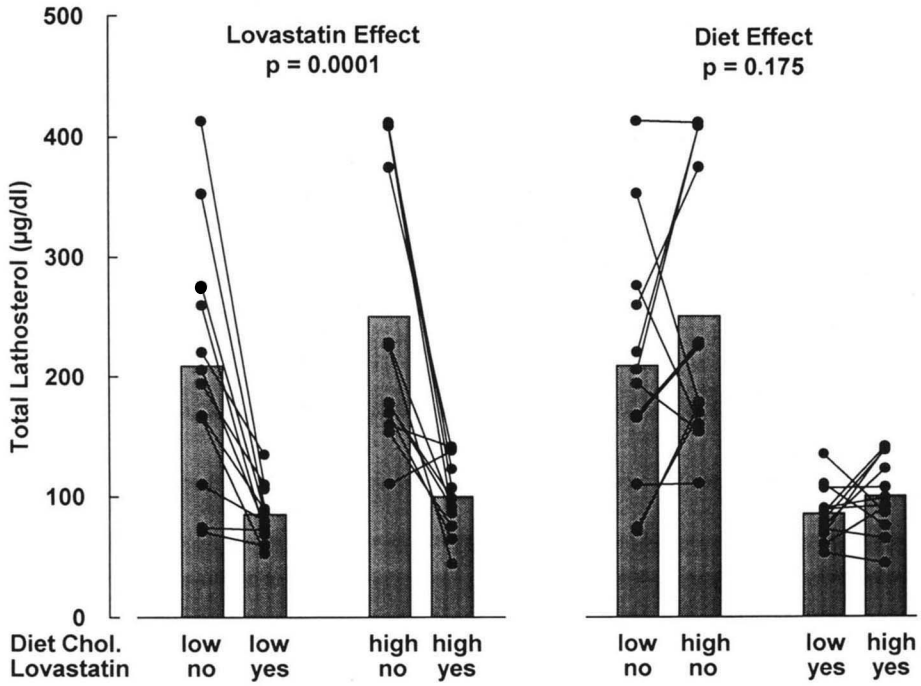

Fig. 1. Serum levels of total lathosterol in 12 subjects studied in four periods representing all combinations of low versus high dietary cholesterol with and without lovastatin, 80 mg/day. Data are shown twice. The set of four bars on the left appose periods with and without lovastatin to demonstrate the lovastatin effect. The set of four bars on the right appose periods of low and high dietary cholesterol to demonstrate the effect of increasing dietary cholesterol. Lovastatin clearly lowered lathosterol levels, but increasing dietary cholesterol tended to increase lathosterol levels despite lowering cholesterol synthesis (Fig. 2).

cholesterol synthesis on high dietary cholesterol was lower than during low dietary cholesterol both on and off lovastatin. ANOVA indicated a highly significant effect of dietary cholesterol ($P = 0.0005$). There was no significant interaction between lovastatin and dietary cholesterol with respect to cholesterol synthesis.

We also analyzed 2 typical days of the low cholesterol diet and four typical eggs used for the high cholesterol diet. Greater than 90% of the cholesterol and lathosterol in diet and eggs was unesterified. The mean ± SD ratio of lathosterol/cholesterol was 0.01070 ± 0.00109 in the low cholesterol diet and 0.00470 ± 0.00038 in the eggs. Thus,

TABLE 1. Summary of means and ANOVA testing

	Means				ANOVA Effects		
Sterol	Low Diet No Lov	High Diet No Lov	Low Diet + Lov	High Diet + Lov	Diet	Lov	Int
Lathosterol			µg/dl			P value	
Free	155	172	70	77	.366	.0001	.694
Esterified	54	79	15	23	.176	.0003	.479
Total	209	251	85	100	.175	.0001	.512
Lathosterol			*µg/100 mg cholesterol*				
Free	272	280	176	195	.555	.0005	.830
Esterified	72	103	30	53	.156	.0184	.832
Total	132	156	74	96	.184	.0013	.946
Serum cholesterol[a]			*mg/dl*				
Total	207	219	138	142	.099	.0001	.404
Low density lipoprotein	133	148	71	79	.047	.0001	.484
Cholesterol synthesis[a]			µmol/day				
	2978	2450	2447	2063	.0005	.0004	.546

Abbreviations: Diet, dietary cholesterol; Lov, lovastatin; Int, interaction between dietary cholesterol and lovastatin.
[a]Data previously published (10).

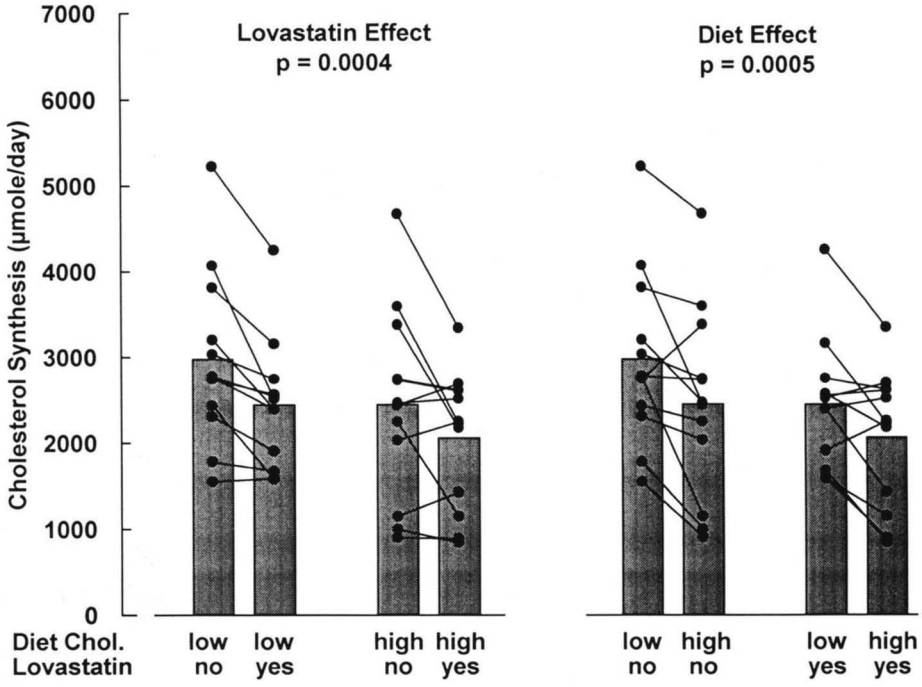

Fig. 2. Whole body cholesterol synthesis determined by sterol balance in the 12 study subjects in four periods representing all combinations of low versus high dietary cholesterol with and without lovastatin 80 mg/day. As in Fig. 1, data are shown twice. The set of four bars on the left appose periods with and without lovastatin to demonstrate the lovastatin effect. The set of four bars on the right appose periods of low and high dietary cholesterol to demonstrate the effect of increasing dietary cholesterol. Both lovastatin and increased cholesterol consumption significantly lowered cholesterol synthesis. These data have been published previously (10).

the low cholesterol diet, which provided on average 246 mg/day cholesterol, also provided about 2.6 mg lathosterol. The high cholesterol diet provided an average of 1071 mg/day of cholesterol, 825 mg of which was derived from eggs, and about 6.5 mg lathosterol.

DISCUSSION

Of the many intermediates in the cholesterol synthetic pathway, serum levels of lathosterol are believed by many investigators to provide the best reflection of cholesterol synthesis (2–6, 13). Lanosterol also appears to correlate well with activity of HMG-CoA reductase and whole body cholesterol synthesis, but has the disadvantage of being less stable in storage than lathosterol (13, 14). Whether optimal correlation with cholesterol synthesis is achieved with total versus free lathosterol or lathosterol expressed as µg/dl versus µg/100 mg cholesterol has been a matter of some disagreement (2–6, 13, 15). For that reason we presented our data expressed in all the various ways used by other investigators. We found, however, that the data showed the same differences and led to the same conclusion regardless of how they were expressed (Table 1).

Serum lathosterol has been shown to change in the same direction as cholesterol synthesis in response to ileal exclusion (16) as well as during administration of cholestyramine (2, 6), chenodeoxycholic acid (2), and inhibitors of HMG-CoA reductase (4–6). The present study confirms and strengthens the reported concordance of serum lathosterol with cholesterol synthesis during administration of an HMG-CoA reductase inhibitor.

Interestingly, in response to lovastatin the change in serum lathosterol was proportionately greater than the change in sterol balance (Figs. 1 and 2). Lathosterol, like cholesterol, is carried on lipoproteins (17). Lovastatin alters LDL receptors and affects lipoprotein levels by influencing both their production and removal from the serum (18). Thus changes in lathosterol induced by lovastatin might have partially reflected altered levels of lipoproteins apart from cholesterol synthesis rate. This possibility is supported by the fact that on lovastatin the disparity between change in lathosterol versus sterol balance was more pronounced for lathosterol expressed as µg/dl compared to µg/100 mg cholesterol (Table 1). Finally, there remains the possibility that other underdetermined factors may have contributed to this disparity.

Our results clearly demonstrate a discordance between changes in serum lathosterol levels and cholesterol synthesis in response to dietary cholesterol consumption. Thus,

while cholesterol synthesis was significantly lowered by ingestion of additional dietary cholesterol, serum lathosterol levels did not change significantly, and in fact mean levels tended to increase on the high cholesterol diet (Table 1, Fig. 1). Little direct attention has been paid to the relationship of serum lathosterol to cholesterol ingestion. Consistent with our findings, Vuoristo and Miettinen (19) recently reported that a group of vegetarians, who consumed much less dietary cholesterol than a control group, had higher rates of cholesterol synthesis but a lower mean level of serum lathosterol compared to the controls. Meijer et al. (15), studying rabbits, found that cholesterol feeding increased serum lathosterol levels, but lowered the ratio of lathosterol to the sum of cholesterol in very low density lipoproteins, intermediate density lipoproteins, and low density lipoproteins. This same group in an earlier article (3) indicated that eggs contained enough lanosterol to obscure the correlation between serum lanosterol and cholesterol synthesis, but that eggs contained little or no lathosterol.

We found that there was an appreciable amount of lathosterol in eggs, and that increasing cholesterol in the basal diet by addition of eggs also substantially increased dietary lathosterol (Results). This increased dietary intake of lathosterol seems the most likely explanation for our observation that cholesterol consumption, while significantly lowering cholesterol synthesis, tended to increase, not decrease, lathosterol levels. Although the amount of dietary lathosterol was low (2.6 mg/day versus 6.5 mg/day on the low versus high cholesterol diets, respectively), serum levels were also low, approximately 1000-fold lower than levels of cholesterol. Because daily input of cholesterol is about 1000 mg/day (10), it is perhaps not surprising that changes in lathosterol input on the order of a few milligrams/day might influence serum lathosterol levels. Alternatively, it is conceivable that cholesterol consumption had some effect on the rate of cellular leakage or rate of removal of lathosterol from serum, although a mechanism by which either of these might have occurred is not obvious. More definitive conclusion about these possibilities would require a study focusing on absorption and turnover of lathosterol.

Regardless of the reason, our data show that changes in cholesterol synthesis induced by cholesterol consumption are not accompanied by commensurate changes in serum lathosterol levels. We conclude, therefore, that serum lathosterol remains a useful indirect indicator of whole body cholesterol synthesis, but that studies using this indicator must carefully control for dietary cholesterol.

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