Dietary and supplementary betaine: acute effects on plasma betaine and homocysteine concentrations under standard and postmethionine load conditions in healthy male subjects1–3

Wendy Atkinson, Jane Elmslie, Michael Lever, Stephen T Chambers, and Peter M George

ABSTRACT

Background: Betaine comes from the diet and from choline, and it is associated with vascular disease in some patient groups. Betaine supplementation lowers plasma total homocysteine.

Objective: We compared the acute effects of dietary and supplementary betaine and choline on plasma betaine and homocysteine under standard conditions and after a methionine load.

Design: In a randomized crossover study, 8 healthy men (19–40 y) consumed a betaine supplement (≈500 mg), high-betaine meal (≈517 mg), choline supplement (500 mg), high-choline meal (≈564 mg), high-betaine and -choline meal (≈517 mg betaine, ≈622 mg choline), or a low-betaine and -choline control meal under standard conditions or postmethionine load. Plasma betaine, dimethylglycine, and homocysteine concentrations were measured hourly for 8 h and at 24 h after treatment.

Results: Dietary and supplementary betaine raised plasma betaine concentrations relative to control (P < 0.001) under standard conditions. This was not associated with raised plasma dimethylglycine concentration, and no significant betaine appeared in the urine. A small increase in dimethylglycine excretion was observed when either betaine or choline was supplied (P = 0.011 and < 0.001). Small decreases in plasma homocysteine 6 h after ingestion under standard conditions (P ≤ 0.05) were detected after a high-betaine meal and after a high-betaine and high-choline meal. Dietary betaine and choline and betaine supplementation attenuated the increase in plasma homocysteine at both 4 and 6 h after a methionine load (P ≤ 0.001).

Conclusions: Dietary betaine and supplementary betaine acutely increase plasma betaine, and they and choline attenuate the postmethionine load rise in homocysteine concentrations. Am J Clin Nutr 2008;87:577–85.

KEY WORDS Betaine, choline, total homocysteine, tHcy, diet, supplementation, postmethionine load, healthy subjects

INTRODUCTION

Betaine (glycine betaine or N, N, N-trimethylglycine) is obtained from the diet (1, 2) or from its metabolic precursor choline (3–5). Betaine is a major tissue osmolyte and plays an important role in the metabolism of homocysteine, with betaine-homocysteine methyltransferase (BHMT) catalyzing the remethylation of homocysteine to methionine. Plasma betaine is a determinant of total plasma homocysteine concentrations in patients with vascular disease under postmethionine load (PML) (6) as well as fasting conditions (7, 8). This may be significant because elevated homocysteine is associated with an increased risk of vascular disease (9–12), although whether this relation is causal is a matter of debate (13). Supplementation with folate and other B vitamins in patients who have had a vascular event does not decrease the incidence of subsequent events, despite a dose-dependent reduction in homocysteine (14, 15). However, the association between homocysteine and vascular disease is well established, and this contradiction highlights the need to explore other factors in homocysteine metabolism such as betaine (13). Folate supplementation would not correct a betaine deficiency.

Pharmacologic doses of betaine (>6 g/d) and its metabolic precursor choline are used to lower homocysteine concentrations in patients with homocystinuria (16–19) and severe hyperhomocysteinemia (20, 21). Similar betaine supplementation during 6–12 wk lowers fasting and PML homocysteine by ≤20% and 50%, respectively, in healthy subjects (22–25). There are few reports of the effects of near-physiologic doses of betaine (25, 26). Supplementation with 1.5 g betaine/d for 6 wk reduces fasting and PML homocysteine by 12% and 23%, respectively (25). Interestingly, a single acute dose of 0.75 g betaine lowered PML homocysteine by 16%, similar to the effect seen after 6 wk of treatment (25), although in another study a single 1-g oral betaine dose had no effect on fasting homocysteine (26). Choline supplementation at a dose of 2.6 g/d for 2 wk lowered fasting and PML homocysteine by 18% and 29%, respectively, in healthy male subjects with mildly elevated homocysteine concentrations, whereas a single dose of choline (1.5 g) reduced PML homocysteine by 15% (27). The acute effect of a single dietary load of betaine or choline on homocysteine concentrations has not been reported. An alternative would be to increase the intake of betaine or choline or both through dietary modification. Oral betaine supplementation, particularly at high doses, has proved
problematic with side effects such as indigestion and diarrhea, along with difficulty ingesting the required dosage, reducing long-term patient compliance (28). Dietary modification may be simpler and more cost-effective. However, dietary betaine or choline may not provide benefits comparable to oral supplementation; eg, increasing the dietary intake of folate is less effective than supplementation for lowering homocysteine (29).

We investigated the acute effects of a physiologic dose of betaine or choline, delivered in dietary form or as an oral supplement, on plasma betaine and homocysteine concentrations under standard and PML conditions in healthy male volunteers. Because previous work shows that urinary betaine excretion is a significant predictor of plasma homocysteine concentrations (8), this was also studied.

SUBJECTS AND METHODS

Subjects

Healthy white male volunteers aged 19–40 y (n = 8) were recruited by local advertisement, with a different group of 8 subjects enrolled for each of the 2 parts of the study. Three subjects participated in both studies. All subjects provided written informed consent after ethical approval of the study by the Canterbury Ethics Committee (Christchurch, New Zealand). Subjects had no current or previous history of vascular or renal disease, were free from acute or chronic illness requiring prescription medication, did not take dietary or vitamin supplements, were nonsmokers, and were in good health based on medical history and physical examination. Subjects were excluded if they displayed any of the following: abnormal hemoglobin concentration (<130 g/L men); abnormal homocysteine concentration (<5 or >15 μmol/L); the presence of vitamin B-12 (<120 pmol/L), vitamin B-6 (<35 nmol/L), or folate (<445 nmol/L) deficiency; or 677C→T polymorphism in the methylene tetrahydrofolate reductase gene. The baseline characteristics of the subjects are presented in Table 1.

Study design

A 2-part randomized crossover study was performed. In the first part of the study subjects received, under standard conditions, the following 6 treatments: 1) a betaine supplement (500 mg of trimethylglycine; Life Extension, Ft Lauderdale, FL) together with a control meal consisting of ½ cup of toasted rice cereal [Rice Krispies or Rice Bubbles; Kellogg’s (Aust Pty, Botany, NSW, Australia), ½ cup canned peaches, ½ cup trim milk (ie, homogenized low-fat milk, 0.5% milkfat, with added low-fat milk solids); 2) a high-betaine meal (providing ~517 mg glycine betaine estimated from food composition databases) consisting of 1 cup extruded bran cereal (All-Bran; Kellogg’s), 1 tablespoon wheat bran, ½ cup trim milk, 2 slices whole-meal toast, two 10-g sachet margarine, two 14-g sachet jam); 3) a choline supplement (500 mg choline, supplied as choline chloride; Vitamin Research Products, Carson City, NV) together with a control meal as described above; 4) a high-choline meal (estimated to provide 517 mg betaine and 622 mg choline based on food composition data) consisting of 4 scrambled eggs, 1 cup extruded bran cereal, 1 tablespoon wheat bran, ½ cup trim milk, 2 slices whole-meal toast, two 10-g sachet margarine, two 14-g sachet jam); and 6) a control meal low in both betaine and choline (as described for treatment 1 above, estimated to provide <1.5 mg betaine and <1 mg choline). In the second part of the study, subjects received a betaine supplement, a high-betaine meal, a high-choline meal, and a control meal as described above, along with a methionine load (0.1 g methionine/kg body weight) which was administered immediately after treatment ingestion, as described previously (30).

Subjects received treatments over consecutive weeks, with 1 d/wk the study day and the rest of the week a wash-out period. Treatments were administered after an overnight fast, with each subject consuming 1 treatment/d and receiving all treatments on completion of the study. Subjects were asked to abstain from foods known to be high in betaine or choline (2, 31), caffeine, and alcohol for 2 d and to fast for 12 h before treatment. Water and food intakes were closely controlled throughout each study day, with subjects receiving 100 mL water hourly and, immediately after the 3-h blood sampling time point, a standard lunch immediately after the 3-h blood sampling time point, a standard lunch immediately after the 3-h blood sampling time point, which was estimated to be low in betaine and choline from food composition databases (2, 31).

Blood collection and biochemical analyses

Fasting venous blood samples were collected at baseline (t = 0 h), by an indwelling cannula in the antecubital fossa, into EDTA-coated tubes and immediately placed on ice. After treatment, subsequent blood samples were collected hourly at 1, 2, 3, 4, 5, 6, 7, 8 h and 24 h after treatment. A fasting urine sample was obtained at baseline, with subsequent sampling every 2 h at 2, 4, 6, 8 h, and an overnight urine collection from 8 h to 24 h after treatment.

Plasma was separated by centrifugation at 2000 × g for 10 min at room temperature within 2 h of blood collection. Urine sample

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Baseline characteristics of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard conditions</strong></td>
<td><strong>Postmethionine load conditions</strong></td>
</tr>
<tr>
<td>Age (y)</td>
<td>$25.3 ± 1.3$ ($19.0, 40.0$)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>$76.8 ± 2.5$ ($65.2, 98.4$)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>$24.5 ± 1.7$ ($19.0, 33.9$)</td>
</tr>
<tr>
<td>Plasma tHcy (μmol/L)</td>
<td>$8.3 ± 0.3$ ($5.6, 10.6$)</td>
</tr>
<tr>
<td>Serum vitamin B-12 (pmol/L)</td>
<td>$278 ± 19$ ($165, 424$)</td>
</tr>
<tr>
<td>Red blood cell folate (nmol/L)</td>
<td>$701 ± 16$ ($574, 854$)</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>$151.5 ± 6.3$ ($141.4, 161.0$)</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM; 10th, 90th percentiles in parentheses. n = 8, tHcy, total homocysteine.
volumes were recorded, and a 10-mL aliquot was removed. All samples were stored at −20 °C before analysis. Plasma concentrations of betaine, dimethylglycine, and homocysteine and urine concentrations of betaine, dimethylglycine, and creatinine were measured. Betaine, dimethylglycine, and carnitine were measured in plasma and urine by HPLC after derivatization with 2-naphthylacyl triflate (32, 33). Plasma homocysteine was measured at baseline and at 4 and 6 h after treatment by fluorescence polarization on an Abbott IMX Analyzer (Abbott Diagnostic Division, Abbott Laboratories, Abbott Park, IL). Urine creatinine was measured with the use of the Jaffé reaction on the Abbott Aeroset Analyzer (Abbott Laboratories), and urine betaine and dimethylglycine excretion were calculated as a ratio to creatinine. Serum vitamin B-12 and red blood cell folate concentrations were measured by separate competitive immunoassays on an automated Chemiluminescence ACS:180 Analyzer (Chiron Diagnostics Corp, Emeryville, CA). Duplicates of all foods and beverages consumed by each subject on all treatment days were assayed for betaine content to confirm the amount ingested as described previously (2, 31). Total choline was estimated in the food by the strong singlet nuclear magnetic resonance of the methyl protons (34, 35), after an extraction based on an optimized coenzyme Q extraction (36, 37). The samples were homogenized with water and with isopropanol and centrifuged, and the supernatant fluids were evaporated to dryness. The extracts were reconstituted in deuterium oxide and in deuterated chloroform for quantification by nuclear magnetic resonance spectroscopy (38), estimating the water and lipid-soluble choline separately. Calibration was against L-α-phosphatidylcholine type XVI-E (99%) and choline iodide, both from Sigma Chemical Co (St Louis, MO), with validation by standard addition of the same materials to food homogenates.

Data analyses

For plotting data, the differences in the concentrations of betaine, dimethylglycine, and total homocysteine in plasma and of urinary betaine and dimethylglycine before and after treatment were calculated for each subject at each time point. This baseline subtraction minimized interindividual variation. The statistical significance of the differences between data at the different time points was assessed with the use of 2-factor repeated-measures analysis of variance (ANOVA), with post hoc comparisons made with the use of Tukey’s honest significant difference test. Standard pharmacokinetic markers were calculated for each individual subject as follows: the ratio to fasting baseline area under the postprandial concentration-time curve (AUC ratio) was calculated for all data obtained from 0 to 8 h as the area under the postprandial concentration curve compared with time, divided by fasting concentration multiplied by 8 (39). The peak concentration (Cmax) and the time taken to reach this peak concentration (Tmax) were estimated for each individual subject directly from the data. Differences between these markers were evaluated between treatments by one-factor repeated-measures ANOVA with post hoc adjustment for multiple comparisons by Tukey’s honest significance test unless otherwise stated. Urine data were not normally distributed and were log-transformed before analysis. Data were analyzed with the use of SIGMASTAT version 3.1 (Systat Software Inc, San Jose, CA) and SPSS version 14 for WINDOWS (SPSS Inc, Chicago, IL). Statistical significance was defined as P < 0.05 unless otherwise stated.

RESULTS

Estimates of actual concentrations of betaine and choline in the food samples

Analysis of the study meals suggested that the betaine and choline contents of both meals were slightly higher than those estimated from food composition tables, although consistent with the expected variation in food samples from different sources. The control meal provided, on analysis, <1 mg betaine and 1.6 mg choline. The measured betaine content of the high-betaine meal was 560 mg, the high-choline meal was estimated to provide 760 mg choline, and the high-choline and -betaine meal provided 630 mg betaine and 846 mg choline. This confirmed that the meals had different betaine and choline contents and that they had similar, although higher, betaine or choline contents than did the supplements.

Standard conditions

Plasma betaine concentration

Plasma betaine concentrations were significantly increased by all treatments except the control meal (Figure 1). The interaction between treatment and time was highly significant (P < 0.001). The increases with the betaine supplement (Xi: 50 μmol/L at 2 h) were significant (compared with the control meal) from 1 to 8 h (P < 0.001), although they were not significant at 24 h. After the high-betaine meal the increases (Xi: 34 μmol/L at 2 h) were significantly different from the control meal from 1 h to 6 h (P < 0.001), at 7 h (P = 0.003), at 8 h (P = 0.008), and not significant at 24 h. At 1 h the increase after the supplement was significantly greater than after the meal (P = 0.004). Increases after the choline supplement were not significantly different from the control meal at 1 h, 8 h, or 24 h, but they were significant from 2 to 5 h (P < 0.001), at 6 h (P = 0.002), and at 7 h (P = 0.016). After a high-choline meal, the increase in plasma betaine was significant at 2 h (P = 0.029), from 3 to 6 h (P < 0.001), and remained significant to 8 h (P = 0.002). After the high-betaine and -choline meal, plasma betaine concentrations were elevated compared with the control meal at all sampling points from 1 to 24 h (P < 0.001).

This pattern was confirmed by the estimates of pharmacologic markers. The overall effect of treatment (diets compared with control meal) on plasma betaine under standard conditions was highly significant (P ≤ 0.0001 for both betaine AUC and betaine Cmax; Table 2). Both a high-choline meal and a choline supplement increased plasma betaine concentrations as shown by greater AUC ratio and Cmax values relative to the control meal (Table 2), although no difference in the Tmax values was observed. As expected, betaine supplementation resulted in higher plasma Cmax values (P < 0.001) and shorter Tmax values (P = 0.021) than did choline supplementation (Table 2).

Plasma dimethylglycine concentrations

The plasma dimethylglycine concentrations did not change in proportion to the increase in betaine. Two-factor repeated-measures ANOVA confirmed an overall difference in plasma dimethylglycine between treatments (P = 0.017), but the interaction between treatment and time was weak (P = 0.064). The changes were not significantly different from the control meal at any time point after any of the treatments, except possibly (if there is an interaction) for the combined high-betaine...
and -choline meal at 8 h \((P = 0.027)\). Similarly, the overall pharmacologic markers suggested that plasma dimethylglycine tended to be slightly higher during all treatments than with the control meal, with an increase in the \(C_{\text{max}}\) from 0.66 \(\mu\text{mol/L}\) (betaine supplement) to 2.16 \(\mu\text{mol/L}\) (high-choline and -betaine meal), but this trend (diets compared with the control meal) for

\[
\begin{array}{lllll}
\text{Treatment} & \text{Baseline} & \text{AUC ratio} & \text{C}_{\text{max}} & \text{T}_{\text{max}} \\
& \mu\text{mol/L} & & \mu\text{mol/L} & \text{min} \\
\hline
\text{Standard conditions} & & & & \\
\text{Betaine supplement} & 40.7 \pm 4.9 & 1.8 \pm 0.1^3 & 92.7 \pm 8.5^3 & 105.0 \pm 0.3 \\
\text{High-betaine meal}^4 & 38.2 \pm 2.4 & 1.6 \pm 0.3^3 & 77.6 \pm 4.1^3,5,6,7 & 157.8 \pm 0.3 \\
\text{Choline supplement} & 35.1 \pm 2.7 & 1.4 \pm 0.1 & 56.2 \pm 3.0^8 & 240.0 \pm 0.3^9 \\
\text{High-choline meal} & 34.1 \pm 2.9 & 1.5 \pm 0.1 & 56.9 \pm 4.1^8 & 240.0 \pm 0.3^9 \\
\text{Betaine- and choline-rich meal} & 37.5 \pm 3.9 & 1.9 \pm 0.2^3 & 81.9 \pm 4.1^3 & 187.5 \pm 0.3 \\
\text{Control meal} & 36.1 \pm 2.7 & 1.1 \pm 0.1 & 41.0 \pm 1.6 & — \\
\text{Postmethionine load conditions} & & & & \\
\text{Betaine supplement} & 35.4 \pm 5.1 & 2.0 \pm 0.2^3 & 102.2 \pm 7.8^3 & 90.0 \pm 18.1 \\
\text{High-betaine meal}^9 & 38.9 \pm 2.6 & 1.8 \pm 0.2^3 & 95.7 \pm 11.8^8,9,11 & 157.8 \pm 24.3 \\
\text{High-choline meal} & 37.9 \pm 3.0 & 1.3 \pm 0.1^{12} & 57.4 \pm 3.6^9 & 232.8 \pm 18.1^{12} \\
\text{Control meal} & 38.1 \pm 3.2 & 1.1 \pm 0.1 & 53.4 \pm 7.2 & — \\
\end{array}
\]

\(^1\) All values are \(\bar{x} \pm \text{SEM}; n = 8\). AUC ratio, ratio to fasting baseline under the postprandial concentration-time curve; \(C_{\text{max}}\), peak concentration; \(T_{\text{max}}\), time taken to reach peak concentration.

\(^2\) For AUC ratio, 1.0 = no change relative to fasting baseline. There were no significant differences at baseline.

\(^3,4\) Significantly different from control meal (one-factor repeated-measures ANOVA, Tukey’s test): \(^3P \leq 0.001\), \(^4P \leq 0.01\).

\(^5\) High-betaine meal concentration maximum significantly different from choline supplement \((P = 0.009)\) and high-choline meal \((P = 0.012)\).

\(^6\) Significantly different from choline supplement, \(P = 0.009\) (one-factor repeated-measures ANOVA, Tukey’s test).

\(^7,8\) Significantly different from high-choline meal (one-factor repeated-measures ANOVA, Tukey’s test): \(^7P = 0.012\), \(^8P = 0.004\).

\(^9,10\) Significantly different from betaine supplement (one-factor repeated-measures ANOVA, Tukey’s test): \(^9P < 0.001\), \(^10P < 0.05\), \(^12P < 0.01\).

\(^11\) High-betaine meal significantly different from high-choline meal \((P = 0.004)\).
plasma dimethylglycine $C_{\text{max}}$ values was not significant with the small numbers in this study ($P = 0.053$).

**Urinary betaine excretion**

Betaine supplementation and the high-betaine meal had a minimal effect on betaine excretion (Figure 2), especially in the first 4 h. Two-factor repeated-measures ANOVA did not indicate that there was a difference in urine betaine excretion associated with the effects of the different meals ($P = 0.369$) with no significant time-by-intervention interaction ($P = 0.302$). The pharmacologic markers also showed no significant differences between the treatments.

**Urinary dimethylglycine excretion**

Despite the minimal effects of the treatments on betaine excretion and plasma dimethylglycine concentrations, urinary dimethylglycine excretion was increased by betaine intake and by the high-choline meals (Figure 2). Two-factor repeated-measures ANOVA confirmed that the differences in the increases were highly significant ($P < 0.001$) between the different treatments and time points. Compared with the control meal, the high-choline and the high-betaine and -choline meals raised mean urinary dimethylglycine most (geometric mean increases 3.5 and 2.9 mmol/mol; $P < 0.001$ and $P = 0.001$) with smaller increases as a result of betaine supplementation or a high-betaine meal (geometric mean increases of 2.4 for supplementation, $P = 0.011$, and 2.2 mmol/mol for the high-betaine meal, $P = 0.030$). The effects were most apparent at 6 h (Figure 2), but significant elevations were still present at 24 h, after the 3 meals with a high-betaine or -choline content. The elevation after choline supplementation was not significant ($P = 0.45$; Figure 2).

The AUC and $C_{\text{max}}$ values (Table 3) showed a significant overall effect of treatment (diets compared with the control meal) on peak urinary dimethylglycine excretion ($P \leq 0.001$), although the effects on urinary dimethylglycine AUC and time to peak excretion were not significant ($P = 0.42$ and $P = 0.71$, respectively). Urinary dimethylglycine excretion was higher (compared with the control meal) after a high-choline meal ($P < 0.001$), a high-betaine meal ($P = 0.006$), a high-betaine and -choline meal ($P = 0.003$), or a betaine supplement ($P = 0.034$), but the increase after a choline supplement was not significant ($P = 0.150$).

**Plasma homocysteine**

The mean plasma total homocysteine decreased in the first 4 h after all treatments except the control meal (Table 4). At 4 h, the mean decrease was $0.33 \mu\text{mol/L}$ ($P = 0.026$), although only the high-choline meal (decrease: $0.77 \mu\text{mol/L}$; $P = 0.002$) and the high-betaine and -choline meal (decrease: $0.59 \mu\text{mol/L}$; $P = 0.021$) were associated with significant decreases, both by 2-factor repeated-measures ANOVA. A significant interaction was observed between treatment and time ($P = 0.026$). The high-betaine meal at 6 h may have had a small effect ($P = 0.044$, Tukey’s test, one-factor repeated-measures ANOVA). With the use of the less stringent Sidak’s test, there appeared to be a significant overall effect of treatment (diets compared with the control meal) on the change in plasma homocysteine concentrations between baseline and 4 h ($P = 0.015$) and between baseline and 24 h ($P = 0.007$) after betaine supplementation was also significant ($P = 0.039$ at 4 h; $P = 0.003$ at 6 h). The interaction between treatment and time was significant ($P = 0.030$) for change in urinary dimethylglycine excretion only.

**FIGURE 2.** Mean ($\pm$ SEM) changes in urinary betaine and urinary dimethylglycine excretion from baseline under standard conditions after the study treatments. $n = 8$ subjects. Significance was assessed by Tukey’s test based on 2-factor repeated-measures ANOVA with the use of log-transformed data. No significant differences were observed in betaine excretion between treatments. Dimethylglycine excretion was elevated ($P < 0.005$) compared with the control meal at 4 and 6 h after the high-choline and the high-betaine and -choline meals and was still elevated ($P < 0.05$) after 24 h with these treatments. Increases after betaine supplementation were also significant ($P = 0.039$ at 4 h; $P = 0.003$ at 6 h). The interaction between treatment and time was significant ($P = 0.030$) for change in urinary dimethylglycine excretion only.
TABLE 3
Urinary dimethylglycine excretion after study treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>AUC ratio†</th>
<th>Cmax</th>
</tr>
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<tr>
<td></td>
<td>nmol/mol mol creatinine</td>
<td></td>
<td>nmol/mol mol creatinine</td>
</tr>
<tr>
<td>Standard conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betaine supplementation</td>
<td>3.1 ± 1.4</td>
<td>4.0 ± 0.8</td>
<td>11.1 ± 2.4</td>
</tr>
<tr>
<td>High-betaine meal</td>
<td>1.2 ± 0.8</td>
<td>4.2 ± 0.6</td>
<td>5.9 ± 1.6</td>
</tr>
<tr>
<td>Choline supplement</td>
<td>1.2 ± 0.2</td>
<td>3.3 ± 0.5</td>
<td>5.7 ± 0.8</td>
</tr>
<tr>
<td>Choline-rich meal</td>
<td>0.9 ± 0.3</td>
<td>2.6 ± 0.8</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>Betaine- and choline-rich meal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control meal</td>
<td>3.1 ± 1.4</td>
<td>4.0 ± 0.8</td>
<td>11.1 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.8</td>
<td>4.2 ± 0.6</td>
<td>5.9 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 0.8</td>
<td>5.3 ± 1.2</td>
<td>6.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>1.4 ± 0.6</td>
<td>7.3 ± 2.8</td>
<td>5.8 ± 1.9</td>
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<td>Postmethionine load conditions</td>
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</tr>
<tr>
<td>Betaine supplementation</td>
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<td>5.9 ± 1.6</td>
</tr>
<tr>
<td>Choline-rich meal</td>
<td>1.6 ± 0.8</td>
<td>5.3 ± 1.2</td>
<td>6.6 ± 1.9</td>
</tr>
<tr>
<td>Control meal</td>
<td>1.4 ± 0.6</td>
<td>7.3 ± 2.8</td>
<td>5.8 ± 1.9</td>
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</tbody>
</table>

† All values are x ± SEM; n = 8. AUC ratio, ratio to fasting baseline under the postprandial concentration-time curve; Cmax, peak concentration.

and 6 h (P = 0.05) under standard conditions, although the magnitude of the change was small.

Postmethionine load

Plasma betaine concentration

The effects of the high-betaine and -choline meals and the betaine supplement on plasma betaine concentrations relative to the control meal after a methionine load were similar to the effects seen under normal (standard) conditions (Figure 1). By 2-factor repeated-measures ANOVA, betaine was significantly higher after supplementation in the 1–3-h samples (P < 0.001 at 1 and 2 h, P = 0.002 at 3 h) and after the high-betaine meal at 2–6 h (P < 0.001 at 2 and 3 h, P = 0.002 at 4 h, P = 0.007 at 5 h, and P = 0.010 at 6 h). The elevations after the high-choline meal were not significant. At 1 h the supplement had a significantly greater effect (P = 0.033) than did the high-betaine meal.

The overall effect of treatment (diets compared with the control meal) on plasma betaine after a methionine load was highly significant, P ≤ 0.0001 for both betaine AUC and betaine Cmax, respectively. The overall effect of treatment on betaine Tmax was also significant, although less so (P = 0.008). Betaine supplementation increased both plasma betaine AUC and plasma betaine Cmax relative to the control meal (Table 2).

Plasma dimethylglycine concentrations

None of the changes in plasma dimethylglycine concentrations were statistically significant, there was not a significant interaction between treatment and time (P = 0.064), and neither were estimates of the AUC and Cmax values significantly different from the control data. The magnitudes of the observed changes were minimal after all treatments, the largest difference from control (1.3 μmol/L) being with betaine supplementation at 5 h, but even this was not statistically significant.

Urinary betaine and dimethylglycine excretion

No significant changes in urine betaine excretions were observed after any of the treatments. Surprisingly, in contrast to the effects of the treatments under the normal standard conditions, the urinary excretion of dimethylglycine was also not significantly different from the control meal after any of the treatments.

TABLE 4
Plasma total homocysteine concentrations after study treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4-h values</th>
<th>6-h values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>μmol/L</td>
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<tr>
<td>Standard conditions</td>
<td></td>
<td></td>
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<tr>
<td>Betaine supplementation</td>
<td>7.9 ± 0.8</td>
<td>-0.3 ± 0.2</td>
</tr>
<tr>
<td>High-betaine meal</td>
<td>7.6 ± 0.6</td>
<td>-0.3 ± 0.1</td>
</tr>
<tr>
<td>Choline supplement</td>
<td>7.8 ± 0.8</td>
<td>-0.1 ± 0.2</td>
</tr>
<tr>
<td>Choline-rich meal</td>
<td>7.9 ± 0.8</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>High-betaine and -choline meal</td>
<td>7.5 ± 0.7</td>
<td>-0.6 ± 0.1</td>
</tr>
<tr>
<td>Control meal</td>
<td>7.8 ± 0.7</td>
<td>0.0 ± 0.2</td>
</tr>
<tr>
<td>Postmethionine load conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betaine supplementation</td>
<td>23.3 ± 1.5</td>
<td>15.1 ± 0.9</td>
</tr>
<tr>
<td>High-betaine meal</td>
<td>21.6 ± 1.2</td>
<td>13.3 ± 0.8</td>
</tr>
<tr>
<td>Choline-rich meal</td>
<td>21.4 ± 1.4</td>
<td>13.2 ± 0.9</td>
</tr>
<tr>
<td>Control meal</td>
<td>28.5 ± 2.1</td>
<td>20.2 ± 1.8</td>
</tr>
</tbody>
</table>

† All values are x ± SEM; n = 8. There were no significant differences at baseline.

† † † † Significantly different from control meal (2-factor repeated-measures ANOVA, Tukey’s test): † P < 0.05, † † P < 0.01, † † † P ≤ 0.001.
Plasma homocysteine

All treatments significantly attenuated the effect of a methionine load on plasma homocysteine compared with the control group (Table 4). The interaction of treatment and time was highly significant (P < 0.001). Both the high-betaine meal and the high-choline meal attenuated the increase in plasma homocysteine concentrations by 6.9–7.6 μmol/L (P < 0.001) at both 4 h and 6 h, slightly (but not statistically significantly) more than the 5.2 μmol/L attenuation 4 or 6 h after betaine supplementation. This latter attenuation was also highly significantly (P = 0.001) different from the control meal.

DISCUSSION

An acute physiologic dose of betaine (=550 mg) or of its metabolic precursor choline (=800 mg), administered by dietary manipulation or as an oral supplement, significantly increased plasma betaine concentrations under standard and PML conditions. As expected, the 2 treatments led to almost identical increases. At these doses, little betaine appeared in the urine; thus, it is presumed that most was stored in tissues, where betaine concentrations are typically higher than in the circulation. Some was metabolized, because dietary betaine and choline administration reduced plasma homocysteine concentrations, and there was a small but significant increase in both circulating and urinary concentrations of dimethylglycine. These data add support to the hypothesis that dietary modification can replenish tissue stores of betaine and lower circulating homocysteine. The magnitude of the acute lowering of homocysteine was small and near the limit of detection.

Most data on betaine supplementation are based on larger doses (>6 g) over long treatment durations. We show that even relatively small loads (=550 mg; the amount in a high-betaine meal) increase plasma betaine concentrations ~2-fold, reaching a peak at ~2 h after either betaine supplementation or a high-betaine meal (Figure 1). This is in agreement with previous reports of betaine being rapidly absorbed and distributed after ingestion (40, 41). The small difference between the betaine content of the supplement and the high-betaine meal was not reflected by a change in plasma betaine concentration. On the contrary, supplementation at the slightly lower concentration led to a seemingly greater increase in plasma betaine than that observed after a high-betaine meal. Plasma betaine concentrations may increase more quickly after betaine supplementation than after ingestion of a betaine-enriched meal. This may reflect fast absorption and entry into the portal circulation from an oral supplement, whereas a high-betaine meal requires mechanical break-up and digestion. Moreover, dietary betaine transport into the gut will face competition from the presence of additional meal components. Despite this, the meal and supplement produced similar Cmax values, suggesting that the difference in time taken to reach maximal plasma concentrations does not represent any real clinical benefit of supplementation over dietary modification. Interestingly, despite similar increases in plasma betaine concentrations, dietary betaine and choline administration appeared more effective than did oral supplementation at lowering homocysteine concentrations, possibly reducing plasma homocysteine under standard conditions and leading to a greater attenuation of the rise in homocysteine after a methionine load. This is the first study to directly compare the homocysteine-lowering ability of dietary with supplementary sources of betaine or choline. It was suggested that large doses of oral betaine are required to achieve even modest reductions in homocysteine (42), and, in line with our findings, Schwab et al (26) found no significant decrease in homocysteine after administration of a 1-g oral betaine supplement to healthy volunteers under standard conditions.

The acute effect of dietary choline on plasma betaine and homocysteine concentrations in healthy volunteers has not previously been reported, but it is in agreement with the reported reduction in fasting and PML homocysteine after choline supplementation in men with mildly elevated homocysteine concentrations (27). The decrease in plasma homocysteine after a high-choline meal may be mediated by its oxidation to betaine and betaine-dependent remethylation of homocysteine to methionine, although a decrease in the endogenous production of phosphatidylcholine resulting from increased choline availability was suggested as an alternative mechanism (27). Because phosphatidylcholine synthesis generates homocysteine at a rate of 3 homocysteine molecules per molecule of choline synthesized, decreased synthesis could lead to a reduction in homocysteine concentrations. The acute effect of a high-choline meal on plasma homocysteine, without a significant rise in plasma betaine, may argue against betaine as an intermediate, but this is not conclusive because both choline oxidation and betaine methyl transfer occur in the liver, where intracellular betaine is higher than circulating concentrations.

The absence of any significant change in urinary betaine excretion under standard or PML conditions after a physiologic dose of betaine is an interesting finding that complements previous work (43) and suggests that a large betaine load is required to elicit any measurable change in urinary betaine concentrations. In health, betaine is freely filtered in the kidney and not normally cleared in the urine, being almost completely reabsorbed in the proximal tubules (44–46). Betaine is mainly eliminated by metabolism to dimethylglycine (40), explaining the increase in plasma and urinary dimethylglycine after betaine administration. This may signify increased flow through the BHMT pathway and support the concept that the BHMT pathway plays an important role in the control of betaine and homocysteine metabolism (47). Urine betaine excretion is normally quite constant (46), but some patients excrete abnormal amounts of betaine (8, 45, 46), and this loss is associated with elevated homocysteine and increased incidence of vascular disease (8). Some patients consistently excrete large amounts of betaine for years (48), and especially these patients could benefit from an increased betaine intake.

Because there are sex-related differences in betaine and choline metabolism (8, 44, 45, 49, 50), we included only male subjects in this study. This, the well-documented sex-related differences in body composition and fat mass (important determinants of homocysteine concentrations in healthy persons [51]), and sex differences in homocysteine metabolism (52) highlight the need for similar studies in women.

In conclusion, an acute physiologic dose of betaine, or its metabolic precursor choline, significantly increases plasma betaine concentrations in healthy volunteers under standard and PML conditions. Ingestion of a meal rich in betaine or choline, but not oral supplementation at a comparable level, led to a small reduction in plasma homocysteine under standard conditions, whereas dietary betaine and choline or a betaine supplement
attenuated the rise in homocysteine after a methionine load. Urinary betaine excretion did not change significantly after a physiological dose of betaine. This study suggests that tissue betaine can be replenished by dietary betaine, and this also acts as a homocysteine-lowering agent. If maintaining tissue betaine concentrations reduces the risk of vascular disease, then increasing oral intake of betaine is a simple and cost-effective strategy with obvious therapeutic potential.

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The author’s responsibilities were as follows—WA: directly supervised the work, carried out the analyses, and wrote the first draft of the manuscript; JE: designed the diets, provided nutritional advice, carried out statistical analyses, and edited the manuscript; ML: supervised the laboratory work, carried out statistical analyses, and revised the manuscript; PMG and STC: were responsible for the initial study design, provided clinical advice, and reviewed and edited the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES


