In Vivo Regulation of Human Mononuclear Leukocyte 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase

Studies in Normal Subjects

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Abstract

In vivo regulation of microsomal HMG CoA reductase activity was investigated in freshly isolated mononuclear leukocytes from 26 healthy adult males. Reductase activity exhibited a diurnal rhythm and decreased during fasting. Enzyme activity was also modulated in vivo by alterations in dietary and plasma cholesterol, suggesting the existence of an operative cholesterol feedback regulatory system. A single, high cholesterol meal decreased reductase activity within 2 h. In addition, rapid depletion of circulating cholesterol levels by plasmapheresis led to an approximately twofold elevation in enzyme activity within 90 min of treatment. Finally, reductase activity was inhibited by dichloroacetate, a compound known to lower plasma cholesterol in man and inhibit the human leukocyte enzyme in vitro. The regulatory mechanisms controlling HMG CoA reductase activity in the human mononuclear leukocyte in vivo thus are similar to those that modulate the mammalian liver enzyme in vivo. Assessment of mononuclear leukocyte reductase activity may provide insight into the in vivo regulation of human cholesterol metabolism.

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase)[1] (E.C. 1.1.1.34) catalyzes the rate-limiting step in cholesterol biosynthesis (1) and plays an important role in maintaining cholesterol homeostasis (2, 3). In normal animal tissues, HMG CoA reductase is regulated by a variety of physiological mechanisms that act to alter rates of enzyme synthesis and degradation (4–8) or enzyme catalytic efficiency (9–16). Animal studies have shown that HMG CoA reductase exhibits a diurnal variation in activity (1, 4, 17, 18), is inhibited by dietary cholesterol through a feedback regulatory mechanism (19, 20), and is decreased by fasting (1, 21, 22).

It has been difficult to assess the in vivo regulatory properties of human HMG CoA reductase for lack of an appropriate tissue from which repetitive samples may be made, or for lack of appropriate methodologies for measurement of enzyme activity in vivo. Several laboratories have estimated enzyme activity in biopsy tissues from liver (23, 24) or intestine (25, 26). This method, however, precludes repetitive sampling and hence prevents detailed study of in vivo enzyme regulatory properties within individuals. Other laboratories have examined rates of cholesterol synthesis from [^{14}C]acetate in human mononuclear leukocytes cultured for 48 h or longer in lipid-depleted medium or in medium supplemented with lipoprotein cholesterol (26–28). Similar experiments have also been conducted using cultured human fibroblasts (19, 29). While these studies have demonstrated important relationships between the availability of exogenous cholesterol and the regulation of endogenous cholesterolgenesis, they do not necessarily mirror conditions in vivo. Using a more direct approach, McNamara and co-workers (30) demonstrated increased cholesterol synthesis from acetate in mononuclear leukocytes following short-term administration of cholestyramine to normal volunteers, and Mistry et al. (31) showed suppression of mevalonate formation in mononuclear cells obtained from healthy subjects equilibrated on a high cholesterol diet. In none of these experiments, however, was enzyme activity measured directly. Furthermore, since only 50% of the acetate converted to mevalonate by HMG CoA reductase in the human mononuclear leukocyte is ultimately converted into cholesterol (32, 33), the relationship between HMG CoA reductase activity and these in vitro perturbations is also uncertain.

We recently developed a method for directly measuring microsomal HMG CoA reductase activity in freshly isolated human leukocytes (32), and have employed this technique to examine the regulation of the human mononuclear leukocyte enzyme in vivo. In this report we demonstrate that mononuclear leukocyte HMG CoA reductase exhibits a diurnal rhythm in activity, is inhibited by fasting and cholesterol feeding, is stimulated by rapid depletion of lipoprotein cholesterol due to plasmapheresis, and is inhibited by dichloroacetate, a compound that decreases plasma cholesterol levels in man (34, 35). The regulatory mechanisms controlling HMG CoA reductase activity in vivo in the human leukocyte, therefore, appear to be similar to those that modulate the mammalian liver enzyme.

Methods

Subjects. 26 healthy adult males (aged 22 to 66 yr) participated. All were within 15% of ideal body weight, based on Metropolitan Life Insurance Tables (36). 24 subjects had fasting lipid and lipoprotein levels within the 95th percentiles of the normal North American population, based on age, sex, and race (37). Two participants had mild hypercholesterolemia.

Experimental design. The protocol was approved by the Institutional Review Board of Shands Hospital, University of Florida. Subjects undergoing dietary or pharmacologic investigations were admitted to the

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1. *Abbreviations used in this paper:* DCA, sodium dichloroacetate; HMG CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; TBS, tris-buffered saline; TEDK, Tris, EDTA, dithiothreitol, and KCl.

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Clinical Research Center at Shands Hospital. Each received a constant diet isocaloric with his normal, outpatient regimen. Caloric distribution was: fat, 40% (polyunsaturated/saturated fat ratio, 1.0); carbohydrate, 45%; and protein, 15%. Daily cholesterol intake was 150 mg. Meals were divided into three calorically equivalent portions, plus a bedtime snack. The ratio of fat/carbohydrate/protein was maintained for the three main meals. After a period of dietary equilibration lasting 1–3 wk, subjects underwent 1–2 d of periodic blood sampling under one of the following protocols: (*a*) diurnal variation of mononuclear leukocyte microsomal HMG CoA reductase activity, (*b*) fasting up to 36 h, (*c*) consumption of a high cholesterol meal, (*d*) plasmapheresis (4L exchange) or (*e*) administration of a single dose of sodium dichloroacetate (DCA). The high cholesterol diet consisted of a single 8:00 a.m. feeding of four hard-boiled eggs (~ 1 g cholesterol) given in lieu of the individual's usual breakfast. DCA (35 mg/kg) was administered by mouth at 8:00 a.m.

Chemicals. Dithiothreitol, glucose-6-phosphate and NADP+ were from U. S. Biochemicals (Cleveland, OH). Glucose-6-phosphate dehydrogenase, EDTA, *Escherichia coli* alkaline phosphatase and Histopaque 1077 were from Sigma (St. Louis, MO). Percoll was from Pharmacia Fine Chemicals (Piscataway, NJ). RPMI 1640 and heat-inactivated fetal bovine serum were from Gibco Laboratories (Grand Island, NY). [3-^{14}C]HMG CoA (57 mCi/mmol) and [5-^{3}H]mevalonolactone (24 Ci/mmol) were from New England Nuclear (Boston, MA). Sodium dichloroacetate was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). All other materials were from previously listed sources (32, 38).

Solutions. Hanks' balanced salt solution contained 80 g/liter NaCl, 4 g/liter KCl, 10 g/liter glucose, 600 mg/liter KH_2PO_4, 475 mg/liter Na_2HPO_4 and 170 mg/liter phenol red (pH 7.4). Tris-buffered saline (TBS) contained 2.4 g/liter Tris (pH 7.5) and 29.1 g/liter NaCl. 55% isoosmotic Percoll contained 55 ml of Percoll, 0.48 g of NaCl and 45 ml of 0.15 M NaCl. TEDK buffer contained 50 mM Tris (pH 7.5), 1.0 mM EDTA, 5.0 mM dithiothreitol, and 70 mM KCl.

Isolation of peripheral blood mononuclear leukocytes. 40 ml blood samples were obtained using tourniquet pressure from an antecubital vein with a 19-gauge needle and a 60-ml syringe. After removing the needle from the syringe, the blood was discharged into a 50-ml conical, polystyrene, centrifuge tube containing 1.0 ml of 250 mM EDTA (pH 7.4) and inverted several times. Care was taken to empty the syringe over 5 to 10 s with a gentle stream of blood coursing along the side of the tube. This was done to minimize hemolysis, which may artifactually elevate leukocyte HMG CoA reductase activity. Within 10 min of blood drawing, the sample was diluted with an equal volume of room temperature Hanks' balanced salt solution. 20-ml aliquots of the diluted blood sample were added to 50 ml plastic, conical, centrifuge tubes, and underlayered with 20 ml of room temperature Histopaque 1077. Following centrifugation at 400 *g* for 40 min to sediment erythrocytes and granulocytes, the mononuclear leukocyte population ("buffy coat") was isolated, washed twice in Hanks' balanced salt solution, once in 0.87% NH_4Cl to lyse contaminating erythrocytes and once in TEDK buffer. The resulting leukocyte pellet was resuspended in 0.3 ml TEDK buffer and frozen in liquid N_2. This fraction typically contained 77±1.3% (±SEM; n = 38) lymphocytes and 23±1.2% (n = 38) monocytes, based on α-naphthylacetate esterase staining, using the method outlined in Sigma Technical Bulletin No. 90 (1983).

Isolation of enriched populations of lymphocytes and monocytes. Enriched populations of lymphocytes and monocytes were isolated from peripheral blood leukocytes from healthy subjects as described by Harwood et al. (32). Peripheral blood leukocytes from 1 U of blood, isolated, and concentrated by leukophoresis (30–50 ml leukocyte suspension per unit of blood), were diluted to 120 ml with Hanks' balanced salts solution. 15-ml aliquots of the diluted suspension were added to each of eight 50-ml conical centrifuge tubes and underlayered with 15 ml of 55% isoosmotic Percoll. After centrifugation for 40 min at 350 *g*, the buffy coat layer was removed, washed twice in RPMI 1640 tissue culture medium containing 10% fetal bovine serum, and resuspended in 10 ml of the same medium. An aliquot (1.25 ml) of the mononuclear cell population was applied to each of eight 24 ml (7 × 2.2 cm) isoosmotic continuous Percoll gradients (d = 1.010 to d = 1.160 g/ml), prepared by centrifuging 55% Percoll in phosphate-buffered saline for 40 min at 21,000 *g* (15,000 rpm) with a 50.2 Ti fixed-angle ultracentrifuge rotor (Beckman Instruments, Inc.). After centrifugation at 1,000 *g* for 20 min, the upper band of leukocytes (d = 1.060), containing primarily (70–89%) monocytes, and the lower band of leukocytes (d = 1.075), containing exclusively (> 99%) lymphocytes, were washed three times in RPMI 1640 supplemented with 10% fetal bovine serum, and twice in TEDK. The final cell pellet was resuspended in a minimal volume of TEDK and frozen in liquid N_2.

Isolation of leukocyte microsomes. Leukocyte microsomes were isolated as previously described (32). Frozen cell suspensions were incubated at room temperature until just thawed. Subsequent operations were at 0–5°C. Suspensions were homogenized 15 times with a ground-glass pestle in a 1-ml Potter-Elvehjem tissue homogenizer and then homogenized five times with a motor-driven Teflon pestle. The homogenate was diluted to 8 ml with TEDK and centrifuged at 2,000 *g* for 15 min. The pellet was discarded and the resulting supernatant liquid was centrifuged at 172,000 *g* for 90 min. Following centrifugation, the microsomal pellet was resuspended in 0.16 ml TEDK per 10^8 cells.

Measurement of human leukocyte HMG CoA reductase activity. Leukocyte HMG CoA reductase activity was measured as described by Harwood et al. (32), with the following exceptions. The specific activity of [3-^{14}C]HMG CoA used for determining enzyme activity in freshly isolated leukocytes was 30 cpm/pmol, compared to a specific activity of 10 cpm/pmol employed for measuring HMG CoA reductase activity in liver (17, 39) or cultured leukocytes (38, 40). 68 mM EDTA was included in all incubations to prevent conversion of mevalonate to phosphomevalonate. HMG CoA reductase activity is expressed as picomoles of mevalonate formed per minute of incubation at 37°C per mg of microsomal protein. Under the standard isolation conditions described above (32), HMG CoA reductase is fully activated with respect to dephosphorylation (40) and thiol-disulfide reduction.[2] No further increases in enzyme activity occur upon treatment with either *E. coli* alkaline phosphatase, rat liver phosphoprotein phosphatase or 20 mM dithiothreitol.[2]

Measurement of protein concentration. Protein was determined by the method of Bradford (41), using bovine serum albumin as standard.

Measurement of serum lipid and lipoprotein concentrations. Serum triglyceride and cholesterol concentrations were determined by standard clinical laboratory techniques (42). High density lipoprotein (HDL) cholesterol concentration was measured directly by heparin-manganese precipitation (42). Very low density lipoprotein (VLDL) cholesterol and low density lipoprotein (LDL) cholesterol concentrations were estimated according to the approximation of Friedewald et al. (43).

Results

Population statistics. Table I summarizes basal (11–14 h fasted) levels of serum lipids and lipoproteins and mononuclear leukocyte microsomal HMG CoA reductase activity obtained at 8:00 a.m. from 26 healthy male volunteers. Mean enzyme specific activity was 9.8±0.6 pmol/min per mg. Age correlated significantly with total (r = 0.49; $P < 0.02$) cholesterol concentrations. There was no significant correlation, however, between basal HMG CoA reductase activity and age, serum lipids or serum lipoproteins.

Diurnal variation in mononuclear leukocyte HMG CoA reductase activity. In mammalian liver (1, 4, 17, 18, 21) and intestine (44) HMG CoA reductase activity exhibits a circadian rhythm in which maximal enzyme activity corresponds to feeding and minimal activity corresponds to times of fasting. As shown in Fig. 1, human mononuclear leukocyte HMG CoA

2. Harwood, H. J., Jr., D. M. Bridge and P. W. Stacpoole. 1986. Stimulation of human leukocyte HMG CoA reductase activity by a factor released during erythrocyte lysis. (Manuscript under review.)

Table I. Serum Lipids and Lipoproteins and Mononuclear Leukocyte Microsomal HMG CoA Reductase Activity in 26 Healthy Adult Males

	Total cholesterol	Total triglycerides	LDL cholesterol	HDL cholesterol	VLDL cholesterol	HMG CoA reductase activity
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*pmol/min per mg*
Mean±SEM	183±8	84±6	124±6	53±2	17±1	9.8±0.6
Range	116–282	48–199	68–219	35–85	10–40	4.1–15.9

45 ml blood samples were obtained at 8:00 a.m. following an 11–14-h overnight fast from 26 healthy adult male subjects. 5 ml was used for determination of serum lipid and lipoprotein concentrations as described in Methods. Mononuclear leukocytes were isolated from the remaining 40 ml by density gradient centrifugation over Histopaque 1077. Leukocyte microsomes were isolated and HMG CoA reductase activity was quantitated as described in Methods. Data are the mean of the average values obtained for the 26 individuals ±SE.

reductase activity also exhibits a circadian rhythm. As with mammalian liver and intestine, the human mononuclear leukocyte enzyme demonstrates peak activity during times of maximal food ingestion and minimal activity during fasting. The rise in HMG CoA reductase activity between 4 a.m. and 8 a.m. occurred before breakfast, suggesting that the diurnal variation in enzyme activity is, to some extent, independent of eating. The repetitive nature of the diurnal cycle is illustrated in one subject studied over two consecutive days (Fig. 1, *inset*). In this individual, reductase activity varied up to 4.6-fold, from 18.4 pmol/min per mg to 4.1 pmol/min per mg during this period.

Fasting decreases mononuclear leukocyte HMG CoA reductase activity. Fasting diminishes HMG CoA reductase activity both in liver (1, 21) and leukocytes of animals (22). Preliminary evidence suggests that fasting may also lower plasma mevalonate levels in humans (45). Fig. 2 shows that fasting also markedly decreases human mononuclear leukocyte HMG CoA reductase activity. Mean basal enzyme activity was 11.3±1.2 pmol/min per mg and decreased progressively over 36 h of fasting to 4.1±1.4 pmol/min per mg, a value 36% of control.

Modulation of enzyme activity by cholesterol feeding or plasma cholesterol depletion. Under steady-state conditions, dietary cholesterol inhibits reductase activity in animals (21, 22) and lowers plasma mevalonate concentrations in man (46). As demonstrated in Fig. 3, administration of a high cholesterol meal (1 g) to individuals equilibrated on a low cholesterol (150 mg/d) diet led to a rapid fall in mononuclear leukocyte HMG CoA reductase activity. Enzyme activity was decreased an average of 30% within 2 h of cholesterol feeding and remained suppressed

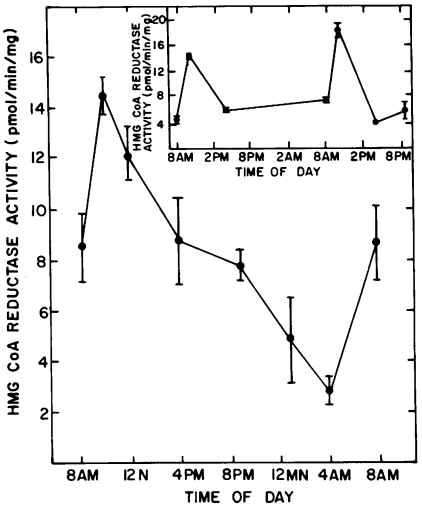

Figure 1. Diurnal variation of human mononuclear leukocyte HMG CoA reductase activity. Seven healthy, normocholesterolemic adult males were equilibrated on a constant, isocaloric diet for 2 wk. Meals were scheduled as follows: breakfast, 8:00 a.m.; lunch, 12:00 p.m.; dinner, 5:30 p.m.; evening snack, 9:00 p.m. On the final day of the constant diet, 45-ml blood samples were obtained at the indicated times. 5 ml of blood were used for measuring serum lipid and lipoprotein concentrations as described in Methods. Mononuclear leukocytes were isolated from the remaining 40 ml of blood by density gradient centrifugation over Histopaque 1077. Leukocyte microsomes were isolated and HMG CoA reductase activity was measured as described in the Methods. Data are the mean of the average HMG CoA reductase activities for the seven subjects ±SE. Shown is HMG CoA reductase activity as a function of the time of day at which the blood sample was obtained. *Inset:* Shown is the relation between HMG CoA reductase activity and time of day through two cycles of the diurnal rhythm in one subject from whom blood samples were obtained during the final 2 d of the constant diet.

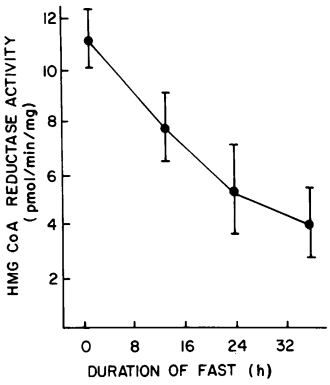

Figure 2. Fasting decreases human mononuclear leukocyte HMG CoA reductase activity. Eight healthy, normocholesterolemic adult male subjects were equilibrated on a constant isocaloric diet for 2 wk. Following the final meal of the last day of the constant diet, subjects were fasted for 36 h. 45-ml blood samples were obtained at 8:00 a.m., 10:00 a.m., 12:00 p.m., 5:30 p.m., and 9:00 p.m. on the last day of the constant diet and at the indicated times during the fast, chosen specifically to minimize differences in enzyme activity by diurnal variation. 5 ml of the blood sample were used for measuring serum lipid and lipoprotein concentrations as described in Methods. Mononuclear leukocytes were isolated from the remaining 40 ml of blood as described in Fig. 1. The zero time HMG CoA reductase activity for each individual was determined by averaging the activities obtained for the five leukocyte samples obtained on the final day of the constant diet. Data shown represent the mean of the average HMG CoA reductase activities for the eight subjects ±SE. Shown is mononuclear leukocyte HMG CoA reductase activity as a function of duration of the fast.

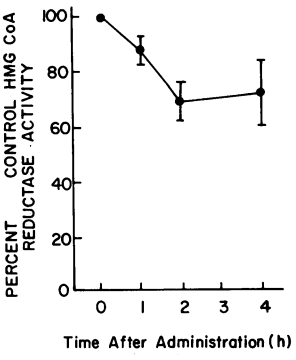

Figure 3. Time-dependent suppression of mononuclear leukocyte HMG CoA reductase activity following a high cholesterol meal. Five healthy, normocholesterolemic adult male subjects were fed a constant isocaloric diet with cholesterol intake averaging 150 mg/d for 2 wk. On the final day of the isocaloric diet, 45 ml of blood were obtained at 8:00 a.m., just before a high cholesterol breakfast consisting of four hard-boiled eggs (~ 1 g cholesterol), 9:00 a.m., 10:00 a.m., and 12:00 p.m., just before lunch. 5 ml of blood were used for measurement of serum lipid and lipoprotein concentrations. The remaining 40 ml of blood were used for isolation of mononuclear leukocytes microsomes and quantitation of microsomal HMG CoA reductase as described in the Methods. The percentage of control HMG CoA reductase activity was calculated for each individual at each time point by dividing the enzyme activity measured following the high cholesterol breakfast by the activity measured at the same time point following the standard breakfast. Shown is the average percentage of control HMG CoA activity for the five subjects (±SE) as a function of the time between cholesterol administration and blood sampling.

for the next 2 h. Serum lipids and lipoproteins, however, remained unchanged during this brief interval (data not shown). The effect of dietary cholesterol on HMG CoA reductase activity was variable, with maximal inhibition ranging between 11 and 49% (Table II).

Two normocholesterolemic subjects underwent combined plasmapheresis and leukophoresis. A 4 liter plasma exchange was conducted over ~ 90 min. Blood was drawn immediately before and after the procedure for measuring mononuclear leukocyte HMG CoA reductase activity. In addition, leukocytes were harvested from the first liter of pheresed blood and enriched subpopulations of monocytes and lymphocytes were isolated to determine baseline (prepheresis) HMG CoA reductase activity. Similarly, enriched monocyte and lymphocyte populations were obtained from the final liter of pheresed blood to estimate postpheresis enzyme activity.

In both subjects, plasmapheresis led to a rapid 60% fall in circulating total and LDL cholesterol (Fig. 4). Similar changes occurred in VLDL cholesterol, HDL cholesterol and triglyceride levels (data not shown). Reduction of plasma cholesterol concentrations was accompanied by a rapid increase in mononuclear leukocyte HMG CoA reductase activity (Fig. 4). This increase appeared to be due entirely to stimulation of the monocyte enzyme. In contrast, lymphocyte HMG CoA reductase activity remained unchanged or decreased slightly following plasmapheresis.

Inhibition of mononuclear leukocyte HMG CoA reductase activity by dichloroacetate. Dichloroacetate reduces cholesterol levels in man (34, 35), inhibits hepatic cholesterol biosynthesis in rats (39) and is a noncompetitive inhibitor of HMG CoA reductase obtained from rat liver (39). Previous studies have also shown that dichloroacetate inhibits human leukocyte HMG CoA reductase activity in vitro (32). As illustrated in Fig. 5, a single 35-mg/kg oral dose of dichloroacetate rapidly decreased

Table II. Variability in the Degree of Suppression of Mononuclear Leukocyte HMG CoA Reductase Activity Following a High Cholesterol Meal

Subject	HMG CoA reductase activity measured 120 min following: Standard breakfast (A)	4 Eggs (B)	Percent of control
	pmol/min per mg	*pmol/min per mg*	*B/A × 100*
1	11.4±0.2	5.8±0.7	51
2	10.3±1.2	5.7±0.5	55
3	8.2	6.2±0.3	75
4	16.6±1.6	13.9±1.0	84
5	7.3±0.7	6.5±1.0	89

Five healthy male subjects received for 2 wk an isocaloric diet with cholesterol intake averaging 150 mg/d. On the final day of the isocaloric diet 40 ml of blood were obtained at 10:00 a.m., 2 h after a standard breakfast. The next day, 40 ml of blood were obtained at 10:00 a.m., 2 h following a high cholesterol breakfast consisting of four hard-boiled eggs (~1 g cholesterol). Mononuclear leukocytes were isolated by density gradient centrifugation over Histopaque 1077. Leukocyte microsomes were isolated and HMG CoA reductase was measured as described in Methods. Data are the mean of triplicate determinations (except for a single determination for subject 3 on a standard breakfast) of HMG CoA reductase activity ±SE.

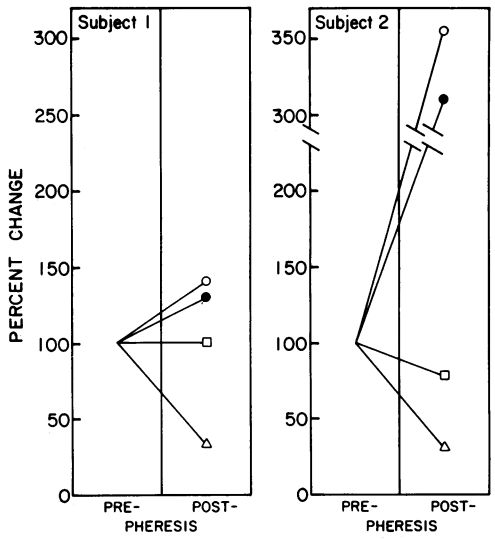

Figure 4. Increased monocyte, but not lymphocyte, HMG CoA reductase activity following plasmapheresis. Two male subjects underwent a 2 h, 4-liter plasma exchanges (plasmapheresis) at the Civitan Regional Blood Center, Gainesville, FL. The initial (prepheresis) and final (postpheresis) units of blood to undergo plasma exchange were also subjected to leukopheresis. 5 ml of blood were also obtained before and after plasmapheresis, for subsequent measurement of serum lipid and lipoprotein concentrations. Mononuclear leukocyte, lymphocyte and monocyte populations were isolated from the preplasmapheresis and postplasmapheresis leukopheresis samples by density gradient centrifugations as described in Methods. Leukocyte microsomes were isolated and microsomal HMG CoA reductase activity was measured as described in Methods. Shown is the percent change in plasma LDL cholesterol concentration (*open triangles*), mononuclear leukocyte HMG CoA reductase activity (*closed circles*), monocyte HMG CoA reductase activity (*open circles*), and lymphocyte HMG CoA reductase activity (*open square*) as a result of plasmapheresis for each of the two subjects.

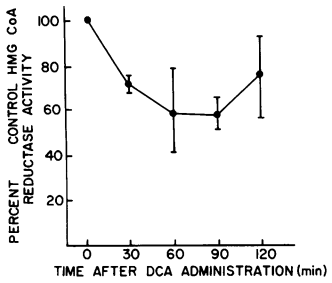

Figure 5. Decreased mononuclear leukocyte HMG CoA reductase activity following dichloroacetate administration. Five healthy, adult male subjects received a single, 35-mg/kg dose of sodium dichloroacetate at 8:00 a.m. after an 11–14-h overnight fast. 45-ml blood samples were obtained immediately before drug administration (8:00 a.m.) and at the indicated times after administration. Subjects were not given breakfast until the end of the study. 5 ml of the blood samples were used for measuring serum lipid and lipoprotein concentrations as described in Methods. Mononuclear leukocytes were isolated from the remaining 40 ml of blood by density gradient centrifugation over Histopaque 1077. Leukocyte microsomes were isolated and microsomal HMG CoA reductase activity was measured as described in Methods. Shown is the mean of the average percent of pre-drug administration HMG CoA reductase activity for the five subjects (±SE) as a function of the time duration between drug administration and blood sampling.

leukocyte enzyme activity in vivo. The onset of inhibition occurred within 30 min. By 1 h, HMG CoA reductase activity was 42% below baseline. Mean enzyme activity was still 20% below basal 4 h after dichloroacetate administration.

Discussion

The data presented in this report provide new insight into the physiological regulatory mechanisms controlling HMG CoA reductase activity in the human leukocyte in vivo and demonstrate that these mechanisms are similar to, if not the same as, those controlling enzyme activity in animal liver and intestine. We have demonstrated for the first time that the activity of the human leukocyte enzyme exhibits a diurnal rhythm, is decreased by fasting or a single, high cholesterol meal and is increased by reducing circulating cholesterol levels. The rapidity of response in enzyme activity to these perturbations is as impressive as is the magnitude of change. In previous studies, we reported that the activity of human leukocyte HMG CoA reductase is also modulated by reversible phosphorylation (40) and thiol-disulfide formation (38). Thus, in many respects, the regulatory properties of the human leukocyte enzyme parallel those described for HMG CoA reductase of mammalian liver or intestine.

Diurnal variation in the activity of HMG CoA reductase is a well-described phenomenon in mammalian liver and small intestine (1, 4, 17, 18, 21, 44) and appears to reflect primarily fluctuations in enzyme protein synthesis (4). Recent evidence has suggested, however, that changes in enzyme catalytic efficiency may also be important to the diurnal variations in enzyme activity (18). Increments in hepatic HMG CoA reductase activity and cholesterol biosynthesis are linked to time of day and feeding schedule, although the exact temporal relationships vary among species (1, 17). In meal-fed rats, for example, maximal hepatic HMG CoA reductase activity normally occurs soon after feeding (21), similar to the changes we observed to occur in the human leukocyte enzyme after the first daytime meal. Indeed, rates of hepatic bile acid synthesis in man also vary diurnally with peak synthesis occurring soon after the first daytime meal (47). In contrast, diurnal maxima in plasma mevalonate levels in humans do not appear to be closely associated with feeding (45), although differences in the timing of meals in those studies and ours make it difficult to compare circadian changes in plasma mevalonate and leukocyte reductase. That diurnal variation in microsomal reductase activity was not observed in mixed rat leukocyte populations (22) is difficult to reconcile with these other data in animals and humans.

Fasting markedly diminishes the activity of hepatic (1, 21) and leukocyte (22) microsomal HMG CoA reductase in rats. In rats fasted 36 h, reductase activity decreases 20- to 25-fold, although the diurnal rhythm in enzyme activity is preserved (21). Fasting in humans also leads to decreased plasma levels of mevalonate (45) and, as shown in this study, leukocyte HMG CoA reductase activity. Taken together, the changes in leukocyte HMG CoA reductase activity and circulating mevalonate imply that fasting leads to suppression of cholesterol biosynthesis in humans. This is consistent with limited studies in healthy animals (48) or patients with anorexia nervosa (49, 50), in whom caloric restriction or fasting is associated with diminished turnover and/or synthesis of cholesterol. In our experiments, healthy subjects fasted up to 36 h showed a progressive fall in mononuclear leukocyte HMG CoA reductase activity, to a level approximately one-third basal. Whether diurnal variation in enzyme activity persisted during fasting cannot be determined with certainty from the few sampling points obtained. It is clear, however, that the expected rise in reductase activity at 24 h was either greatly attenuated or abolished. No significant variation in serum cholesterol, triglycerides, VLDL, LDL, or HDL cholesterol were noted during these investigations of diurnal rhythm or fasting. This implies that the suppression of human leukocyte reductase activity during fasting may not be due to lipoprotein uptake and processing by these cells.

Numerous studies in healthy subjects have demonstrated positive correlations between dietary cholesterol intake and circulating total and LDL cholesterol concentrations (31, 50, 56). Sterol balance experiments have shown that chronic administration of a high cholesterol diet is frequently associated with suppression of whole body cholesterol biosynthesis (49, 57, 58), presumably by feedback inhibition of HMG CoA reductase. Furthermore, wide variations among individuals exist in the magnitude of change in circulating cholesterol in response to cholesterol intake (59). Similarly, our results demonstrate that a feedback mechanism by dietary cholesterol operates quickly, and to a variable degree, in suppressing HMG CoA reductase activity in circulating mononuclear leukocytes. The effect appears to be too rapid to be mediated by cellular uptake of circulating LDL cholesterol. A possible alternative mechanism in feedback control of mononuclear leukocyte HMG CoA reductase by dietary cholesterol might involve chylomicron remnants or cholesterol-enriched β-VLDL lipoproteins (60, 61). The time course of enzyme inhibition coincides with the expected appearance of these particles in the circulation after a fat- or cholesterol-rich meal (62). Also consistent with such a mechanism is the transient nature of the inhibition of HMG CoA reductase by dietary cholesterol, since chylomicron remnants (and probably β-VLDL) are cleared rapidly from the circulation in healthy individuals (63). An additional question, however, concerns itself with the chemical nature of the inhibitory molecule. If cholesterol per se is involved, then it would seem necessary to invoke net cholesterol uptake by the mononuclear cells in order to achieve down regulation of HMG CoA reductase. On the other hand, cholesterol in food may be converted to oxidized derivatives by processes such as exposure to atmosphere or cooking (64–66). In-

deed, some of these derivatives are more potent inhibitors of HMG CoA reductase than native cholesterol (2). Oxygenated sterols could exchange with cholesterol in leukocyte membranes and thereby suppress HMG CoA reductase without requiring net gain of intracellular sterol. Although stimulation of mononuclear leukocyte HMG CoA reductase activity by plasmapheresis affects only the monocyte enzyme, a mechanism involving membrane lipid exchange would need to exist for both blood monocytes and lymphocytes. This is because the magnitude of mononuclear leukocyte HMG CoA reductase inhibition (up to 49%) in some of our subjects was too great to represent an effect solely on the relatively few monocytes in our mixed cell populations (23% monocytes vs. 77% lymphocytes). Clearly, this hypothesis requires further investigation and is unlikely to provide the only explanation for down regulation of leukocyte HMG CoA reductase induced by dietary cholesterol.

Mononuclear leukocyte HMG CoA reductase is sensitive to pharmacologic, as well as nutritional perturbations. Our studies show that the cholesterol-lowering drug, DCA, at a single dose lower than that reported to decrease cholesterol levels in patients with diabetes (34) or homozygous familial hypercholesterolemia (35), rapidly but transiently inhibited enzyme activity. These results are consistent with those obtained in rat liver (39) and cultured human lymphoid (IM-9) cells (32).

Our experiments thus are consistent with the concept, originally proposed by Young and Rodwell (22), that determination of microsomal HMG CoA reductase activity from freshly isolated mononuclear leukocytes may provide useful information in evaluating changes in rates of in vivo cholesterol biosynthesis in man. However, to demonstrate that basal (or peak) levels of mononuclear leukocyte HMG CoA reductase activity provide an accurate measurement of whole body cholesterol biosynthesis, enzyme activity must be correlated with standard methods of cholesterol production. If this hypothesis is substantiated by subsequent investigations, the methodology may offer certain advantages over the classical, but laborious and expensive, methods of fecal sterol balance (49, 67), radioactive tracer analysis (69–72) and the more recently described technique for measuring plasma mevalonate (45, 46, 72), and is particularly well suited for exploring early changes in HMG CoA reductase activity induced by various effectors of cholesterol metabolism. Finally, measurements of the human mononuclear leukocyte enzyme may also prove useful in investigating certain genetic or acquired metabolic disorders thought to be influenced by abnormal HMG CoA reductase activity or regulation. Preliminary results of such studies have recently been reported (73, 74).

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