

# Urinary excretion of mevalonic acid as an indicator of cholesterol synthesis

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**Abstract** Urinary excretion of mevalonic acid was investigated as an indicator of cholesterol synthesis. In normolipemic volunteers, excretion of mevalonic acid averaged  $3.51 \pm 0.59$  (SD)  $\mu\text{g}/\text{kg} \times \text{day}^{-1}$ ; ( $n = 24$ ) and was not different from patients with hypercholesterolemia ( $3.30 \pm 0.92 \mu\text{g}/\text{kg} \times \text{day}^{-1}$ ;  $n = 24$ ). In patients with cerebrotendinous xanthomatosis, the excretion was significantly higher ( $8.55 \pm 1.92 \mu\text{g}/\text{kg} \times \text{day}^{-1}$ ;  $n = 6$ ,  $P < 0.001$ ) but comparable to volunteers treated with cholestyramine ( $6.69 \pm 2.6 \mu\text{g}/\text{kg} \times \text{day}^{-1}$ ;  $n = 5$ ). A significant correlation was found between 24-h excretion of mevalonic acid and cholesterol synthesis ( $r = 0.835$ ;  $n = 35$ ;  $P < 0.001$ ). The coefficient of variation of excretion of mevalonic acid during 3 consecutive days was small (9.8%;  $n = 7$ ). However, urinary output of mevalonic acid was significantly higher during the night ( $164 \pm 14 \mu\text{g}/12\text{-h}$ ) than during the day ( $129 \pm 9 \mu\text{g}/12\text{-h}$ ;  $n = 11$ ;  $P < 0.05$ ). In patients treated with simvastatin (40 mg/day) for 6 weeks, the ratio of mevalonic acid to creatinine in a morning urine sample decreased significantly compared to pretreatment values ( $110 \pm 25 \mu\text{g}/\text{g}$  vs.  $66 \pm 25 \mu\text{g}/\text{g}$ ;  $P < 0.001$ ). Furthermore, the ratio of mevalonic acid to creatinine in a morning urine sample correlated with the ratio from the 24-h collection period ( $r = 0.714$ ;  $n = 34$ ;  $P < 0.001$ ). ■ The results indicate that the analysis of urinary mevalonic acid, either in 24-h collection or in a single morning sample, is an attractive method for evaluation of long and very short term changes of the rates of cholesterol synthesis.—Lindenthal, B., A. Simatupang, M. T. Dotti, A. Federico, D. Lütjohann, and K. von Bergmann. Urinary excretion of mevalonic acid as an indicator of cholesterol synthesis. *J. Lipid Res.* 1996. **37**: 2193–2201.

**Supplementary key words** simvastatin • lovastatin • pravastatin • cholestyramine • hypercholesterolemia • diurnal variation of cholesterol synthesis • fecal balance • stable isotope

The conversion of 3 $\beta$ -hydroxy-3 $\beta$ -methylglutaryl coenzyme A (HMG-CoA) into mevalonic acid regulated by HMG-CoA reductase is the rate-limiting step in cholesterol biosynthesis (1). In the metabolic steady state, the serum concentrations of mevalonic acid reflect the activity of hepatic HMG-CoA reductase, including its peak concentrations at night and low concentrations during the day that may vary 2- to 5-fold over 24 h (2). Despite

this diurnal variation in serum concentrations of mevalonic acid, a significant correlation between mevalonic acid in fasting serum and total cholesterol synthesis measured by the fecal balance method has been observed under metabolic ward conditions (3). Under outpatient conditions, however, Popjak et al. (4) and Parker et al. (2) noted a large variation on different days in the serum concentrations of individual subjects. For this reason, it may be difficult to estimate cholesterol synthesis rates in outpatients by measuring serum concentrations of mevalonic acid. As mevalonic acid from serum is excreted rapidly into urine, a 24-h urinary excretion of mevalonic acid provides a measurement of the integrated serum concentrations of mevalonic acid (5, 6). Therefore, daily excretion of mevalonic acid might be a better indicator of total cholesterol synthesis (7). Indeed, it has been demonstrated that inhibition of cholesterol synthesis by HMG-CoA reductase inhibitors reduces urinary outputs of mevalonic acid (8, 9), whereas stimulation of bile acid synthesis by administration of a bile acid binding resin has the opposite effect (2, 9).

In the present study we selected healthy normolipemic volunteers and patients with primary hypercholesterolemia and tested the hypothesis that the 24-h urinary excretion of mevalonic acid reflects cholesterol synthesis measured by fecal balance. Twenty-four-hour urinary excretion rates of mevalonic acid were also measured in patients with the rare inherited disease of cerebrotendinous xanthomatosis (CTX) who are known to have elevated cholesterol synthesis rates (10). Further-

Abbreviations: HMG-CoA, 3 $\beta$ -hydroxy-3 $\beta$ -methylglutaryl coenzyme A; CTX, cerebrotendinous xanthomatosis; GLC/MS, gas-liquid chromatography-mass spectrometry; SIM, selected ion monitoring; HDL, high density lipoprotein; LDL, low density lipoprotein.

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more, we investigated whether the diurnal rhythm of serum mevalonic acid can also be detected in urinary output, when the sampling time is divided into day and night time. Finally, we tested the hypothesis that the ratio of mevalonic acid to creatinine in a single fasting urine sample could be used to indicate changes in cholesterol synthesis.

## PATIENTS AND METHODS

### Subjects

Twenty-four normolipemic male volunteers (controls) aged 22 to 32 years, 24 patients with primary hypercholesterolemia (15 men and 9 women) aged 26 to 68 years, and 6 patients with CTX (3 men and 3 women) aged 29 to 45 years participated in the study. Diabetes, renal/liver disease, or thyroid dysfunction were excluded by history and laboratory means, and none of the volunteers or patients were treated with drugs known to influence cholesterol metabolism. In patients with CTX, chenodeoxycholic acid administration was stopped 4 weeks before the study. The clinical profile and lipoprotein concentrations of the different groups of subjects are summarized in **Table 1**.

### Study design

Seven different kinds of studies were performed. 1) Measurement of 24-h urinary mevalonic acid was obtained in all normolipemic subjects, patients with hypercholesterolemia, and CTX. 2) In 12 of the volunteers and in 23 of the patients with hypercholesterolemia, fecal balance measurements for total cholesterol synthesis were carried out during the week of the 24-h urine collections. 3) Seven subjects collected urine for 3 consecutive days under metabolic ward conditions and again 4 weeks later for 3 consecutive days as outpatients. 4) In 11 volunteers the 24-h urinary collections were divided into two 12-h periods, the first from 7.00 AM–7.00 PM (day) and the second one from 7.00 PM–7.00 AM (night). 5) Seven volunteers collected urine for 24-h before and after 6 weeks of administration of pravastatin (20 mg at bedtime), and 5 volunteers collected urine before and during the last day of cholestyramine treatment (4 g b.i.d. for 2 weeks). 6) In 21 of the volunteers and in 13 of the patients with hypercholesterolemia, an additional urine fasting specimen was collected after the completion of the 24-h urine collection period. 7) Mevalonic acid concentrations were measured in a morning urine specimen in 12 patients with hypercholesterolemia before and after 6 weeks of treatment with simvastatin (40 mg/day). A morning urine sample was also obtained in patients treated with different doses of an HMG-CoA reductase inhibitor. Six patients were

treated with a low dose of 10 mg q.d. or 10 mg b.i.d., 19 with a medium dose (20 mg q.d. or 20 mg b.i.d.), and 9 with a high dose (40 mg q.d. or 40 mg b.i.d.) of simvastatin or lovastatin, respectively. Patients received the treatment for at least 6 weeks. The results were compared to 18 patients without lipid-lowering drugs, matched for age, sex, and body weight. All subjects and patients gave informed consent and all the studies were approved by the local ethical committee.

### Measurement of mevalonic acid

Mevalonic acid in urine was determined by method 3 previously reported (11). Briefly, 60.2 ng of [ $^2\text{H}_3$ ]mevalonic acid (MSD Isotopes, Montreal, Canada) was added as internal standard to 400  $\mu\text{l}$  urine. After extraction, purification, and derivatization with methyl-tertiary-butyl-dimethylsilyl-trifluoroacetamide, the concentration of mevalonic acid was analyzed by gas-liquid chromatography/mass spectrometry (GLC/MS) on a Hewlett-Packard (HP) GLC 5890 combined with an HP 5970 quadrupole type MS. Selected ion monitoring (SIM) was performed by cycling the quadrupole mass filter between  $m/z$  values at a rate of 5.2 cycles per second. In the SIM mode, the ion  $m/z$  317 (base peak) was scanned for authentic mevalonic acid and the ion  $m/z$  320 for [ $^2\text{H}_3$ ]mevalonic acid. Creatinine in urine was measured by a standard laboratory procedure.

### Cholesterol synthesis

Fecal samples for sterol balance measurements were collected and analyzed as described elsewhere (12). For this purpose, subjects received sitostanol (30 mg t.i.d.) for 7 days as fecal flow and recovery marker and stools were collected on days 5, 6, and 7 for GLC analysis of neutral and acidic sterols. Daily cholesterol intakes were calculated from 7-day food records using a computer program for food composition (13). The rate of cholesterol synthesis was then estimated by subtracting the daily intake of cholesterol from total daily fecal sterol excretion (12).

### Lipid and lipoprotein analysis

Blood was drawn for serum lipid and lipoprotein analysis in the morning after an overnight fast. Total cholesterol and triglycerides in serum were measured by enzymatic methods (Boehringer, Mannheim, Germany). High density lipoprotein (HDL) cholesterol was also determined enzymatically in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with heparin-manganese (Boehringer, Mannheim, Germany). Low density lipoprotein (LDL) cholesterol was calculated by the method of Friedewald, Levy, and Fredrickson (14).

TABLE 1. Clinical data and lipoprotein concentrations in normolipemic volunteers, patients with hypercholesterolemia, and patients with cerebrotendinous xanthomatosis (CTX)

| Variable                 | Controls              | Hypercholesterolemic               | CTX                                  |
|--------------------------|-----------------------|------------------------------------|--------------------------------------|
|                          | n = 24                | Patients<br>n = 24                 | Patients<br>n = 6                    |
| Age (yr)                 | 27 ± 3<br>(21–32)     | 50 ± 12 <sup>a</sup><br>(26–68)    | 39 ± 6 <sup>b</sup><br>(29–45)       |
| Body weight (kg)         | 83 ± 14<br>(62–122)   | 76 ± 14 <sup>a</sup><br>(50–98)    | 54 ± 7 <sup>b</sup><br>(46–65)       |
| BMI (kg/m <sup>2</sup> ) | 25 ± 4<br>(20–40)     | 25 ± 3<br>(22–30)                  | 20 ± 2 <sup>a</sup><br>(17–23)       |
| Cholesterol (mg/dl)      | 182 ± 21<br>(147–229) | 314 ± 54 <sup>a</sup><br>(237–452) | 143 ± 16 <sup>a,c</sup><br>(120–160) |
| LDL-cholesterol (mg/dl)  | 109 ± 19<br>(75–146)  | 243 ± 56 <sup>a</sup><br>(160–396) | nd                                   |
| HDL-cholesterol (mg/dl)  | 55 ± 12<br>(34–74)    | 44 ± 10 <sup>a</sup><br>(29–62)    | nd                                   |
| Triglyceride (mg/dl)     | 83 ± 18<br>(46–108)   | 133 ± 38 <sup>a</sup><br>(65–212)  | nd                                   |
| Cholesterol (mg/dl)      | nd                    | nd                                 | 2.4 ± 1.2 <sup>c</sup><br>(1.1–4.4)  |

Values are given as means ± SD; range in parentheses; n, number of subjects; nd, not done.

<sup>a</sup>Significantly different from controls,  $P < 0.05$ .

<sup>b</sup>Significantly different from controls and patients with hypercholesterolemia,  $P < 0.05$ .

<sup>c</sup>Measured by gas-liquid chromatography.

### Analysis of cholesterol and cholestanol by gas-liquid chromatography

Analysis of cholesterol and cholestanol was performed as described previously (15). Briefly, to 100  $\mu$ l of serum 50  $\mu$ g of 5 $\alpha$ -cholestane (Serva Feinbiochemica, Heidelberg, Germany) was added as internal standard. After alkaline hydrolysis and extraction with n-hexane, cholesterol and cholestanol were derivatized to their trimethylsilyl-ethers and quantified by GLC.

### Calculation of the amount of mevalonic acid excreted in urine and not used for cholesterol synthesis

As 6 moles of mevalonic acid are incorporated into cholesterol (16) it was possible from the present results to calculate the percentage of mevalonic acid excreted in urine and not used for cholesterol synthesis by the following equations:

$$\frac{\text{Mevalonic acid (C) (mol/day)}}{\text{cholesterol synthesis (mol/day)}} \times 6 \quad \text{Eq. 1}$$

$$\frac{\text{Mevalonic acid (U) (mol/day)}}{\text{mevalonic acid (C) (mol/day)}} \times 100\% \quad \text{Eq. 2}$$

where (C) is the amount used for cholesterol synthesis and (U) the amount excreted in urine.

### Statistical analysis

The results are expressed as mean ± standard deviation (mean ± SD) to show variations in a group or as standard error of the mean (SEM) to compare different groups. Differences between paired or unpaired results were calculated using the Student's *t*-test and considered significant at a level of  $P < 0.05$ . The correlation between parameters was tested by Pearson's correlation coefficient. Coefficients of variation (CV) were calculated as SD/mean × 100%. All calculations were done with the statistical software of SPSS/Windows (SPSS Inc.).

## RESULTS

### Twenty-four-hour urinary excretion of mevalonic acid in different groups of subjects

Twenty-four-hour urinary excretions of mevalonic acid in normolipemic volunteers, in patients with hypercholesterolemia, and patients with CTX are summarized in Table 2. In the control subjects, excretion of mevalonic acid averaged 288  $\mu$ g/day, and was slightly (but significantly) higher (14%) than in patients with hypercholesterolemia (249  $\mu$ g/day;  $P < 0.05$ ). As the weights of the volunteers were also slightly (but significantly) higher than those of the hypercholesterolemic patients, no significant difference could be detected after correction for body weight or when expressed as ratio to creatinine (Table 2). These results demonstrate that there were no differences in the rates excretion of

TABLE 2. Excretion of mevalonic acid in 24-h urine of normolipemic volunteers (controls), patients with hypercholesterolemia, and patients with cerebrotendinous xanthomatosis (CTX)

| Subjects                               | Mevalonic Acid<br>$\mu\text{g/day}$ | Mevalonic Acid<br>per Body Weight<br>$\mu\text{g/kg} \times \text{day}^{-1}$ | Mevalonic Acid<br>per Creatinine<br>$\mu\text{g/g} \times \text{day}^{-1}$ |
|--|-------------------------------------|--|--|
| Volunteers (n = 24)                    | $288 \pm 54$<br>(167–428)           | $3.51 \pm 0.59$<br>(2.57–4.43)   | $142 \pm 22$<br>(93–177)   |
| Hypercholesterolemic patients (n = 24) | $249 \pm 82^a$<br>(89–398)          | $3.30 \pm 0.92$<br>(1.53–5.04)   | $158 \pm 52$<br>(89–266)   |
| CTX patients (n = 6)                   | $467 \pm 141^b$<br>(312–678)        | $8.55 \pm 1.92^b$<br>(5.67–10.43)  | $424 \pm 37^b$<br>(350–449)  |

Values are given as means  $\pm$  SD; range in parentheses.

<sup>a</sup>Significantly different from volunteers,  $P < 0.05$ .

<sup>b</sup>Significantly different from volunteers and hypercholesterolemic patients,  $P < 0.001$ .

mevalonic acid between normolipemic volunteers and patients with hypercholesterolemia. Although dietary cholesterol intake was significantly higher in volunteers ( $291 \pm 70$  mg/day) than in patients with hypercholesterolemia ( $179 \pm 89$  mg/day;  $P < 0.05$ ) this difference did not affect mevalonic acid excretion. In contrast, the rates of daily urinary excretion in patients with CTX were on the average 1.6- and 1.9-fold higher than those in volunteers and in hypercholesterolemia patients, and even 2.5-fold higher when expressed to body weight or as ratio to creatinine ( $P < 0.001$ ).

#### Correlation between urinary excretion of mevalonic acid and cholesterol synthesis

Twenty-four-hour urinary excretion of mevalonic acid was compared to the rate of total cholesterol synthesis in 12 normolipemic volunteers and 23 patients with hypercholesterolemia. Total cholesterol synthesis was significantly correlated to excretion of mevalonic acid ( $r = 0.835$ ;  $P < 0.001$ ; Fig. 1). There was no difference in the slope of the line between normolipemic volunteers ( $y = 0.18x + 80$ ;  $r = 0.655$ ;  $P < 0.02$ ) and patients with hypercholesterolemia ( $y = 0.21x + 19$ ;  $r = 0.897$ ;  $P < 0.001$ ). No relation between mevalonic acid excretion and dietary intake of cholesterol was seen, but excretion of mevalonic acid was also related to body weight ( $r = 0.607$ ;  $P < 0.001$ ;  $n = 48$ ) (data not shown). From the combined measurements of total cholesterol synthesis and 24-h urinary excretion of mevalonic acid, the percentage of mevalonic acid not used for cholesterol synthesis and that spilled over into urine was calculated. Normolipemic volunteers excreted a calculated average of  $0.011 \pm 0.002\%$  (SD), that was identical to that in male ( $0.011 \pm 0.002\%$ ), and female patients with hypercholesterolemia ( $0.010 \pm 0.001\%$ ). The data indicate that the percentage of mevalonic acid that escapes cholesterol synthesis is extremely constant over the whole range of total cholesterol synthesis (560 mg/day to 1790

mg/day) and is independent of hypercholesterolemia and gender.

#### Reproducibility of daily excretion of mevalonic acid

To investigate the reproducibility of the daily excretion of mevalonic acid, the day to day variation in urinary excretion of mevalonic acid was evaluated in 7 volunteers over 3 consecutive days, first under metabolic ward conditions and second 4 weeks later as outpatients. The results of this study are given in Table 3. The coefficient of variation of excretion of mevalonic acid was low and remained the same whether collected under metabolic ward conditions (range: 3.5% to 14.3%, mean 9.2%) or as outpatients (range: 2.2% to 16.2%; mean 9.8%). Although there were some differences in total daily output from subject to subject, the overall excretion rates were not different between metabolic ward or outpatient conditions ( $285$   $\mu\text{g/day}$  vs.  $287$   $\mu\text{g/day}$ ).

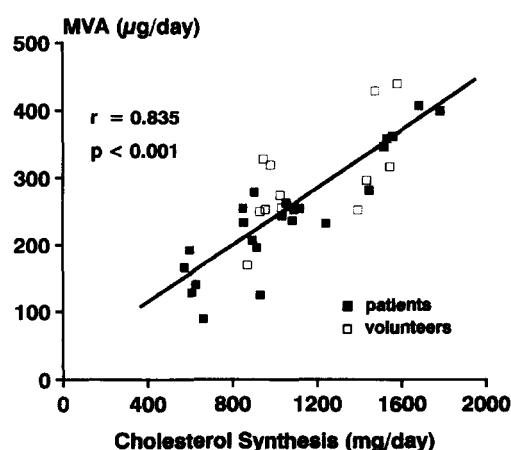


Fig. 1. Comparison of cholesterol synthesis measured by fecal balance and 24-h urinary excretion of mevalonic acid in 12 normolipemic healthy volunteers and 23 patients with hypercholesterolemia.



TABLE 3. Excretion of mevalonic acid in 24-h urine from seven normolipemic volunteers (controls) on 3 consecutive days under metabolic ward and outpatient conditions

| Volunteer      | Mevalonic Acid         |                        |
|----------------|------------------------|------------------------|
|                | Metabolic Ward         | Outpatient             |
|                | $\mu\text{g/day}$      |                        |
| 1              | 254 $\pm$ 36<br>(14.3) | 312 $\pm$ 36<br>(11.5) |
| 2              | 286 $\pm$ 39<br>(13.5) | 254 $\pm$ 40<br>(16.2) |
| 3              | 272 $\pm$ 9.5<br>(3.5) | 321 $\pm$ 31<br>(4.8)  |
| 4              | 253 $\pm$ 24<br>(9.2)  | 263 $\pm$ 20<br>(5.7)  |
| 5              | 283 $\pm$ 14<br>(5.0)  | 348 $\pm$ 21<br>(2.2)  |
| 6              | 326 $\pm$ 42<br>(12.8) | 356 $\pm$ 17<br>(15.9) |
| 7              | 321 $\pm$ 19<br>(5.8)  | 223 $\pm$ 27<br>(12.0) |
| Mean $\pm$ SEM | 285 $\pm$ 11           | 287 $\pm$ 19           |

Values are given as mean  $\pm$  SD; coefficient of variation (SD/mean  $\times$  100[%]) in parentheses.

#### Diurnal variation in excretion of mevalonic acid

Dividing 24-h urine collections in two periods of time, a significantly higher output of mevalonic acid was seen during the night than during the day ( $P < 0.05$ ; Fig. 2). However, in 3 out of 11 volunteers, the excretion during the day was slightly higher than during the night (4%, 18%, and 20%). In the 8 subjects where the output was higher during the night than during the day, the excretion of mevalonic acid was 46% higher at night (range 6% to 122%).

#### Effect of pravastatin and cholestyramine on excretion of mevalonic acid

As expected, pravastatin decreased 24-h excretion of mevalonic acid in all 7 volunteers, on the average by 38% from  $293 \pm 65$  mg/day to  $196 \pm 52$  mg/day ( $P < 0.02$ ). The bile acid binding resin, cholestyramine, increased urinary output on the average from  $305 \pm 97$  mg/day to  $487 \pm 137$  mg/day (+60%;  $P < 0.02$ ;  $n = 5$ ).

#### Excretion of mevalonic acid during 24 h and concentrations measured in a morning urine sample

To study the possibility that measurement of mevalonic acid in a spontaneous fasting urine sample collected in the morning is suitable as an indicator of changes in cholesterol synthesis rates, we compared the results with those from the 24-h collections. The results of 24-h urinary excretion of mevalonic acid in volunteers and patients with hypercholesterolemia compared to those from a single fasting urine sample from the same subject showed a significant positive correlation when expressed as ratio to creatinine ( $r = 0.714$ ;  $P < 0.001$ ;  $n =$

34; Fig. 3). Furthermore, the ratio of mevalonic acid to creatinine in the morning urine specimen also correlated to the daily excretion of mevalonic acid ( $r = 0.541$ ;  $P < 0.001$ ). Thus, even the ratio of mevalonic acid to creatinine in a single urine sample can give an indication of the cholesterol synthesis in individual subjects.

#### Effect of simvastatin and lovastatin on mevalonic acid concentrations in a morning urine sample

In order to test further this hypothesis that mevalonic acid in a single urine specimen is valid for showing changes in cholesterol synthesis, we compared the ratio of mevalonic acid to creatinine in the morning urine specimens in 12 hypercholesterolemic patients before and after 6 weeks of treatment with simvastatin (40 mg/day). The ratio of mevalonic acid to creatinine was lowered in all subjects, on the average by 40%, from  $110 \pm 25$   $\mu\text{g/g}$  to  $66 \pm 25$   $\mu\text{g/g}$  ( $P < 0.001$ ). The individual results are shown in Fig. 4. In addition, the ratio of mevalonic acid to creatinine was measured in patients treated with different doses of simvastatin or lovastatin and compared to matched patients with hypercholesterolemia without lipid lowering therapy (Table 4). A significantly lower ratio of mevalonic acid to creatinine was seen with all three different doses compared to no lipid lowering therapy ( $P < 0.01$ ). However, no further significant reduction was seen between the low, medium or high dose of the two HMG-CoA reductase inhibitors.

## DISCUSSION

Measuring cholesterol synthesis provides us with important information on cholesterol homeostasis under various physiological conditions, disease states, and

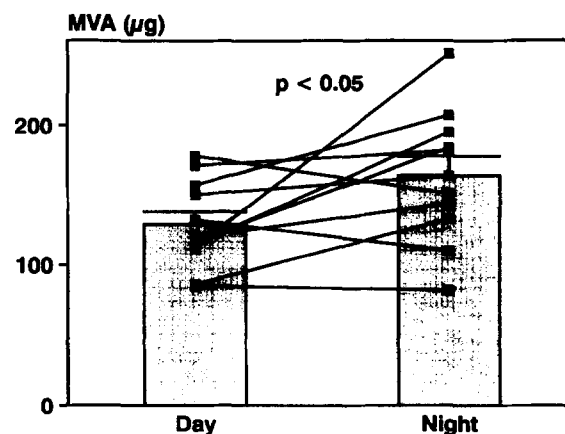


Fig. 2. Urinary excretion of mevalonic acid during the day (7.00 AM–7.00 PM) and the night (7.00 PM–7.00 AM) in 11 normolipemic volunteers.

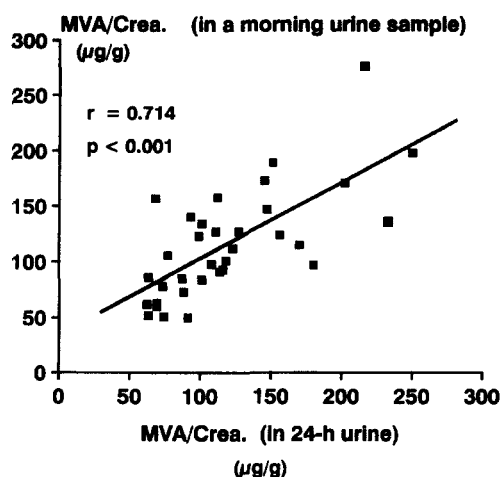


Fig. 3. Comparison of the ratio of mevalonic acid to creatinine in urine collected for 24 h and in a morning specimen collected immediately after the 24-h collection period in 34 subjects.

drug treatment. Previous studies have shown that serum concentrations of mevalonic acid might be useful in estimating the rate of cholesterol synthesis under metabolic ward conditions. However, quantification of mevalonic acid in serum is more difficult than in urine, because of lower concentrations and high serum proteins. In addition, serum concentrations of mevalonic acid show a circadian rhythm and outpatients have large intraindividual variations, making an evaluation more difficult (2, 4). Measurements of 24-h urinary excretion reflect the integrated serum concentration and could overcome these pitfalls. Therefore, we focused the present study on the urinary excretion of mevalonic acid as an indicator of cholesterol metabolism under various conditions in humans, using a simple method developed in our laboratory (11).

#### Twenty-four-hour excretion of mevalonic acid in different groups of subjects

The results of urinary excretion of mevalonic acid in normolipemic volunteers and in patients with hypercholesterolemia obtained during the present study are consistent with earlier reports by Kopito and Brunnengraber (6), Parker et al. (3), and Pappu and Illingworth (9). No significant differences of mevalonic acid excretion could be detected between the two groups when the results were expressed to body weight or to creatinine excretion. The 24-h excretion of mevalonic acid was independent of dietary cholesterol intake. This is in contrast to previous studies where low and high cholesterol intakes were compared (3), but might be the result of the small range of dietary cholesterol intake during the present study (only three subjects had a cholesterol intake above 400 mg/day). Total cholesterol synthesis

also revealed no difference between normolipemic controls and patients with hypercholesterolemia ( $14.2 \text{ mg/kg} \times \text{day}^{-1}$  vs.  $14.3 \text{ mg/kg} \times \text{day}^{-1}$ ). Previous studies have also indicated that patients with heterozygous familial hypercholesterolemia do not or only slightly differ in their total cholesterol synthesis from controls (17), or in their biliary secretion rates of cholesterol and bile acids (18, 19). Thus, the present results of excretion of mevalonic acid are in accordance with studies performed previously on cholesterol metabolism in patients with hypercholesterolemia (17–19).

Patients with CTX showed a 2.5-fold higher secretion rate of mevalonic acid per body weight compared to volunteers and patients with hypercholesterolemia. Previous studies using the fecal balance method also showed a higher total cholesterol synthesis in two patients with CTX compared to five controls ( $18.2 \text{ mg/kg} \times \text{day}^{-1}$  vs.  $11.1 \text{ mg/kg} \times \text{day}^{-1}$ ) (10), but bile alcohols were not included in the calculation. Whereas fecal balance is the standard procedure for measuring total cholesterol synthesis (20, 21), it is more difficult to perform in patients with CTX for at least two reasons. First, patients with this inherited metabolic disease excrete not only fecal neutral and acidic sterols but also different bile alcohols; quantification of bile alcohols is more difficult to perform. Second, bile alcohols are also excreted in urine in considerable amounts as glucuronides and sulfates. In order to calculate the total cholesterol synthesis, bile alcohols from the feces and urine must be added to neutral and acidic fecal sterols. This was not done by Salen and Grundy (10) during their fecal balance study. Measuring the excretion of mevalonic acid overcomes this problem. Serum levels of lathosterol also correlate with total cholesterol synthesis under various conditions (22) but not all (23). Indeed, Wolthers et al. (24) found elevated serum concentrations of lathosterol

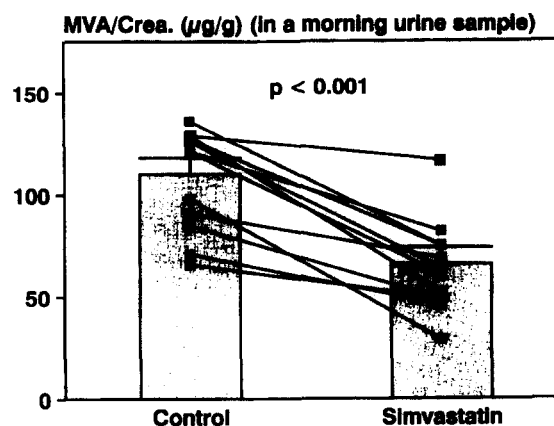


Fig. 4. Ratio of mevalonic acid to creatinine in a morning urine sample before and after 6 weeks of treatment with simvastatin (40 mg/day) in 12 patients with hypercholesterolemia.

in patients with CTX, another indication of high synthesis rates in this disease. Assuming that 0.01% of total cholesterol synthesis is also excreted as mevalonic acid in urine in patients with CTX, the calculated total synthesis in our patients would be  $34 \text{ mg/kg} \times \text{day}^{-1}$ . Compared to the results of Salen and Grundy (10) almost half of total cholesterol synthesized must have been converted into bile alcohols. Thus, the higher excretion of mevalonic acid measured in the six patients with CTX is in line with the considerable amounts of bile alcohols produced by the patients. The high mevalonic acid output and total cholesterol synthesis in patients with CTX is probably due to the reduced feedback inhibition of cholesterol 7 $\alpha$ -hydroxylase and subsequent inhibition of HMG-CoA reductase in the liver. As the synthesis of bile acids is regulated by the quantity of these substances that return to the liver in the enterohepatic circulation, a blockage of bile acid synthesis reduces this quantity and releases the feedback inhibition. The huge amounts of bile alcohols formed by these patients do not down-regulate the key enzymes for bile acid and cholesterol synthesis. The mechanism of increased urinary excretion of mevalonic acid in patients with CTX is comparable to treatment with a bile acid binding resin. During the present study the administration of cholestyramine resulted in excretion rates of mevalonic acid similar to patients with CTX ( $487 \mu\text{g/day}$  vs.  $467 \mu\text{g/day}$ ).

#### **Correlation between excretion of mevalonic acid and cholesterol synthesis**

Results from the present study confirm the close relationship seen between cholesterol synthesis and mevalonic acid metabolism. Previous investigators compared the plasma concentration of mevalonic acid with the results of fecal balance under metabolic ward conditions (3). However, Popjak et al. (4) and Parker et al. (2) noted a large variation in the concentration of plasma mevalonic acid during outpatient conditions. We, therefore, in the present study used 24-h excretion rates. Daily excretion rates may be more reliable for this purpose because they provide a measure of the integrated serum concentrations of mevalonic acid (5, 6). Although, it has previously been documented that measuring cholesterol synthesis by the fecal balance method is reproducible under outpatient conditions (12), it is still necessary to use markers for several days to reach steady state fecal excretion and to assess dietary cholesterol intake. It is thus an advantage to obtain synthesis rates by a 24-h excretion of mevalonic acid. The present results convincingly demonstrate for the first time that 24-h urinary output of mevalonic acid is highly correlated to rates of cholesterol synthesis (Fig. 1). As cholesterol synthesis increases, urinary output of mevalonic acid also becomes greater. This relationship between cholesterol

synthesis and excretion of mevalonic acid was seen over a wide range of cholesterol synthesis in both normolipemic volunteers and patients with hypercholesterolemia. As total body weight is an important factor in regulating cholesterol synthesis (25–27), it is obvious that excretion of mevalonic acid is also related to body weight. From the combined measurements of total cholesterol synthesis and 24-h urinary excretion of mevalonic acid it was possible to calculate the percentage of mevalonic acid excreted in urine and not converted to cholesterol. The percentage was extremely low but constant, and independent of age, weight, sex, and cholesterol synthesis. It should be mentioned that this calculation does not include the incorporation of mevalonic acid into nonsterol isoprenoids (1).

#### **Reproducibility of daily excretion of mevalonic acid**

The small intraindividual variations in the daily urinary output over 3 consecutive days, measured either under metabolic ward or outpatient conditions, support our hypothesis that urinary mevalonic acid is an excellent marker for measuring short term changes in cholesterol synthesis. Even minor changes in the rates of cholesterol synthesis could be detected by this method.

#### **Diurnal variation in excretion of mevalonic acid**

The diurnal variations in cholesterol synthesis, measured either by the activity of the HMG-CoA reductase or the serum concentration of mevalonic acid, have not been explained in detail. Parker et al. (3) demonstrated that fasting and cholesterol feeding affects the diurnal rhythm. In addition, Pappu and Illingworth (28) noted similar diurnal variations in patients with homozygous hypobetalipoproteinemia. Their cholesterol absorption is negligible (29); thus, mechanisms other than dietary cholesterol must also play a role in the diurnal variation. The present investigation tested whether the diurnal variation can also be observed in urinary output of mevalonic acid. The results confirm previous observations in animals and humans, according to other methods that show cholesterol synthesis has a circadian rhythm (2, 28, 30, 31). It is of interest to note that not every subject exhibited greater excretion rates at night than during the day, reported also by other investigators using mass isotope distribution analysis (31). Thus, it might be worthwhile to investigate in more detail those subjects with a weakened or an inverse diurnal rhythm. The 2-part 24-h urine sampling for measurements of mevalonic acid provides a simple approach for investigating the mechanism(s) that cause the diurnal periodicity of cholesterol synthesis in humans. In addition, it can be performed under outpatient conditions and no blood sampling at night is necessary.

TABLE 4. Effect of different doses of simvastatin and lovastatin on the ratio of mevalonic acid to creatinine in patients with primary hypercholesterolemia in one urine specimen obtained between 8.00 AM and 10.00 AM

| Drug                            | n  | Dose<br>mg/day   | Mevalonic Acid/Creatinine<br>μg/g |
|---------------------------------|----|------------------|-----------------------------------|
| None                            | 18 |                  | 169 ± 40                          |
| Simvastatin<br>or<br>Lovastatin | 6  | 1 × 10<br>2 × 10 | 108 ± 17 <sup>a</sup>             |
| Simvastatin<br>or<br>Lovastatin | 19 | 1 × 20<br>2 × 20 | 98 ± 7 <sup>a</sup>               |
| Simvastatin<br>or<br>Lovastatin | 9  | 1 × 40<br>2 × 40 | 84 ± 13 <sup>a</sup>              |

Values are given as means ± SEM; n, number of patients.

<sup>a</sup>Significantly different from no treatment,  $P < 0.01$ .

### Effect of pravastatin and cholestyramine on excretion of mevalonic acid

The finding of reduced excretion of mevalonic acid during treatment with an HMG-CoA reductase inhibitor and increased output by administration of cholestyramine confirms previous results (2, 8, 9, 32). Thus, these measurements can be used to investigate the mechanism of action of lipid-lowering drugs (33).

### Excretion of mevalonic acid during 24 h and concentrations measured in a morning urine sample

The close relationship between the ratio of mevalonic acid to creatinine in 24-h urine collections with this ratio in a spontaneous urine sample measured in the same subject after an overnight fast and the relationship between 24-h urinary output of mevalonic acid offers new opportunities to investigate changes in the rate of cholesterol synthesis during short- and long-term studies.

### Effect of simvastatin and lovastatin on mevalonic acid concentration measured in a morning urine sample

The ratio of mevalonic acid to creatinine before and during treatment with simvastatin in urine samples collected in the morning in outpatients with hypercholesterolemia (Fig. 4) proves that this ratio is an excellent parameter to evaluate changes in rates of cholesterol synthesis. The magnitude of reduction of this ratio is in line with previous reports of 24-h excretions rates (3, 9, 32) and with the results of pravastatin during the present study in healthy volunteers (-33%). Low dose administration of lovastatin and simvastatin also resulted in a striking decrease in excretion of mevalonic acid (-36%) compared to results in patients without lipid-lowering drugs. A 2- or 4-fold higher dose

resulted only in a marginally greater reduction in the ratio that was not significant. Pappu et al. (9, 32) did identical studies with different doses of lovastatin, but measured daily output of mevalonic acid. They also found the largest decrease in mevalonic acid output during the administration of lovastatin in a dose of 10 mg twice daily. The higher doses (20 mg and 40 mg b.i.d.) did not lead to a further reduction in excretion of mevalonic acid. The results indicate that measuring mevalonic acid in a spontaneous urine sample in the morning is a simple and reliable way to measure the effects of HMG-CoA reductase inhibitors on changes in cholesterol synthesis. It can also be used in clinical studies to control patients' compliance of drug intake.

In conclusion, our simplified GLC/MS method of measuring urinary excretion of mevalonic acid provides important information on cholesterol synthesis and can be used under diverse conditions to investigate disorders in cholesterol synthesis.

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