Betaine and Folate Status as Cooperative Determinants of Plasma Homocysteine in Humans

Pål I. Holm, Per M. Ueland, Stein Emil Vollset, Øivind Midttun, Henk J. Blom, Miranda B.A.J. Keijzer, Martin den Heijer

Objective—Two published studies have demonstrated that betaine in the circulation is a determinant of plasma total homocysteine, but none had sufficient power to investigate the possible effect modification by folate status.

Methods and Results—We measured homocysteine, betaine, folate, vitamin B6, and related compounds in serum/plasma from 500 healthy men and women aged 34 to 69 years before (fasting levels) and 6 hours after a standard methionine loading test. Choline, dimethylglycine, and folate were determinants of plasma betaine in a multiple regression model adjusting for age and sex. The increase in homocysteine after loading showed a strong inverse association with plasma betaine and a weaker inverse association with folate and vitamin B6. Fasting homocysteine showed a strong inverse relation to folate, a weak relation to plasma betaine, and no relation to vitamin B6. Notably, adjusted (for age and sex) dose-response curves for the postmethionine increase in homocysteine or fasting homocysteine versus betaine showed that the inverse associations were most pronounced at low serum folate, an observation that was confirmed by analyses of interaction.

Conclusions—Collectively, these results show that plasma betaine is a strong determinant of increase in homocysteine after methionine loading, particularly in subjects with low folate status. (Arterioscler Thromb Vasc Biol. 2005;25:1-7.)

Key Words: betaine ■ choline ■ homocysteine ■ folate ■ methionine

The concentration of total homocysteine (tHcy) in serum/plasma is associated with risk of atheroembolic cardiovascular vascular disease (CVD).¹ Most studies have investigated fasting tHcy, often obtained after an overnight fasting (fasting tHcy). The high concentration of tHcy detected after a standard methionine loading dose (postmethionine load [PML] tHcy) seems to be a risk factor independent of fasting tHcy,² and fasting and PML tHcy identify overlapping but also different subjects with hyperhomocysteinemia and increased CVD risk.² The PML increase in tHcy (PML ΔtHcy) can be calculated as the difference between PML tHcy and fasting tHcy, but its separate role in CVD risk assessment is not settled.

Determinants of fasting tHcy include genetic and a variety of lifestyle factors and pathologies.³ Strong predictors of fasting tHcy include renal function and cobalamin and folate status.² The methylenetetrahydrofolate reductase 677C→T polymorphism is the most influential common genetic factor.⁴ PML tHcy and ΔtHcy have some determinants in common with fasting tHcy, including folate status and the methylenetetrahydrofolate reductase 677C→T polymorphism, whereas renal function seems to have minor impact. Vitamin B6 status has been reported to have a moderate effect on PML tHcy and a weaker or essentially no effect on fasting tHcy,² which is in agreement with the role of the vitamin B6–dependent transulfuration pathway in degrading superfluos homocysteine.⁵ The effect of these B vitamins are explained by their role in homocysteine metabolism, as depicted in Figure 1.

Betaine (trimethylglycine) is obtained in small amounts from foods or is generated from endogenous choline.⁵,⁶ It serves as a methyl donor in a reaction converting homocysteine to methionine, catalyzed by the enzyme betaine-homocysteine methyltransferase (DHMT; EC 2.1.1.5; Figure 1). This pathway is confined to liver and kidney and represents an alternative route of homocysteine remethylation, a reaction that is also performed by the ubiquitous folate-dependent methionine synthase.⁷ Treatment with high doses of betaine (≥6 g per day) has been used for years to reduce fasting (basal) tHcy in homocystinurics⁸ but also has a marked effect (10% to 15% tHcy reduction) in healthy individuals.⁷,⁸ PML tHcy seems to be more responsive to betaine, and high doses of betaine markedly reduced PML tHcy in healthy subjects⁹ and in renal patients treated with folic acid and vitamin B6.¹⁰ In comparison, folic acid supplementation reduces fasting tHcy more than betaine, but it has a marginal and nonsignificant effect on PML tHcy.⁹ Notably, low betaine doses in the range of dietary intake have been shown recently to reduce fasting and, in particular PML, tHcy.¹¹
performed as described. The study was approved by the ethics committee of the Leyenburg Hospital, The Hague, Holland. These subjects were healthy controls in a published study previously. and plasma betaine has been shown to be a strong predictor of PML tHcy in 90 patients enrolled in a B vitamin intervention trial. We conducted a large study of 500 subjects undergoing methionine loading to investigate the relationship between plasma betaine and basal and PML tHcy and the possible effect modification by folate status.

Methods

Subjects and Protocol

Subjects were recruited through a general practice in The Hague, Holland. These subjects were healthy controls in a published study on homocysteine and venous thrombosis. Details on recruitment, inclusion criteria, and data collection have been published previously. A 6-hour standard oral methionine loading test (0.1 g L-methionine per kg body weight in 200 mL orange juice) was performed as described. The study was approved by the ethics committee of the Leyenburg Hospital, The Hague, Holland, and informed consent was obtained from all study participants.

Blood Collection and Biochemical Analyses

The EDTA plasma was stored at −20°C until analysis and serum kept at −70°C. Plasma tHcy, serum creatinine, serum vitamin B12, serum folate, vitamin B6 (sum of pyridoxal 5'-phosphate and pyridoxal), betaine, choline, and dimethylglycine (DMG) were determined with published methods.

Statistics

Data are presented as medians with 10th to 90th percentiles. Between-group comparisons of continuous variables were done by the Mann–Whitney U test. Spearman rank correlation and multiple regression analyses were used to evaluate associations between individual variables. Multiple linear regression analysis was used to assess the simultaneous relationship between various predictors of tHcy. Plasma tHcy was the dependent variable, whereas the independent variables were presented in the model as quartiles of betaine, folate, cobalamin, and creatinine. Thus, the regression coefficient was used to estimate the difference in mean tHcy between the reference and the other 3 quartiles. tHcy across quartiles was tested for homogeneity of means and for linear trend. Estimates were adjusted for age and sex, or B vitamin levels and creatinine, in addition to age and sex. We investigated the possible interaction between plasma betaine and folate and between betaine and vitamin B6 by including a product term between the 2 variables in multiple linear regression models with tHcy as the dependent variable, retaining betaine and the B vitamin as independent variables. Because tHcy values (fasting and the increase after methionine loading) were not normally distributed, the multiple regression analyses, when appropriate, were also carried with log-transformed tHcy as outcome measures. The dose-response relationships between metabolites were also estimated with Gaussian generalized additive models (GAMs), as implemented in S-PLUS and R. For other analyses, we used SPSS version 11.0 (SPSS).

Results

Subject Characteristics

A total of 500 subjects (292 females and 208 males) with a mean age of 50 years (range 34 to 69) was investigated. Their blood indices before and after methionine loading and according to gender are given in Table 1. Fasting plasma tHcy, betaine, choline, DMG, methionine, creatinine and vitamin B6 and PML betaine, choline, and DMG were all significantly higher in men than in women.

The distribution of plasma betaine in terms of median, 10th to 90th percentiles, and range were 30.3, 18.8 to 45.3, and 9.4 to 94.9 μmol/L. The corresponding values for serum folate were 12.7, 7.0 to 23.6, and 3.0 to 54.4 nmol/L, respectively. Median serum cobalamin was 217 pmol/L.

Loading caused a 20-fold increase in overall median plasma methionine and a 4-fold increase in tHcy. Loading was not associated with any change in median betaine, choline, or DMG (Table 1).

Bivariate Correlations

Spearman rank correlation coefficients are listed in supplemental Table 1 (available online at http://atvb.ahajournals.org). PML ΔtHcy was weakly and inversely related to the folate, cobalamin, and vitamin B6 but showed a moderate inverse relation to fasting (r = −0.27) and PML betaine (r = −0.32; all P < 0.001). Fasting tHcy showed a moderate positive relation to age (r = 0.30) and creatinine (r = 0.37), an inverse relation to folate (r = −0.29) and cobalamin (r = 0.27; all P < 0.001), and a weak but significant positive relation to choline and DMG.

Betaine (fasting and PML) was strongly and positively related to choline and DMG (r = 0.41 to 0.50; P < 0.001) and showed a weak but significant positive relation to folate and vitamin B6 (r = 0.18 to 0.22; P < 0.001).

Folate, Choline, and DMG as Determinants of Betaine

We investigated the relationship between betaine and indices, showing a simple correlation with betaine such as folate,
TABLE 1. Subject Characteristics and Blood Indices at Baseline According to Gender*

<table>
<thead>
<tr>
<th></th>
<th>Total n=500</th>
<th>Men n=208</th>
<th>Women n=292</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50 (34–69)</td>
<td>50 (31–69)</td>
<td>50 (34–68)</td>
<td>0.7</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>10.7 (6.7–15.5)</td>
<td>11.7 (7.2–16.7)</td>
<td>10.3 (6.4–15.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PML tHcy, μmol/L</td>
<td>37.0 (25.5–57.2)</td>
<td>37.0 (26.2–51.6)</td>
<td>37.2 (25.1–61.5)</td>
<td>0.3</td>
</tr>
<tr>
<td>Betaine, μmol/L</td>
<td>30.3 (18.8–45.3)</td>
<td>34.7 (25.9–50.9)</td>
<td>26.8 (15.6–38.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PML betaine, μmol/L</td>
<td>28.6 (18.6–42.6)</td>
<td>34.2 (24.8–48.2)</td>
<td>24.8 (16.3–37.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Choline, μmol/L</td>
<td>7.8 (5.9–10.5)</td>
<td>8.2 (6.3–11.0)</td>
<td>7.6 (5.7–9.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PML choline, μmol/L</td>
<td>7.2 (5.5–8.8)</td>
<td>7.7 (5.8–10.4)</td>
<td>6.9 (5.3–9.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DMG, μmol/L</td>
<td>3.1 (2.0–4.9)</td>
<td>3.4 (2.4–5.2)</td>
<td>2.8 (1.9–4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PML DMG, μmol/L</td>
<td>3.6 (2.5–5.4)</td>
<td>3.8 (2.7–5.9)</td>
<td>3.4 (2.4–5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Methionine, μmol/L</td>
<td>23.6 (19.1–30.7)</td>
<td>25.1 (18.8–28.6)</td>
<td>23.0 (18.8–28.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PML Methionine, μmol/L</td>
<td>469 (308–657)</td>
<td>478 (341–638)</td>
<td>458 (293–671)</td>
<td>0.4</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>74 (55–99)</td>
<td>87 (71–107)</td>
<td>66 (52–84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td>12.7 (7.0–23.6)</td>
<td>12.9 (7.3–22.7)</td>
<td>12.5 (6.9–24.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>Cobalamin, pmol/L</td>
<td>217 (125–389)</td>
<td>216 (131–385)</td>
<td>219 (123–393)</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin B6, nmol/L</td>
<td>27.7 (15.8–54.8)</td>
<td>32.7 (18.0–56.3)</td>
<td>25.3 (15.3–54.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Data are given as medians with 10th to 90th percentiles in parentheses.
†Mann–Whitney U test.

choline, DMG, creatinine, vitamin B6, and age, using a Gaussian generalized additive regression (GAM), which produces dose-response curves adjusted for age, sex, and other parameters (Figure 2). Serum folate, choline, and DMG showed a positive relation to betaine, but the curve for choline leveled off at 14 μmol/L (99.5 percentile) and that for DMG at 4 μmol/L (80 percentile; Figure 2). Betaine showed no relation to vitamin B6 and was inversely related to creatinine in this regression model. The associations obtained by GAM were essentially in agreement with those obtained by multiple linear regression (Figure 2 legend).

Estimated Change in PML ΔtHcy and Fasting tHcy by Betaine and Other Determinants

Determinants of ΔtHcy were estimated by multiple regression analysis (Table 2). For all variables, we estimated the difference in mean ΔtHcy between each quartile and the reference quartile. The mean ΔtHcy difference across the extreme quartiles adjusted for age and sex was highest (8.3 μmol/L) for betaine, intermediate (5.7 μmol/L; data not shown) for creatinine, and moderate (4.3 to 4.9 μmol/L) for folate cobalamin and vitamin B6; all P values were <0.001. The betaine–tHcy relationship remained strong (P<0.001) after additional adjustment for all blood indices (including folate and vitamin B6), whereas multiple adjustments weakened the associations of tHcy with folate, cobalamin, vitamin B6, and creatinine (P=0.006 to 0.03; Table 2). Betaine (P<0.001), creatinine (P=0.02), folate (P=0.1), cobalamin (P=0.02), and vitamin B6 (P=0.005) showed nearly identical associations after log transformation of ΔtHcy (in a model containing age, sex, and all blood indices), and inclusion of the product term of betaine and vitamin B6 in this regression model demonstrated no interaction between betaine and vitamin B6 (P for interaction=0.8).

We performed the same calculation of the differences in mean fasting tHcy between quartiles of betaine, folate, cobalamin, vitamin B6, and creatinine (Table 2). The tHcy difference across the extreme quartiles adjusted for age and sex was moderate (2.2 μmol/L) for betaine, weak (0.6 μmol/L) for vitamin B6, intermediate (2.6 μmol/L) for cobalamin, and highest (3.7 to 3.9 μmol/L) for folate and creatinine; all P values were <0.001. Additional adjustment for all blood indices reduced the tHcy difference across the extreme betaine quartiles to 0.9 μmol/L, which now became of borderline significance (P=0.07). This adjustment had essentially no effect on the tHcy change across the quartiles of folate, cobalamin, and creatinine but attenuated the tHcy–vitamin B6 relationship (Table 2). Again, essentially the same associations with betaine (P=0.03), folate (P>0.001), cobalamin (P>0.001), vitamin B6 (P=0.8), and creatinine (P>0.001) were obtained after log transformation of tHcy (data not shown). Additional adjustment for smoking, alco-
<table>
<thead>
<tr>
<th>Blood Indices</th>
<th>Adjusted for Age and Sex</th>
<th>Adjusted for all Parameters*</th>
</tr>
</thead>
</table>

**TABLE 2. Estimated Change in PML Increase in tHcy and Fasting tHcy According to Quartiles of Betaine and B-Vitamins**

<table>
<thead>
<tr>
<th>Betaine (μmol/L)</th>
<th>Change in PML Increase in tHcy (μmol/L) (mean (95% CI))</th>
<th>P</th>
<th>P&lt;0.001†</th>
<th>( P&lt;0.001 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>vs &gt;37.0, n = 125</td>
<td>3.2 (0.2–6.2)</td>
<td>2.6 (−0.3–5.6)</td>
<td>2.6 (−0.3–5.6)</td>
<td></td>
</tr>
<tr>
<td>30.4–37.0 (n = 125)</td>
<td>6.4 (3.3–9.4)</td>
<td>6.0 (2.9–9.0)</td>
<td>6.0 (2.9–9.0)</td>
<td></td>
</tr>
<tr>
<td>24.8–30.3 (n = 126)</td>
<td>8.3 (5.0–11.6)</td>
<td>7.2 (3.9–10.6)</td>
<td>7.2 (3.9–10.6)</td>
<td></td>
</tr>
</tbody>
</table>

**Folate (nmol/L)**

| vs >18.1, n = 125 | 2.1 (−0.9–5.1) | 1.0 (−1.8–4.0) | 1.0 (−1.8–4.0) |
| 12.8–18.1 (n = 127) | 3.9 (0.9–6.9) | 1.8 (−1.2–4.7) | 1.8 (−1.2–4.7) |
| 9.4–12.7 (n = 127) | 4.9 (1.8–7.9) | 3.2 (0.2–6.2) | 3.2 (0.2–6.2) |

**Cobalamin (pmol/L)**

| vs >291, n = 125 | 3.6 (0.6–6.6) | 2.9 (0.0–5.9) | 2.9 (0.0–5.9) |
| 218–291 (n = 124) | 4.5 (1.5–7.5) | 3.7 (0.8–6.6) | 3.7 (0.8–6.6) |
| 168–217 (n = 126) | 4.3 (1.3–7.3) | 3.2 (0.3–6.1) | 3.2 (0.3–6.1) |

**Vitamin B6 (nmol/L)**

| vs >37.4, n = 125 | 1.3 (−1.6–4.3) | 0.7 (−2.2–3.6) | 0.7 (−2.2–3.6) |
| 27.7–37.4 (n = 125) | 4.5 (1.5–7.6) | 3.2 (0.2–6.2) | 3.2 (0.2–6.2) |
| 20.8–27.7 (n = 125) | 4.9 (1.9–5.0) | 3.7 (0.7–6.8) | 3.7 (0.7–6.8) |

*All parameters (betaine, folate, cobalamin, vitamin B6, creatinine, age, and sex) are included in the model.
†P for trend.
PML indicates postmethionine load.
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d to perform methionine loading. The logistics are even more
complicated for methionine testing, with a sampling interval
of 6 hours used in the present study compared with short 2-
or 4-hour tests. However, the 6-hour test has been recom-
mended because the homocysteine response has lower
within-subject variability than for the short tests.26 This has
been attributed to variable rate of methionine absorption.

**Plasma Levels and Determinants of Betaine,**
**Choline, and DMG**

The median concentrations of betaine (30.3 μmol/L), choline
(7.8 μmol/L), and DMG (3.1 μmol/L) determined for this
study population of healthy adult men and women were
similar to the concentration of betaine,12,17,21 choline,17,22,23
and DMG17,21,24 reported previously by us and others.

The size of the study population investigated here allowed
detailed assessment of predictors of betaine in regression
model, including sex, age, and all blood indices (Figure 2).
Choline showed a linear relation to betaine and was the
strongest metabolic predictor of betaine, which could be
explained by choline being the immediate precursor. Folate
also showed a linear relation to betaine, suggesting common
dietary sources25 or a mutual sparing effect. Finally, the initial
linear relationship between DMG and betaine at low DMG
may reflect DMG production from betaine, whereas the
plateau phase could be attributable to product inhibition of
BHMT by DMG.26

**Betaine as Determinant of PML and Fasting tHcy**

This study demonstrated that betaine is a strong determinant
of PML tHcy. This effect was only slightly reduced after
multiple adjustments (including folate and vitamin B6; Table
2). The observation that betaine is a strong determinant of
PML ΔtHcy confirms similar results from a recent small
study13 and is in agreement with consistent reports that oral
intake of betaine markedly reduces PML tHcy.9,11

We also observed a significant relationship between betaine
and fasting tHcy, which became of borderline significance
after multiple adjustments, including folate (Table 2). The
weak overall association between betaine and fasting
tHcy is consistent with such association in 120 cardiovascular
patients12 and the observation of moderate reduction in
fasting tHcy by betaine supplementation.5,9,11

**Vitamin B6**

We measured vitamin B6 in this study because of the
prevailing view that vitamin B6 status is an important deter-
minant of PML tHcy. This idea is based mainly on experi-
ments with vitamin B6-deficient rats27 and studies of subjects
with low vitamin B6 status.28 However, in most studies of
humans without overt deficiency, vitamin B6 is not related to
PML tHcy24,29–32 or is a weaker determinant than folate.33,34
In a previous study on cardiovascular patients,13 we observed
no association between vitamin B6 and tHcy (fasting and
increase after loading). The present study demonstrates that
in healthy subjects, vitamin B6 is not a predictor of fasting tHcy
and is a weaker determinant of the PML increase in tHcy than
betaine (Table 2).

**Discussion**

We investigated the role of endogenous plasma betaine as a
determinant of fasting and PML tHcy in 500 healthy subjects
undergoing methionine loading. Plasma betaine was a stron-
ger predictor of the increase in PML tHcy (ΔtHcy) than other
parameters investigated, including folate, vitamin B6, and
cobalamin, and the inverse association was particularly
pronounced in subjects with low serum folate. We also observed
that plasma betaine was a predictor of fasting tHcy but only
at low folate status.

**Study Design**

The strength of this study is the number of subjects included,
which is high considering the resources and logistics required

---

**Figure 3.** Dose-response relationship between plasma betaine
and the increase in tHcy after methionine loading (top panels)
or fasting tHcy (bottom panels) according to tertiles of serum
folate. The curves are obtained by additive Gaussian regression
analysis and are adjusted for cobalamin, vitamin B6, creatinine,
sex, and age. The solid lines indicate the estimate dose-
response curves and the shaded areas 95% CI.

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Effect Modification by Folate Status

We observed that betaine was a strong predictor of PML ΔtHcy in subjects with low serum folate and a weaker predictor at high folate (Figure 3). The effect modification by folate status is the most notable finding in the present study. It elaborates the preliminary observation demonstrating attenuation of the betaine–PML ΔtHcy relationship in subjects supplemented with combinations of B vitamins (cobalamin, folate, and vitamin B6).13

We also observed that the weak overall association between betaine and fasting tHcy (Table 2) was attributable to such association in subjects with serum folate in the lower tertile (Figure 3). We are pursuing latter observation in a large ongoing study of the betaine as a determinant of tHcy in folate-deficient subjects.

Mechanisms

The strong association between betaine and PML ΔtHcy at low folate suggests increased catalytic activity of the BHMT at low 5-methyltetrahydrofolate, which is supported by measurement of rat liver enzyme activity35 and by a mathematical model, based on known enzyme kinetics.36 The increased catalytic activity is accomplished by increasing homocysteine availability and by lowering S-adenosylmethionine, which relieves S-adenosylmethionine–mediated BHMT inhibition.36

An important message from this and a previous work,13 as well as from studies of betaine supplementation,8,9,11,12 is that betaine reduces tHcy under conditions of high methionine. This seems to be in conflict with the prevailing view based on rat and chick experiments, suggesting that BHMT conserves homocysteine under conditions of methionine deficiency.3 However, in pigs, BHMT activity in liver increases in response to methionine.37 Thus, in some species at least, including humans, homocysteine accumulating during folate deficiency may be directed into the BHMT pathway, even in the presence of superfluous methionine.

Betaine is more strongly associated with PML ΔtHcy than with fasting tHcy. This may indicate a role for betaine in regulating postprandial homocysteine status. However, the PML tHcy probably reflects a massive first-pass homocysteine export from the liver after uptake of excess methionine. One may speculate whether high betaine directs the first-pass homocysteine metabolism after loading into betaine–tHcy probably reflects a massive first-pass homocysteine before and after B-vitamin supplementation. Arterioscler Thromb Vasc Biol. 2004;24:301–307.


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| TABLE I. Bivariate Associations in Terms of Spearman Correlation Coefficients |
|---------------------------------|--------|--------|-----------|-----------|--------|--------|--------|--------|--------|
|                                 | Age    | Cr     | ΔPMLarethcy | Betaine betaine | Choline | DMG    | PMLmet | Folate | Cbl    | B6     |
| tHcy                            | 0.30‡  | 0.37‡  | 0.28‡       | -0.03         | -0.03   | 0.11*  | 0.15†  | -0.29‡ | -0.27‡ | -0.08  |
| ΔPMLarethcy                     | 0.07   | 0.02   | -0.28‡      | -0.32‡        | -0.08   | -0.09* | 0.05   | -0.13† | -0.15† | -0.17‡ |
| Betaine                         | 0.13†  | 0.27‡  | 0.88‡       | 0.50‡         | 0.44‡   | 0.17†  | 0.21‡  | 0.06   | 0.22‡  |
| PMLbetaine                      | 0.13†  | 0.27‡  | 0.41‡       | 0.47‡         | 0.32‡   | 0.18‡  | 0.06   | 0.21‡  |
| Choline                         | 0.31‡  | 0.24‡  | 0.36‡       | 0.28          | 0.12†   | 0.00   | 0.06   |
| DMG                             | 0.07   | 0.27‡  |             | 0.20‡         | -0.09*  | 0.03   | 0.03   |

* p<0.05  
† p<0.01  
‡ p<0.001

B6, vitamin B6; Cbl, cobalamin; Cr, creatinine; ΔPML, post methionine load increase; DMG, dimethylglycine; Met, methionine; PML, post-methionine load; tHcy, total homocysteine.