Intestinal HMG-CoA reductase activity is low in hypercholesterolemic patients and is further decreased with lovastatin therapy

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Abstract Significant cholesterol synthesis occurs in gut mucosa of animals and humans. However, the role of gut synthesis in hypercholesterolemia and the effect of drugs on this function have not been defined. We obtained jejunal biopsies and bile samples from 21 Type II hypercholesterolemic subjects (mean serum cholesterol = 331 mg/dl) on a low fat diet after an overnight fast. Whole gut mucosal homogenate was assayed for activity of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the rate-determining enzyme of cholesterol synthesis. Mean reductase activity (pmol/mg per min) was 5.5 ± 1.0 (n = 21) in hypercholesterolemic subjects versus 11.3 ± 1.0 in 52 normal subjects ($P < 0.01$). This is consistent with the hypothesis that the primary defect in these patients is not excessive cholesterol synthesis but decreased low density lipoprotein (LDL) clearance. It implies that high LDL levels down-regulate gut reductase activity. After treatment of 7 patients with lovastatin (40–80 mg/day for at least 6–13 weeks), gut reductase activity decreased from 7.7 ± 2.6 to 3.6 ± 0.5 ($P < 0.05$), in biopsies obtained 12 hr after the last drug dose. Inhibition of reductase activity by this drug was detected 12 hr after a dose, and the enzyme was not measurably induced during 6–13 weeks of therapy. In keeping with the decrease in serum cholesterol (332→239 mg/dl) and mucosal reductase activity during lovastatin therapy, mean gallbladder bile cholesterol saturation index also decreased (1.045 ± 0.112 vs. 0.883 ± 0.109, n = 7). During treatment with cholestyramine (16–24 g/day for ≥8 weeks), gut reductase tended to increase (4.7 ± 1.0 vs. 10.4 ± 3.9 pmol/mg per min), consistent with its effect on cholesterol synthesis in animal studies, but the changes did not reach statistical significance. We conclude that intestinal cholesterol synthesis is low in Type II hypercholesterolemic patients and is modified by lovastatin. — **Freeman, M. L., W. F. Prigge, D. B. Hunninghake, W. C. Duane, and R. L. Gebhard.** Intestinal HMG-CoA reductase activity is low in hypercholesterolemic patients and is further decreased with lovastatin therapy. *J. Lipid Res.* 1988. **29:** 839–845.

Supplementary key words intestinal cholesterol synthesis • bile lithogenic index • mevinolin • cholestyramine

In humans, de novo synthesis appears to contribute two or three times more cholesterol to the total body pool than does absorption of dietary cholesterol (1), depending upon dietary intake. Mucosa of the gastrointestinal tract is responsible for cholesterol absorption and is an active site of cholesterogenesis (2, 3). Measurements of cholesterol synthesis by individual organs show that intestine is second only to liver in most species, and may be the predominant organ in other species (4). Synthetic activity in human intestinal mucosa may be comparable to that of liver (5). In gut mucosa, as in other tissues, the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-determining step in cholesterol synthesis and its activity correlates with synthetic rate (6, 7). We have previously shown that significant activity of HMG-CoA reductase is present throughout the human gut (5). Intestinal cholesterol synthesis can be regulated by luminal factors such as cholesterol and bile salts, and may also be subject to feedback regulation by circulating low density lipoprotein (LDL) cholesterol (8–11).

The importance of total body cholesterol synthetic rate in hypercholesterolemic patients is unclear; most studies of familial Type II hypercholesterolemics (both heterozygous and homozygous) have shown normal or decreased total synthesis despite dramatic elevations in circulating LDL levels (1). One study has shown decreased activity of HMG-CoA reductase in leukocytes (12), but little information is available on cholesterol synthetic rates or HMG-CoA reductase activity in either liver or intestine.

In the current study we have measured HMG-CoA reductase activity in intestinal mucosa of hypercholesterolemic patients and compared it to activity in mucosa of normal subjects. We studied the effect of two important hypolipidemic agents. The drug lovastatin (formerly

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; LDL, low density lipoproteins

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mevinolin) is a competitive inhibitor of HMG-CoA reductase (13). Inhibition of this enzyme results in an augmentation of LDL catabolism and modest reduction or no effect on total body cholesterol synthesis (14). In contrast, the drug cholestyramine binds bile acids and increases their excretion, resulting in enhanced conversion of cholesterol to bile acids with induction of LDL receptor activity. Most animal studies of cholestyramine administration show a resultant increase in total body and hepatic cholesterol synthesis, but variable effects on intestinal cholesterol synthesis (15–19). Therefore, we have studied the effect of these two drugs on intestinal HMG-CoA reductase activity in human hypercholesterolemic subjects. Additionally, since lovastatin might alter biliary cholesterol output and bile lithogenicity, we have studied its effect on bile composition.

MATERIALS AND METHODS

Materials

[3-^{14}C]HMG-CoA and DL-[5-^{3}H]mevalonic acid were obtained from New England Nuclear (Boston, MA). Cofactors and other chemicals were obtained from Sigma Chemical (St. Louis, MO). Lovastatin (mevinolin) was provided by Merck, Sharp & Dohme (Rahway, NJ) as part of an investigational review board-approved study of its lipid-lowering efficacy.

Methods

Hypercholesterolemic patients with markedly elevated LDL cholesterol (Type IIa) or LDL and VLDL cholesterol (Type IIb), as determined by ultracentrifugation, were recruited at the University of Minnesota Lipid Research Clinic and the Minneapolis VA Medical Center. Sixteen patients appeared to have familial hypercholesterolemia (as indicated by concomitant xanthomata or positive family history of early myocardial infarction or cholesterol >350 mg/dl) and five did not. All hyperlipemic patients had been maintained on a low-saturated fat, low-cholesterol American Heart Association Phase II diet for 8–10 weeks prior to study. Adherence to diet was evaluated by a dietitian using food diaries, diet recall, and food frequency reports. Noncompliant subjects were excluded. After overnight fast, a Carey Capsule was passed into the jejunum between 8:00–10:00 AM. Positioning of the capsule was evaluated by X-ray or by placement at least 70 cm from the incisors and distal to biliary drainage at pH 7. In separate studies, this technique yielded histologic jejunal mucosa in 17 of 18 biopsies. Cholecystokinin octapeptide obtained from Squibb (0.01 μg/kg) was administered intravenously, and gallbladder bile was collected on ice. Finally, a jejunal biopsy was obtained and a buffered homogenate of washed whole mucosa was immediately prepared. Mucosal biopsies averaged 1–2 mg of total protein. Some patients underwent mucosal biopsy only, without cholecystokinin administration, and some patients had bile sampling only. For patients initiating drug therapy, studies were performed on two occasions. *1*) Control: subjects were on diet only, with no lipid-altering medications (n = 21). *2*) Treatment: after at least 6 weeks of therapy with either cholestyramine (12–24 g/day) or lovastatin (40–80 mg/day) (n = 16), biopsies were taken at least 12 hr after the last dose of medication. For treatment studies, only paired analysis was performed; patients having inadequate biopsy samples in one period were excluded from HMG-CoA reductase analysis, and patients with inadequate bile samples in one period were excluded from lithogenicity analysis.

The control values for jejunal HMG-CoA reductase activity have been reported previously (15). They were obtained from a group of 52 normolipemic individuals with jejunal mucosa obtained at surgery or by X-ray confirmed placement of biopsy capsule. These subjects were similarly fasted prior to mucosal biopsy. Most were taking no drugs and had normal jejunal histology as part of a diarrhea evaluation (functional bowel disease was common) or were undergoing elective ulcer surgery. Biopsies were also obtained from three contemporary controls using methodology identical to that used for hypercholesterolemics.

Enzyme assay. HMG-CoA reductase activity was measured in whole mucosal homogenates by a modification of the method of Shapiro et al. (20) as previously described (5). Tissue fragments were homogenized in a Dounce homogenizer with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.2 M sucrose and 30 mM ethylenediaminetetraacetic acid. Reductase assays were done in duplicate and pairs generally agreed within 10%. Each assay for HMG-CoA reductase contained 0.1–0.2 mg of mucosal protein and cofactors in a volume of 0.16 ml. Final cofactor concentrations were 25.5 mM glucose-6-phosphate, 1 U/ml of glucose-6-phosphate dehydrogenase, 3 mM NADP, 15 mM dithiothreitol, and 70 mM NaCl. Samples were preincubated at 37°C for 5 min prior to the addition of [^{14}C]HMG-CoA (31 mM; sp act 12 nCi/nmol). Incubations were terminated after 15 min by addition of 0.025 ml of concentrated HCl followed by DL-[5-^{3}H]mevalonic acid [2 pmol (20,000 dpm/pmol)]. Mevalonate was separated from HMG-CoA by silica gel G chromatography in benzene–acetone 1:1 and radioactivity was measured by liquid scintillation counting. Protein was determined by the method of Lowry et al. (21). Reductase activity was expressed as picomoles of mevalonate formed per milligram of whole mucosal protein per minute. HMG-CoA reductase can exist in both active and inactive forms (22). The conditions of this assay have been considered to measure total activity of HMG-CoA reductase,

but we have previously provided evidence that most enzyme is present in an active form in intestine (5, 23). **Table 1** shows good reproducibility of mucosal reductase values when measurements were made in individual subjects at different times or different locations in the upper gut.

For some experiments, murine hepatic microsomes were prepared during the mid-dark period by standard techniques (6). Apparent HMG-CoA reductase activity in 0.1 mg of microsomal protein was then measured in the presence and absence of added patient gut mucosal homogenate. These experiments were performed in order to ascertain whether competitive inhibitor activity for reductase (i.e., lovastatin) was present in patient intestinal mucosa.

Bile lithogenicity. Upon completion of bile sampling, the chilled bile was combined with an equal volume of methanol. Duplicate aliquots of the bile–methanol mixture were immediately combined with equal volumes of water and extracted with chloroform–methanol 8:3. Phospholipid was determined on aliquots of the chloroform phase using standard techniques based on spectrophotometric assay of inorganic phosphorus (24). Two more duplicate aliquots of bile–methanol mixture were made to 70% methanol and extracted with hexane. Cholesterol was determined on the hexane phase by gas–liquid chromatography of the trimethylsilyl ether using 5α-cholestane as an internal standard. The aqueous methanol phase of this extraction was analyzed for total bile salt using an automated procedure based on Talalay's enzymatic assay (25). Cholesterol saturation index of bile was calculated from these data using the equations of Carey and Small (26) assuming a total solid concentration of 10 g/dl. Our procedures for analyzing biliary lipids have been described in more detail in a previous publication (27).

Statistical methods. Values given are means ± SEM. Statistics were done using Student's two-tailed *t*-test or Wilcoxon's matched-pairs signed-ranks test for paired variants.

TABLE 1. Reproducibility of mucosal HMG-CoA reductase activity

Subject	Date	Tissue Pieces	Reductase Values[a]
1 (Normal)	6/17	3, jejunum	19.5, 19.2, 21.7
2 (Low cholesterol)	7/20	2, jejunum	35.6, 37.7
3 (Normal)	12/7	2, duodenum	11.9, 13.4
(Normal)	12/10	1, jejunum	14.2
4 (Type IV HLP)[b]	12/10	1, jejunum	11.3
(Type IV HLP)	3/24	1, jejunum	11.5

HMG-CoA reductase was measured in separate pieces of jejunal or duodenal mucosa obtained at different levels of the intestinal tract or on different dates. Reproducibility of values is demonstrated.

[a] Values are given as pmol/mg protein · min.

[b] Type IV hyperlipoproteinemia.

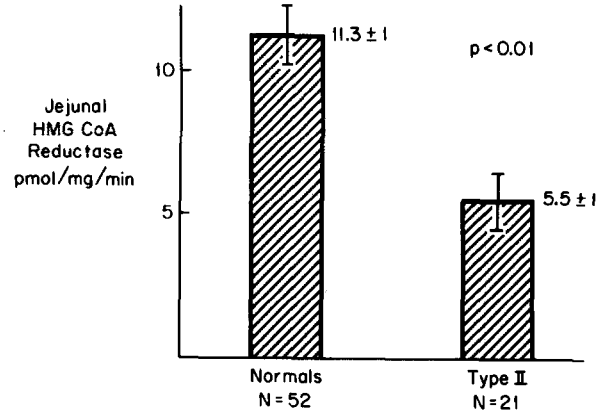

Fig. 1. Total HMG-CoA reductase activity in biopsies of gut mucosa from normal subjects and hypercholesterolemic subjects.

RESULTS

Fig. 1 shows that mean jejunal total HMG-CoA reductase activity in 21 Type II hypercholesterolemic subjects was significantly lower than activity previously found in 52 control patients studied in our laboratory (5). Total HMG-CoA reductase activity measured in mucosa of Type II hypercholesterolemic subjects is seen to be one-half that of normal subjects. The historic control group consisted of 50 males and 2 females with an average age of 52.0 ± 2.1 years and average total blood cholesterol level of 170 ± 6 mg/dl. This compared to an average age of 51.5 ± 2.3 years for the 19 male and 2 female hyperlipemic subjects who had total blood cholesterol levels of 336 ± 13 mg/dl and triglyceride levels of 172 ± 17 mg/dl. Only 5 study patients had triglyceride levels greater than 230 mg/dl and none had a level greater than 293 mg/dl.

In order to exclude an effect of variable biopsy technique between historic controls and hypercholesterolemic patients, three additional normolipemic control subjects underwent mucosal biopsy under identical circumstances. Values of 10.6, 13.1, and 11.2 pmol/mg per min were comparable to the historic normal values of 11.3 ± 1.0, demonstrating no effect of cholecystokinin, bile flow, or capsule biopsy technique. In addition, jejunal mucosa from four patients with total cholesterol greater than 300 mg/dl and triglycerides less than 200 mg/dl, obtained without cholecystokinin octapeptide, gave low values of 2.2, 1.5, 2.3, and 2.7 pmol/mg per min.

Seven hyperlipemic patients underwent 6 to 13 weeks of treatment with 40–80 mg of lovastatin daily. These subjects' cholesterol levels fell from a mean of 322 ± 30 mg/dl to a mean of 239 ± 11 mg/dl. In paired biopsies from the seven patients, total measured gut HMG-CoA reductase activity was decreased by 55% (as shown in **Fig. 2**). This demonstrates in vivo that the drug lovastatin does inhibit the HMG-CoA reductase enzyme and that this effect per-

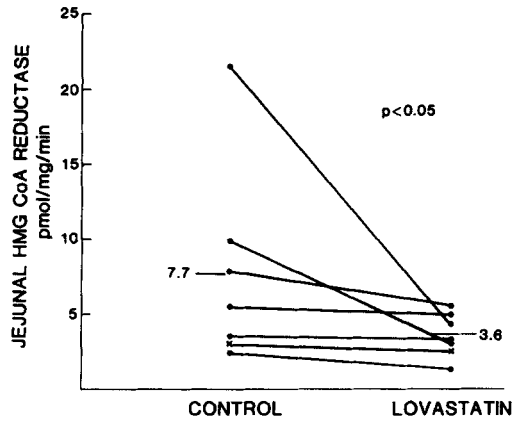

Fig. 2. Effect of lovastatin therapy on total gut reductase activity. The same patients were studied before and after therapy. Patient "x—x" was treated with probucol for a 3-month interval, but initial biopsy was taken on diet therapy only and the final biopsy was taken at the end of 6 weeks of lovastatin only.

sists during long-term continuous treatment, for as long as 12 hr after the last dose of drug. Statistical significance at the $P < 0.05$ level was achieved by the Wilcoxon matched-pairs signed-rank test (utilized because of non-normality of the data distribution). There was, however, no apparent correlation between degree of lowering of intestinal reductase activity and lowering of serum cholesterol levels. There was no apparent relationship between family history of hypercholesterolemia (four patients positive, three negative) and change in reductase activity, although the greatest change (21.7 pmol/mg per min to 4.4 pmol/mg per min) occurred in a patient with negative family history.

To determine whether drug inhibitory activity persisted in gut mucosa at the time of biopsy, mucosal homogenates from five lovastatin-treated patients were added to assay tubes with rat hepatic microsomes and reductase activity in these microsomes was measured. Whole homogenates from patients prior to lovastatin therapy had either no effect on rat microsomal reductase activity or increased the measured activity. Homogenates from three treated patients inhibited activity by 20–64%, while two treated patients' mucosa increased measured reductase activity. Gut mucosal reductase of the three patients with evidence for presence of inhibitory activity showed changes of 9.8 to 3.0, 5.5 to 4.9, and 7.9 to 5.5 pmol/mg per min before and during therapy. The two patients without apparent inhibitory activity in the mucosal homogenate had changes of 21.7 to 4.4 and 2.3 to 1.4 pmol/mg per min.

Fig. 3 shows data for biliary saturation index of seven patients treated with 40–80 mg of lovastatin per day. Along with the dramatic decrease in blood cholesterol and the decrease in gut reductase activity, mean gallbladder bile saturation index was significantly reduced following treatment (1.045 ± 0.112 vs. 0.883 ± 0.109, n = 7, $P < 0.001$ by paired analysis). **Table 2** shows the molar percent of bile salt, lecithin, and cholesterol for these seven subjects before and after lovastatin therapy. It is apparent that a decrease in molar percent of gallbladder bile cholesterol was the most consistent finding during the therapy.

Fig. 4 shows that after 8 weeks of treatment with cholestyramine in nine hypercholesterolemic patients, mean total gut HMG-CoA reductase activity increased. However, there was substantial variability in this response. By paired *t*-test, the increase in mean activity reached a *P* value of 0.1. There was no apparent correlation between change in reductase activity and change in serum cholesterol. It should be noted that mean cholesterol level did not change substantially beyond the effect of diet in these nine patients, reflecting the relatively modest cholesterol-lowering effect of this drug. Initial cholesterol levels were 354 ± 16 mg/dl, while post treatment levels were 344 ± 39 mg/dl.

DISCUSSION

Total activity of intestinal HMG-CoA reductase in Type II hypercholesterolemic subjects in our study was less than one-half that of normal subjects. This can reasonably be assumed to reflect a decreased rate of intestinal cholesterol synthesis. Information pertaining to the diet of the control group was not obtained. However, the low-cholesterol, low-saturated fat diet in the hypercho-

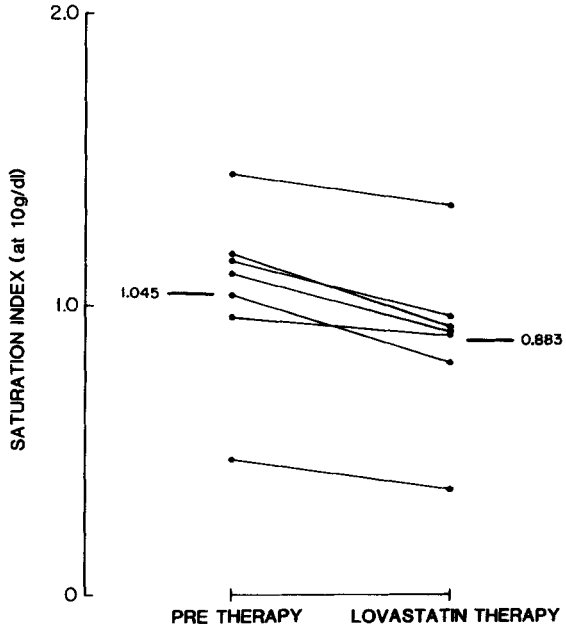

Fig. 3. Effect of lovastatin on bile lithogenic index before and after 6 to 13 weeks of therapy. A significant reduction in lithogenic index was seen ($P < 0.001$).

TABLE 2. Gallbladder bile lipid composition

Subject	Molar Percent		
	Bile Salt	Lecithin	Cholesterol
1 7/9/85 (Control)	68.6	21.1	10.33
9/17/85 (Lovastatin)	68.7	21.6	9.72
2 6/21/85 (Control)	65.7	30.4	3.86
8/29/85 (Lovastatin)	75.3	22.2	2.54
3 6/14/85 (Control)	84.1	11.5	4.38
9/4/85 (Lovastatin)	77.0	17.6	5.50
4 6/14/85 (Control)	64.0	26.8	9.24
9/13/85 (Lovastatin)	63.6	28.7	7.76
5 8/15/85 (Control)	66.0	25.1	9.00
10/30/85 (Lovastatin)	60.6	31.8	7.59
6 10/17/85 (Control)	56.0	35.4	8.58
2/9/85 (Lovastatin)	64.6	28.9	6.49
7 10/22/85 (Control)	70.4	21.7	7.90
2/19/85 (Lovastatin)	76.4	18.0	5.68

Molar percent of gallbladder bile lipids from seven patients before and after lovastatin therapy. The most consistent change with treatment is reduction in molar percent cholesterol.

lesterolemic group would be expected to raise rather than lower intestinal reductase and total body cholesterol synthesis in these patients (19). Therefore, the uncontrolled variable of diet would actually minimize rather than maximize a difference. All subjects were fasted overnight prior to biopsy at 8:00–10:00 AM to avoid variations in circadian rhythm (28).

The observation that intestinal reductase activity, and therefore cholesterol synthesis, is low in hypercholesterolemics is consistent with data from other studies. In a review of previous studies, total body cholesterol synthesis in Type II hypercholesterolemics has been observed to be lower than in normals (1). Stacpoole et al. (12) found decreased apparent HMG-CoA reductase activity in freshly isolated leukocytes of patients with familial hypercholesterolemia. Similar to our results, they observed that this activity was one-half that of normal subjects. They reported that the difference in apparent activity was not due to phosphorylation–dephosphorylation status of the enzyme. These data collectively suggest that some degree of feedback regulation of cholesterol synthesis by high levels of LDL is taking place in several tissues of these patients.

At present, it is unclear whether cholesterol synthesis by gut mucosal cells is regulated by LDL cholesterol levels (via LDL receptors) as is the case for other tissues. Animal studies by Purdy and Field (10) provide evidence for plasma lipoprotein regulation of gut HMG CoA reductase, but Stange and Dietschy (11) failed to show a relationship between gut LDL uptake and cholesterol synthesis. Studies in our laboratory have suggested that mucosal cells do not normally possess large numbers of LDL receptors but are able to increase LDL sensitivity during periods of cholesterol deprivation (8). The data presented in this study would suggest that high circulating levels of LDL can inhibit mucosal cholesterol synthesis in hypercholesterolemics.

Our data from patients before and after drug therapy show that lovastatin inhibited apparent activity of intestinal HMG-CoA reductase at least 12 hr after the last dose of medication, providing additional evidence for its pharmacologic effect. This suppression of gut reductase activity persisted beyond 8 weeks of treatment. In rodent studies, lovastatin has been shown to rapidly increase the total quantity of hepatic HMG-CoA reductase enzyme in cells by inhibiting its degradation (29). The induction of total enzyme quantity in rodents is able to overcome the competitive inhibition and blunt the cholesterol-reducing effect of the drug. If similar induction of the enzyme occurs in man, it either does not occur in gut or it cannot overcome the net competitive inhibition of enzyme activity produced by lovastatin. The reduced apparent enzyme activity also suggests that some drug is still present in mucosa 12 hr after the last dose. This was confirmed in some, but not all, subjects by coassay with rat hepatic microsomes. Our finding of failure to induce measured enzyme activity in human intestine correlates with the observed persistence of the drug's efficacy in man, i.e., the hypocholesterolemic effect is seen during at least 3 months of therapy.

Lovastatin has striking effects on cholesterol metabolism and homeostasis. Secretion of cholesterol into bile

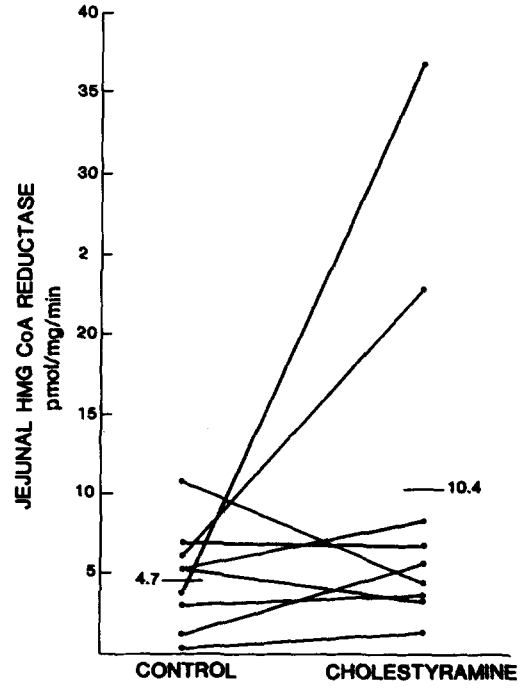

Fig. 4. Effect of cholestyramine on gut HMG-CoA reductase activity.

and conversion of cholesterol into bile acids influence the formation of cholesterol gallstones, and these parameters might be predicted to be altered by lovastatin. We found that the mean lithogenic index of gallbladder bile was reduced during lovastatin treatment. Molar percent of cholesterol in gallbladder bile fell in six of seven patients, without consistent effect on molar percent of bile salt or lecithin. This finding suggests that the risk of cholesterol gallstone formation is reduced during lovastatin therapy. The possibility that the drug might actually promote cholesterol gallstone dissolution warrants further investigation.

In our study, cholestyramine caused a variable increase in intestinal cholesterol synthesis, but this did not reach statistical significance. In rats, cholestyramine treatment has resulted in increased intestinal HMG-CoA reductase activity and rate of cholesterol synthesis in most studies to date (15–17). However, in two studies of swine and guinea pigs, respectively, ileal HMG-CoA reductase activity and cholesterol synthesis were not affected by cholestyramine feeding (18, 19). In our study, failure to show significant rise in intestinal reductase activity with cholestyramine may reflect species variability in capability of intestine to augment cholesterol synthesis, or it may be a result of the relatively mild cholesterol-lowering effect achieved in our patients.

Intestinal mucosa is an important organ of cholesterol synthesis. Analysis of intestinal HMG-CoA reductase activity by capsule biopsy affords the opportunity to study gut cholesterogenesis in humans and to determine the effect of drugs on this function. The manner in which gut cholesterol synthesis correlates with hepatic or total body cholesterol body synthesis remains to be investigated.

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