

Betaine concentration as a determinant of fasting total homocysteine concentrations and the effect of folic acid supplementation on betaine concentrations^{1–3}

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ABSTRACT

Background: Remethylation of homocysteine to methionine can occur through either the folate-dependent methionine synthase pathway or the betaine-dependent betaine-homocysteine methyltransferase pathway. The relevance of betaine as a determinant of fasting total homocysteine (tHcy) is not known, nor is it known how the 2 remethylation pathways are interrelated.

Objective: The objectives of the study were to examine the relation between plasma betaine concentration and fasting plasma tHcy concentrations and to assess the effect of folic acid supplementation on betaine concentrations in healthy subjects.

Design: A double-blind randomized trial of 6 incremental daily doses of folic acid (50–800 µg/d) or placebo was carried out in 308 Dutch men and postmenopausal women (aged 50–75 y). Fasted blood concentrations of tHcy, betaine, choline, dimethylglycine, and folate were measured at baseline and after 12 wk of vitamin supplementation.

Results: Concentrations of tHcy were inversely related to the betaine concentration ($r = -0.17$, $P < 0.01$), and the association was independent of age, sex, and serum concentrations of folate, creatinine, and cobalamin. Folic acid supplementation increased betaine concentration in a dose-dependent manner (P for trend = 0.018); the maximum increase (15%) was obtained at daily doses of 400–800 µg/d.

Conclusions: The plasma betaine concentration is a significant determinant of fasting tHcy concentrations in healthy humans. Folic acid supplementation increases the betaine concentration, which indicates that the 2 remethylation pathways are interrelated. *Am J Clin Nutr* 2005;81:1378–82.

KEY WORDS Total homocysteine, tHcy, betaine, folate, folic acid supplementation, healthy population, humans

INTRODUCTION

High concentrations of total homocysteine (tHcy) have been suggested as a possible risk factor for cardiovascular disease (CVD) (1). Dietary supplementation with folic acid (vitamin B-11) is highly effective in reducing tHcy concentrations, and this effect is mediated by remethylation of homocysteine to methionine (2). Dietary supplementation with cobalamin (vitamin B-12) can also reduce tHcy concentrations but to a much lower extent than does supplementation with folic acid (3, 4).

Plasma tHcy concentrations can also be lowered by betaine (trimethylglycine). Betaine is derived endogenously from the oxidation of choline and exogenously from dietary sources (5). Betaine serves as methyl donor for the reaction catalyzed by betaine-homocysteine methyltransferase (BHMT) that converts homocysteine to methionine and betaine to dimethylglycine (**Figure 1**). Methylation through the BHMT pathway is confined to the liver and kidney (6), whereas methylation of homocysteine catalyzed by methionine synthase (MTR) occurs in all cells. Several studies have shown that concentrations of both fasting and postmethionine-load tHcy in plasma can be lowered by betaine supplementation in homocystinuric patients (7, 8) and healthy humans (9, 10). Plasma betaine concentration was inversely associated with postmethionine tHcy concentrations in coronary disease patients, but this association was attenuated after supplementation with B vitamins for 1 y (11), which may indicate that increased remethylation of homocysteine via MTR down-regulates BHMT activity.

The hypothesis that the 2 remethylation pathways for homocysteine are interrelated is supported by data from animal research. After consumption of a choline-deficient diet for 2 wk, the hepatic folate content in rats had decreased by 31% (12, 13). Moreover, rats maintained on a folate-deficient diet showed depletion of hepatic choline (14). This suggests that the limitation of one pathway increases remethylation via the other pathway.

The aims of the current study were to examine the relation between plasma betaine concentration and fasting plasma tHcy concentrations and to investigate whether lowering tHcy concentrations with various doses of folic acid had an effect on plasma betaine concentrations.

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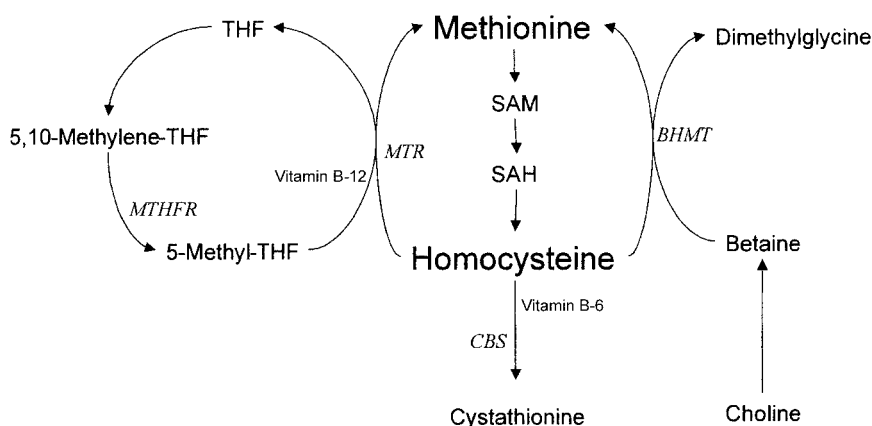


FIGURE 1. Remethylation of total homocysteine by either methionine synthase (MTR) or betaine homocysteine methyltransferase (BHMT). THF, tetrahydrofolate; MTHFR, 5,10-methylenetetrahydrofolate reductase; CBS, cystathionine β -synthase; SAM, *S*-adenosyl methionine; SAH, *S*-adenosyl homocysteine.

SUBJECTS AND METHODS

Subjects

Healthy men and women aged 50–75 y were recruited by means of postal questionnaires from a random sample of people living in the community near Wageningen, Netherlands, and from a database of people who previously expressed interest in participating in such studies (15). People who had a history of CVD, renal or thyroid disease, or cancer were excluded, as were those who were taking medication that could interfere with folate or homocysteine metabolism. Users of B vitamin supplements ≤ 3 mo before study were also excluded from participation. All of the women were required to be postmenopausal. In total, 425 persons returned their questionnaires, of whom 353 persons were found to be eligible; 331 participants underwent biochemical screening. On the basis of this screening, 15 subjects were excluded because of high concentrations of plasma tHcy ($> 26 \mu\text{mol/L}$) or serum creatinine ($> 125 \mu\text{mol/L}$) or low serum concentrations of cobalamin ($< 160 \text{pmol/L}$).

All participants gave written informed consent. The study protocol was approved by the Medical Ethical Committee of Wageningen University.

Study design

Subjects visited the research unit on 3 separate occasions. Biochemical screening took place 4 wk before randomization, and further data were collected at randomization and at the end of the 12-wk intervention. Randomization implied allocation to 6 different doses of folic acid or placebo after stratification for plasma tHcy concentrations measured at the screening visit. The daily doses of folic acid (50, 100, 200, 400, 600, or 800 μg) and placebo were prepared in identical capsules, so that the subjects and staff were kept blinded to the allocated treatment. Subjects were asked to adhere to their habitual diet and to refrain from eating liver, yeast extracts, or supplements containing B vitamins during the trial. In addition, they were asked to refrain from consuming liver products (eg, liver paste) for 3 d before each blood collection. Subjects were asked to keep a diary during the study and to record the daily intake of capsules, illnesses experienced, and the use of any medication.

Blood collections and biochemical analyses

Fasting venous blood samples were collected from subjects at each visit. Plasma (EDTA) or serum was separated from blood cells by centrifugation at $2600 \times g$ for 10 min at 4°C and stored at -80°C until analysis.

Betaine, choline, and dimethylglycine concentrations in plasma were measured at the Department of Pharmacology, University of Bergen, Norway, by using normal-phase chromatography–tandem mass spectrometry (16). Intraassay and interassay CVs for betaine, choline, and dimethylglycine were 3–6% for all 3 metabolites. The tHcy concentrations in plasma were measured at the Division of Human Nutrition, Wageningen University, Netherlands, by using HPLC with fluorimetric detection (17, 18). Intraassay and interassay CVs of tHcy analyses were 2% and 7%, respectively. Serum folate and cobalamin concentrations were measured by using a commercial chemiluminescent immunoassay analyzer (Immulite 2000; Diagnostic Products Company, Los Angeles, CA). Serum creatinine concentrations were measured with a modification of the kinetic Jaffé reaction (Dimension; DuPont, Wilmington, DE).

Statistical analysis

Because tHcy concentrations were higher in men than in women, data are reported separately for men and women when appropriate. Spearman correlation coefficients for associations between concentrations of tHcy, betaine, choline, dimethylglycine, folate, and creatinine were calculated. Correlation coefficients with a P value < 0.01 were considered to be significant. Linear regression models were used to assess associations between tHcy, betaine, and folate concentrations at baseline. For the model evaluating determinants of tHcy, we included conventional variables such as age, sex, folate, creatinine, and cobalamin in the model and tested the additional predictive capacity of betaine in the model. Similarly, after adjustment for age, sex, and choline, folate was added to the model to assess its additional predictive value with respect to betaine. Log transformations were made to normalize the distribution of tHcy, folate, and cobalamin concentrations. General linear models were used to assess trends in changes in betaine concentration with increasing

TABLE 1

Plasma concentrations of total homocysteine (tHcy), betaine, and related metabolites and vitamins in men and women at the time of random assignment

| | Men (<i>n</i> = 182) | Women (<i>n</i> = 126) | <i>P</i> ¹ |
|----------------------------------|------------------------------|----------------------------|-----------------------|
| tHcy (μmol/L) | 11.3 (9.0–16.2) ² | 10.6 (7.8–14.2) | <0.0001 |
| Betaine (μmol/L) | 35.3 (25.6–46.6) | 32.4 (24.1–44.5) | 0.008 |
| Choline (μmol/L) | 7.8 (5.7–9.7) | 7.2 (5.3–9.9) | 0.02 |
| Dimethylglycine (μmol/L) | 3.4 (2.4–4.7) | 3.0 (2.3–4.5) | 0.01 |
| Folate (nmol/L) | 11.8 (7.8–17.7) | 13.1 (8.7–19.3) | 0.002 |
| Cobalamin (pmol/L) ³ | 279 (182–499) | 292 (196–508) | 0.5 |
| Creatinine (μmol/L) ³ | 82 (69–98) | 69 (57–86) | <0.0001 |

¹ Mann-Whitney *U* test.

² Median; 10–90th percentiles in parentheses (all such values).

³ Measured at screening.

doses of folic acid supplementation and their effect on the proportional reductions in tHcy concentrations. All statistical procedures were performed with SPSS for WINDOWS software (version 11.01; SPSS Inc, Chicago, IL).

RESULTS

Subject characteristics and blood indexes at baseline

The study population included 316 subjects (59% men) with a median age of 60 y (range: 50–75 y) as described previously (15). Complete data for the current analyses were available for 308 subjects. Median values for selected characteristics before treatment separately for males and females are shown in **Table 1**. The median (10th–90th percentiles) betaine concentration was 34.8 μmol/L (range: 24.6–45.3 μmol/L), and that of tHcy was 11.1 μmol/L (8.2–15.4 μmol/L). Concentrations of betaine, choline, dimethylglycine, tHcy, and creatinine were all ≈10% higher in the men than in the women, but folate concentrations were higher in the women than in the men (Table 1). Mean (±SD) betaine concentrations at baseline for the groups receiving placebo or 50, 100, 200, 400, 600, and 800 μg of folic acid were 34.1 ± 7.8, 35.6 ± 7.6, 34.2 ± 8.1, 34.8 ± 6.4, 35.3 ± 11.2, 33.9 ± 9.6, 37.4 ± 10.6 μmol/L, respectively.

TABLE 2

Betaine as a determinant of plasma total homocysteine (tHcy) concentrations at randomization by multiple linear regression

| Dependent variable | Independent variables | β | Standardized β | Adjusted R^2 |
|-------------------------------|--------------------------------------|---------|----------------------|----------------|
| tHcy (ln μmol/L) ¹ | Intercept | 3.24 | | |
| | Age (y) ² | 0.005 | 0.13 | |
| | Males | 0.007 | 0.02 | |
| | Folate (ln nmol/L) ^{1,3} | −0.24 | −0.33 | |
| | Creat (μmol/L) ^{3,4} | 0.006 | 0.33 | |
| | Cobalamin (ln pmol/L) ^{3,4} | −0.15 | −0.22 | |
| | Betaine (μmol/L) ^{1,3} | −0.005 | −0.20 | 0.34 |

¹ Determined at the time of random assignment.

² *P* < 0.01.

³ *P* < 0.001.

⁴ Measured only at screening because these variables were not outcome measures of the study.

TABLE 3

Folate as a determinant of plasma betaine concentrations at the time of random assignment by multiple linear regression

| Dependent variable | Independent variables | β | Standardized β | Adjusted R^2 |
|-------------------------------|---------------------------------|---------|----------------------|----------------|
| Betaine (μmol/L) ¹ | Intercept | 8.86 | | |
| | Choline (μmol/L) ^{1,2} | 1.84 | 0.36 | |
| | Age (y) | 0.10 | 0.07 | |
| | Males ³ | 2.28 | 0.13 | |
| | Folate (ln nmol/L) ¹ | 1.79 | 0.07 | 0.17 |

¹ Measured at the time of random assignment.

² *P* < 0.001.

³ *P* < 0.05.

Betaine as a determinant of baseline tHcy

At baseline, tHcy concentrations were inversely related to the concentration of betaine ($r = -0.17$, $P < 0.01$). As expected, baseline tHcy concentrations were inversely related to the folate concentration ($r = -0.40$, $P < 0.001$) and directly related to the creatinine concentration ($r = 0.35$, $P < 0.001$). In linear regression, folate and creatinine were the strongest determinants of tHcy (standardized β : −0.33 and 0.33, respectively). The betaine concentration (standardized β : −0.20) was shown to be as strongly related to tHcy concentrations as was the cobalamin concentration (standardized β : −0.22) (**Table 2**).

Betaine and folic acid supplementation

The betaine concentration at baseline was not related to folate concentration ($r = 0.05$, $P = 0.36$) but was positively related to concentrations of choline ($r = 0.40$, $P < 0.001$) and dimethylglycine ($r = 0.52$, $P < 0.001$). In linear regression analysis, choline concentration was the strongest predictor of baseline betaine concentration (standardized β : 0.36), but serum folate concentration was not predictive (standardized β : 0.07) (**Table 3**).

As previously reported, folic acid supplementation significantly decreased tHcy concentrations in a dose-dependent manner, up to 25% at a dose of 800 μg/d (15). Plasma betaine concentrations increased dose-dependently with increasingly larger doses of folic acid (P for trend = 0.018; **Figure 2**). The proportional decrease in tHcy after folic acid supplementation was associated with an increase in betaine concentration ($r = -0.18$, P for trend < 0.001; **Figure 3**). Doses of 50 to 200 μg folic acid

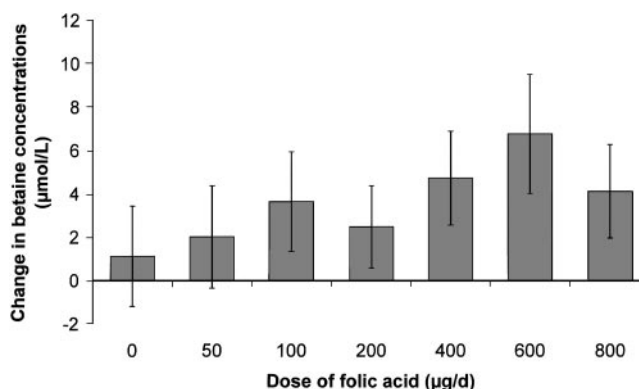


FIGURE 2. Mean (95% CI) changes in plasma betaine concentrations after supplementation with increasing doses of folic acid. *P* for trend = 0.018.

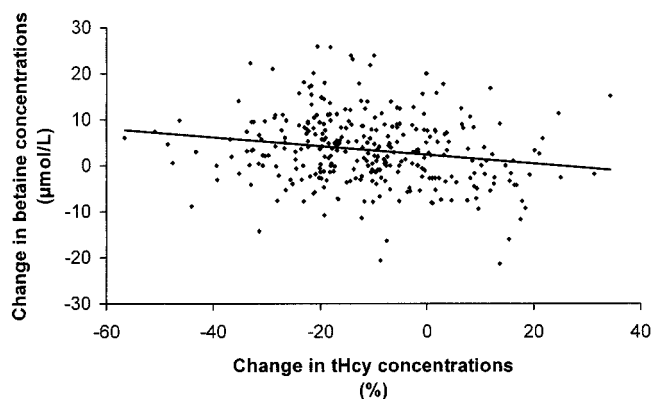


FIGURE 3. Association between changes in plasma concentrations of betaine and percentage changes in homocysteine concentrations. $r = -0.18$, P for trend < 0.001 .

induced an increase in betaine concentration of $\approx 8\%$, whereas doses of 400 to 800 μg induced an increase of $\approx 15\%$ ($P < 0.05$). After folic acid supplementation, betaine remained associated with tHcy concentrations as strongly as before supplementation ($r = -0.17$, $P < 0.01$). Concentrations of betaine and folate became related after supplementation ($r = 0.25$, $P < 0.001$).

DISCUSSION

The current trial showed that plasma betaine concentrations were inversely associated with fasting concentrations of tHcy in plasma. The median betaine concentration was 34.8 $\mu\text{mol/L}$ in this population, which is consistent with results from other studies (11, 19–21). Intervention trials have shown that betaine supplementation can lower tHcy concentrations by 10–20% (9). Hence, diseases associated with high plasma tHcy concentrations, such as neural tube defects (22), CVD (1), and dementia (23), may be associated with low betaine and choline concentrations as well as with low folate concentrations. Betaine presumably plays a significant role in the remethylation of homocysteine to methionine in healthy humans.

The current trial showed that supplementation with folic acid ($< 800 \mu\text{g/d}$) increased plasma betaine concentrations by $\approx 15\%$. Moreover, folate concentration was associated with betaine concentration only after folic acid supplementation. This may indicate that the increased flux through MTR in response to folic acid supplementation diminishes the flux through BHMT in healthy humans, thereby sparing betaine. These findings extend published data showing associations between betaine and postmethionine-loading tHcy concentrations before and after B vitamin supplementation (11).

In contrast, dietary betaine supplementation does not affect folate status, as shown in adults with mildly elevated tHcy concentrations supplemented with 6 g betaine/d (9). This may indicate that the flux through MTR is unaffected when the flux through BHMT is increased. Another explanation is that, because it receives its methyl group from serine, folate is not affected because it is not a primary methyl donor.

In conclusion, plasma betaine concentration is a determinant of fasting plasma tHcy concentrations in a healthy population of older adults. Enhanced remethylation of tHcy through MTR increases plasma betaine concentrations, which indicates that both

pathways may be more interrelated in healthy subjects than previously believed. 

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AM-B, PV, and RC contributed to the study design; AM-B supervised the data collection; PMU and PH performed the additional biochemical analyses; all authors contributed to data analysis; AM-B drafted the paper, and all other authors critically revised the manuscript. None of the authors had any financial or personal conflict of interest.

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