Urinary Excretion and Serum Concentration of Mevalonic Acid During Acute Intake of Alcohol

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The influence of 2 different alcoholic beverages containing an equal amount of alcohol (48 g), 1 with mevalonic acid (beer) and 1 without (vodka), on the urinary excretion and serum concentration of mevalonic acid was investigated in 7 healthy subjects. Drinking 1 L of beer at night containing 608 μg/L mevalonic acid more than doubled the urinary excretion of mevalonic acid the following 12 hours, on average from 103 ± 15 μg/12 h to 211 ± 17 μg/12 h ($P < .001$; 18% of the administered dose). Drinking the same amount of alcohol as vodka had no effect, but urinary mevalonic acid output increased slightly the following day (7 AM to 7 PM) after ingestion of both alcoholic beverages. Serum concentrations of mevalonic acid were significantly increased the following morning after ingestion of beer (from 3.22 ± 0.20 ng/mL to 6.79 ± 0.58 ng/mL) or vodka (from 3.23 ± 0.37 ng/mL to 5.36 ± 0.55 ng/mL, $P < .002$ for both). An increase in the ratio of lathosterol to cholesterol in serum, another indicator of 3β-hydroxy-3β-methylglutaryl coenzyme A reductase activity in the liver, was also observed (+18% and +25%, respectively). After oral administration of [$^{13}C_2$] mevalonic acid at night, 20% ± 0.7% of the dose was excreted in urine the following 12 hours, and only trace amounts thereafter. No [$^{13}C_2$] mevalonic acid could be detected in serum the following morning. We conclude that the absorption of dietary mevalonic acid and alcohol-induced mevalonic acid synthesis affects the urinary excretion and serum concentration of this cholesterol precursor. Therefore, studies using mevalonic acid as a marker of cholesterol synthesis must be carefully monitored regarding dietary mevalonic acid intake and alcohol consumption.

THE 3β-HYDROXY-3β-METHYLGLUTARYL coenzyme A reductase, which catalyzes the formation of mevalonic acid, is the rate-limiting enzyme of cholesterol synthesis.[1] Trace amounts of mevalonic acid are detectable in serum, with peak concentrations at night and lower concentrations during the day.[2] Approximately 0.01% of daily synthesized mevalonic acid is excreted in urine.[3] Despite the low amount of mevalonic acid escaping cholesterol synthesis, the fasting serum concentration and 24-hour urinary excretion of mevalonic acid are significantly correlated with total cholesterol synthesis.[2,3] Therefore, measurements of mevalonic acid in serum or urine are indicators of short- and long-term changes in cholesterol synthesis.[2-7]

Although beer and wine contain mevalonic acid,[8] no studies have considered the influence of these widely used beverages on mevalonic acid metabolism in humans. Furthermore, although alcohol was identified as a substrate for cholesterol synthesis,[9,10] the influence of alcohol on cholesterol synthesis is controversial.[11-13] Therefore, in the current study, the urinary excretion and serum concentration of mevalonic acid were measured after ingestion of beer containing mevalonic acid. The results were compared against the intake of an equimolar amount of alcohol (vodka) without mevalonic acid and ingestion of [$^{13}C_2$] mevalonic acid under identical conditions.

SUBJECTS AND METHODS

Subjects and Study Design

Seven healthy subjects (6 men and 1 woman, aged 22 to 34 years; body mass index, 24.4 ± 2.3 kg/m^2; range, 20.9 to 27.5) participated in the study. None were treated with medication, and none were consuming alcohol during the study except where indicated. All subjects collected urine from 7 PM to 7 AM (night) and from 7 AM to 7 PM (day), first during a control period and second after drinking 1 L of beer at night (between 9 PM to 10 PM) containing 608 μg/L mevalonic acid. Blood was drawn the following morning after an overnight fast. One week later, a second control period was followed by drinking the same amount of alcohol (48 g) in the form of vodka (38% vol/vol ethanol) together with 1 L carbonated water. Five subjects consumed 1 mg [1,2-^{13}C] mevalonic acid (Campro Scientific, Emmerich, Germany) dissolved in 100 mL carbonated water between 9 PM and 10 PM. Two of them drank the [$^{13}C_2$] mevalonic acid with 1 L carbonated water and 3 with 1 L of beer. Urine and serum samples were collected as described before.

The study was performed in accordance with the declaration of Helsinki and was approved by the local ethics committee. All subjects provided written informed consent.

Measurement of Mevalonic Acid in Urine

Mevalonic acid in urine was measured as reported previously.[14] Briefly, 140 ng [$^2H_7$] mevalonic acid (MSD Isotopes, Montreal, Quebec, Canada) was added as an internal standard to 1 mL urine. After extraction, purification, and derivatization with methyl *tert*-butyldimethylsilyl-trifluoracetamide, the concentration of mevalonic acid was determined by gas-liquid chromatography/mass spectrometry on a Hewlett-Packard (Palo Alto, CA) GLC 7573A combined with an HP 5972 quadrupole-type mass spectrometer. Selected ion monitoring was performed by cycling the quadrupole mass filter between *m/z* values at a rate of 5.2 cycles per second. In the selected ion monitoring mode, the ion *m/z* 317 (base peak) was scanned for authentic mevalonic acid and the ion *m/z* 324 for [$^2H_7$] mevalonic acid as internal standard. For measurement of [$^{13}C_2$] mevalonic acid, a different fragment that contained both labeled ^{13}C atoms had to be scanned (435 *m/z*). Therefore, the internal standard was scanned on the same fragment (440 *m/z*). All calculations were made with standard calibration curves. The creatinine level in urine was measured by a standard laboratory procedure.

Measurement of Mevalonic Acid in Serum

Serum concentrations of mevalonic acid were measured by the slightly modified method 2 described previously.[14] One milliliter of serum (adjusted to pH 3.5 with phosphoric acid) was used for sample

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Submitted February 22, 1999; accepted July 1, 1999.

Supported by research grants from the Bundesministerium für Forschung und Technologie (01EC9402) and the Deutsche Forschungsgemeinschaft (BE 1673/1-1).

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0026-0495/00/4901-0021$10.00/0

Table 1. Urinary Excretion of Mevalonic Acid During the Control Period and After Drinking 1 L of Beer or an Equal Amount of Alcohol as Vodka at Night (mean ± SEM)

	Mevalonic Acid Excretion			
	7 PM to 7 AM		7 AM to 7 PM	
Study Period	μg/12 h	μg/g Creatinine	μg/12 h	μg/g Creatinine
	mean ± SEM (n = 7)			
Control	103 ± 15	104 ± 14	65 ± 9	69 ± 9
Beer intake	211 ± 17*	218 ± 14*	92 ± 11†	97 ± 9‡
Control	112 ± 21	116 ± 17	71 ± 10	75 ± 6
Vodka intake	120 ± 14	122 ± 16	89 ± 7‡	92 ± 9‡

*$P < .001$ *v* control.
†$P = .060$ *v* control.
‡$P < .05$ *v* control.

preparation. Seven nanograms of [$^2H_7$] mevalonic acid was used as internal standard. After precipitation of proteins with 2 mL acetone, lipids were removed by extraction with 2 mL cyclohexane. Mevalonolactone was extracted 3 times into a mixture of 3 mL ethylacetate: acetone (2:1 vol/vol) using a salt-out effect with sodium chloride. From combined extracts, the solvent was removed by a slight stream of nitrogen. Thereafter, derivatization was performed as described for urine samples. The derivative was extracted into 80 μL *n*-decane and measured by gas-liquid chromatography/mass spectrometry as already described.

Lipid and Lipoprotein Analysis

Total cholesterol and triglyceride serum levels were measured using an enzymatic kit from Boehringer (Mannheim, Germany). High-density lipoprotein cholesterol was also determined enzymatically in the supernatant after precipitation of apolipoprotein B–containing lipoproteins with heparin-manganese (Boehringer). Low-density lipoprotein cholesterol was calculated by the equation of Friedewald et al.[15]

Analysis of Lathosterol and Cholesterol in Serum

Analyses of lathosterol and cholesterol in serum were performed as described previously.[16] Briefly, 50 μg 5α-cholestane (Serva Feinbiochemica, Heidelberg, Germany) was added to 0.1 mL serum as internal standard. After alkaline hydrolysis and extraction with *n*-hexane and derivatization to their trimethylsilyl ethers, the sterols were quantified by gas-liquid chromatography.

Statistical Analysis

The results are expressed as the mean ± SEM to show variations in a group. Differences between paired results were calculated using the paired Student *t* test and are considered significant at a *P* level less than .05. All calculations were performed with the statistical software SPSS/Windows (SPSS, Chicago, IL).

RESULTS

Mevalonic Acid in Beer

Mevalonic acid content in 4 different kinds of beer (4.8% alcohol) was 608 to 741 μg/L, and was extremely constant in individual beer brands.

Urinary Excretion of Mevalonic Acid

Urinary excretion of mevalonic acid from 7 PM to 7 AM during the control period was 103 ± 15 μg/12 h, and increased after drinking 1 L of beer containing 608 μg mevalonic acid to 211 ± 17 μg/12 h ($P < .001$). This increment is equal to 18% of the administered dose. Ingestion of vodka containing a similar amount of alcohol but no mevalonic acid had no effect (112 ± 21 *v* 120 ± 14 μg/12 h; Table 1). Individual results are depicted in Fig 1.

Urinary excretion of mevalonic acid from 7 AM to 7 PM the next day was slightly increased after both beverages compared with the control. The increase after drinking vodka was significant (71 ± 10 *v* 89 ± 7 μg/12 h, $P < .05$), whereas the increase after beer was of borderline significance (65 ± 9 *v* 92 ± 11 μg/12 h, $P = .060$; Table 1). Corrected for urinary creatinine, this ratio increased significantly after ingestion of both beverages, from 69 ± 9 to 97 ± 9 μg/g creatinine after ingestion of beer and from 75 ± 6 to 92 ± 9 μg/g creatinine after intake of vodka ($P < .05$ for both; Table 1). After oral intake of 1 mg [$^{13}C_2$] mevalonic acid, 201 ± 7 μg (20% ± 0.7%

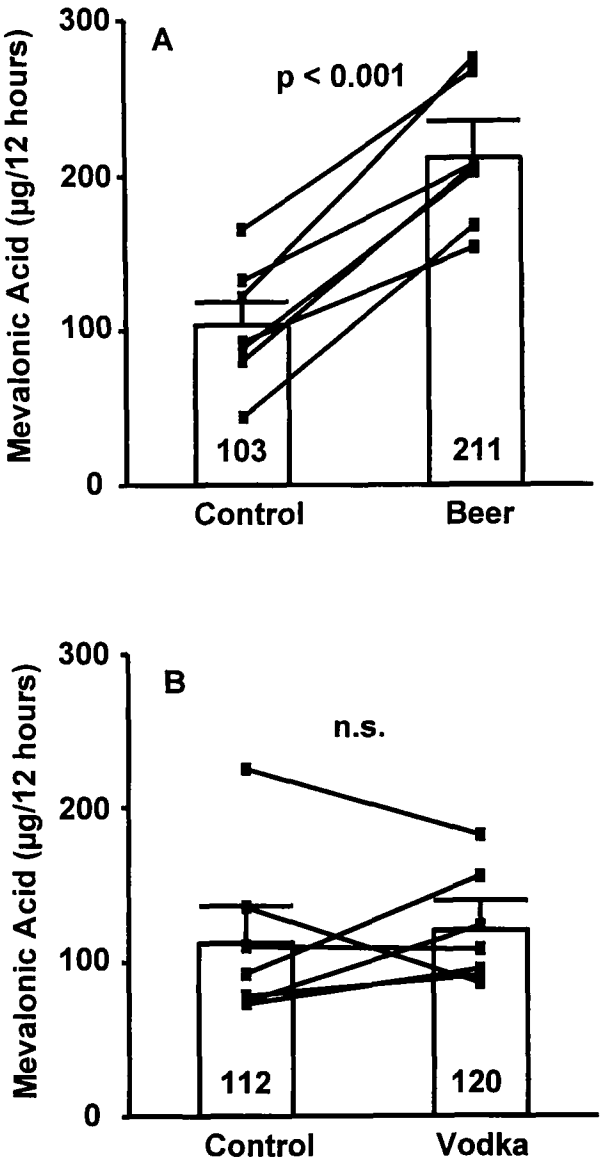

Fig 1. Urinary excretion of mevalonic acid in 7 subjects from 7 PM to 7 AM during the control period and after drinking 1 L of beer (A) or an equal amount of alcohol as vodka (B) at night.

of the dose) was recovered in urine during the following collection period (7 PM to 7 AM), and trace amounts the following 12-hour period (23 ± 6 μg, or 2.3% ± 0.83% of the dose; Table 2). No differences were detected whether [$^{13}C_2$] mevalonic acid was administered with carbonated water or beer.

Serum Concentration of Mevalonic Acid

Drinking 1 L of beer or an equal amount of alcohol at night was followed by a significant increase of mevalonic acid in serum the following morning. On average, the concentration of mevalonic acid increased from 3.22 ± 0.20 to 6.79 ± 0.58 ng/mL after beer and from 3.23 ± 0.37 to 5.36 ± 0.55 ng/mL after vodka ($P < .002$ for both). The increase was found in every subject (Fig 2). Similar results were obtained when the subjects ingested [$^{13}C_2$] mevalonic acid with and without beer (Table 2). However, [$^{13}C_2$] mevalonic acid could not be detected in serum.

Lathosterol and Serum Lipoproteins

The ratio of lathosterol to cholesterol, a marker of 3β-hydroxy-3β-methylglutaryl coenzyme A reductase activity in the liver, increased after consumption of beer from 1.21 ± 0.18 to 1.43 ± 0.19 μg/g ($P < .05$) and after vodka from 1.24 ± 0.22 to 1.55 ± 0.39 μg/g ($P = .089$; Table 3). Total cholesterol (200 ± 33 mg/dL; range, 148 to 261), low-density lipoprotein cholesterol (130 ± 27 mg/dL; range, 76 to 179), high-density lipoprotein cholesterol (51 ± 8 mg/dL; range, 33 to 64), and triglycerides (95 ± 34 mg/dL; range, 38 to 163) in serum were not altered by intake of beer or vodka.

DISCUSSION

Mevalonic acid in serum and urine is an indicator of short- and long-term changes in cholesterol synthesis under various physiological conditions, eg, during drug treatment and disease states.[3-7] Whether dietary intake of mevalonic acid contributes to serum and/or urinary mevalonic acid has not been studied, although it was reported previously by Popjak et al[8] that beer and wine contain mevalonic acid, probably related to yeast fermentation.[17] Indeed, the present study confirms that 1 L of beer contains a large amount of mevalonic acid, which is 3.5 times higher than the average 24-hour excretion during this study (Table 1).

Table 2. Urinary Excretion and Serum Concentration of [$^{13}C_2$] Mevalonic Acid After Oral Administration of 1 mg [$^{13}C_2$] Mevalonic Acid at Night With 1 L Water or Beer

Subject No.	Dose (1 mg)	Urinary [$^{13}C_2$] Mevalonic Acid, μg (% of dose)		Serum Endogenous Mevalonic Acid, 7AM (ng/mL)
		7 PM to 7 AM	7 AM to 7 PM	
1	Water	200 (20)	35 (3.5)	2.67
2	Water	203 (20)	18 (1.8)	1.54
3	Beer	191 (19)	13 (1.3)	5.33
4	Beer	196 (20)	23 (2.3)	6.42
5	Beer	216 (22)	26 (2.6)	4.52
Mean ± SEM		201 (20) 6.6 (0.7)	23 (2.3) 6 (0.6)	

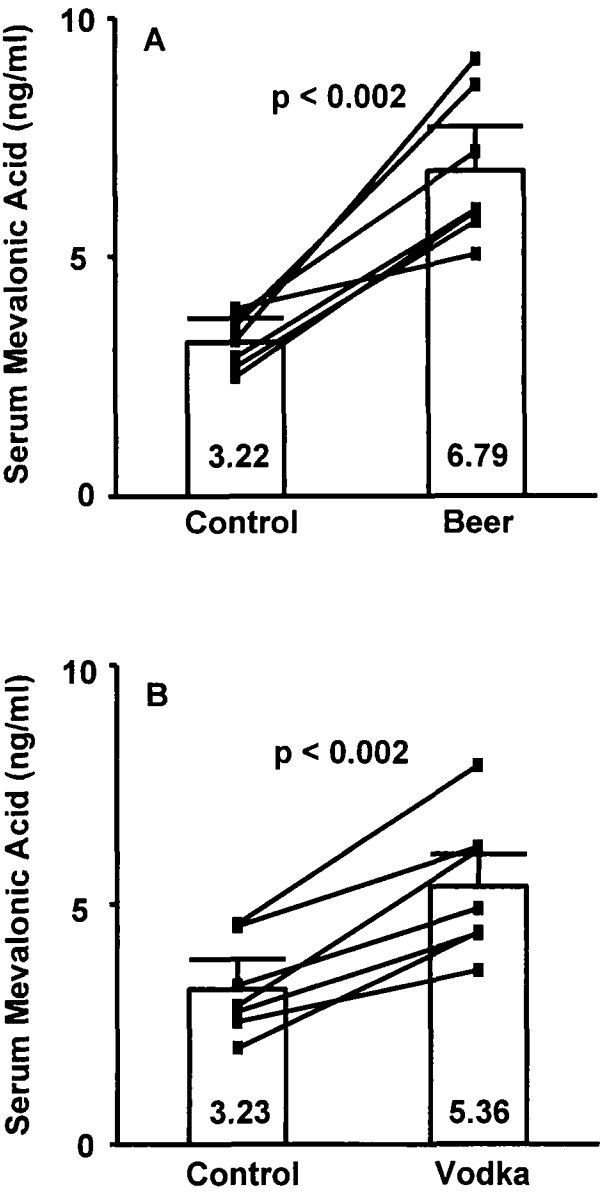

Fig 2. Serum concentration of mevalonic acid on the morning after an overnight fast during the control period and after drinking 1 L of beer (A) or an equal amount of alcohol as vodka (B) at night.

The results of the present study clearly indicate that beer influences mevalonic acid metabolism in 2 ways. First, dietary mevalonic acid is absorbed and 18% to 20% of the dose is excreted rapidly into urine. This was demonstrated during the present study by beer consumption and oral ingestion of [$^{13}C_2$] mevalonic acid. The urinary excretion of dietary mevalonic acid after oral intake is in agreement with the rapid absorption (peak concentration after 15 minutes) and short terminal half-life of about 1 hour.[8] Second, acute intake of alcohol is associated with an increase of mevalonic acid and probably cholesterol synthesis. This is indicated during the present study by 3 independent observations. Serum concentrations of mevalonic acid were

Table 3. Ratio of Lathosterol to Cholesterol in Serum on the Morning After an Overnight Fast During the Control Period and After Ingestion of 1 L of Beer or an Equal Amount of Alcohol as Vodka at Night (mean ± SEM, n = 7)

Study Period	Lathosterol/Cholesterol (μg/g)
Control	1.21 ± 0.18
Beer intake	1.43 ± 0.19*
Control	1.24 ± 0.22
Vodka intake	1.55 ± 0.39†

*$P < .05$ *v* control.
†$P = .089$ *v* control.

significantly higher on the morning after intake of alcoholic beverages, either beer or vodka. In contrast, stable-labeled mevalonic acid could not be detected the next morning after ingestion of [$^{13}C_2$] mevalonic acid, even though the administered dose of [$^{13}C_2$] mevalonic acid was 1.5 times higher than the amount administered as beer. Thus, the increased serum concentration must be a direct effect of alcohol ingestion. Furthermore, the ratio of lathosterol to cholesterol, an indicator of the activity of 3β-hydroxy-3β-methylglutaryl coenzyme A reductase in the liver,[18] also increased after intake of alcoholic beverages. Finally, after alcohol ingestion, a small but significant increase in urinary output of mevalonic acid 12 to 24 hours later was observed. Despite the almost 2-fold serum concentration of mevalonic acid 12 hours after alcohol intake, the increase in urinary output compared with control was low (5% to 10%). This is in line with measurements of total cholesterol synthesis with and without alcohol intake during a metabolic ward study by Crouse and Grundy.[13] During daily intake of 90 g alcohol for 11 to 28 days in 12 subjects, cholesterol synthesis increased nonsignificantly by 11% on average. However, the high level of mevalonic acid in serum on the morning after alcohol intake suggests that the serum concentration of mevalonic acid is not always a reliable marker of cholesterol synthesis. Direct effects of alcohol on mevalonic acid excretion, the mevalonic acid shunt pathway, or influences of alcohol on nonhepatic tissues cannot be ruled out by our study.

Whether increased levels of circulating mevalonic acid due to dietary intake interfere with other functions of mevalonic acid and mevalonic acid–derived products in the circulation has yet to be elucidated.[19-21] Furthermore, mevalonic acid has recently been investigated in renal disease,[22-24] and knowledge of the nutritional effects on mevalonic acid might prevent misinterpretations in these studies and studies on the influence of the apolipoprotein E genotype[25] or on the estimation of hepatic defects of cholesterol and bile acid synthesis by plasma mevalonic acid.[26] In addition, certain genetic disorders such as heterozygous mevalonic aciduria[27] and cerebrotendinous xanthomatosis[3] show elevated physiological concentrations of mevalonic acid comparable to the levels found after the intake of beer or pure alcohol in our study.

The present findings have major implications. Studies using serum or urinary mevalonic acid as a marker for cholesterol synthesis must be carefully monitored with respect to alcohol consumption and dietary intake of mevalonic acid. High concentrations of mevalonic acid were detected not only in different kinds of beer and wine but also in some yogurts and fruit juices (Lindenthal et al, unpublished results, May 1996).

ACKNOWLEDGMENT

The excellent technical assistance of H. Prange and K. Wilmersdorf is gratefully acknowledged.

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