Lovastatin treatment inhibits sterol synthesis and induces HMG-CoA reductase activity in mononuclear leukocytes of normal subjects

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Abstract The mechanism by which competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase decrease serum cholesterol is incompletely understood. The few available data in humans suggest that chronic administration of the competitive inhibitor, lovastatin, decreases serum cholesterol with little or no change in total body sterol synthesis. To further define the effect of lovastatin on cholesterol synthesis in normal subjects, we investigated the effect of a single oral dose of lovastatin and a 4-week treatment period of lovastatin on mononuclear leukocyte (ML) sterol synthesis as a reflection of total body sterol synthesis. In parallel, we measured serum lipid profiles and HMG-CoA reductase activity in ML microsomes that had been washed free of lovastatin. ML sterol synthesis did not significantly decrease (23 ± 5%, mean ± SEM) at 3 h after a single 40-mg dose of lovastatin. With a single oral 80-mg dose, ML sterol synthesis decreased by 57 ± 10% ($P<0.05$) and remained low for the subsequent 6 h. With both doses, total HMG-CoA reductase enzyme activity in microsomes prepared from harvested mononuclear leukocytes was induced 4.8-fold ($P<0.01$) over baseline values. Both the 20-mg bid dose and the 40-mg bid dose of lovastatin administered for a 4-week period decreased serum cholesterol by 25–34%. Lovastatin at 20 mg bid decreased ML sterol synthesis by 23 ± 6% ($P<0.02$) and increased ML HMG-CoA reductase 3.8 times ($P<0.001$) the baseline values. Twenty four hours after stopping lovastatin, ML sterol synthesis and HMG-CoA reductase enzyme activity had returned to the baseline values. The higher dose of lovastatin (40 mg bid) decreased ML sterol synthesis by 16 ± 3% ($P<0.05$) and induced HMG-CoA reductase to 53.7 times ($P<0.01$) the baseline value at 4 weeks. Stopping this higher dose effected a rebound in ML sterol synthesis to 140 ± 11% of baseline ($P<0.01$), while HMG-CoA reductase remained 12.5 times baseline ($P<0.01$) over the next 3 days. No rebound in serum cholesterol was observed. From these data we conclude that in normal subjects lovastatin lowers serum cholesterol with only a modest effect on sterol synthesis. The effect of lovastatin on sterol synthesis in mononuclear leukocytes is tempered by an induction of HMG-CoA reductase enzyme quantity, balancing the enzyme inhibition by lovastatin. The speed (within 3 h) and the magnitude of enzyme induction (53.7-fold) with lovastatin administration demonstrate the exquisite nature of the feedback regulation mechanism of HMG-CoA reductase enzyme in human mononuclear leukocytes. —**Stone, B. G., C. D. Evans, W. F. Prigge, W. C. Duane, and R. L. Gebhard.** Lovastatin treatment inhibits sterol synthesis and induces HMG-CoA reductase activity in mononuclear leukocytes of normal subjects. *J. Lipid Res.* 1989. **30:** 1943–1952.

Supplementary key words cholesterol synthesis • hypercholesterolemia • hydroxymethylglutaryl CoA reductase regulation • HMG-CoA reductase inhibitors

In 1988 the National Cholesterol Education Program advocated new guidelines for the detection and treatment of high blood cholesterol levels in adults (1). This and the timely emergence of a new class of cholesterol-lowering agents have sparked renewed interest in the treatment of hypercholesterolemia. This class of agents, which includes compactin, mevinolin, and synvinolin, all appear to act through competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting step of cholesterol synthesis (2). In humans, serum cholesterol is routinely lowered by 20–30% after 1 week of therapy and little further decrease occurs with longer administration (3–5). The decrease occurs almost exclusively in the cholesterol contained in low density lipoproteins (LDL) while high density lipoprotein (HDL) cholesterol levels are maintained. Despite demonstrated efficacy (3, 5), the mechanism of the cholesterol-lowering action of these agents is not completely understood.

In microsomal preparations and in tissue culture these agents are potent inhibitors of HMG-CoA reductase activity (6–8). The in vivo effects are species-dependent and vary with the length of administration of the drug. In rats a single dose of mevinolin inhibits hepatic cholesterol synthesis and decreases serum cholesterol within a 2- to 3-h period (9–11). With chronic administration of lovastatin to rats, the amount of HMG-CoA reductase protein in-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ML, mononuclear leukocyte; LDL, low density lipoprotein; HDL, high density lipoprotein; EBS, Earle's balanced salt solution; FH, familial hypercholesterolemia.

creases in the face of continued exposure to its competitive inhibitor (11). The induction of HMG-CoA reductase by lovastatin balances its inhibition so that hepatic cholesterol synthesis and serum cholesterol are no longer decreased after 1 week of lovastatin administration. The subsequent withdrawal of lovastatin effects an overshoot in cholesterol synthesis as the increased HMG-CoA reductase protein is no longer inhibited (12). In distinction, chronic lovastatin administration to hamsters maintains a decrease in serum cholesterol despite a comparable induction of HMG-CoA reductase (13). In this species the decrease in serum cholesterol results in part from an induction of LDL receptor activity and an enhanced removal of lipoprotein-bound cholesterol from the plasma in response to the lovastatin-mediated inhibition of cholesterol synthesis (13).

In humans, the mechanism of the cholesterol-lowering effect of lovastatin has been partially but not completely characterized. Lovastatin administration to patients with heterozygous familial hypercholesterolemia (FH) has been shown to increase receptor-mediated clearance of LDL from plasma (14). In contrast, a study of patients with primary hypercholesterolemia suggested that the serum cholesterol-lowering effect of lovastatin resulted from decreased LDL production without a change in the fractional catabolic rate of LDL (15). Both of these effects of lovastatin on lipoprotein-bound cholesterol are thought to be triggered by the inhibition of cholesterol synthesis (14, 15). However, convincing evidence of a decrease in cholesterol synthesis in humans in response to lovastatin has not been demonstrated.

Using sterol balance methods to measure cholesterol synthesis, Grundy and Bilheimer (16) have demonstrated a modest reduction in cholesterol synthesis in three subjects and no change in synthesis in two subjects treated with lovastatin. Other studies using urinary mevalonate excretion as a measure of cholesterol synthesis have suggested that lovastatin in a dose of 25–50 mg bid for 4 weeks inhibited total body cholesterol synthesis by greater than 80% (17).

Several groups have advocated measuring either HMG-CoA reductase activity (18, 19) or sterol synthesis (20) in mononuclear leukocytes (ML) as an indirect measure of cholesterol synthesis. Parallel changes in hepatic and ML HMG-CoA reductase activity have been demonstrated both in response to dietary (fasting, cholesterol feeding) and drug (dichloroacetate, cholestyramine) manipulation (20–23). Human ML sterol synthesis has also been shown to mirror changes in total body cholesterol synthesis as measured by the sterol balance methods in response to cholesterol feeding, cholestyramine administration, and probucol administration (19, 20, 23–25). To better define the effect of lovastatin on cholesterol synthesis in humans, we measured sterol synthesis in mononuclear leukocytes harvested from subjects treated with lovastatin. In addition, we made parallel measurements of HMG-CoA reductase activity in washed and diluted ML microsomes in order to determine the quantity of uninhibited HMG-CoA reductase enzyme activity contained within these cells (7). The comparison of total HMG-CoA reductase activity and sterol synthesis rates should demonstrate whether inhibition of sterol synthesis induces HMG-CoA reductase enzyme in human mononuclear leukocytes. Finally, studying the temporal nature of HMG-CoA reductase enzyme induction will further clarify the effect of lovastatin treatment on cholesterol synthesis in humans.

METHODS

Subjects

Study protocols were approved by the Human Subjects Committee of the Minneapolis Veterans Administration Medical Center. Informed consent was obtained from all subjects prior to the initiation of the study. No subject had a known lipid disorder, liver disease, or was taking any medications known to alter lipid metabolism. All subjects were counseled to continue their prestudy dietary patterns. The ages and baseline values for the groups prior to chronic lovastatin treatment are given in **Table 1**. The group administered lovastatin as an acute single dose ($n = 6$) ranged in age from 31 to 44 years (mean age of 37) and had serum cholesterol of 183 $\pm$ 3 (mean $\pm$ SE).

Study protocols

Two separate study protocols were utilized to investigate the effect of lovastatin administration on serum lipids, ML sterol synthesis, and HMG-CoA reductase activity.

Acute lovastatin administration. ML sterol synthesis, serum cholesterol, and HMG-CoA reductase activity were determined after a single dose of either 40 mg or 80 mg of lovastatin in separate groups of subjects. For these studies fasting baseline values were determined at 7 AM, 10 AM, and 1 PM. On a separate day a single dose of lovastatin was administered at 4 AM and blood samples were obtained 3 (7 AM), 6 (10 AM), and 9 h (1 PM) after administration.

Chronic lovastatin administration. To determine the effect of chronic lovastatin administration and withdrawal, baseline values of ML sterol synthesis, ML HMG-CoA reductase activity, and serum lipid levels were measured in each subject before initiation of lovastatin therapy. Then either 20 mg or 40 mg of lovastatin was administered orally bid at approximately 6 AM and 6 PM. Measurements of ML sterol synthesis, HMG-CoA reductase, and serum cholesterol were repeated at intervals within the 4-week treatment period and for 3 days after discontinuing the drug. All blood samples were obtained between 7 and 9 AM after a 12-h fast and, except where specifically stated, 2 h after the last dose of lovastatin.

TABLE 1. Patient profile of subjects prior to treatment with lovastatin for 4 weeks

Statistics	Lovastatin Dose	
	20 mg bid	40 mg bid
Number of patients	5	6
Age		
Mean ± SE	27 ± 3	45 ± 7
Range	21 - 40	29 - 70
Serum cholesterol (mg/dl)		
Mean ± SE	166 ± 17	189 ± 6
Range	104 - 197	179 - 217
Serum LDL cholesterol (mg/dl)		
Mean ± SE	49 ± 13	115 ± 9
Range	31 - 112	94 - 147
Serum HDL cholesterol (mg/dl)		
Mean ± SE	49 ± 10	38 ± 4
Range	38 - 88	28 - 50
Serum triglycerides (mg/dl)		
Mean ± SE	186 ± 12	174 ± 41
Range	167 - 209	75 - 322
ML sterol synthesis (pmol/10^6 cells/hr)		
Mean ± SE	1.3 ± 0.2	2.4 ± 0.4
Range	1.0 - 2.1	1.2 - 3.4
ML HMG-CoA reductase activity (pmol/mg/min)		
Mean ± SE	1.2 ± 0.2	0.9 ± 0.3
Range	0.3 - 1.6	0.2 - 1.7
Mononuclear leukocyte harvest		
Yield (10^6 cells/ml blood)	1.5 ± 0.2	1.6 ± 0.3
Lymphocytes (% of total)	78 ± 3	76 ± 3
Monocytes (% of total)	22 ± 3	24 ± 3
Viability (% of total)	97.4 ± 0.6	96.8 ± 0.4

After an overnight fast, 60 ml of blood was obtained between 7:00 AM and 9:00 AM from subjects before the initiation of oral lovastatin treatment at a dose of 20 mg bid (five subjects) or 40 mg bid (six subjects) for 4 weeks. Mononuclear leukocytes (ML) were harvested by density gradient centrifugation over Histopaque 1077. The rate of ML sterol synthesis, ML microsomal HMG-CoA reductase activity, and serum lipid profiles were determined as described in Methods. The differentials for the baseline harvest of mononuclear leukocytes were determined on a cytospin preparation stained with a Wright stain or α-naphthylacetate esterase. Viability was determined by trypan blue exclusion. The baseline values presented are those determined just prior to the initiation of lovastatin therapy.

Isolation and characterization of peripheral blood mononuclear leukocytes

Mononuclear leukocytes were obtained from 30 ml of blood collected in sterile heparinized Vacutainer tubes (Becton Dickinson, Rutherford, NJ) and isolated by density gradient centrifugation adapted from Boyum (26) as follows. Fifteen ml of peripheral blood was carefully aliquoted into 50-ml conical plastic tubes. The blood was diluted to 35 ml with chilled Earle's Balanced Salt Solution (EBS, Gibco, Grand Island, NY) and underlayered with 10 ml of Histopaque 1077 (Sigma, St. Louis, MO). After centrifugation (400 *g*, 30 min) at 15°C, the mononuclear leukocytes at the Histopaque–plasma interface were removed with a plastic pipette, washed with 40 ml of chilled EBS, and reisolated by centrifugation (400 *g*, 10 min) at 4°C. Red blood cell contamination was eliminated by treatment with 0.87% NH_4Cl at room temperature for 5 min (27) following which the mononuclear leukocytes were reisolated and washed twice with EBS. The final cell pellet was resuspended in EBS to a concentration of between 1.2 and 6.3 × 10^7 cells/ml. An aliquot was stained with trypan blue to assess viability and counted using a hemocytometer. Separate aliquots were taken for cytospin cell preparations and stained with a Wrights stain and α-naphthylacetate esterase (Sigma Procedure #90, St. Louis, MO) to assess lymphocyte and monocyte ratios (28). Mononuclear leukocyte recovery averaged 1.5 ± 0.2 × 10^6/ml of blood, with a differential of 76 ± 2% lymphocytes and 24 ± 2% monocytes. Viability by trypan blue exclusion routinely exceeded 96%. Lovastatin administration did not significantly alter the recovery, differential, or viability of the harvested mononuclear leukocytes. Duplicate aliquots were utilized for ML culture and an additional aliquot was pelleted, resuspended in 0.5 ml of 0.25 M sucrose, frozen in liquid N_2, and stored at −70°C for subsequent HMG-CoA reductase activity measurements.

Measurement of mononuclear leukocyte sterol synthesis

Freshly isolated mononuclear leukocytes were incubated in 5 ml of autologous serum (obtained at the time of the blood collection) supplemented with 25 mM HEPES buffer, 10,000 units/ml penicillin, 10 mg/ml streptomycin, and 4.46 μmol [^{14}C]acetate (2.24 mCi/mmol). Duplicate samples were incubated with shaking at 37°C in 95% O_2–5% CO_2. The incorporation of [^{14}C]acetate into sterols was terminated by the addition of 8 N KOH in 70% ethanol, after which [^{3}H]cholesterol (5000 dpm) was added to assess recovery.

The total incubation mixtures were saponified for 90 min at 70°C. After saponification, normal saline was added to adjust the samples to 50% ethanol and sterols were extracted with hexane (10 ml × 2). The hexane extract was back-washed with water, evaporated, and the sterols were redissolved in 6 ml of ethanol–ether 3:1. The sterols were then precipitated by the addition of an equal volume of 1% digitonin in ethanol–water 1:1, after which the digitonin-precipitated sterols were sequentially washed as described by Popjak (29). The sterols were split from the digitonin by the addition of pyridine, and after heating for 5 min at 70°C, the sterols were extracted with diethyl ether into scintillation vials (30). The ether was evaporated and the radiolabeled sterols were counted in Aquasol (New England Nuclear, Boston, MA). All samples were corrected for recovery and background determined by a parallel incubation of the subjects' serum without the added mononuclear cells. ML sterol synthesis was expressed as pmol [^{14}C]acetate incorporated into digitonin-precipitable sterols/10^6 cells per h. The rate of ML sterol synthesis was linear for 4 h and linear with regard to cell number from 7×10^6–16×10^6 cells/incubation. A 2-h incubation of 10^7 cells was routinely used. The precision of repeated measurements of ML sterol synthesis in an untreated subject determined over a 4-week period varied by an average of 6.2 ± 1.5% (n = 6).

Mononuclear leukocyte microsomal HMG-CoA reductase activity

Frozen mononuclear leukocytes were thawed, diluted to 9 ml with 0.25 M sucrose, and disrupted with Polytron homogenization for 10 sec at a setting of 3–4. Microsomes were then prepared after the method of Harwood, Schneider, and Stacpoole (27) by low speed centrifugation (500 $g \cdot h$) to pellet cell fragments, followed by centrifugation of the supernatant at 172,000 g for 90 min. The resultant microsomal pellet was resuspended in TEDK buffer (50 mM Tris, 68 mM EDTA, 70 mM KCl, 5 mM dithiothreitol, pH 7.5) to a protein concentration of 2 mg/ml. An aliquot (50 μl) of microsomal protein was incubated in TEDK buffer (final volume 75 μl) containing a final concentration of 1 mM $NADP^+$, 20 mM dithiothreitol, 10 mM glucose-6-phosphate, and 0.2 U glucose-6-phosphate dehydrogenase. In selected instances, samples were incubated with 3 units of alkaline phosphatase to maximize ML HMG-CoA reductase activity, or ML were disrupted and microsomes were prepared in the presence of 50 mM sodium fluoride to inhibit activation of the enzyme by dephosphorylation (31). The HMG-CoA reductase assay was initiated by addition of [3-^{14}C]HMG-CoA (52 mCi/mmol, final concentration 30 μM) and [^{3}H]mevalonate as an internal standard. After incubation at 37°C, the reaction was terminated by the addition of 10 μl of 6 N HCl and the [^{14}C]mevalonate formed was recovered as previously described (32). HMG-CoA reductase enzyme activity was calculated after correcting for recovery and subtracting background activity determined by a parallel incubation mixture without the added microsomal protein. HMG-CoA reductase activity was expressed as pmol [^{14}C]mevalonate formed/mg microsomal protein/min and was linear between 30 and 90 min and 50 and 150 μg of microsomal protein. Sixty minutes and 100 μg of microsomal protein were routinely utilized to measure ML HMG-CoA reductase activity.

Serum lipid measurements

Serum total and high density lipoprotein (HDL) cholesterol levels were measured by the cholesterol oxidase method (33, 34) and serum triglycerides were determined by the method of Kreutz (35), both using assay kits from Sigma (St. Louis, MO). Low density lipoprotein (LDL) cholesterol levels were calculated by the method of Friedewald, Levy, and Fredrickson (36).

Statistical analysis

The baseline values for serum lipids, ML sterol synthesis, and HMG-CoA reductase activity in each subject were compared to his/her values obtained at various times after lovastatin administration and withdrawal. In addition, the values after 4 weeks of lovastatin administration were compared to values 24, 48, and 72 h after discontinuing the drug. Differences in these comparisons at each time point were considered significant if *1*) a significant difference was demonstrated by analysis of variance, and *2*) the difference was statistically significant at $P < 0.05$ as assessed by paired t-test after correcting for a type I error introduced by multiple comparisons (37). Unless stated, all values are expressed as the mean ± SEM.

RESULTS

Effect of a single dose of lovastatin on ML sterol synthesis and HMG-CoA reductase activity

Two groups of three subjects were given a single oral dose of lovastatin (40 mg or 80 mg) and the rate of sterol synthesis in their ML was compared to baseline values

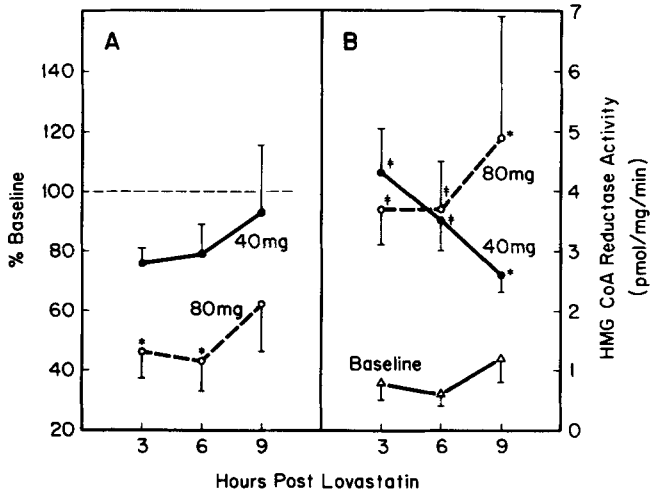

Fig. 1. Effect of acute lovastatin administration on ML sterol synthesis and HmG-CoA reductase activity. ML sterol synthesis and HMG-CoA reductase activity were determined 3, 6, and 9 h after a single oral dose of lovastatin (40 or 80 mg) as described in the Methods. These values were compared to ML sterol synthesis and HMG-CoA reductase activities determined at the same times (7:00 AM, 10:00 AM, 1:00 PM) on a separate day. A: Effect on ML sterol synthesis. The values are expressed as a percent of a baseline determined at the corresponding time but without ingestion of lovastatin. B: Effect on HMG-CoA reductase. HMG-CoA reductase activity was determined in microsomes washed free of lovastatin. The values are expressed as pmol/mg per min and compared to the baseline values obtained in the same subject on a day when lovastatin had not been given. There was no significant difference between the baseline values for the two groups and these data have been combined. Therefore, each point represents the mean ± SE of three subjects except for the baseline HMG-CoA reductase values which are the mean ± SE of six subjects; *, different from baseline by paired *t*-test, $P<0.05$; †, different from baseline by paired *t*-test, $P<0.01$.

measured at the same time of day (**Fig. 1A**). Baseline values for ML sterol synthesis averaged 1.7 ± 0.2 pmol [^{14}C]acetate incorporated/10^6 cells per h and ranged from 0.5 to 3.4 pmol/10^6 cells per h. Three hours after a 40-mg dose of lovastatin there was a 23 ± 5% decrease (NS) in ML sterol synthesis. A greater decrease (57 ± 10%, $P<0.05$) in ML sterol synthesis was observed 3 h after administration of 80 mg of lovastatin, and values remained low for the subsequent 6 h. A single subject ingesting 120 mg of lovastatin did not show a further reduction in ML sterol synthesis compared to the 80-mg dose (data not shown).

Administration of a single dose of either 40 mg or 80 mg of lovastatin resulted in a four- to fivefold increase in ML HMG-CoA reductase activity at 3 h compared to baseline values (Fig. 1B). The majority (74%) of the induced HMG-CoA reductase enzyme was in the active form as determined by assays in the presence and absence of sodium fluoride. Addition of alkaline phosphatase (3 units/assay) to the assay did not further stimulate apparent HMG-CoA reductase activity above that measured without alkaline phosphatase either in the baseline period or after lovastatin administration (data not shown). With the 40-mg dose, a decrease in ML HMG-CoA reductase activity was observed over the subsequent 6-h period from the 3-h post-lovastatin value of 4.3 ± 0.8 pmol/mg per min to 2.6 ± 0.3 pmol/mg per min measured 9 h after lovastatin administration. The induction of reductase activity was sustained with the 80-mg dose so that 9 h after administration, ML HMG-CoA reductase activity was still 4.9 ± 2.0 pmol/mg per min compared to the paired baseline value of 1.2 ± 0.4 pmol/mg per min ($P<0.05$). No change in serum cholesterol occurred over the 9 h with either single dose of lovastatin (data not shown).

Effect of chronic lovastatin administration on ML sterol synthesis, HMG-CoA reductase activity, and serum cholesterol

Since acute lovastatin administration resulted in a prompt increase in total HMG-CoA reductase, chronic exposure to lovastatin might result in an induction of HMG-CoA reductase great enough to counteract the inhibitory effect of the drug on ML sterol synthesis. We therefore administered two doses of lovastatin to groups of normal subjects for a 4-week period and measured serum cholesterol, ML sterol synthesis, and total ML HMG-CoA reductase activity. The oral administration of 20 mg of lovastatin bid to five subjects decreased serum cholesterol to 75 ± 5% of baseline ($P<0.01$) by 7 days with little further decrease over the next 3 weeks (**Fig. 2**). The

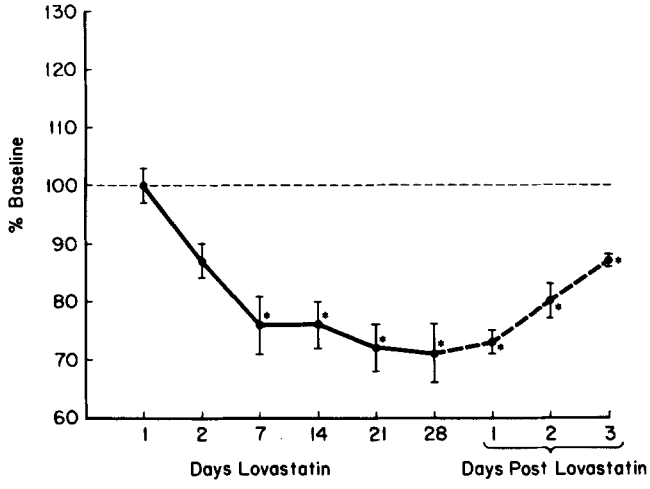

Fig. 2. Effect of chronic lovastatin (20 mg bid) administration on serum cholesterol. In five normal subjects, serum cholesterol was determined after a 12-h fast on the day prior to and on days 1, 2, 7, 14, 21, and 28 after initiation of a 4-week treatment period with lovastatin. In addition, a serum cholesterol level was determined in the same subjects for 3 days after the discontinuation of lovastatin. The values are expressed as the percent of the baseline value obtained before initiating therapy (dotted line). Statistical significance for multiple comparisons between the baseline value and the values obtained after initiation and discontinuation of lovastatin were determined as described in the Methods; *, different from baseline by paired analysis, $P<0.01$.

decrease in serum cholesterol could all be accounted for by a decrease in the LDL fraction from a baseline value of 80 ± 13 to 34 ± 9 ($P<0.01$) at 28 days. No change in HDL cholesterol (49 ± 10 baseline vs 52 ± 9 at 28 days; NS) or serum triglycerides (186 ± 12 baseline vs 170 ± 19 at 28 days; NS) occurred over the treatment period.

One day after initiating treatment, a decrease comparable to that measured with the acute administration of 40 mg of lovastatin was observed (86 ± 7% of baseline; **Fig. 3**). A consistent decrease in ML sterol synthesis was observed between days 7 and 28 of treatment from an average of 1.3 ± 0.2 pmol/10^6 cells per h at baseline to 1.0 ± 0.1 pmol/10^6 cells per h ($P<0.02$). No individual time point over the 28-day period significantly differed from baseline, however. Chronic lovastatin at this dose significantly increased total ML HMG-CoA reductase activity at 2 days compared to the baseline value with little further stimulation of ML HMG-CoA reductase over the next 4 weeks (**Fig. 4**).

One day after discontinuing lovastatin, ML sterol synthesis recovered from the 28-day value of 1.0 ± 0.2 pmol/10^6 cells per h to 1.4 ± 0.2 pmol/10^6 cells per h ($P<0.05$; Fig. 3). HMG-CoA reductase activity promptly returned to baseline values within 24 h of discontinuing lovastatin (Fig. 4). Serum cholesterol also recovered, although not as quickly, and 72 h after discontinuing lovastatin, serum cholesterol increased from the 28-day value of 118 ± 16 to 143 ± 14 ($P<0.05$; Fig. 2).

The chronic administration of a higher daily dose of lovastatin (40 mg bid) to six subjects decreased serum cholesterol to 66 ± 4% of baseline at 4 weeks ($P<0.01$; **Fig. 5**), a change comparable to the 20-mg bid dose (Fig. 2). LDL cholesterol decreased from 116 ± 9 to 55 ± 12 at 4 weeks (n = 5, $P<0.01$) and HDL cholesterol increased slightly from 38 ± 4 to 42 ± 3 (n = 5, $P<0.02$). Although serum triglycerides decreased an average of 14 ± 8%, this failed to reach statistical significance. This higher dose (40 mg bid) of lovastatin induced the HMG-CoA reductase activity in washed ML microsomes to a level 53.7 times the baseline value (**Fig. 6**). Due to the striking induction of HMG-CoA reductase activity, a modest decrease (16 ± 3%, $P<0.05$) in ML sterol synthesis was observed (Fig. 5). This decrease with the higher dose of lovastatin was comparable to the decrease (20 ± 14%, Fig. 3) in ML sterol synthesis after 4 weeks of treatment with the 20-mg bid dose.

Discontinuing high dose lovastatin resulted in a prompt reduction in ML HMG-CoA reductase activity (Fig. 6). In contrast to the results with the 20-mg bid dose (Fig. 4), HMG-CoA reductase activity remained significantly increased at 12.5 times the baseline by 72 h after stopping lovastatin. ML sterol synthesis rebounded after discontinuing lovastatin, and 24 h after the last dose ML sterol synthesis had increased 48 ± 15% above the 4-week level ($P<0.05$, Fig. 5). This increase was sustained throughout the 72 h observation period. After stopping lovastatin, each subject also had a significantly increased ML sterol synthesis over the prelovastatin baseline value and the maximum measured value of ML sterol synthesis was 3.2 ± 0.4 pmol/10^6 cells per h compared to a baseline of 2.4 ± 0.4 pmol/10^6 cells per h ($P<0.01$). Serum cholesterol levels increased over the 3 days after stopping

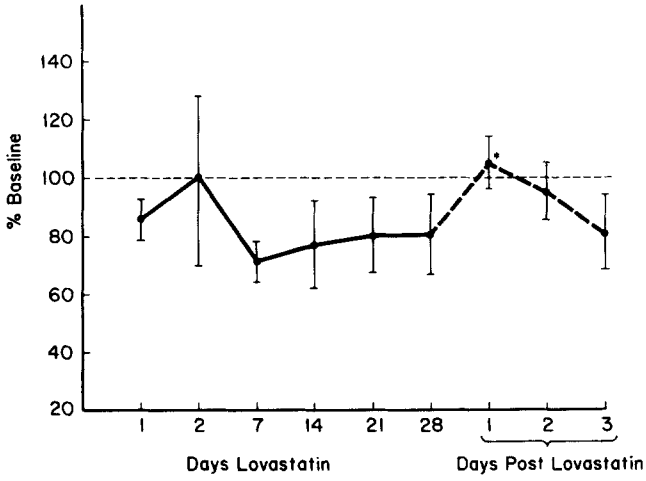

Fig. 3. Effect of chronic lovastatin (20 mg bid) administration on ML sterol synthesis. In five normal subjects, ML sterol synthesis was determined prior to and on the days noted after the initiation and subsequent discontinuation of lovastatin treatment. The values are expressed as a percent of the baseline value (dotted line). The value obtained 1 day after discontinuing lovastatin therapy was significantly increased over the last day of lovastatin therapy (day 28); *, different from the 28-day value by paired analysis, $P<0.05$.

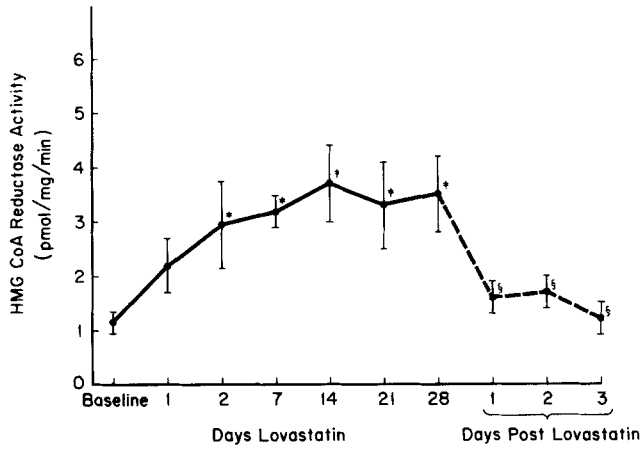

Fig. 4. Effect of chronic lovastatin (20 mg bid) administration on ML HMG-CoA reductase activity. HMG-CoA reductase activity was determined in washed microsomes prepared from five normal subjects before (baseline), during, and after treatment with lovastatin for a 4-week period. Statistical significance for multiple comparisons between the baseline value and the values obtained after initiation and discontinuation of lovastatin was determined as described in the Methods; *, different from baseline value by paired analysis, $P<0.05$; ‡, different from baseline value by paired analysis, $P<0.001$; §, different from the 28-day value by paired analysis, $P<0.05$.

lovastatin but did not rebound above baseline values (Fig. 5).

To test for the presence of reductase inhibitor activity in the plasma of subjects taking lovastatin, we performed parallel incubations of ML from three untreated subjects in both autologous plasma and in plasma from a subject taking lovastatin (40 mg bid for 4 weeks). Sterol synthesis in ML incubated in plasma from treated subjects averaged 58 ± 8% (n = 3, $P<0.01$) of the rate of sterol synthesis in the same cells incubated in autologous (untreated) plasma. This decrease in ML sterol synthesis was comparable to the observed inhibition of ML sterol synthesis after a single 80-mg dose (Fig. 1).

DISCUSSION

A major effect of lovastatin treatment is to decrease LDL cholesterol both by enhancing receptor-mediated uptake (14) and decreasing LDL formation (15). The results of the two studies investigating the effect of lovastatin on cholesterol synthesis in humans are conflicting (16, 17). We therefore utilized the rate of sterol synthesis in harvested mononuclear leukocytes as a reflection of total body cholesterol synthesis to attempt to better define the action of lovastatin on cholesterol synthesis. Our results suggest that a modest inhibition of sterol synthesis may effect major changes in LDL cholesterol metabolism and serum LDL cholesterol.

Previous studies of human fibroblasts cultured in the presence of the HMG-CoA reductase inhibitor, compactin, demonstrated a marked increase in sterol synthesis when the compactin was subsequently removed from the culture media (7). Removal of the mononuclear leukocytes from the lovastatin-containing serum might result in a similar stimulation of sterol synthesis. In a single experiment, we found that ML sterol synthesis in a subject treated with lovastatin (40 mg bid) increased 16% when incubated in serum from a subject not on lovastatin compared to the autologous incubation. To avoid potential changes in ML sterol synthesis, we isolated mononuclear leukocytes rapidly at 4°C and reincubated them in autologous plasma during the ML sterol synthesis assay. Our results demonstrate that a single oral dose of lovastatin decreased ML cholesterol synthesis as early as 3 h after dosing, that the inhibition appeared to be dose-related, and that this inhibition lasted for a further 6 h (Fig. 1).

In distinction, chronic lovastatin treatment resulted in less change in ML sterol synthesis than the single dose. The effect of chronic lovastatin administration on ML sterol synthesis appears to be tempered by the marked induction of HMG-CoA reductase activity which serves to counteract the inhibitory effect of lovastatin on the enzyme.[1] Furthermore, the enzyme induction appears to be dose-related as the higher dose (40 mg bid) induced tenfold greater levels of ML HMG-CoA reductase than did the lower dose. We have previously reported that measured HMG-CoA reductase activity in gut mucosa of lovastatin-treated patients was not induced (38). Since those measurements were made in whole gut homogenates rather than washed microsomes, inhibitor may have been present in the assay. Therefore, it is unclear whether gut response to lovastatin differs from other tissues or whether, in that study, enzyme induction in gut was masked by residual drug.

In tissue culture and in rat liver, lovastatin induces HMG-CoA reductase enzyme both by increased transcription of reductase mRNA and by reduced cellular degradation of the protein (39–41). A final mechanism that has been reported to increase enzyme activity in rat

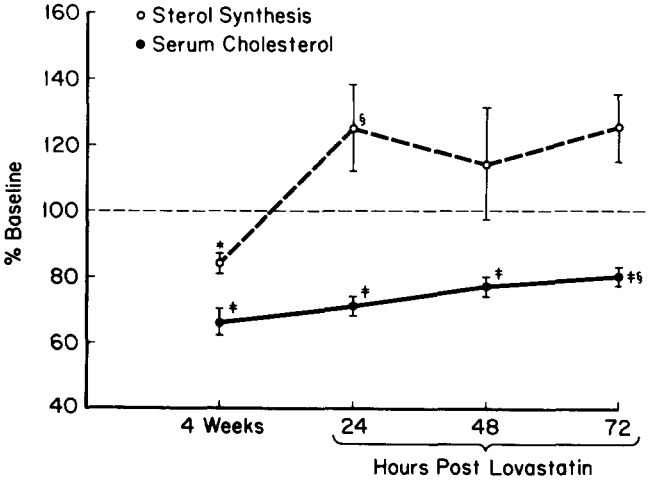

Fig. 5. Effect of chronic lovastatin (40 mg bid) therapy on ML sterol synthesis and serum cholesterol. In six normal subjects, ML sterol synthesis (○) and serum cholesterol (●) were determined before and after a 4-week treatment period with lovastatin. Additional determinations of ML sterol synthesis and serum cholesterol were made in the same subjects at various times within 72 h of stopping lovastatin. The values are expressed as the percent of the baseline value obtained before initiating therapy (dotted line). Statistical significance for multiple comparisons between the baseline value, the 4-week value, and the values after the discontinuation of lovastatin was determined as described in the Methods; *, different from baseline by paired analysis, $P<0.05$; ‡, different from baseline by paired analysis, $P<0.01$; §, different from the 4-week value by paired analysis, $P<0.05$.

[1]Since submission of this manuscript, studies by Hagemenas and Illingsworth (46) reported a twofold induction of HMG-CoA reductase activity in mononuclear leukocytes of FH patients treated with lovastatin for 4 weeks. In contrast to our finding of a modest decrease in ML sterol synthesis with chronic lovastatin treatment (Figs. 3, 5), these authors found a dose-related increase (up to 39% over baseline) in ML sterol synthesis when measured 12 h after the last dose of lovastatin. It is not clear whether this difference is due to intrinsic differences in lovastatin action between normal and FH subjects or whether timing of last dose or other methodological differences are involved. Time course studies on these FH subjects after discontinuing chronic lovastatin treatment and after an acute dose of lovastatin as were done on our subjects might clarify this difference.

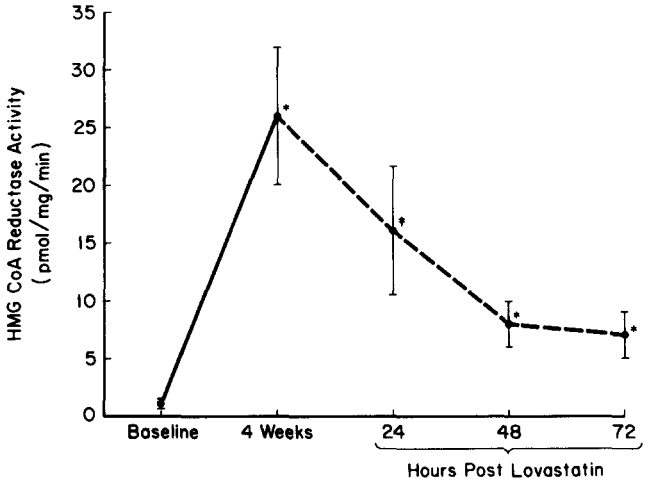

Fig. 6. Effect of chronic lovastatin (40 mg bid) administration on ML HMG-CoA reductase activity. HMG-CoA reductase activity was determined in washed microsomes prepared from six normal subjects before (baseline), after treatment with lovastatin (40 mg bid) for 4 weeks, and for a 72-h period after stopping lovastatin therapy. Statistical significance for multiple comparisons between the baseline and the 4-week value in each subject was determined as described in the Methods; *, different from baseline value by paired analysis, $P<0.01$; ‡, different from baseline value by paired analysis, $P<0.05$.

liver in response to a stimulus is the activation (dephosphorylation) of inactive preformed enzyme (42). The rapidity of the fourfold increase in ML HMG-CoA reductase after a single oral dose (Fig. 1) is striking and at first glance suggests activation of latent enzyme. However, the fluoride and alkaline phosphatase experiments demonstrate that we are measuring an increase in total enzyme protein rather than activation of preformed enzyme. Whether the observed increase is due to induction of enzyme protein synthesis or decrease in degradation is not known at this time.

Less than 5% of an orally administered dose of lovastatin reaches the systemic circulation as the native compound or an active metabolite (43). From our mixed incubation studies of normal cells in lovastatin-treated serum, it appears that this 5% is more than an adequate quantity to inhibit ML sterol synthesis. Furthermore, serum lovastatin concentrations decrease to very low levels within 24 h of discontinuing the drug (3). After stopping the lower (20 mg bid) lovastatin dose, ML sterol synthesis (Fig. 3) and HMG-CoA reductase activity (Fig. 4) returned to baseline values within 24 h, suggesting that competitive inhibition of HMG-CoA reductase and total body cholesterol synthesis was no longer present. With the higher dose (40 mg bid), a greater induction of HMG-CoA reductase enzyme activity occurred and HMG-CoA reductase activity was not back to baseline even after 72 h (Fig. 6). This sustained increase in uninhibited enzyme resulted in a rebound of ML sterol synthesis over the baseline value. This overshoot in sterol synthesis after lovastatin withdrawal has also been noted in tissue culture and in the rat (7, 12). The increased rate of sterol synthesis with discontinuation of lovastatin did not result in a rebound increase in serum cholesterol levels.

Fogelman et al. (44) have demonstrated that the rate of sterol synthesis in monocytes is five times that of lymphocytes, and cautioned that studies of ML sterol synthesis must control for variations in monocyte:lymphocyte ratios. In our studies, no changes in monocyte:lymphocyte ratios were observed with lovastatin treatment or withdrawal. Furthermore, the marked elevation of HMG-CoA reductase activity cannot be explained merely by variability in the ratio of monocytes to lymphocytes, since only a fivefold increase would be possible if lovastatin changed the differential to 100% monocytes.

In our study of normal subjects, little additional serum cholesterol-lowering effect was noted by increasing the lovastatin dose from 20 mg bid to 40 mg bid, as this served merely to further induce HMG-CoA reductase activity. These results suggest that the net effect of lovastatin on cellular LDL metabolism is inversely related to the cells' ability to maintain sterol synthesis by increasing HMG-CoA reductase enzyme concentrations. The potential for induction of HMG-CoA reductase activity to levels that balance the inhibitory effect of lovastatin may explain the mechanism behind reports of selective patients resistant to the cholesterol-lowering effect of lovastatin (45).

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