Influence of mevinolin on metabolism of low density lipoproteins in primary moderate hypercholesterolemia

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Abstract Mevinolin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, was used for treatment of 12 patients with moderate hypercholesterolemia, but not classical familial hypercholesterolemia. For most patients, measurements of turnover of low density lipoprotein-apolipoprotein B (LDL-apoB) were made on placebo and during treatment with two doses of mevinolin. LDL turnover was determined after injection of autologous ^{125}I-labeled radioiodinated LDL. Compared to placebo, a low dose of mevinolin (10 mg, twice daily (BID)) caused reductions of plasma total cholesterol and LDL-cholesterol averaging 15% and 20%, respectively; corresponding reductions on high doses of mevinolin (20 mg BID) were 22% and 31%, respectively. Triglyceride levels were unchanged by the drug. High density lipoprotein cholesterol levels rose significantly on the high dose, but not on the low dose. Neither dose produced a statistically significant change in fractional catabolic rate (FCR) for LDL-apoB for the whole group, although several patients had increases in FCR on both doses. In contrast, both doses of mevinolin caused decreases in production rates of LDL-apoB. Thus, the fall in LDL levels in patients with moderate hypercholesterolemia can be explained more by a reduction in the input rate of LDL-apoB than by enhanced fractional removal of LDL from the circulation. —**Grundy, S. M., and G. L. Vega.** Influence of mevinolin on metabolism of low density lipoproteins in primary moderate hypercholesterolemia. *J. Lipid Res.* 1985. **26:** 1464–1475.

Supplementary key words apoB • fractional catabolic rate • cholesterol • LDL turnover

Mevinolin and compactin belong to a new class of cholesterol-lowering drugs that appear highly effective in relatively low doses. The primary action of these drugs is to competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol synthesis (1, 2). Clinical studies have shown that mevinolin (and compactin) effectively reduce plasma levels of low density lipoproteins (LDL) in normal subjects (3) and in patients with heterozygous familial hypercholesterolemia (4–8). The inhibition of cholesterol synthesis by mevinolin appears to trigger a compensatory increase in LDL receptors that can account for the lowering of LDL levels (9). Recent studies in heterozygotes for familial hypercholesterolemia (7) have demonstrated that mevinolin enhances the fractional clearance of LDL, and the increment in clearance occurs via the LDL receptor pathway.

Most patients with primary hypercholesterolemia do not have heterozygous familial hypercholesterolemia. Yet patients with other forms of primary hypercholesterolemia constitute a significant proportion of all cases of premature coronary heart disease (CHD) (10, 11). If mevinolin is to have a broad usefulness in treatment of elevated cholesterol levels for prevention of CHD, it would have to be effective for primary hypercholesterolemia of types other than familial hypercholesterolemia. Since the mechanisms responsible for elevated LDL levels in primary hypercholesterolemia are not understood, it cannot be assumed that mevinolin will be effective in such patients. The current study, therefore, was carried out in patients with primary hypercholesterolemia who did not have classical familial hypercholesterolemia. The study was designed to determine the actions of mevinolin on concentrations of plasma lipoproteins and on metabolism of LDL in these patients.

METHODS

Twelve middle-aged men were studied on the metabolic ward of the Veterans Administration Medical Center, Dallas, TX. Clinical characteristics of each patient are shown in **Table 1**. All patients had plasma total cholesterol concentrations exceeding 250 mg/dl at time of

Abbreviations: LDL, low density lipoproteins; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; CHD, coronary heart disease; HDL-C, high density lipoprotein cholesterol; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; TG, triglyceride; FCR, fractional catabolic rate.

TABLE 1. Clinical data

No.	Age	Weight[a]	%IBW[a]	Plasma[a] TC	Plasma[a] TG	Family[b] CHD	Family[b] HCH	Family[b] HTG
	yrs	*kg*	*%*	*mg/dl*				
1	48	68	116	259	195	–	n.a.	n.a.
2	61	75	107	289	167	–	0/2	0/2
3	59	86	105	276	125	+	0/5	0/5
4	56	85	129	259	128	+	0/5	0/5
5	64	82	117	287	135	–	0/1	0/1
6	59	89	119	284	108	+	0/3	0/3
7	69	76	110	265	94	+	0/2	0/2
8	55	102	152	274	176	+	1/3	0/3
9	59	59	91	316	190	+	0/9	0/9
10	69	61	87	266	164	+	0/2	0/2
11	63	80	112	253	112	+	0/2	0/2
12	51	99	132	303	185	+	1/3	0/3
Mean ± SEM	59 ± 2	79 ± 4	113 ± 5	268 ± 8	157 ± 13			

[a]Clinical data obtained at time of selection for study. %IBW, Percent ideal body weight estimated from the Metropolitan Life Insurance Company Statistical Bulletin. No 40, 1959.

[b]Family history of CHD and hyperlipidemia: the numerator indicates the number of subjects in the family with hypercholesterolemia (HCH) or hypertriglyceridemia (HTG). The denominator indicates the total number of first-degree relatives screened. Hyperlipidemia was defined as total cholesterol or triglyceride exceeding the 95th percentile for the patients' age and sex according to Lipid Research Clinic criteria (29).

entrance into the study. All had coronary heart disease (CHD) defined as previous myocardial infarction documented on clinical grounds or previous coronary artery bypass graft; however, neither had occurred during the 6 months prior to study. The average percent of ideal body weight (%IBW) was 113 ± 5 (SEM)%, although one patient (No. 4) was 129% and another (No. 8) was 152% IBW. None of the patients had taken hypolipidemic agents for 6 months preceding the study; none had unstable angina, congestive heart failure, or disorders of the gastrointestinal or endocrine systems. No secondary causes of hypercholesterolemia were detected.

None of the patients had classical familial hypercholesterolemia. For example, none had a history of CHD before age 45 or tendon xanthomata, nor were either reported for family members. Screening of first-degree relatives for hyperlipidemia was carried out to the extent possible, as described before (12) (Table 1). None of the relatives had hypertriglyceridemia, and only 2 relatives of 37 screened had hypercholesterolemia; this again is uncharacteristic of classical familial hypercholesterolemia. However, 9 of the 12 patients gave a family history of CHD in first-degree relatives. The patients were designated as having primary moderate hypercholesterolemia. The qualifying term "moderate" was employed because cholesterol levels at time of screening were between 250 and 325 mg/dl, which generally are lower than the severely elevated levels of familial hypercholesterolemia. Informed consent was obtained from each patient and the protocol was approved by the appropriate institutional review board.

Experimental design

Patients having a history of CHD in the outpatient department of the Veterans Administration Medical Center were screened for hypercholesterolemia. They were selected for study on the basis of having a plasma total cholesterol (TC) over 250 mg/dl on an ad lib diet. For most patients, studies of LDL metabolism were carried out three times: on placebo, on mevinolin 10 mg twice daily (BID) (low-dose mevinolin), and on mevinolin 20 mg BID (high-dose mevinolin). The periods of placebo and treatment were randomized, and each period lasted 6–7 weeks. Patients received the drug for 3 to 4 weeks before starting turnover studies of LDL, and each turnover study lasted 20 days. Two patients (Nos. 6 and 10) had only two turnover measurements, one on placebo and the other on mevinolin, either 10 or 20 mg BID.

Five days before starting the LDL turnover study, 250 ml of plasma was removed by plasmapheresis. Two days later the patients were started on 0.5 to 0.9 g of potassium iodide orally in divided doses to suppress uptake of radioiodine by the thyroid; this was continued throughout the study. LDL was isolated, radioiodinated, and on the fifth day reinjected into the patient. Disappearance of radioactivity from plasma was monitored for 20 days, and levels of plasma lipids, lipoprotein-cholesterol, and LDL-apoB were measured every 3 days throughout the study.

Diets

On the metabolic ward the patients were fed a repetitive, solid-food diet containing 40% of calories as fat (18%

saturates, 17% monounsaturates, and 5% polyunsaturates), 45% as carbohydrate, and 15% as protein. Daily intake of cholesterol was 300 mg. The fat-carbohydrate-protein composition of the diet was designed to resemble the "typical" American diet. Inpatients' weights were monitored daily throughout the study, and caloric content was adjusted to maintain a constant body weight. Throughout the study, weights did not change more than 2 kg, and mevinolin had no effect on body weight.

LDL-apoB turnover procedure

Following plasmapheresis, LDL (d 1.019–1.063 g/ml) was isolated by ultracentrifugation (13) and radioiodinated with ^{125}I by the iodine-monochloride method of McFarlane (14) as modified by Langer, Strober, and Levy (15) and Bilheimer, Eisenberg, and Levy (16). ^{125}I-Labeled LDL prepared by this procedure was mixed with 5–10 mg of autologous unlabeled LDL and 5% human serum albumin. The mixture was filtered through pyrogen-free 0.22-μm and 0.45-μm Millipore filters. Thirty to 50 μCi of labeled LDL was injected intravenously. Blood samples (7 ml) were collected at 10 and 20 min and at 1, 4, 8, 12, and 24 hr. Thereafter, 7 ml blood was collected every 12 hr for the subsequent 3 days, and finally every 24 hr from the 4th through the 20th day of the turnover study. Radioactivity was measured at each time interval, and plasma lipids, lipoprotein cholesterol, and LDL-apoB were measured every 3 days. Urine was collected quantitatively throughout the study for estimation of daily excretion of radioactivity.

Plasma total cholesterol and triglycerides (TG) were determined enzymatically (17, 18). The cholesterol standard supplied by Boehringer-Mannheim was calibrated using pooled reference plasma as standard; the cholesterol in the standard was measured by gas–liquid chromatography. HDL-cholesterol (HDL-C) was measured enzymatically on whole plasma after precipitation of apoB-containing lipoproteins by phosphotungstic acid–Mg^{2+} (19). This procedure may precipitate HDL containing apolipoprotein E, and it has been reported to give slightly lower values (e.g., about 3–5 mg/dl lower) than HDL-C determined after precipitation of apoB-containing lipoprotein with heparin–Mn^{2+} followed by chemical measurement (20). Very low density and intermediate density lipoproteins (VLDL + IDL) (d < 1.019 g/ml) were removed by ultracentrifugation, and cholesterol was measured in the infranatant. LDL-cholesterol (LDL-C) equaled total infranatant cholesterol minus HDL-C. LDL (d 1.019–1.063 g/ml) was isolated by ultracentrifugation of the infranatant; on this fraction, cholesterol was measured enzymatically and apoB was determined by a modification of the procedure of Lowry et al. (21) using selective precipitation of apoB by isopropanol (22). The LDL-apoB concentration was calculated by multiplying the ratio of LDL-apoB/LDL-C on the isolated fraction by the LDL-C concentration obtained as described above.

The fractional catabolic rates (FCR) and transport rates of LDL-apoB were determined from the plasma die-away curves. The FCR was estimated using a two-pool mammillary model described by Matthews (23). The kinetic parameters were estimated with the CONSAM program of Berman et al. (24) based on the SAAM program of Berman and Weiss (25). The data were analyzed on a VAX 11/780 computer (Digital Equipment Corp., Maynard, MA). For all patients, the die-away curves for the three study periods were compared by Fisher's F statistic (26). The transport rate was calculated by multiplying the pool size of LDL-apoB by the FCR for LDL-apoB. This calculation assumes that LDL is a kinetically homogenous class of lipoproteins. The pool size of LDL-apoB was the product of the LDL-apoB level and plasma volume, the latter being estimated by isotope dilution at 10 min after injection of labeled LDL. In subsequent discussion, the term "production rate" will be used synonymously with "transport rate."

Urine was collected daily and counted for radioactivity. The fraction of the injected dose excreted in the urine each day was divided by the fraction of dose remaining in plasma to give the urine/plasma (U/P) ratio (27, 28). The mean U/P ratio was estimated by averaging daily ratios for the first 14 days, as typically done for LDL turnover studies (28). After 2 weeks, less than 1% of the injected dose was excreted daily, and U/P ratios tended to decline; the validity of the ratios after 14 days might be questioned and thus were not used in the calculations. The U/P ratios were used as a check on the accuracy of FCR for LDL-apoB determined from the die-away curve, but they were not used in estimation of transport rates.

Statistical analysis

Linear statistical procedures available as Interactive Statistical Programs (ISP) were used for comparison of means. One-way analysis of variance (ANOVA) was used for comparisons of all parameters among the study periods for each patient. The paired *t*-test was used for comparison of means for each study period. Two patients were not studied for all three periods, i.e., placebo, low-dose mevinolin; patients 6 and 10 did not receive the low and high dose, respectively. Therefore, for paired *t*-test, comparisons between mevinolin therapy and placebo were made for only 11 patients, and the placebo value was omitted for the corresponding absent value for mevinolin therapy. Comparisons between the two mevinolin doses employed only 10 patients. In the paired *t*-tests, the mean differences in value were tested against zero. Percentage differences quoted in the Results section represent the same comparisons as used for statistical analysis. The statistical packages were used at the Medical Computing Resource

Center, University of Texas Health Science Center at Dallas, and analyses were carried out on a DEC-10 computer.

RESULTS

Plasma lipid and lipoprotein concentrations

Plasma total cholesterol and triglycerides. For the 12 patients of this study, the plasma total cholesterol during the placebo period averaged 263 ± 8 (SEM) (**Table 2**). Total cholesterol levels declined significantly by 15% on low-dose mevinolin and by 22% on the higher dose. Although the percentage reduction was greater on the higher dose, the difference in total cholesterol levels between high- and low-dose periods was not statistically significant. The mean level of plasma triglycerides on placebo (136 ± 10 mg/dl) was not significantly different from that on either low-dose or high-dose mevinolin (**Table 3**).

Plasma LDL-C. The mean concentration of LDL-C on placebo was 197 ± 7 mg/dl (**Table 4**). In all patients, LDL-C levels exceeded the 75th percentile for the patients' ages during the placebo period, and in all but three (Nos. 1, 3, and 4), they exceeded the 90th percentile (29). Low-dose mevinolin reduced LDL-C levels by an average of 20%. An apparently greater response was noted during high-dose mevinolin. On the high dose, the mean level of LDL-C was 31% lower than on placebo; however, this percentage was not statistically significantly greater than the 20% reduction on the low dose.

Plasma HDL-C. On placebo, the HDL-C level averaged 40 ± 4 mg/dl (**Table 5**). The mean HDL-C on low-dose mevinolin was somewhat higher but not significantly different from that of the placebo period. On high-dose mevinolin, HDL-C levels rose significantly by 20% compared to placebo.

Kinetics of LDL-apoB

The mean concentration of plasma LDL-apoB on placebo was 114 ± 4 mg/dl (**Table 6**). On low-dose mevinolin, LDL-apoB levels fell significantly by 21%, and on the high dose, levels were 31% lower than on placebo; the mean on the high dose was significantly lower than the low-dose mean. The LDL-apoB/LDL-C ratio in the placebo period averaged 0.57 ± 0.02, and this ratio was unchanged by low-dose mevinolin (0.59 ± 0.02) or high-dose mevinolin (0.55 ± 0.01).

The mean FCR for LDL-apoB on placebo was 0.25 ± 0.01 pools/day (**Table 7**). Average FCRs for LDL-apoB on low-dose mevinolin (0.27 ± 0.01 pools/day) and on high-dose mevinolin (0.27 ± 0.01 pools/day) were not significantly different from placebo. However, for individual patients, the responses were more variable. In nine of 12 patients, FCRs for LDL-apoB were significantly higher, by Fisher's F statistic (26), in either one or both periods of mevinolin therapy, compared to placebo. However, in two patients (Nos. 7 and 12), FCRs for LDL-apoB were significantly lower during treatment with mevinolin. Urine/plasma (U/P) ratios for radioactivity following injection of labeled LDL-apoB were similar to FCRs for

TABLE 2. Effects of mevinolin on plasma cholesterol concentrations

Patient	Plasma Total Cholesterol		
	Placebo	Low-Dose Mevinolin	High-Dose Mevinolin
	mg/dl ± SEM (%Δ)[a] (n = 7)		
1	222 ± 3	190 ± 1 (−14)[b]	184 ± 10 (−17)[b]
2	235 ± 15	184 ± 8 (−22)[b]	212 ± 2 (−10)[c]
3	248 ± 5	248 ± 13 (0)[c]	184 ± 3 (−26)[b]
4	246 ± 6	224 ± 6 (−9)[b]	202 ± 5 (−18)[b]
5	256 ± 7	209 ± 6 (−18)[b]	226 ± 3 (−12)[b]
6	257 ± 14		173 ± 4 (−33)[b]
7	260 ± 12	228 ± 3 (−12)[b]	216 ± 5 (−17)[b]
8	273 ± 7	238 ± 5 (−13)[b]	178 ± 4 (−35)[b]
9	277 ± 14	213 ± 4 (−23)[b]	197 ± 6 (−29)[b]
10	277 ± 8	250 ± 7 (−10)[b]	
11	287 ± 9	211 ± 5 (−26)[b]	189 ± 10 (−34)[b]
12	314 ± 7	265 ± 13 (−16)[b]	289 ± 7 (−8)[b]
Mean ± SEM (vs placebo)	263 ± 7	223 ± 8 (−15)[d] (vs 263 ± 7)	204 ± 10 (−22)[e] (vs 261 ± 7)

[a] Percent change from control period.
[b] Significantly lower than placebo ($P < 0.03$).
[c] Not significantly different from placebo (at $P < 0.05$).
[d] Low-dose mevinolin significantly lower than placebo at $P < 0.001$ (paired *t*-test). Placebo mean (n = 11; patient 6 omitted) shown in parentheses.
[e] High-dose mevinolin significantly lower than placebo at $P < 0.001$ (paired *t*-test). Placebo mean (n = 11; patient 10 omitted) shown in parentheses.

TABLE 3. Effect of mevinolin on plasma triglyceride concentrations

Patient	Plasma Triglycerides		
	Placebo	Low-Dose Mevinolin	High-Dose Mevinolin
	mg/dl ± SEM (%Δ)[a] (n = 7)		
1	80 ± 1	86 ± 3 (+8)[b]	94 ± 3 (+18)[c]
2	151 ± 8	149 ± 6 (−1)[b]	128 ± 8 (−15)[b]
3	117 ± 3	154 ± 13 (+32)[c]	147 ± 5 (+26)[c]
4	212 ± 39	228 ± 8 (+8)[c]	188 ± 17 (−11)[d]
5	156 ± 6	150 ± 10 (−4)[c]	182 ± 12 (−14)[b]
6	107 ± 4		83 ± 7 (−22)[d]
7	113 ± 8	101 ± 6 (−11)[c]	122 ± 10 (+8)[b]
8	114 ± 8	125 ± 6 (−10)[c]	131 ± 7 (+15)[b]
9	121 ± 5	89 ± 5 (−26)[d]	126 ± 12 (+4)[b]
10	161 ± 11	148 ± 6 (−8)[b]	
11	141 ± 6	128 ± 6 (−9)[b]	101 ± 4 (−28)[d]
12	126 ± 13	124 ± 10 (−2)[b]	159 ± 4 (−26)[c]
Mean ± SEM (vs placebo)	136 ± 10	135 ± 12 (0)[e] (vs 135 ± 10)	133 ± 10 (−1)[f] (vs 131 ± 10)

[a] Percent change from control period.
[b] Not significantly different from placebo (at $P < 0.05$).
[c] Significantly higher than placebo (at $P < 0.03$).
[d] Significantly lower than placebo ($P < 0.03$).
[e,f] Low-dose and high-dose mevinolin not significantly lower than placebo mean (n = 11, patients 6 and 10 omitted, respectively) by paired *t*-test.

LDL-apoB; these ratios provided a check on the reliability of FCRs estimated from the radioactivity decay curves of plasma LDL-apoB.

Fig. 1 compares plasma decay curves and U/P ratios in a typical patient. A similarity of the decay curves was noted for placebo, low-dose mevinolin, and high-dose mevinolin; also the mean U/P ratios were comparable for each period. The values for U/P ratios represent mean values for the first 14 days; thereafter, there was a tendency for ratios to decline, but at this time less than 1% of the injected dose was excreted in the urine each day. Overall, no differences in patterns of U/P ratios were noted for mevinolin therapy compared to placebo.

The transport rate of LDL-apoB averaged 12.2 ± 0.6 mg/kg-day on placebo. This decreased significantly on low-dose mevinolin to 9.8 ± 0.6 mg/kg-day and on the high dose to 8.6 ± 0.2 mg/kg-day. In contrast to variable changes in FCRs, reductions in transport rates of LDL-

TABLE 4. Effects of mevinolin on LDL-cholesterol concentrations

Patient	Plasma LDL-Cholesterol		
	Placebo Control	Low-Dose Mevinolin	High-Dose Mevinolin
	mg/dl ± SEM (%Δ)[a] (n = 7)		
1	182 ± 3	139 ± 1 (−24)[b]	135 ± 6 (−26)[b]
2	186 ± 15	122 ± 7 (−34)[b]	138 ± 3 (−26)[b]
3	175 ± 4	166 ± 8 (−5)[c]	114 ± 4 (−35)[b]
4	172 ± 8	145 ± 5 (−16)[b]	132 ± 5 (−23)[b]
5	199 ± 7	145 ± 4 (−27)[b]	143 ± 4 (−28)[b]
6	205 ± 10		120 ± 4 (−41)[b]
7	195 ± 9	159 ± 3 (−18)[b]	146 ± 4 (−25)[b]
8	194 ± 5	162 ± 9 (−16)[b]	110 ± 3 (−43)[b]
9	204 ± 11	153 ± 5 (−25)[b]	122 ± 5 (−40)[b]
10	197 ± 6	173 ± 5 (−12)[b]	
11	210 ± 7	156 ± 17 (−26)[b]	125 ± 7 (−40)[b]
12	259 ± 9	219 ± 11 (−15)[b]	241 ± 7 (−7)[c]
Mean ± SEM (vs placebo)	197 ± 7	158 ± 7 (−20)[d] (vs 197 ± 7)	134 ± 11 (−32)[e] (vs. 197 ± 7)

[a] Percent change from control period.
[b] Significantly lower than placebo ($P < 0.03$).
[c] Not significantly different from placebo (at $P < 0.05$).
[d,e] Low-dose and high-dose mevinolin significantly lower than placebo mean at $P < 0.001$ by paired *t*-test. For comparison with placebo, patients 6 and 10 were omitted for the two doses of mevinolin, respectively.

TABLE 5. Effects of mevinolin on HDL-cholesterol concentrations

Patient	Plasma HDL-Cholesterol		
	Placebo	Low-Dose Mevinolin	High-Dose Mevinolin
	mg/dl ± SEM (%Δ)[a] (n = 7)		
1	29 ± 1	43 ± 1 (+33)[b]	41 ± 3 (+29)[b]
2	25 ± 1	41 ± 1 (+39)[b]	37 ± 1 (+48)[b]
3	41 ± 2	47 ± 4 (+15)[c]	54 ± 1 (+24)[b]
4	36 ± 2	39 ± 1 (+8)[c]	46 ± 2 (+28)[b]
5	32 ± 2	45 ± 1 (+41)[b]	55 ± 2 (+42)[b]
6	41 ± 4		44 ± 3 (+7)[c]
7	40 ± 1	54 ± 2 (+35)[b]	54 ± 1 (+35)[b]
8	46 ± 4	39 ± 2 (−15)[d]	54 ± 3 (+17)[c]
9	59 ± 4	51 ± 2 (−16)[c]	61 ± 3 (+3)[c]
10	58 ± 1	61 ± 4 (+5)[c]	
11	55 ± 2	53 ± 3 (−4)[c]	54 ± 6 (−2)[c]
12	24 ± 2	30 ± 1 (+25)[b]	30 ± 2 (+25)[b]
Mean ± SEM (vs placebo)	40 ± 4	46 ± 3 (+15)[e] (vs 40 ± 4)	48 ± 3 (+23)[f] (vs 39 ± 3)

[a] Percent change from control period.
[b] Significantly higher than placebo ($P < 0.03$).
[c] Not significantly different from placebo (at $P < 0.05$).
[d] Significantly lower than placebo ($P < 0.03$).
[e] Low-dose mevinolin not significantly different from placebo by paired *t*-test.
[f] High-dose mevinolin significantly different from placebo mean at $P < 0.001$ by paired *t*-test (placebo value for patient 10 omitted).

apoB were noted in almost all patients, especially on the high dose.

Mevinolin was well tolerated by all the patients throughout the study. None developed symptoms or abnormal physical signs related to the drug. Also, there were no abnormalities in the hemogram, liver function tests, electrolytes, blood urea nitrogen, or serum creatinine.

DISCUSSION

The effectiveness of mevinolin in lowering plasma LDL in heterozygotes for familial hypercholesterolemia raises the question of how effective the drug is for treatment of more moderate forms of primary hypercholesterolemia that are much more common than familial hypercholes-

TABLE 6. Effects of mevinolin on LDL-apoB concentrations and LDL-apoB/LDL-C ratios

Patient	LDL-apoB Concentrations and LDL-apoB/Cholesterol Ratios[a]					
	Placebo		Low-Dose Mevinolin		High-Dose Mevinolin	
	mg/dl	*ratio*	*mg/dl*	*ratio*	*mg/dl*	*ratio*
1	108 ± 5	0.59 ± 0.11	84 ± 1[b]	0.59 ± 0.02[c]	79 ± 19[b]	0.58 ± 0.07[c]
2	135 ± 25	0.73 ± 0.05	74 ± 11[b]	0.61 ± 0.05[b]	75 ± 7[b]	0.54 ± 0.05[b]
3	119 ± 8	0.68 ± 0.13	91 ± 11[b]	0.55 ± 0.03[b]	59 ± 8[b]	0.52 ± 0.05[b]
4	119 ± 16	0.65 ± 0.07	80 ± 14[b]	0.54 ± 0.05[b]	77 ± 11[b]	0.59 ± 0.05[b]
5	107 ± 2	0.56 ± 0.03	78 ± 7[b]	0.54 ± 0.03[c]	78 ± 6[b]	0.54 ± 0.03[c]
6	113 ± 21	0.55 ± 0.05			66 ± 5[b]	0.55 ± 0.03[c]
7	112 ± 5	0.62 ± 0.05	107 ± 12[c]	0.67 ± 0.05[c]	72 ± 6[b]	0.50 ± 0.05[b]
8	112 ± 17	0.58 ± 0.05	96 ± 16[b]	0.59 ± 0.05[c]	73 ± 7[b]	0.66 ± 0.03[e]
9	113 ± 13	0.57 ± 0.05	99 ± 7[b]	0.65 ± 0.03[e]	60 ± 8[b]	0.49 ± 0.03[b]
10	95 ± 9	0.49 ± 0.05	97 ± 9[c]	0.55 ± 0.03[e]		
11	98 ± 10	0.46 ± 0.05	70 ± 8[b]	0.49 ± 0.03[c]	64 ± 11[b]	0.51 ± 0.03[c]
12	136 ± 13	0.52 ± 0.05	117 ± 13[b]	0.53 ± 0.03[c]	126 ± 11[c]	0.51 ± 0.05[c]
Mean ± SEM	114 ± 4	0.59 ± 0.02	90 ± 4[f] (vs 114 ± 4)	0.57 ± 0.02[d] (vs 0.59 ± 0.02)	75 ± 5[f] (vs 116 ± 3)	0.55 ± 0.01[d] (vs 0.59 ± 0.02)

[a] All results are means ± SD (n = 7).
[b] Significantly lower than placebo ($P < 0.03$).
[c] Not significantly different from placebo ($P < 0.05$).
[d] Low-dose and high-dose mevinolin not significantly different from placebo by paired *t*-test.
[e] Significantly higher than placebo ($P < 0.03$).
[f] Low-dose and high-dose mevinolin significantly lower than placebo at $P < 0.001$ by paired *t*-test (patients 6 and 10, respectively, were omitted from placebo period).

TABLE 7. Effects of mevinolin on LDL-apoB kinetic parameters

Patients	Placebo			Low-Dose Mevinolin			High-Dose Mevinolin		
	Pool	FCR (U/P)	Transport	Pool	FCR (U/P)	Transport	Pool	FCR (U/P)	Transport
	mg	*pool/d*	*mg/kg-d*	*mg*	*pool/d*	*mg/kg-d*	*mg*	*pool/d*	*mg/kg-d*
1	2821	0.25 (0.24)	10.2	2194	0.24 (0.27)	9.1	2063	0.26 (0.25)	7.8
2	4556	0.24 (0.24)	14.6	2498	0.28[a] (0.29)	9.3	2531	0.26 (0.25)	7.8
3	4460	0.27 (0.25)	14.3	3411	0.28 (0.28)	11.3	2211	0.33[a] (0.33)	8.7
4	3776	0.28 (0.28)	12.6	2538	0.28 (0.29)	8.5	2443	0.30[a] (0.28)	8.7
5	3453	0.29 (0.31)	12.6	2517	0.33[a] (0.33)	10.4	2517	0.28 (0.26)	8.9
6	4475	0.22 (0.24)	11.2				2614	0.26[a] (0.26)	7.7
7	3728	0.26 (0.24)	13.0	3562	0.18[b] (0.19)	8.6	2397	0.24[b] (0.26)	7.7
8	5168	0.27 (0.26)	13.6	4429	0.33[a] (0.30)	14.3	3368	0.28 (0.29)	9.2
9	2704	0.23 (0.22)	10.6	2369	0.25[a] (0.27)	10.1	1435	0.33[a] (0.33)	8.1
10	2955	0.22 (0.23)	10.8	3017	0.35[a] (0.33)	17.5			
11	3174	0.20 (0.21)	8.8	2267	0.25[a] (0.27)	7.9	2073	0.27[a] (0.24)	7.8
12	5651	0.23 (0.22)	12.8	4861	0.18[b] (0.17)	8.6	5235	0.19[b] (0.19)	9.8
Mean ± SE (vs placebo)	3910 ± 275	0.25 ± 0.01	12.1 ± 0.5	3060 ± 274[c] (vs 3895 ± 283)	0.27 ± 0.02 (vs 0.25 ± 0.01)	10.5 ± 0.9[d] (vs 12.1 ± 0.5)	2444 ± 335[c] (vs 3997 ± 273)	0.27 ± 0.01 (vs 0.25 ± 0.01)	8.5 ± 0.2[d] (vs 12.1 ± 0.5)

[a] FCR for LDL-apoB higher than placebo by Fisher F test (26) at $P < 0.05$.
[b] FCR for LDL-apoB lower than placebo by Fisher F test (26) at $P < 0.05$.
[c] Pool size of LDL-apoB lower than placebo by paired *t*-test ($P < 0.05$) (placebo n = 11; patients 6 and 10 omitted for two doses of mevinolin).
[d] Transport rate of LDL-apoB lower than placebo by paired *t*-test ($P < 0.01$) (placebo n = 11; patient 10 omitted).

terolemia. Major epidemiological studies (10, 11) indicate that patients with moderate hypercholesterolemia are at increased risk for CHD. The recent report of the Lipid Research Clinic (LRC) Coronary Primary Prevention Trial (CPPT) (30, 31) provides strong evidence that reduction of LDL levels in such patients will significantly reduce the risk for CHD. However, bile acid sequestrants, as employed in the CPPT, are poorly accepted by many patients, and better tolerated drugs for cholesterol lowering are needed. Mevinolin may be such a drug. It is effective in relatively low doses and, so far, significant side effects have not been observed.

Causes of primary moderate hypercholesterolemia

The genetics of primary moderate hypercholesterolemia are not well understood. Goldstein et al. (32, 33) used the term "polygenic" hypercholesterolemia for elevated concentrations of LDL-C that do not originate from a single mutant gene, as occurs with familial hypercholesterolemia; instead, abnormally high levels of LDL apparently result from the interaction of multiple genetic and perhaps environmental factors. Epidemiological surveys suggest that this category of hypercholesterolemia is ten to fifteen times more common than familial hypercholesterolemia (29, 32, 33). Polygenic hypercholesterolemia can be distinguished from familial hypercholesterolemia in two ways (34): *a*) hypercholesterolemia is present in no more than 10% of first-degree relatives, in contrast to 50% in heterozygous familial hypercholesterolemia, and *b*) tendon xanothomata are absent in polygenic hypercholesterolemia. The patients of this study had polygenic hypercholesterolemia by these criteria. Therefore, multiple genetic factors may have contributed to their hypercholesterolemia. However, it is possible that a single metabolic defect was a predominant factor in some or most patients. This is to say, they may have had a monogenic disorder producing only moderate hypercholesterolemia in the propositus, and failure to detect a monogenic mode of inheritance may have been the result of incomplete penetrance of the genetic defect. The latter is possible because the defect need not cause as severe an elevation of LDL levels as in familial hypercholesterolemia, and affected family members may have had only mild increases in LDL that are not classified as frank hypercholesterolemia.

We can consider the possible metabolic defects responsible for hypercholesterolemia in our current patients. To facilitate this discussion, the kinetic parameters of LDL metabolism in these patients will be compared with those obtained previously in our laboratory for 11 normal men of similar age and for 10 heterozygotes for familial hypercholesterolemia (**Table 8**). All patients were studied by

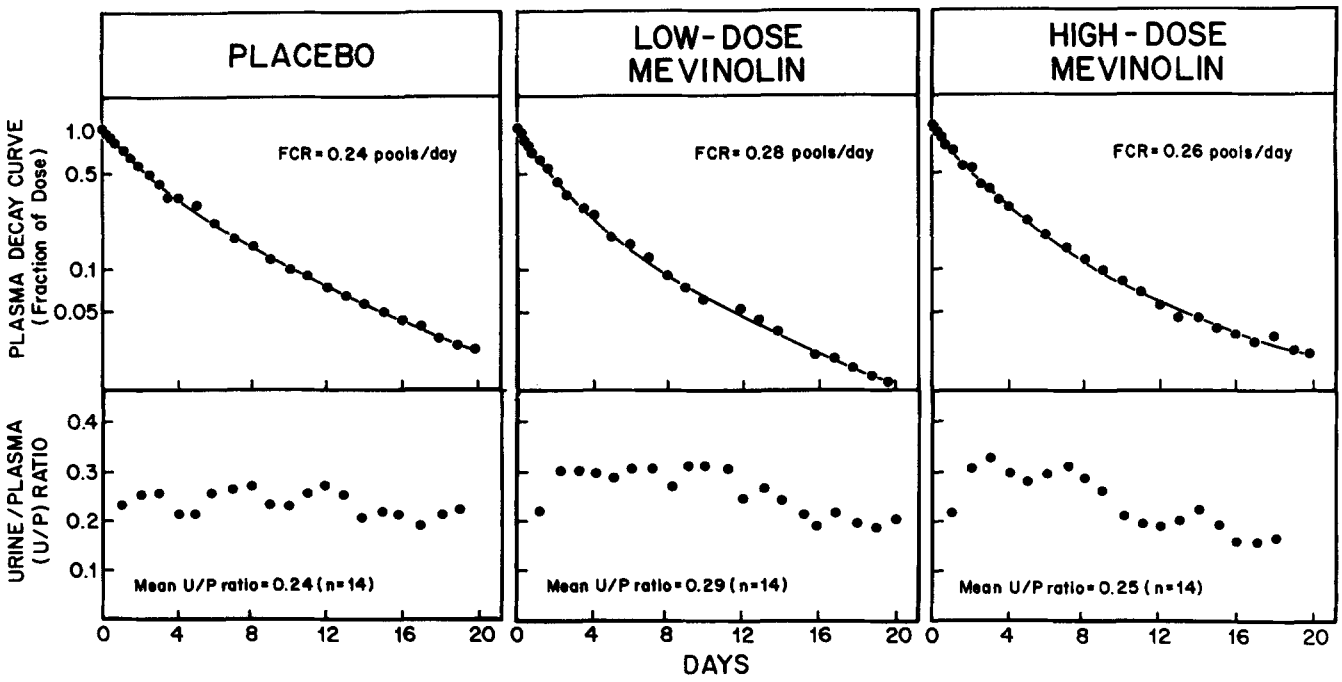

Fig. 1. Comparison of plasma radioactivity decay curve and urine/plasma (U/P) ratio for three periods: placebo, low-dose mevinolin, and high-dose mevinolin for patient 2. The mean U/P ratio was calculated from the first 14 days. Near the end of each period, U/P ratios tended to fall, and after 14 days they were not used in the calculation.

the same techniques (7, 35). The normal men had mean levels of total cholesterol and LDL-C near those reported for normal American men in the Lipid Research Clinic population study (29).

One cause for moderate elevations of LDL levels could be a defective clearance of LDL. Indeed, the mean FCR for LDL-apoB in our current patients (0.25 ± 0.01 pools/day) was significantly lower than that of the normocholesterolemic men of similar age (0.31 ± 0.01 pools/day). This difference undoubtedly contributed to higher LDL levels in the affected patients. Furthermore, the reduced FCR in these patients was similar to that noted in heterozygotes for familial hypercholesterolemia (0.27 ± 0.02 pools/day). This raises the possibility that the decrease in fractional clearance of LDL-apoB in patients with primary moderate hypercholesterolemia was related

TABLE 8. LDL-apoB kinetic parameters of hypercholesterolemic patients compared to normal subjects and patients with familial hypercholesterolemia

Group	Number of Patients	Plasma Levels			LDL-B	
		TC	LDL-C	LDL-apoB	FCR	Transport
		mg/dl ± SEM			*pools/d ± SEM*	*mg/kg-d ± SEM*
"Polygenic" hypercholesterolemia[a]	12	263 ± 8[d]	197 ± 7[d]	114 ± 4[d]	0.25 ± 0.01[e]	12.2 ± 0.6
Familial hypercholesterolemia[b]	10	358 ± 25	304 ± 28	208 ± 18	0.27 ± 0.02	19.4 ± 1.5[f]
Normal men[c]	11	202 ± 9[g]	136 ± 5[g]	81 ± 3[g]	0.31 ± 0.01	11.3 ± 1.6

[a] Patients with moderate hypercholesterolemia in the current study.
[b] Ten patients with classical heterozygous familial hypercholesterolemia, five of each sex. Ages ranged from 32 to 55 yr. Results in five have been presented previously (7). LDL-apoB turnovers were performed by the same methods as in the current study.
[c] Eleven normolipidemic control men; ages ranged from 38 to 68 yr (mean = 54 ± 9(SD) yr). LDL-apoB turnovers were performed by the same methods as in the current study. Results have been published previously (35).
[d] Significantly lower than familial hypercholesterolemia, but higher than normal ($P < 0.05$).
[e] Significantly lower than normal ($P < 0.05$).
[f] Significantly higher than two other groups ($P < 0.05$).
[g] Significantly lower than two other groups ($P < 0.05$).

to a decrease in LDL receptor activity. If so, a reduced receptor activity may be the result of defective regulation of LDL receptor synthesis; it presumably cannot be an abnormality in the primary structure of LDL receptors, which by definition is the defect in familial hypercholesterolemia (36). A defect in the regulation of receptor synthesis could be secondary to an abnormality in cholesterol metabolism because synthetic mechanisms for both cholesterol and LDL receptors are closely linked (7, 9). A reduction in LDL receptor activity, however, is not the only possible mechanism for a low FCR for LDL-apoB. For example, a decreased fractional clearance of LDL also could have been due to an abnormality in the apoB molecule (or LDL structure) that reduces the affinity of LDL for receptors.

Another factor that can raise LDL-C levels is increased production of LDL. Previous studies have shown that variability of LDL-C concentrations over the normal range, from low to high, is determined in part by production rates of LDL-apoB (37). Also, in familial hypercholesterolemia, production rates of LDL-apoB are distinctly elevated; in the heterozygote patients shown in Table 8, production rates of LDL-apoB averaged 19.4 ± 1.5 mg/kg-day versus 11.3 ± 1.6 mg/kg-day for normal men. Recent evidence (38, 39) suggests that overproduction of LDL-apoB in familial hypercholesterolemia is due to decreased hepatic uptake of VLDL remnants secondary to a reduced number of LDL receptors; this is because VLDL remnants can also be removed by LDL receptors. Consequently, more VLDL remnants are converted to LDL. It might have been anticipated that most of our patients with moderate hypercholesterolemia likewise would have had an increased production of LDL-apoB, especially if their underlying defect was a reduced activity of LDL receptors. Surprisingly, this was not the case. Their production rate for LDL-apoB averaged 12.1 ± 0.6 mg/kg-day which was not significantly higher than that of the normal men (11.3 ± 1.6 mg/kg-day). The most obvious explanation for this finding is that the LDL in these patients had a reduced affinity for the LDL receptor; the lack of overproduction of LDL-apoB suggests that VLDL remnants were cleared normally by LDL receptors. Other explanations, however, are possible, as will be discussed below.

Effects of mevinolin

Mevinolin is highly effective in treatment of heterozygous familial hypercholesterolemia (7, 8). Patients with this disorder have one normal gene for the LDL receptor, but one defective gene is also present that manufactures a nonfunctioning receptor. The good response to mevinolin can be explained by enhanced synthesis of LDL receptors encoded by the single normal gene. The drug inhibits the synthesis of cholesterol which triggers an increase in LDL receptor synthesis (7, 9). In familial hypercholesterolemia, the response linking decreased cholesterol synthesis and increased receptor synthesis (for the one normal gene) is fully intact. If primary moderate hypercholesterolemia is characterized by a defect in this linkage, such that a decrease in cholesterol synthesis does not evoke an increase in receptor synthesis, mevinolin might not be as effective in raising the activity of LDL receptors as in heterozygous familial hypercholesterolemia. On the other hand, if the defect lies in the affinity of LDL for its receptor, mevinolin should increase the synthesis of LDL receptors, enhance the clearance of VLDL remnants, lower the production of LDL-apoB, but have a lesser effect on fractional clearance of LDL. These possibilities prompted the current investigation; its primary aim was to determine whether mevinolin is also highly effective in hypercholesterolemic patients without familial hypercholesterolemia. A relatively low dose of the drug (10 mg twice daily) as well as a higher dose was tried, because of the previous report that 6.5 mg of mevinolin twice daily produced a maximum lowering of LDL in normal subjects (3). We therefore asked whether a similar low dose is maximally effective in patients with primary moderate hypercholesterolemia.

Plasma lipids and lipoproteins

In our patients, mevinolin therapy caused significant reductions of plasma levels of both total cholesterol and LDL-C at doses of 10 mg and 20 mg twice daily. Although there was not a statistically significant difference between the two doses for most plasma components, the overall response appeared better for the higher dose. The average decrease in LDL-C levels of 31% on high-dose therapy was similar to that reported previously for familial hypercholesterolemia heterozygotes given a similar dose (4–8) and for normal subjects at a lower dose (3). This reduction in LDL-C levels occurred without a detectable change in LDL composition as revealed by constancy of LDL-apoB/LDL-C ratios (Table 6).

Of particular interest, the levels of HDL-C were raised significantly by high-dose mevinolin ($P < 0.001$). On placebo, most patients had levels below the 50th percentile for their age and sex (mean = 40 ± 4 mg/dl), but on high-dose mevinolin, the mean level rose to the range of the 50th percentile or above (29). A rise in HDL-C levels on mevinolin was not reported previously in normal subjects (3), but neither were their levels low before treatment. This unexpected rise in HDL-C concentrations in patients with moderate hypercholesterolemia is in contrast to reductions in plasma HDL-C observed for other cholesterol-lowering drugs, e.g., neomycin (40) and probucol (41); it should be noted, however, that small increases in HDL-C levels were reported for patients treated with cholestyramine in the Coronary Primary Prevention Trial (30, 31).

Kinetics of plasma LDL-apoB

A surprising finding in our current patients was that mevinolin did not dramatically increase the FCR for LDL-apoB for most patients on either dose. An increase might have been expected to be the major response, because mevinolin is thought to stimulate the synthesis of LDL receptors. In accord with this concept, Bilheimer et al. (7) observed that mevinolin consistently enhanced FCRs for LDL-apoB in patients with heterozygous familial hypercholesterolemia, and this change was primarily responsible for their LDL lowering. Therefore we might consider the reasons for the apparent difference in kinetic responses to mevinolin between these two forms of hypercholesterolemia.

First, most of our patients with primary moderate hypercholesterolemia *did* have small but significant increases in FCR for LDL-apoB on one or both doses of mevinolin. Therefore, it seems likely that mevinolin *did* raise LDL receptor activity, at least to some extent. Still, in these patients, the increase in receptor activity could have been relatively small; if so, they might have carried a regulatory defect that prevented the expected increase in receptor synthesis in response to an inhibition in cholesterol synthesis. If receptor activity was not enhanced by mevinolin, the fall in LDL levels on the drug would have to be explained by a decrease in production of VLDL or IDL, the precursors of LDL. A reduced synthesis of these precursor lipoproteins is compatible with the observed fall in production rates of LDL on mevinolin therapy. To detect such a change it would be necessary to carry out turnover studies of VLDL simultaneously with those of LDL.

Second, the relatively small increases in FCRs for LDL-apoB on mevinolin therapy could have been due to enhanced uptake of VLDL remnants. Recent work indicates that VLDL remnants have a higher affinity for LDL receptors than LDL itself because of the presence of apoE on remnants (42, 43). An increase in receptor activity secondary to mevinolin therapy thus should preferentially remove VLDL remnants from the circulation. If so, enhanced binding of remnants to receptors could compete with uptake of LDL and thus prevent the expected rise in FCR for LDL-apoB. This mechanism also is consistent with the decrease in production rates of LDL-apoB noted during treatment with mevinolin. An enhanced uptake of VLDL remnants would leave fewer remnants to be converted to LDL, and thus the production rates of LDL should fall. Since mevinolin previously has been shown to raise the activity of LDL receptors (7, 9), we suggest that this mechanism better explains the decrease in LDL production rates than does an inhibition of VLDL secretion.

Third, the failure of mevinolin to enhance FCRs for LDL-apoB as much as expected could have been due in part to differences in affinities of subpopulations of LDL for LDL receptors. This inhomogeneity could occur in the absence of a true metabolic defect. Such a mechanism has been proposed by Witztum et al. (44) on the basis of their studies of LDL metabolism in cholestyramine-fed guinea pigs. Their work suggested that an increase in receptor activity associated with cholestyramine treatment promoted removal of some LDL more than others. If this is true, the poorer binding LDL should accumulate in plasma, and they would be mainly labeled in turnover studies. As a result, the fractional clearance of LDL would be relatively slow. This response could also be responsible for an apparent decrease in production rates of LDL. Such a mechanism for decreased LDL production is similar to that discussed above for VLDL remnants. In one case, an increased receptor activity would preferentially remove VLDL remnants, and in the other, a subfraction of LDL.

In the studies of Witztum et al. (44), cholestyramine treatment of guinea pigs actually altered the composition of LDL. The particles remaining in plasma of treated animals were smaller than normal and had abnormally high apoB/cholesterol ratios. A similar change has been noted for human LDL during cholestyramine therapy (45) and, consequently, Witztum et al. (44) have postulated that LDL present during sequestrant therapy may have a reduced affinity for LDL receptors. Of interest, we found no changes in LDL-apoB/cholesterol ratios during mevinolin therapy despite the claim that both drugs increase the activity of LDL receptors (46).

Finally, the mechanisms postulated above might explain why mevinolin therapy caused less of an increase in FCRs for LDL-apoB than expected, but they do not explain why the current patients seemingly differ from those with heterozygous familial hypercholesterolemia in their response to mevinolin. Thus, we must consider the possibility that in some patients with primary moderate hypercholesterolemia the whole LDL fraction has a reduced affinity for LDL receptors. If there was an abnormality in the apoB molecule so that it was a poor ligand for the LDL receptor, this could account for the failure of mevinolin to raise the FCR. We can provide no direct evidence for such a defect; however, the apoB molecule contains many sites at which amino acid substitutions might occur and thus alter its receptor binding properties (47–49).

Limitations of the study

The present investigation suffers from the limitations inherent in multicompartmental analysis of LDL kinetics. Estimations of production rates of LDL-apoB depend on the unproven assumption that LDL behaves kinetically as a homogeneous pool, which may not be true (45). Furthermore, the lack of a simultaneous study of VLDL metabolism further limits our ability to interpret the findings. Thus, we are uncertain of the reasons for the unexpectedly small rises in FCRs for LDL-apoB during mevinolin therapy, whether due to *a*) limited enhance-

ment of LDL receptor activity, *b*) competition for clearance of LDL by stimulated removal of VLDL remnants, *c*) preferential removal of a subpopulation of LDL having higher affinity for the LDL receptor, *d*) a genetic defect causing poor affinity of plasma LDL for LDL receptors, or *e*) a combination of these factors. In any case, the data are not in accord with simplistic concept that the fall in LDL levels on mevinolin therapy is due merely to enhanced receptor activity and increased clearance of whole plasma LDL. The results thus bring into sharper focus a variety of questions about mechanisms regulating LDL levels.

Regardless of the mechanism by which mevinolin lowers LDL levels in patients with primary moderate hypercholesterolemia, our results indicate that the degree of decrease in LDL levels was similar to reductions reported for normal subjects (3) and for heterozygotes for familial hypercholesterolemia (4–8). Thus, mevinolin seemingly offers equal promise for the treatment of patients with this most prevalent form of hypercholesterolemia.

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