Orally Administered Betaine Has an Acute and Dose-Dependent Effect on Serum Betaine and Plasma Homocysteine Concentrations in Healthy Humans

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ABSTRACT Betaine, i.e., trimethylglycine, is linked to homocysteine metabolism. A 3-mo daily betaine supplementation decreased even normal plasma total homocysteine (tHcy) concentrations in humans. The pharmacokinetic characteristics and metabolism of betaine in humans have not been investigated in detail. The aim of this study was to assess the pharmacokinetics of orally administered betaine and its acute effect on plasma tHcy concentrations. Healthy volunteers (n = 10; 3 men, 7 women) with normal body weight (mean ± SD, 69.5 ± 17.0 kg), 40.8 ± 12.8 y old, participated in the study. The betaine doses were 1, 3, and 6 g. The doses were mixed with 150 mL of orange juice and ingested after a 12-h overnight fast by each volunteer according to a randomized double-blind crossover design. Blood samples were drawn for 24 h and a 24-h urine collection was performed. Orally administered betaine had an immediate and dose-dependent effect on serum betaine concentration. Single doses of 3 and 6 g lowered plasma tHcy concentrations (P = 0.019 and P < 0.001, respectively), unlike the 1-g dose. After the highest dose, the concentrations remained low during the 24 h of monitoring. The change in plasma tHcy concentration was linearly associated with betaine dose (P = 0.006) and serum betaine concentration (R² = 0.17, P = 0.025). The absorption and elimination of betaine were dose dependent. The urinary excretion of betaine seemed to increase with an increasing betaine dose, although a very small proportion of ingested betaine was excreted via urine. In conclusion, a single dose of orally administered betaine had an acute and dose-dependent effect on serum betaine concentration and resulted in lowered plasma tHcy concentrations within 2 h in healthy subjects. J. Nutr. 136: 34–38, 2006.

KEY WORDS: • Betaine • cysteine • dimethylglycine • homocysteine • human

Betaine, i.e., trimethylglycine, is formed by oxidation of choline in mammals including humans. Minor amounts of betaine may also be obtained from food. Betaine is an effective methyl-donor and is metabolized further in vivo to dimethylglycine (DMG),3 sarcosine, and finally to glycine.

Betaine has been used as a supplement in animal nutrition, e.g., in feed for salmon and pigs (1). In pigs, betaine was shown to decrease the amount of body fat (2). However, in obese middle-aged humans, a 6-g daily betaine supplementation for 3 mo did not affect body weight, but it was shown that betaine supplementation significantly decreased even normal plasma homocysteine concentrations in humans (3). In a recent study, plasma betaine concentration was found to be a significant determinant of fasting plasma homocysteine concentration in healthy humans (4).

Homocysteine is a thiol-containing amino acid formed by demethylation of methionine. Whether an elevated plasma total homocysteine (tHcy) concentration is an independent cardiovascular risk factor is still under investigation (5). However, it is a marker of the risk of thrombosis and might have a role in the progression of Alzheimer’s disease (6). An association with depressive symptoms was also reported (7).

Two pathways of homocysteine metabolism occur via the key enzymes, methionine synthase and cystathione β-synthase.
with the help of the cofactors folate-cobalamin and vitamin B-6, respectively. In the third pathway, homocysteine metabolism is linked to betaine by the enzyme betaine-homocysteine methyl transferase, which is most abundant in liver and kidney tissues (8). Homocysteine is remethylated to methionine, whereas betaine is converted to DMG.

Betaine supplementation was included in the folic acid and vitamin B-6 treatments of selected patients with the inherited disease, homocystinuria, caused by an enzyme deficiency in the main homocysteine metabolic pathway, i.e., cystathionine β-synthase deficiency (9). Despite the use of betaine in this clinical condition, there are few pharmacokinetic data for betaine. Sakura et al. (10), Schwahn et al. (11), and Olthof et al. (12) presented some data on healthy subjects. Schwahn et al. (11) showed that administration of betaine at a dose of 50 mg/kg body weight resulted in maximal serum concentration of betaine 0.5–1.5 h after the ingestion of the betaine load. They also estimated values for absorption, distribution and elimination half-lives (T1/2abs 0.28 ± 0.17 h, T1/2dist 0.59 ± 0.22 h, and T1/2elim 14.38 ± 7.17 h, respectively). Matthews et al. (13) examined the pharmacokinetics of betaine in the treatment of homocystinuria (cystathionine β-synthase deficiency). A single oral dose of 100 mg/kg was given to 6 patients; betaine concentrations and the mean absorption and elimination half-lives of betaine were reported to be in agreement with those reported by Schwahn et al. (11).

The aim of the present study was to examine the linearity of oral betaine administration at doses of 1, 3, and 6 g on serum betaine kinetics and plasma homocysteine concentrations in healthy subjects. The urinary excretion of DMG was measured to examine the metabolism of betaine.

**SUBJECTS AND METHODS**

**Subjects.** Healthy Caucasian volunteers (n = 10; 3 men and 7 women) participated in the study. They had normal liver, kidney, and thyroid functions. They also had normal fasting plasma glucose concentrations. The inclusion criterion for fasting serum cholesterol concentration was <7.5 mmol/L and for triglycerides it was <3.5 mmol/L. The baseline characteristics of the subjects are presented in Table 1.

**Study design.** Each of the subjects ingested betaine in doses of 1, 3, and 6 g in random order using a double-blind crossover design. Each of the betaine doses was mixed with 150 mL of orange juice. The juice contained ~38 μg folate/100 g.

The betaine doses were ingested 7 d apart after a 12-h overnight fast. The subjects were not allowed to ingest anything during the first 4 h after the ingestion of the betaine dose. After 4 h, the subjects were allowed to eat according to their normal food habits except for foodstuffs containing moderate amounts of betaine, e.g., spinach, shellfish, liver, and kidney. The use of wheat products was limited to 3 g/d. The laboratory personnel were unaware of the dose assignments.

Fasting serum total cholesterol concentration in the whole serum was analyzed using a commercial kit (Boehringer GmbH Kit 237574) and Kone Pro Clinical Chemistry Analyzer (Thermo Clinical Lab-systems). Plasma glucose was analyzed with the enzymatic photometric method (Granutest 250; Merck) using a Kone Pro Clinical Analyzer. Concentrations of serum alanine aminotransferase and creatine were analyzed at the Kuopio University Hospital using standardized methodology. The laboratory personnel were unaware of the dose assignment.

**Pharmacokinetic parameters and statistical analyses.** To depict the kinetic behavior of betaine in serum, the actual concentration data were used. When assessing the effect of the doses in detail, however, the concentration data were corrected for the endogenous synthesis of betaine by subtracting the baseline value from the individual data (18), assuming a relatively invariable rate of synthesis during the serum sample collection period. The pharmacokinetic parameters for the actual and the corrected data were calculated as follows: peak concentration [actual or corrected, Cmax or Cmax(corr), respectively] and time to reach it (Tmax) were taken directly from the data. The area under the curve from 0 to 24 h [AUC0–24betaine or AUC0–24betaine(corr)] was calculated using the trapezoidal method. The linear regression line based on the least-squares method (Systat & version 10 program; SPSS) was fitted to the data. The goodness of fit and the most appropriate model were determined by assessing the randomness of the scatter of actual data points around the fitted function, and using Akaike's information criteria (19). The best fit for all of the data was calculated using the 2-compartmental model with absorption phase, enabling the estimation of rate constants for absorption, distribution, and elimination [kabs, kdist and klim, or kabs(corr), kdist(corr) and klim(corr), respectively]. The data (actual or corrected) of 1 subject after the 3- or
RESULTS

Serum betaine concentrations, dose response, and the effect on plasma homocysteine concentrations. The serum betaine concentration was 47 ± 10 μmol/L at the beginning of the study. Pharmacokinetic parameters calculated from the actual and the corrected data are shown in Table 2.

Orally administered betaine had an immediate effect on serum betaine concentrations (Figure 1). After the 1-g betaine dose, a Cmax of 284 ± 131 μmol/L was reached at a Tmax of 40 (40–60) min; after the 3-g dose, Cmax was 599 ± 190 μmol/L at a Tmax of 60 (40–80) min, and after the 6-g dose, Cmax was 1015 ± 231 μmol/L at a Tmax of 80 (60–80) min. The parameters Cmax(corr) and AUCO–24betaine(corr) increased dose dependently (P < 0.001 for both parameters, ANOVA for repeated measures) in a linear manner (P < 0.001 for both parameters, contrast analysis).

Betaine readily absorbed and distributed with T1/2abs and T1/2dist ranging between 0.17 and 0.39 h and 0.57 and 0.86 h, respectively, whereas elimination occurred with T1/2elim ranging between 13.0 and 25.9 h. In the corrected data, both T1/2abs(corr) and T1/2dist(corr) increased with the dose (P < 0.05 for both parameters, ANOVA for repeated measures), indicating linear change (P < 0.01 for both parameters, contrast analysis). T1/2elim(corr), however, did not change significantly with dose (although in the actual data T1/2elim seemed to decrease with dose). In this study, with a limited number of subjects, age or sex did not affect serum betaine concentrations.

The urinary excretion of betaine seemed to accelerate with an increase in the betaine dose, whereas the excretion of DMG was more constant. The urinary excretion of betaine was 86 ± 43 mmol at the 1-g dose, 638 ± 637 mmol at the 3-g dose, and 2212 ± 1359 mmol at the 6-g dose. The respective values for DMG were 91 ± 49, 160 ± 80, and 261 ± 178 mmol, respectively. A very small proportion of ingested betaine was excreted via urine during the first 24 h because the calculated sum of the excreted betaine and the first metabolic product of betaine, DMG, accounted for only 3.2, 4.3, and 7.4% of the ingested amount of betaine (1, 3, and 6 g, respectively).

The effect of betaine on plasma homocysteine concentrations was immediate and dose dependent (Figure 2). The betaine doses of 3 and 6 g, but not 1 g, lowered homocysteine concentrations compared with the baseline concentrations (P = 0.019 and P < 0.001, respectively) with a maximum response of ~10%. Using the baseline concentration at time 0 h

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<td>Pharmacokinetic parameters after single oral doses of 1, 3, and 6 g of betaine in healthy humans&lt;sup&gt;1&lt;/sup&gt;</td>
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| Pharmacokinetic parameters calculated from actual betaine concentrations | Pharmacokinetic parameters calculated from corrected betaine concentrations<sup>2</sup> |
| --- |
| Dose, g | 1 | 3 | 6 | 1 | 3 | 6 | P<sup>3</sup> |
| Cmax or Cmax(corr), μmol/L | 284 ± 131 | 599 ± 190 | 1015 ± 231 | 239 ± 128 | 551 ± 191 | 966 ± 234 | < 0.001 |
| Tmax, min | 40 (40–60) | 60 (40–80) | 80 (60–80) | 63 ± 40 | 167 ± 57 | 318 ± 76 | < 0.001 |
| AUCO–24betaine or AUCO–24betaine(corr) mmol/(L*min) | 127 ± 46 | 235 ± 57 | 389 ± 76 | 63 ± 40 | 167 ± 57 | 318 ± 76 | < 0.001 |
| T1/2abs or T1/2abs(corr), min | 10 ± 5 | 16 ± 7 | 23 ± 7 | 11 ± 5 | 16 ± 8 | 23 ± 6 | < 0.05 |
| T1/2dist or T1/2dist(corr), min | 34 ± 11 | 47 ± 23 | 52 ± 17 | 28 ± 7 | 45 ± 23 | 49 ± 12 | < 0.05 |
| T1/2elim or T1/2elim(corr), min | 1555 ± 374 | 1197 ± 393 | 779 ± 168 | 452 ± 355 | 611 ± 137 | 526 ± 162 | NS<sup>4</sup> |

<sup>1</sup>Values are mean ± SD or median (min–max), n = 10.
<sup>2</sup>The actual concentration data corrected for endogenous synthesis of betaine were calculated by subtracting the baseline value from the individual data (18).
<sup>3</sup>ANOVA for repeated measures, data calculated from corrected betaine concentrations used in analysis.
<sup>4</sup>NS, P > 0.05.
as the reference, the effect of the 3-g dose lasted for at least 7 h (P < 0.001 between 0 and 2 h, and P = 0.03 between 0 and 7 h, contrast analysis between time points), whereas the 6-g dose was effective throughout the 24-h follow-up period (P < 0.001 between 0 and 2 h, P < 0.001 between 0 and 7 h, and P = 0.007 between 0 and 24 h). When assessed in terms of $AOC_{0\rightarrow24}^{\text{homocysteine}}$ changes in plasma homocysteine concentrations were related to betaine doses and to changes in serum betaine concentrations, i.e., $AOC_{0\rightarrow24}^{\text{homocysteine}}$ increased with dose ($P = 0.037$, ANOVA for repeated measures) in a linear manner ($P = 0.006$, contrast analysis), and there was a weak but significant linear relation between $AOC_{0\rightarrow24}^{\text{homocysteine}}$ and $AUC_{0\rightarrow24}^{\text{homocysteine}}$ (dependent and independent variables, respectively; $R^2 = 0.17$, P = 0.025, linear regression), with 1 case detected as an outlier (studentized residual = 4.061; see Figure 3). Plasma tCys concentrations were not affected by betaine intake (data not shown).

FIGURE 2 Plasma homocysteine concentrations in healthy humans after oral betaine doses of 1, 3, and 6 g. Values are means ± SD, n = 10. The betaine doses of 3 and 6 g lowered homocysteine concentrations compared with baseline ($P = 0.019$ and $P < 0.001$, respectively).

FIGURE 3 Linear relation between serum betaine concentration under the curve [AUC$_{0\rightarrow24}^{\text{betaine}}$] (mol/(L·min)) and plasma total homocysteine concentration over the curve [AUC$_{0\rightarrow24}^{\text{homocysteine}}$] (mol/(L·min)) in humans. $Y = 0.0023319 \times + 349.331$. *This outlier was not included in the linear regression analysis (studentized residual = 4.061). AOC$_{0\rightarrow24}^{\text{homocysteine}}$ increased with dose ($P = 0.037$, ANOVA for repeated measures) in a linear manner ($P = 0.006$, contrast analysis). There was a significant linear relation between AOC$_{0\rightarrow24}^{\text{homocysteine}}$ and $AUC_{0\rightarrow24}^{\text{homocysteine}}$ (dependent and independent variables, respectively; $R^2 = 0.17$, P = 0.025, linear regression).

DISCUSSION

According to Lever et al. (14), physiological betaine serum values (20–60 μmol/L) are quite steady in humans and are not affected by diet. However, serum betaine concentrations were reported to increase 10- to 20-fold after a betaine supplementation of 6 g/d for a longer period of time (3). Currently, limited data are available on the pharmacokinetics of orally administered betaine in humans.

In the present study, orally administered single doses of 1, 3, and 6 g betaine increased serum betaine concentrations linearly. The betaine doses of 3 and 6 g decreased plasma tHcy concentrations within 2 h in healthy adults. After the highest dose, the concentrations remained low during the 24 h of monitoring. The change in plasma tHcy concentration was associated linearly with the betaine dose and the serum betaine concentration. The present study is the first study showing that betaine decreases plasma homocysteine concentrations in as little as 24 h in healthy subjects.

Brief reports of the pharmacokinetics of betaine in healthy subjects were presented by Schwahn et al. (11) and Sakura et al. (10). Schwahn et al. (11) studied 12 healthy men (1 single 3.5-g administration of betaine) and Sakura et al. (10), 3 adult volunteers (1 single 7-g administration of betaine). The data from these studies are in quite good agreement with the present study.

In the present study, we showed that the absorption and elimination of betaine are dose dependent. The absorption and distribution of betaine appeared to slow down as the dose increased, but the rate-limiting step responsible for this cannot be deduced from the data. Instead, the elimination seemed to accelerate first with increasing dose, but when assessed with the data corrected for endogenous synthesis of betaine, this acceleration of the elimination process was no longer evident. Thus, it appears that longer half-lives with lower doses in the actual data reflect the fact that concentrations are approaching the balance between synthesis and elimination, rather than the existence of dose-related changes in the elimination mechanisms.

We observed a fast reduction in plasma tHcy and slightly increased urinary DMG values, which indicate an immediate catabolism of betaine in healthy subjects. DMG is further metabolized to sarcosine, which was not analyzed in this study. Schwahn et al. (11) concluded that betaine is eliminated mainly by metabolism. However, the decline in plasma betaine concentrations after a single dose with minor urinary excretion could also result from distribution into tissues, instead of metabolic degradation. Lever et al. (14) showed that orally administered betaine tends to accumulate in the medulla of rabbits.

The present study showed an immediate and acute plasma tHcy–lowering effect by betaine in healthy subjects. Olthof et al. (12) reported that betaine supplementation lowers plasma tHcy concentrations in healthy subjects in the long run and attenuates the increase in plasma tHcy after a methionine loading test. In patients with homocysteinuria, Schwahn et al. (11) noted that the maximum concentration of serum betaine was achieved 0.8–2.1 h after the intake of betaine (50 mg/kg body weight), and there was an immediate and significant reduction in plasma tHcy concentration. In our study, the immediate maximum reduction in plasma tHcy was about 10% (~0.8 μmol from an average of 8 μmol/L). Interestingly, after
the single 6-g dose, plasma tHcy concentrations remained low during the 24 h of monitoring.

Elevated plasma levels of tHcy > 15 μmol/L are present in <5% of the general population, but as many as 50% of the patients with stroke and other atherothrombembolic vascular diseases (20). Some studies indicate that there is also a sharp elevation of tHcy during acute myocardial infarction and stroke (21,22). Furthermore, in patients with acute myocardial infarction, elevated plasma tHcy concentration was reported to be associated with a higher risk of recurrent coronary events and death (23,24). However, it is unknown whether these elevated concentrations of tHcy have to be lowered to gain clinical benefit.

In conclusion, an orally administered single dose of betaine had an acute dose-dependent effect on serum betaine concentration, and acutely lowered plasma tHcy concentrations in healthy subjects. The lowering effect of betaine on plasma tHcy concentration persisted for the 24-h follow-up period after the highest (6 g) dose.

LITERATURE CITED


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