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Mevalonic acid in human plasma: Relationship of concentration and circadian rhythm to cholesterol synthesis rates in man

(sterol balance/cholestyramine resin/fasting/mononuclear leukocytes/dietary cholesterol)

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ABSTRACT **We tested the hypothesis that the rate of cholesterol synthesis in tissues determines the concentrations of mevalonic acid (MVA) in plasma. We found that plasma MVA concentrations were correlated (*i*) with increased rates of whole-body cholesterol synthesis (measured by sterol-balance methods) in patients treated with cholestyramine resin and (*ii*) with decreased rates of whole-body sterol synthesis (indicated by conversion of labeled acetate to sterol in freshly isolated mononuclear leukocytes) in out-patients after 4 weeks on a cholesterol-rich diet. In addition, a diurnal rhythm of plasma MVA concentrations was observed in patients whose activities were strictly controlled on a metabolic ward. At the peak of the rhythm (between midnight and 3 a.m.) MVA concentrations were 3–5 times greater than at the nadir (between 9 a.m. and noon). Furthermore, a relationship between the diurnal rhythm of plasma MVA and endogenous cholesterol synthesis is suggested by our finding that the plasma MVA rhythm was suppressed by cholesterol feeding (1,200 mg/day) and abolished by a 12-day fast. The presence in human plasma of MVA, an obligate precursor of cholesterol, in amounts apparently related to the rate of cholesterol synthesis offers a noninvasive, nonisotopic method for studying cholesterol synthesis in man.**

Human cholesterol metabolism, as revealed by the sterol-balance method or by analysis of cholesterol kinetics, is controlled by a system of homeostatic mechanisms that resists expansion or depletion of body cholesterol pools (1–3). The recent demonstration of rapid short-term regulation of the rate-limiting enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (NADPH) (E.C. 1.1.1.34), in animals by regulation of enzyme synthesis (4) and by a phosphorylation cascade (5) has created a need for new methods capable of measuring rapid changes in the rate of cholesterol synthesis in man. Existing methods are either invalid in the metabolic unsteady state (e.g., sterol balance or analysis of cholesterol kinetics) or they respond only to long-standing changes [e.g., squalene kinetics (6) and sterol synthesis in freshly isolated mononuclear leukocytes (7)].

In this report we confirm the observations of Hagenfeldt and Hellstrom (8) and of Popjak *et al.* (9) that mevalonic acid (MVA) can be detected in human plasma. Furthermore, we demonstrate that the concentration of MVA is related to the rate of whole-body cholesterol synthesis. In addition, our studies show the existence of a diurnal rhythm of plasma MVA that is abolished by fasting and is reduced in amplitude by cholesterol feeding. Sources of variation in the relationship between whole-body cholesterol synthesis and plasma MVA concentration are considered in order to evaluate the usefulness of measuring plasma MVA levels as a sensitive and noninvasive method for estimating cholesterol synthesis rates in human subjects without the need for *in vivo* administration of radioactive materials.

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MATERIALS AND METHODS

Out-Patient Volunteers and Their Diets. Ten male patients were recruited from the Center for Prevention of Premature Arteriosclerosis at the Rockefeller University Hospital from a group of 150 patients previously described (10). Clinical data and lipid phenotypes for the group are given in Table 1. These patients had no symptoms of ischemic heart disease, gallbladder disease, or diabetes; they had discontinued lipid-lowering medications at least 1 month prior to entering the study. They were trained by a nutritionist in portion size estimation, menu selection, and the keeping of dietary records and were instructed to maintain a diet with 35% of calories as fat (polyunsaturated/saturated ratio $\approx$2.0) and low in cholesterol (<250 mg/day). Actual diet compositions and daily cholesterol intakes were calculated from 9-day food records as described (11). During the second phase of the study, each out-patient added three eggs per day to his original diet to increase daily cholesterol intake to about 800 mg/day; daily caloric intake and polyunsaturated/saturated ratio were held constant by appropriate adjustments in quality and intake of dietary fat. These out-patients made three clinic visits after 4–6 weeks on the low-cholesterol diet in order to obtain diet records (11) and to furnish blood samples for measurement of plasma lipids (12, 13), estimation of sterol synthesis rates in mononuclear leukocytes (7), quantitation of plasma MVA (9), and measurement of cholesterol absorption (14). The same schedule of three visits was repeated after a second 4- to 6-week period on the high-cholesterol diet.

In-Patients, Their Diets, and Experimental Design. The relevant clinical data and lipoprotein phenotypes of patients admitted to the metabolic ward of the Rockefeller University Hospital are given in Table 1. None received medication for hyperlipidemia during control periods. One patient (no. 2) had cholesterol malabsorption [10% of dietary cholesterol by the isotope-ratio method (14)] but absorbed fats well; he had no demonstrable nutritional deficiency.

Caloric intakes were adjusted to maintain body weights constant within $\pm$1.5 kg over study periods of 3–5 weeks. Five liquid-formula meals were given in equal portions at 8 and 11 a.m. and at 1, 5, and 7 p.m. except where specifically noted. Preparation of liquid-formula diets and supplementation with vitamins and minerals have been described (15). For preparation of formulas high in cholesterol, cholesterol (Sigma) was recrystallized from ethanol and dissolved in the oil phase during formula preparation.

In-patients were studied after an adaptation period of at least

Abbreviation: MVA, mevalonic acid.

Table 1. Clinical information*

Patient no.	Age, yr	Body weight, kg	Height, cm	% ideal weight†	Diet	LP phenotype and diagnosis
			Out-patients			
n = 10	48	80.9	—	110	Solid food‡	Normal, 3; HTG, 3; HChol, 3; combined, 1
	[32–63]	[68–99]		[95–124]		
			In-patients§			
1 P.W.	35	91	175	135	31% cottonseed oil	HTG, CHO induced; obesity
2 R.M.	57	67	160	118	62% corn oil	HTG, CHO induced
3 C.H.	53	73	174	110	49% corn oil	HChol
4 R.C.	55	57	159	118	62% corn oil	HChol with ISHD
5 E.K.	66	120	177	173	50% corn oil	HTG, obesity, and maturity-onset diabetes
6 E.F.	45	82	180	115	32% corn oil	Familial HChol with ISHD

HTG, hypertriglyceridemia; CHO, carbohydrate; HChol, hypercholesterolemia; ISHD, ischemic heart disease; LP, lipoprotein.
* All patients were male. Mean values for the out-patient group are shown with the range given in brackets.
† Percent ideal body weight was determined from Metropolitan Life Insurance Tables.
‡ Polyunsaturated/saturated ratio = 1.8 [0.63–3.69]; cholesterol intake = 190 mg/day [82–309].
§ These six patients were maintained at constant body weight by ingestion of liquid formula diets with 15% of calories as protein, the indicated amount of fat, and the remainder as carbohydrate.

2 weeks of formula-feeding on the metabolic ward. Blood samples for routine MVA determination were obtained by venipuncture between 7 and 8 a.m. after an overnight fast. When samples were obtained throughout the day for circadian rhythm studies, a heparin lock was placed in the antecubital vein (heparin dose = 0.01 unit/kg·hr); no alteration in blood coagulation or triglyceride clearance was detected.

Informed consent was obtained from all in-patients and out-patient volunteers after appropriate review and approval of study protocols by the Rockefeller University Hospital Institutional Review Board.

MVA Assay. MVA was assayed in protein-free plasma ultrafiltrates prepared from freshly drawn heparinized blood. The enzymatic assay described by Popjak *et al.* (9) was used except that the gravity-flow 16-column system was replaced by a 20-column system driven by two 10-channel peristaltic pumps (Technicon). The coefficient of variation, measured in 10 replicate samples, was 6%. Plasma ultrafiltrates from two healthy men were pooled for use as a quality control reference that was measured with each assay (44.4 ± 10.8 nM; n = 274), with a day-to-day coefficient of variation of 24.3%.

Statistical Analysis. Statistical analysis of differences between paired and unpaired means was carried out by using Student's t test. All data are presented as the mean ± SEM for the number of measurements given in parentheses.

RESULTS

Plasma MVA Concentrations in Human Subjects. Plasma MVA concentrations in healthy male volunteers ranged from 12 to 102 nM, with a mean value of 43 ± 13 nM (n = 40). No significant relationship was observed between plasma MVA and patients' age, body weight, or plasma level of cholesterol or triglycerides. In three patients with familial hypercholesterolemia, plasma MVA concentrations were increased but within the normal range: homozygote male child, 68 nM; heterozygote father, 56 nM; and heterozygote mother, 63 nM.

Effect of Cholestyramine Administration on Plasma MVA. Plasma MVA concentrations were compared to the rate of whole-body cholesterol synthesis, measured by the sterol balance method, in three patients maintained on the metabolic ward for two 4-week periods, before and during administration of cholestyramine resin (Table 2). All three patients had rates higher than we have seen in familial hypercholesteremia (11 ± 6 mg/kg·day, n = 71) or in normolipidemia (11 ± 7 mg/kg·day, n = 45). Plasma MVA concentrations in these three patients also were higher than the mean found in our normal controls: 72, 67, and 63 nM versus 43 ± 13 nM (n = 40). Nevertheless, the administration of cholestyramine to these patients (8 g twice daily) caused still further increases in whole-body sterol synthesis rates and plasma MVA concentrations.

The time course of changes in sterol balance and plasma MVA was studied in patient 1 during the transition from the control period through the early weeks of cholestyramine treatment (Fig. 1). During the control phase, plasma MVA concentrations averaged 72 ± 7 nM (n = 10). Initiation of cholestyramine treatment caused an immediate increase in sterol excretion with little change in excretion of neutral steroids. Simultaneously, plasma MVA levels increased to reach a new steady-state concentration of ≈200 nM.

Effect of Dietary Cholesterol. Ten volunteers were studied as out-patients on a low-cholesterol diet and then again on a high-cholesterol diet. The data obtained are summarized in Table 3.

The mean intake of dietary cholesterol was increased from 173 to 781 mg/day by adding three eggs per day to the low-cholesterol diet. If one takes into account the percentage of absorption of dietary cholesterol in the two dietary periods, egg feeding increased the mass of cholesterol absorbed from 96 to 450 mg/day. There was no systematic change in plasma cholesterol level in any of the patients. However, the greater input of exogenous cholesterol caused a 34% decrease in the rate of sterol synthesis by freshly isolated mononuclear leukocytes and

Table 2. Effect of cholestyramine on plasma lipid levels, cholesterol balance, and plasma MVA concentrations

Patient	Treatment*	Plasma lipids, mg/dl		Chol balance,† mg/kg·day	Plasma MVA, nM
		Chol	Triglyceride		
1	Control	165 ± 15 (5)	423 ± 80 (5)	20.7 ± 1.2 (5)	72 ± 7 (10)
	Cholestyramine	149 ± 11 (5)	420 ± 53 (5)	47.0 ± 12‡ (5)	192 ± 21‡ (5)
2	Control	2.8 ± 12 (7)	913 ± 152 (7)	17.6 ± 0.9 (7)	67 ± 13 (4)
	Cholestyramine	344 ± 100‡ (7)	2,184 ± 842‡ (7)	53.2 ± 8‡ (5)	203 ± 28‡ (7)
3	Control	302 ± 14 (8)	150 ± 17 (8)	16.2 ± 2 (8)	62.6 ± 6 (6)
	Cholestyramine	274 ± 15‡ (8)	180 ± 13‡ (8)	27.6 ± 7‡ (8)	175 ± 10‡ (3)

Chol, cholesterol.

* Studies 1 and 3 were performed in weeks 3–5 of a control period and during the first 2 weeks of cholestyramine treatment. Measurements in study 2 were made during weeks 4 and 5 of cholestyramine treatment when a new metabolic steady state was reached. All patients received 8 g of cholestyramine twice daily.

† Cholesterol balance data are the mean ± 1 SD of fecal steroid excretion measurements made on 4-day stool collections (number of consecutive collections in parenthesis), after daily cholesterol intakes were deducted.

‡ Significantly different from control at $P < 0.01$.

a 21% decrease in plasma MVA concentration. The direction and extent of these changes were concordant in 8 of 10 patients.

Circadian Rhythm of Plasma MVA. Kopito and Brunengraber (16) have reported that rhythmic changes of plasma MVA were not observed in human volunteers eating three meals per day but were observed only after adaptation for 2 weeks to a one-meal-per-day schedule. We examined the possibility that such rhythms might occur in our patients under the strictly controlled regimen of the metabolic ward.

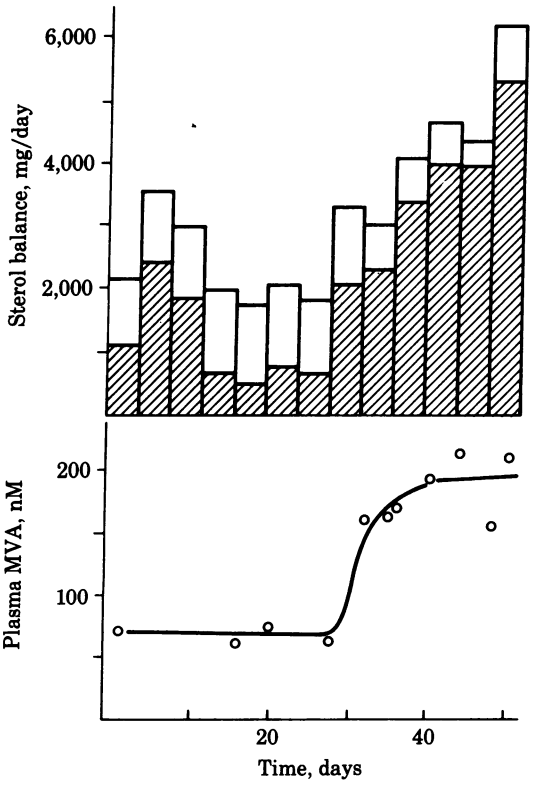

FIG. 1. Sterol balance (*Upper*) and plasma MVA (*Lower*) data during control and cholestyramine treatment periods in patient 1. Total sterol (□, neutral; ▨, acidic) excretion in successive 4-day fecal collections is presented after subtraction of dietary cholesterol intake. MVA was measured in quadruplicate at each time point.

Patient 4 had been adapted for 3 weeks to a schedule of four equal-portion meals of liquid formula daily (8 and 10 a.m. and 1 and 7 p.m.; shown by arrows in Fig. 2). Blood was drawn at 3-hr intervals for 3 consecutive days. The concentration of MVA was found to change over a 3-fold range: from nadirs of 35 and 50 nM to peak levels above 120 nM. The changes were periodic—sharp increases occurring between midnight and 3 a.m. on 3 successive days—and unexpectedly rapid—increasing from 60 nM to 125 nM and returning to 57 nM in 6 hr. The highest concentrations (125 and 123 nM on days 2 and 3, respectively) differed from the mean of all time points (72 ± 23 nM; $n = 24$) by more than 2 SD.

Pattern of MVA Rhythm and Supression by Fasting and by Cholesterol Feeding. In two additional patients, these rhythms were defined in greater detail. Patients 5 and 6 were fed eucaloric levels of a liquid-formula diet in five equal portions daily (8 and 10 a.m. and 1, 5, and 7 p.m.) for 3 weeks. Blood samples were drawn hourly for 24 hr and assayed for MVA. Plasma MVA concentrations varied significantly in both patients (see open circles in Fig. 3 *Upper* and *Lower*). Each increase of plasma MVA defined a smooth, continuous peak through several time points. Although there were individual differences in the patterns, peak levels of plasma MVA occurred between midnight and 3 a.m. in all three patients studied.

To determine whether the MVA rhythms could be suppressed by measures known to decrease cholesterol synthesis rates, patient 5 was restudied after a 12-day fast; patient 6 was studied on low- and high-cholesterol intakes.

Fig. 3 *Upper* shows the effect of fasting: the MVA rhythm seen during eucaloric feeding was completely abolished by fasting this patient for 12 days. The time-averaged values of the two tests were 115 ± 33 nM and 52 ± 14 nM for control and fasting, respectively—a 55% decrease ($P < 0.01$; $n = 24$). At the same time, sterol synthesis by freshly isolated blood mononuclear leukocytes was suppressed by 60%—from 7.9 ± 2.7 pmol ($n = 9$) to 3.2 ± 0.4 pmol ($P < 0.001$; $n = 6$) of acetate incorporated into sterols per hour per 10^6 cells.

Table 3. Effect of dietary cholesterol on cholesterol synthesis rates and plasma MVA levels in 10 out-patients*

Assay	Diet: Low Chol	Diet: High Chol†	P
Dietary Chol intake, mg/dl	173 ± 60	781 ± 122	<0.001
Dietary Chol absorption, %	57 ± 10	53 ± 13	NS
Dietary Chol input, mg/day	96 ± 26	405 ± 108	<0.001
Plasma Chol‡, mg/dl	252 ± 49	251 ± 40	NS
Mononuclear leukocyte sterol synthesis, pmol/hr§	9.4 ± 2.9	6.2 ± 1.4	<0.01
Plasma MVA, nM	45 ± 17	35 ± 14	<0.05

Chol, cholesterol; NS, not significantly different.
* All values are the mean ± SD for 10 patients except as noted in ‡.
† In the high-cholesterol dietary period, the polyunsaturated/saturated ratio and caloric intake were held constant.
‡ Mean ± SD of the average cholesterol concentrations obtained during three clinic visits per patient.
§ Per 1×10^6 cells.

If fasting lowers the amplitude of rhythmic changes in the rate of cholesterol synthesis in man as it does in animals (17), then the coefficient of variation in plasma MVA for all time points on day 12 of the fast (27%) provides an estimate of the baseline variability (physiologic plus methodologic) in plasma MVA. The amplitude and timing of variations in plasma MVA levels observed during eucaloric feeding are too large to be explained by baseline variation; they must eventually be explained in physiological terms.

Fig. 3 (*Lower*) shows the effect of cholesterol supplementation in patient 6: full-maintenance formula feedings, moderate (550 mg/day) and rich (1,200 mg/day) in dietary cholesterol content, were administered for 3 weeks prior to each 24-hr test period. The suppression of peak values of MVA is clearly shown: the highest values were 196 nM on the low-cholesterol intake and 92 nM on the high-cholesterol intake. The time-averaged values obtained on the two regimens were 88 ± 38 and 59 ± 16 nM, respectively ($P < 0.05$; $n = 24$).

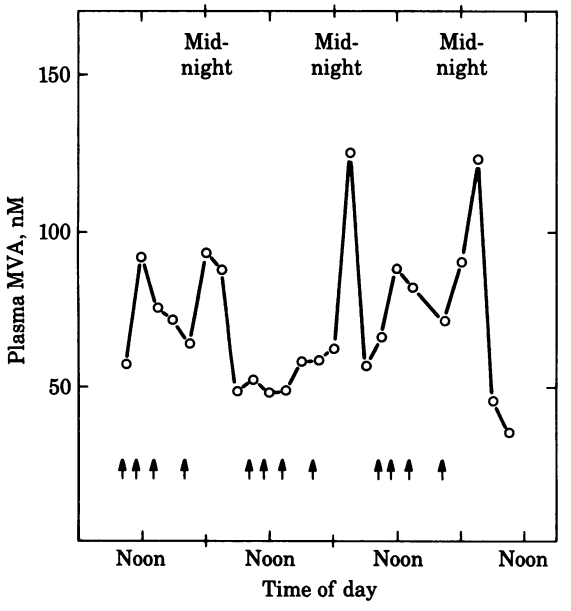

FIG. 2. Variations in plasma MVA concentrations over a 3-day period in patient 4 after 2 weeks on a four-meal-per-day feeding schedule (arrows). Plasma MVA levels were measured every 3 hr.

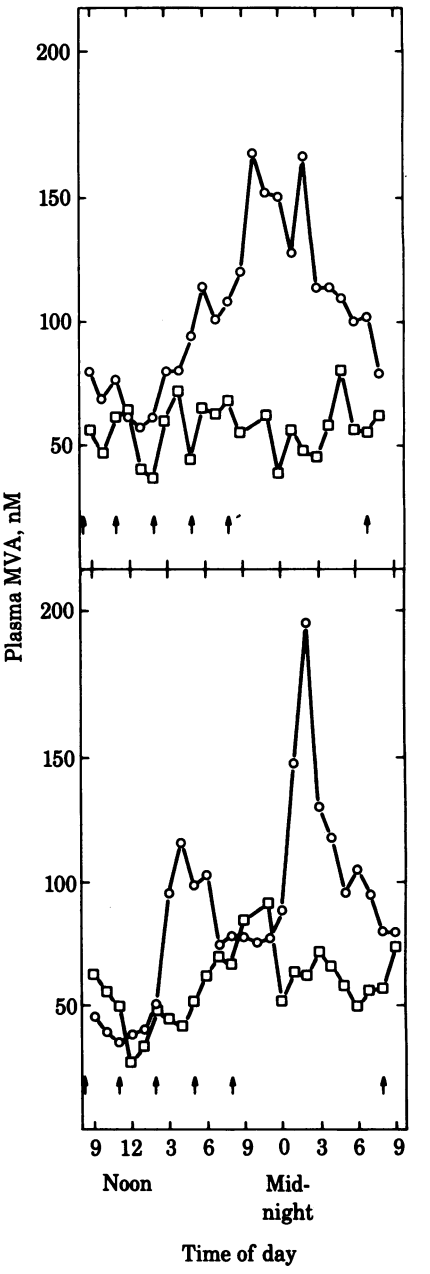

FIG. 3. Effects of a total fast and of cholesterol feeding on plasma MVA rhythms. MVA data in patient 5 (*Upper*) were obtained during eucaloric feeding (○) and after a 12-day total fast (□). MVA data in patient 6 (*Lower*) were obtained on a cholesterol intake of 500 mg/day (○) and 1,200 mg/day (□); the rhythm data were determined after 3 weeks on each regimen.

DISCUSSION

Proposed Model of Plasma MVA Turnover. The data reported here clearly demonstrate a relationship between the concentration of MVA in human plasma and the rate of endogenous sterol synthesis: (*i*) increased plasma MVA in three patients whose rate of whole-body cholesterol synthesis was stimulated by cholestyramine treatment and (*ii*) decreased plasma MVA in 10 patients in whom whole-body cholesterol synthesis was suppressed by feeding a cholesterol-enriched diet. These findings indicate that plasma MVA concentrations shift directly

and in parallel with changes in endogenous cholesterol synthesis.

On the basis of this observation, we propose as a working hypothesis the following model for the turnover of plasma MVA: the steady-state concentration of MVA in plasma is determined by a balance between MVA input (driven by intracellular MVA synthesis in response to factors regulating cholesterol production) and output (mediated by concentration-dependent mechanisms).

This scheme proposes that MVA enters the plasma compartment from those tissues engaged in active MVA synthesis and that dietary sources of MVA are quantitatively unimportant. The MVA content of the American diet is unknown but is probably minor (9); indeed, plasma MVA levels measured in outpatients were not strikingly different from those of in-patients fed liquid-formula diets devoid of MVA.

Only a small amount of MVA must pass out of the cells in order to account for daily turnover in the plasma compartment. Using the data of Popjak *et al.* (9), we estimate that about 36 μmol/day or 0.6% of the MVA needed for daily cholesterol synthesis is lost to the plasma compartment each day in an adult man.

Finally, our model includes the specification that clearance of MVA from plasma be proportional to concentration over the physiological range. MVA is cleared from plasma by several routes; none appears to be saturated at physiological concentrations: (*i*) removal by the kidney, either through resorption and metabolism or by excretion into the urine (18, 19), and (*ii*) removal by conversion to sterols or metabolism to nonsterol products primarily in the liver and intestine (20). There is no suggestion from available evidence that clearance mechanisms would be affected by cholestyramine administration or cholesterol feeding.

Diurnal Rhythm of Plasma MVA. The data presented here demonstrate that plasma MVA concentrations vary 2- to 5-fold during the course of each 24-hr period. The amplitude of this diurnal rhythm was suppressed by cholesterol feeding and by a 12-day fast. Because these maneuvers are known to suppress cholesterol synthesis in man, our observations are consistent with the notion that the diurnal changes in plasma MVA reflect changes in the rate of endogenous cholesterol synthesis.

A number of studies have shown diurnal rhythms in hepatic and intestinal cholesterol synthesis in animals (21), with the peak of cholesterol synthesis coinciding with peak feeding activity. However, in our patients, the peak MVA concentrations were observed when the patients were at rest, at least 5 hr after the last meal: we cannot explain this timing or its relationship to meals. In this respect, it is interesting to note that Kopito and Brunengraber (16) have described a similar diurnal rhythm in ambulatory adult volunteer students who ingest each day's food requirement in a single meal.

Plasma MVA Concentrations and Endogenous Cholesterol Synthesis. Miettinen (22) has described a direct relationship between the concentration of methyl sterols in human serum and whole-body cholesterol synthesis as measured by sterol balance. Thus, the agreement between independent studies of two cholesterol precursors (MVA and methyl sterols) provides further support for the concept that concentrations of cholesterol precursors in plasma can be significantly affected by changes in intracellular rates of cholesterol synthesis.

Problems and Potential Applications. Popjak *et al.* (9) noted large variations over time in the concentration of plasma MVA in free-living individuals. Diurnal rhythms of plasma MVA may account for part of this variation. Indeed, we have observed greater variation between samples obtained on successive days from free-living out-patients (±10–100%) than in similarly obtained samples from patients maintained on the metabolic ward and fed either solid foods (±10–30%) or liquid-formula diets (±5–25%). Thus, we urge that single determinations of plasma MVA be interpreted with caution. The possibility of using 24-hr urine samples for estimating daily MVA production needs study.

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1. Grundy, S. M., Ahrens, E. H., Jr., & Davignon, J. (1969) *J. Lipid Res.* **10**, 304–315.
2. Nestel, P. J. & Poyser, A. (1976) *Metabolism* **25**, 1591–1599.
3. Quintao, E., Grundy, S. M. & Ahrens, E. H., Jr. (1971) *J. Lipid Res.* **12**, 233–247.
4. Higgins, M. & Rudney, H. (1973) *Nature (London) New Biol.* **246**, 60–61.
5. Nordstrom, J. L., Rodwell, V. W. & Mitschelen, J. J. (1977) *J. Biol. Chem.* **252**, 8924–8934.
6. Liu, G. C., Ahrens, E. H., Jr., Schreibman, P. H., Samuel, P., McNamara, D. J. & Crouse, J. R. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4612–4616.
7. McNamara, D. J., Davidson, N. O. & Fernandez, S. (1980) *J. Lipid Res.* **21**, 65–71.
8. Hagenfeldt, L. & Hellstrom, K. (1972) *Life Sci.* **11**, 669–676.
9. Popjak, G., Boehm, G., Parker, T. S., Edmond, S., Edwards, P. A. & Fogelman, A. M. (1979) *J. Lipid Res.* **20**, 716–728.
10. McNamara, D. J., Davidson, N. O., Samuel, P. & Ahrens, E. H., Jr. (1980) *J. Lipid Res.* **21**, 1058–1064.
11. White, E. C., McNamara, D. J. & Ahrens, E. H., Jr. (1981) *Am. J. Clin. Nutr.* **34**, 199–203.
12. Bronzert, T. S. & Brewer, H. B., Jr. (1977) *Clin. Chem.* **23**, 2089–2098.
13. *Lipid Research Clinics Program Manual of Laboratory Operations* (1974) (Dept. Health, Education, and Welfare, Washington, DC), Vol. 1, Publications no. (NIH) 75–628.
14. Samuel, P., Crouse, J. R. & Ahrens, E. H., Jr. (1978) *J. Lipid Res.* **19**, 82–92.
15. Ahrens, E. H., Jr. (1970) in *Advances in Metabolic Disorders*, eds. Levine, R. & Luft, R. (Academic, New York), Vol. 4, pp. 297–332.
16. Kopito, R. R. & Brunengraber, H. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1720 (abstr.).
17. Shapiro, D. J. & Rodwell, V. W. (1972) *Biochemistry* **11**, 1042–1048.
18. Kopito, R. R. & Brunengraber, H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5738–5740.
19. Brunengraber, H., Weinstock, S. B., Story, D. L. & Kopito, R. R. (1981) *J. Lipid Res.* **22**, 916–920.
20. Schwarz, C. C., Berman, M., Vlaheevic, Z. R., Halloran, D., Gregory, H. & Swell, L. (1978) *J. Clin. Invest.* **61**, 408–423.
21. Rodgers, D. H., Kim, D. N., Lee, K. T., Reiner, J. M. & Thomas, W. A. (1981) *J. Lipid Res.* **22**, 811–819.
22. Miettinen, T. A. (1969) *Life Sci.* **8**, 713–721.