

Betaine as a Determinant of Postmethionine Load Total Plasma Homocysteine Before and After B-Vitamin Supplementation

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Objective—Betaine is a substrate in the betaine–homocysteine methyltransferase reaction, converting homocysteine to methionine. There are only sparse data on plasma betaine as a determinant of the plasma total homocysteine (tHcy) concentration.

Methods and Results—Ninety patients undergoing coronary angiography were randomized into 4 groups administered oral: (1) folic acid (0.8 mg), vitamin B12 (0.4 mg), and vitamin B6 (40 mg); (2) folic acid and vitamin B12; (3) vitamin B6 alone; or (4) placebo. Nonfasting blood samples were collected at baseline and 3, 14, and 28 days and 3, 6, and 12 months after treatment start. A 4-hour methionine-loading test (0.1 g/kg) was performed at baseline and after 3 months. At baseline, median (interquartile range) plasma betaine was 36.9 $\mu\text{mol/L}$ (range: 30.3 to 46.8) and was increased by 15% after methionine loading. The postmethionine load (PML) increase in tHcy was inversely related to plasma betaine ($\beta = -0.29$, $P = 0.02$) and even more strongly to PML betaine ($\beta = -0.47$, $P < 0.001$). After 3 months of intervention, the relation between the PML increase in tHcy and PML betaine was weakened ($\beta = -0.33$, $P = 0.007$).

Conclusions—Plasma betaine is a strong determinant of the PML increase in tHcy in subjects not supplemented with B-vitamins. (*Arterioscler Thromb Vasc Biol.* 2004;24:301-307.)

Key Words: betaine ■ homocysteine ■ folate ■ vitamin B12 ■ vitamin B6

Homocysteine is an established risk factor for occlusive vascular disease.¹ High plasma concentration is also associated with impaired cognitive function, Alzheimer disease, adverse pregnancy outcomes, and congenital malformations, particularly neural tube defects.²

The plasma concentration of total homocysteine (tHcy) is determined by a variety of physiological, lifestyle, and genetic factors and disease states.³ Among these, renal function, folate, and cobalamin status are particularly influential.⁴ The vitamin effects are explained by the functions of 5-methyltetrahydrofolate as substrate and cobalamin as cofactor in the ubiquitous methionine synthase reaction, which catalyzes the remethylation of homocysteine to methionine.⁵ Vitamin B6, however, has no^{6,7} or a moderate^{8,9} effect on plasma tHcy.

Methionine loading, which involves measurement of tHcy after a standard oral methionine dose, was originally designed to reveal defects in the vitamin B6-dependent transsulfuration pathway.¹⁰ Plasma postmethionine load (PML) tHcy has since been associated with cardiovascular risk independent of basal tHcy.¹¹ Folate, as well as vitamin B6 status,^{9,11} are significant predictors of PML tHcy. These vitamin effects in

humans are at variance with the idea, based on enzymological data,⁵ animal,¹² and human studies,⁶ that PML tHcy is determined by the activity of vitamin B6-dependent enzyme, cystathionine β -synthase, whereas the folate-dependent homocysteine remethylation regulates basal tHcy.

Betaine (trimethylglycine) is obtained in small amounts through the diet or is generated in liver and kidney from choline through the sequential action of choline oxidase (EC 1.1.3.17) and betaine-aldehyde dehydrogenase (EC 1.2.1.8).^{5,13} Betaine serves as methyl donor of the zinc metalloenzyme, betaine-homocysteine methyltransferase (BHMT; EC 2.1.1.5). This enzyme is mainly confined to the liver and kidney and catalyzes an alternative route of homocysteine remethylation to methionine⁵ (Figure 1). The role of betaine as a methyl donor explains the observations that betaine supplementation reduces tHcy in homocystinurics¹⁴ and in healthy individuals.^{15–18} Betaine also attenuates the tHcy increase after methionine loading in healthy subjects^{16,18} and in cardiovascular¹⁹ and renal patients.²⁰ However, except for a recent Canadian study reporting on an inverse relation between fasting tHcy and betaine in cardiovascular patients,²¹ no study has addressed the question of the role of endogenous betaine as a determinant of basal and PML tHcy.

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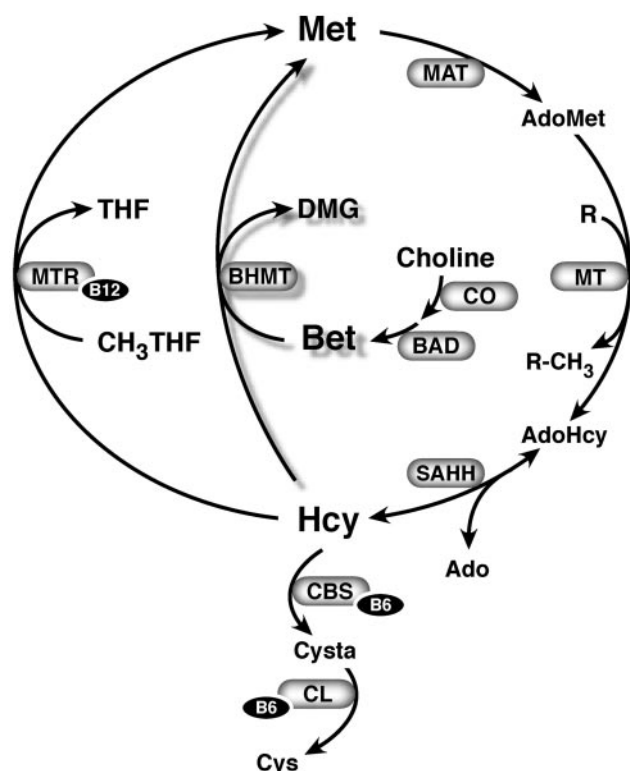


Figure 1. Betaine metabolism, homocysteine remethylation, and transsulfuration. Ado indicates adenosine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; BAD, betaine aldehyde dehydrogenase; Bet, betaine; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β -synthase; CL, cystathionine lyase; CH₃THF, methyltetrahydrofolate; CO, choline oxidase; DMG, dimethylglycine; Cys, cysteine; Cysta, cystathionine; Hcy, homocysteine; MAT, methionine adenosyltransferase; Met, methionine; MT, methyltransferase; MTR, methionine synthase; SAHH, S-adenosylhomocysteine hydrolase; THF, tetrahydrofolate.

Using a newly developed liquid chromatography–tandem mass spectrometry method,²² we investigated the association between plasma betaine and tHcy before and after methionine loading in 90 subjects. The subjects were enrolled in the Western Norway B-Vitamin Intervention Trial (WENBIT) and followed-up for 1 year of B-vitamin intervention as implemented in this trial.

Methods

Subjects

We investigated 90 consecutive patients enrolled in the WENBIT, an ongoing study on the effects of tHcy-lowering therapy on mortality and cardiovascular events in 3000 patients. Eligible subjects were adult patients (older than 18 years) undergoing coronary angiography for suspected coronary artery disease or aortic valvular stenosis. Exclusion criteria were malignant disease, alcohol abuse, mental illness, patient unwillingness to participate in long-term follow-up, and participation in other studies. The subjects were recruited at Haukeland University Hospital from April 1999 to September 1999. Informed consent was obtained from all patients. The study protocol was approved by the regional ethics committee and Norwegian Medicines Agency.

Protocol

Recruited patients were randomized into 4 groups in a 2×2 factorial block design. Group FB (n=22) was administered daily folic acid

(0.8 mg), vitamin B12 (cyanocobalamin, 0.4 mg), and vitamin B6 (pyridoxine, 40 mg). Group F (n=23) was administered folic acid and vitamin B12. Group B (n=21) was administered vitamin B6. Group P (n=24) was administered placebo. For the first 2 weeks, the groups (FB and F) receiving folic acid were administered an additional loading dose of folic acid (5 mg/d). Packages of trial tablets were prepared and given serial number in random order in blocks of 20 by Alpharma A/S (Copenhagen, Denmark). Compliance monitoring was accomplished by tablet counting and by determination of vitamin B concentrations in plasma.

The subjects underwent a full routine medical examination at baseline before coronary angiography. Coronary angiography was performed 3 days after starting vitamin therapy.

Blood Collection and Biochemical Analyses

Nonfasting (basal) blood samples were collected at baseline, after 3 days, at 2 and 4 weeks, and at 3, 6, and 12 months of B-vitamin intervention. A methionine loading test (0.1 g/kg body weight) was performed at baseline and after 3 months. PML blood samples were drawn at 4 hours after loading. EDTA blood samples were immediately placed on ice, centrifuged within 30 minutes, and EDTA plasma stored at -80°C until analysis.

Routine blood analyses, including serum creatinine, were analyzed at the Central Laboratory of Haukeland University Hospital on Technicon Chem 1 (Bayer, Leverkusen, Germany). The tHcy,²³ betaine, choline, di-MethylGlyoxime (DMG),²² cobalamin²⁴ plasma folate,²⁵ and pyridoxal phosphate²⁶ were analysed by published methods.

Statistics

Because of skewed distribution of values for tHcy and some metabolites, data are presented as median values with interquartile ranges. Associations between variables were evaluated by Spearman rank correlation and multiple linear regression analyses. Variables considered likely to influence the outcome parameters were included in the multivariate models. Mann-Whitney *U* test or Kruskal-Wallis test was used for between-group comparisons of continuous variables, and Wilcoxon signed rank test was performed for comparison within groups at baseline and after 3 months. Data were analyzed using SPSS 11.0 (SPSS Inc.).

Results

Subject Characteristics and Blood Indices at Baseline

A total of 90 patients were included. Their median age was 62 years (range 38 to 80); 77% were males. Blood indices at baseline for the whole study group are given in Table 1. Median betaine was 36.9 $\mu\text{mol/L}$ and median PML betaine was 44.5 $\mu\text{mol/L}$. One patient (in group P) had plasma cobalamin (117 pmol/L) below the lower reference limit (200 pmol/L), along with elevated MMA (0.77 $\mu\text{mol/L}$), and was considered cobalamin-deficient. Plasma folate was below the lower reference limit (4.5 nmol/L) in 10 patients.

Table 1 also shows the expected marked increase in tHcy and also a moderate increase in median betaine (15%; $P<0.001$), choline (7%; $P=0.001$), and DMG (18%; $P<0.001$) after methionine loading.

The concentrations of basal and PLM betaine in particular, but also of choline and creatinine, were markedly higher in males than in females (Table 1). No parameters differed significantly according to treatment group, except basal tHcy (18% difference; $P=0.04$) and basal choline (15%; $P=0.01$) and PML choline (21%; $P=0.04$) (data not shown). Details on plasma pyridoxal phosphate²⁶ and cobalamin²⁷ in this population have been given in separate publications.

TABLE 1. Subject Characteristics and Blood Indices at Baseline According to Gender*

	Total n=90	Men n=69	Women n=21	P Value†
tHcy, μmol/L	11.0 (9.3–12.9)	11.4 (9.8–13.0)	9.6 (7.7–12.9)	0.09
PML tHcy, μmol/L	31.3 (27.0–37.6)	30.6 (27.1–36.0)	32.1 (24.3–39.0)	0.9
Betaine, μmol/L	36.9 (30.3–46.8)	39.2 (33.2–48.0)	27.9 (22.8–33.0)	<0.001
PML betaine, μmol/L	44.5 (34.1–53.9)	47.2 (40.1–58.3)	29.3 (27.0–38.8)	<0.001
Choline, μmol/L	9.0 (7.7–10.7)	9.4 (7.9–11.0)	8.0 (7.3–8.9)	0.008
PML choline, μmol/L	10.3 (8.3–11.4)	10.4 (8.4–12.0)	9.6 (7.0–11.0)	0.02
DMG, μmol/L	3.64 (3.17–4.58)	3.67 (3.27–4.63)	3.32 (2.79–4.10)	0.1
PML DMG, μmol/L	4.54 (3.58–5.44)	4.58 (3.72–5.42)	4.17 (3.42–6.11)	0.4
Age, y	62 (54–68)	62 (55–68)	62 (53–70)	1.0
Creatinine, μmol/L	94 (87–101)	96 (89–103)	80 (73–93)	<0.001
Folate, nmol/L	8.2 (6.1–11.1)	7.8 (6.1–10.9)	8.8 (6.6–12.2)	0.4
Cobalamin, pmol/L	369 (311–481)	373 (319–437)	355 (297–425)	0.6
Vitamin B6‡, nmol/L	23.0 (17.7–37.1)	22.9 (15.7–37.0)	23.0 (18.3–39.7)	0.5

*Data are given as medians with interquartile ranges (25th–75th percentiles) in parentheses.

†Mann-Whitney *U* test.

‡Pyridoxal phosphate.

tHcy indicates total homocysteine; PML, postmethionine load; DMG, dimethylglycine.

Simple Correlations at Baseline

Simple correlations at baseline are shown in Table 2. Baseline tHcy was positively related to age, creatinine, and basal DMG, and were inversely related to plasma folate and cobalamin.

The increase in tHcy after methionine loading (Δ PML tHcy) was not related to the levels of B-vitamins. The Δ PML tHcy showed a strong inverse association with PML betaine ($r = -0.37, P < 0.001$) and a moderate association with basal betaine ($r = -0.23, P = 0.03$), as also depicted in Figure 2.

The concentrations of betaine, choline, and DMG, measured before and after loading (PML), were significantly and positively correlated (Table 2; data for choline and DMG not shown).

Determinants of Betaine, Choline, and Dimethylglycine

We assessed age, gender, creatinine, and the B-vitamins as predictors of basal betaine, choline, and DMG at baseline by multiple linear regression (Table 3). Betaine showed a strong relation to sex, whereas choline in particular, but also DMG, was positively related to creatinine. The metabolites were not significantly related to any B vitamin, ie, folate, cobalamin, or vitamin B6 (Table 3). Betaine, choline, and DMG mea-

sured after methionine loading showed similar relations to gender, age, creatinine, and the B-vitamins as observed for the basal concentrations (data not shown).

Betaine as a Determinant of PML tHcy

The Δ PML tHcy was inversely associated with basal betaine (model I), PML betaine (model II) (Table 3), and the PML increase in betaine ($\beta = -0.27, P = 0.02$) by multiple linear regression. PML betaine was a stronger predictor than basal betaine of Δ PML tHcy. The regression models also included age, sex, creatinine, and the B-vitamins, and these factors were not significantly related to Δ PML tHcy (Table 3). Additional adjustment for basal or PML choline and basal or PML DMG in the respective models had no effect (data not shown).

Betaine, Choline, and DMG According to Duration and Type of Intervention

Figure 3 depicts plasma concentrations for choline, betaine, and DMG measured at intervals from baseline to 12 months. PML concentrations at baseline and after 3 months are included. The levels for betaine in particular, but also for choline and DMG, were higher in men than in women

TABLE 2. Spearman Correlations at Baseline

	Age	Creatinine	Δ PML tHcy	Betaine	PML betaine	Choline	DMG	Folate	Cobalamin	Vitamin B6
tHcy	0.50†	0.44†	0.40†	0.07	0.07	0.18	0.32*	-0.21*	-0.21*	-0.08
Δ PML tHcy	0.14	0.08		-0.23*	-0.37†	0.01	0.03	-0.06	-0.13	0.02
Betaine	0.13	0.07			0.79†	0.49†	0.42†	-0.06	0.02	-0.05
PML betaine	0.15	0.18				0.34*	0.33*	-0.08	0.11	0.00
Choline	0.17	0.28*					0.50†	0.05	-0.12	-0.19
DMG	0.28*	0.15						-0.14	-0.1	-0.09

* $P < 0.05$.

† $P < 0.001$.

tHcy indicates total homocysteine; Δ PML, postmethionine load increase; DMG, dimethylglycine.

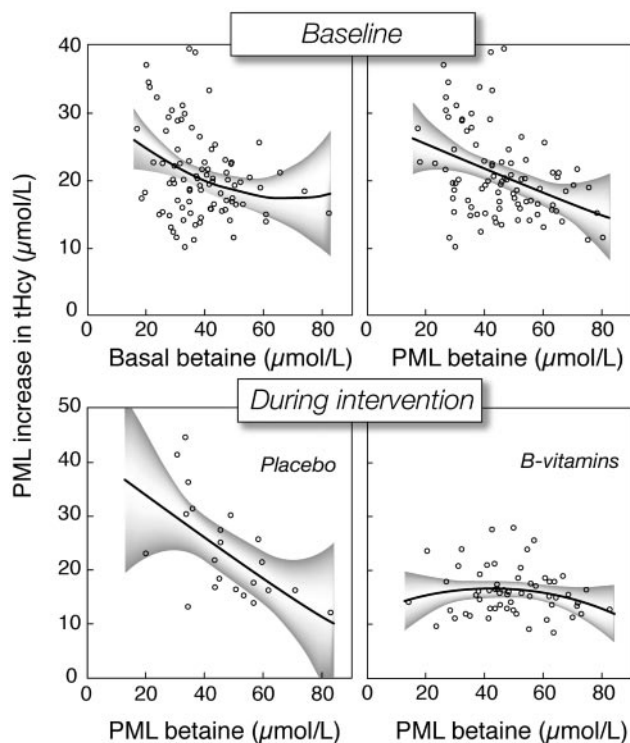


Figure 2. The relation between plasma betaine and the PML increase in plasma tHcy at baseline and after 3 months of B-vitamin supplementation. The upper panels show the relations between betaine (basal and PML) and PML increase in plasma tHcy before intervention. The lower left panel shows PML betaine versus PML increase in tHcy in 22 subjects (group P) administered placebo and the lower right panel shows this relationship in the remaining 68 subjects (groups FB, F, and B) treated with B-vitamins. The curves were obtained by quadratic regression, and the shaded areas represent the 95% confidence interval of mean regression line.

throughout the intervention period and showed a transient increase after methionine loading (Figure 3).

We compared the basal and PML concentrations of betaine, choline, and DMG at 3 months according to the treatment groups FB, F, B, and P. The metabolite concentrations, except PML choline ($P=0.03$), were not significantly different between the groups ($P>0.4$) (data not shown).

B-Vitamin Supplementation and the Betaine–PML tHcy Relationship

After 3 months of intervention, plasma cobalamin and plasma folate were higher than 277 pmol/L and 25 nmol/L, respectively, in all patients in groups FB and F, and pyridoxal phosphate was higher than 142 nmol/L in groups FB and B. No significant changes in median levels of these vitamins occurred in the nonsupplemented groups.

Δ PML tHcy ($\mu\text{mol/L}$; median [interquartile range]) decreased in the groups FB (from 19.4 [15.5 to 22.7] to 14.7 [11.6 to 17.2], $P=0.004$), F (from 19.7 [16.6 to 24.6] to 15.8 [12.9 to 18.9], $P=0.002$), and B (from 19.3 [15.3 to 23.6] to 16.3 [13.5 to 17.9], $P=0.005$) given B-vitamins. Δ PML tHcy did not change in the placebo group P (from 20.9 [17.0 to 30.0] to 21.8 [16.3 to 30.2], $P=0.5$).

After 3 months of intervention, the inverse association between Δ PML tHcy and PML betaine in the subjects

TABLE 3. Determinants at Baseline of Basal Betaine, Choline, Dimethylglycine, and the increase in tHcy After Methionine Loading by Multiple Linear Regression*

Dependent Variable	Independent Variables	β	P
Basal betaine	Age	0.12	0.06
	Sex	-0.48	<0.001
	Creatinine	-0.17	0.1
	Folate	0.14	0.2
	Cobalamin	-0.09	0.4
	Vitamin B6	-0.005	0.9
Basal choline	Age	0.08	0.4
	Sex	-0.12	0.2
	Creatinine	0.37	0.001
	Folate	0.11	0.3
	Cobalamin	-0.15	0.2
	Vitamin B6	-0.08	0.5
Basal DMG	Age	0.16	0.2
	Sex	-0.07	0.5
	Creatinine	0.24	0.04
	Folate	-0.08	0.5
	Cobalamin	-0.17	0.1
	Vitamin B6	0.10	0.4
tHcy increase (Model I)	Age	0.19	0.1
	Sex	0.02	0.9
	Creatinine	0.02	0.8
	Folate	-0.13	0.3
	Cobalamin	0.06	0.6
	Vitamin B6	-0.08	0.5
tHcy increase (Model II)	Age	0.20	0.07
	Sex	-0.10	0.4
	Creatinine	0.06	0.6
	Folate	-0.17	0.1
	Cobalamin	0.09	0.4
	Vitamin B6	-0.02	0.9
	PML betaine	-0.47	<0.001

*All variables are included in each of the models.

DMG indicates dimethylglycine; tHcy, total homocysteine.

receiving B-vitamins was essentially abolished but was still present in the subgroup (P) receiving placebo, as depicted in Figure 2. Multiple linear regression analyses of the association between Δ PML tHcy and PML betaine in the separate intervention groups demonstrated that the association was absent in the groups FB and F administered folic acid ($P>0.6$), showed a trend in the group B administered vitamin B6 ($P=0.09$), and was still strong and significant in the group P administered no B-vitamins ($\beta=-0.66$, $P=0.02$). All these models were adjusted for age, sex, creatinine, cobalamin, folate, and vitamin B6.

Predictors of Δ PML tHcy at 3 months in the whole study group were assessed by multiple regression (Table I, available online at <http://atvb.ahajournals.org>). The model contained the B-vitamins, in addition to creatinine, age, and sex.

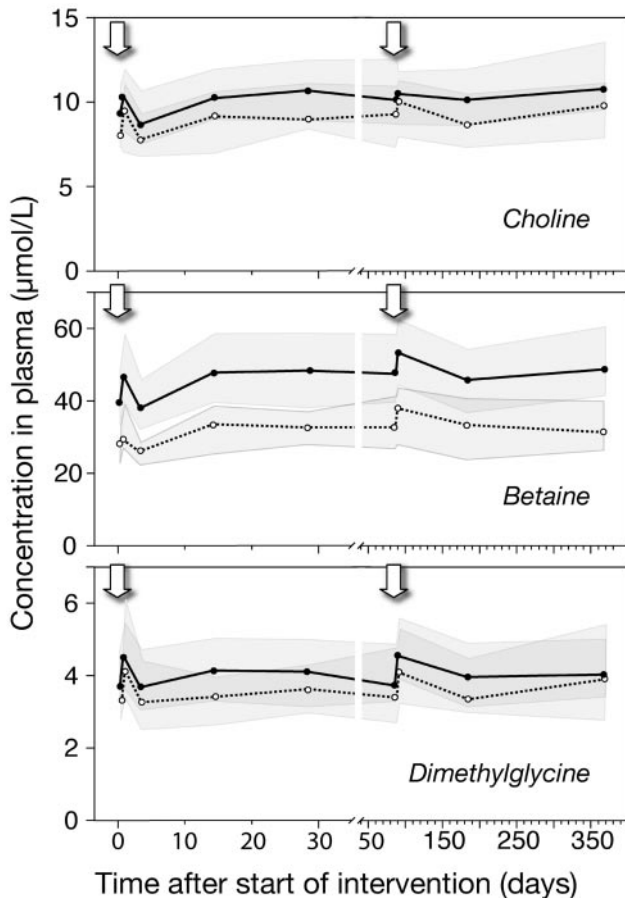


Figure 3. Plasma concentration of choline, betaine, and dimethylglycine during B-vitamin supplementation and after methionine loading. The metabolite concentrations are given separately for men (filled circles) and women (open circles) according to time after start of intervention. Arrows indicate methionine loading, which was performed on day 0 and on day 84, and the values obtained 4 hours after loading are included in the graph. Data are given as medians and the shaded areas indicate the 25 to 75 percentiles.

PML betaine was strongly associated with Δ PML tHcy, but the association was weaker ($\beta = -0.33$, $P = 0.007$) than before treatment ($\beta = -0.47$, $P < 0.001$; Table 3). Plasma folate and, to a lesser extent, vitamin B6 were also significant predictors.

Substitution of B-vitamin concentration with treatment category in the regression model essentially did not change the association between Δ PML tHcy and PML betaine (data not shown).

Discussion

We present data on the relation between betaine and plasma tHcy in 90 patients with cardiovascular disease before and during long-term intervention with folate, cobalamin, and vitamin B6, as implemented in the WENBIT. We demonstrate, for the first time to our knowledge, that the increase in tHcy after methionine loading (Δ PML tHcy) was determined by PML betaine. Basal betaine was a weaker predictor. In 68 subjects supplemented with B-vitamins for 3 months, Δ PML tHcy was reduced, and its inverse relation to betaine was weakened, particularly in the subgroups administered folic acid.

Study Design

The strength of the study is its longitudinal, prospective design, which allows comparison of betaine effects before and after B-vitamin intervention in the same individuals. It was clearly shown that in the combined group treated with B-vitamins, the betaine-PML-tHcy association was essentially abolished, but the small sample size provided limited statistical power to delineate the effects of individual B-vitamins.

We measured PML tHcy 4 hours after methionine ingestion. This short interval was chosen for logistic reasons. A recent study demonstrated that at this time point, PML tHcy has higher within-subject and between-subject coefficients of variation (CV) than the PML tHcy measured at 6 or 8 hours after methionine intake, possibly because of variable methionine absorption a short time after loading.¹⁰ Furthermore, we observed that betaine showed a stronger association with Δ PML tHcy than with PML tHcy. However, analytical CV increases when PML tHcy is adjusted for basal tHcy to obtain Δ PML tHcy, because the variance has the property of being additive.¹⁰ Thus, increased biological and analytical variations may actually lead to underestimation of the “true” association between betaine status and PML tHcy.

Concentrations of Betaine, Choline, and Dimethylglycine

The median plasma concentration of betaine reported here (≈ 37 $\mu\text{mol/L}$; Table 1) is similar to the concentration previously reported by us in healthy blood donors²² and by others.^{21,28} We observed a strong relation between betaine and gender, with higher levels in males than in females (Table 1, Figure 3), which has also been reported by others.²⁹ The presence of consensus sites for steroid hormones, including estrogen and androgen binding sites, in the human betaine homocysteine methyltransferase gene³⁰ may represent a molecular basis of sex steroid effects on plasma betaine.

We observed a median concentration of plasma choline (9 $\mu\text{mol/L}$) that is equal to the concentration (≈ 10 $\mu\text{mol/L}$) previously measured in healthy subjects^{22,31,32} The median plasma DMG (3.6 $\mu\text{mol/L}$) is somewhat higher than the DMG levels (≈ 2 to 3 $\mu\text{mol/L}$) previously reported by us and others.^{22,28,33} Slightly elevated plasma DMG may be related to the possibility that the cardiovascular patients have moderately impaired renal function, which is known to affect the DMG concentration.³³ Conceivably, methodological difference may also account for different metabolite (DMG) levels between various study populations.

Human Studies on Betaine and tHcy

The strong inverse association between Δ PML tHcy and plasma betaine, particularly PML betaine, in subjects not receiving B-vitamin supplementation is the most important result of the present study. The data are in agreement with consistent observations that betaine supplementation reduces PML tHcy and also fasting tHcy in healthy subjects^{16,18} and tHcy in renal patients²⁰ and in homocystinurics.^{34–36} However, our data (Table 2) do not confirm a recent observation of a significant inverse relation between (basal) plasma betaine and tHcy in 122 Canadian cardiovascular patients.²¹

The apparent discrepancy may be related to different size, B-vitamin status, or renal function of the two study populations. We are currently addressing the possible relation between betaine and fasting tHcy in large population-based studies.

Mechanisms and Role of Betaine in Homocysteine Remethylation

Methionine loading caused a 3-fold increase in tHcy, a 15% increase in plasma betaine and DMG, and also a significant increase in choline (Table 1, Figure 3). The choline response may reflect enhanced supply of choline for the synthesis of betaine or a choline-sparing effect from superfluous methionine. The elevation of plasma betaine and DMG levels, however, indicates an increased flux through the BHMT pathway, which is in accordance with the observation that Δ PML tHcy is strongly and inversely related to PML betaine and also to PML increase in betaine. Thus, increased metabolic flux through the choline-betaine-DMG pathway may occur in response to high levels of homocysteine and/or methionine.

After treatment with combinations of folic acid, vitamin B12, and vitamin B6 for 3 months, the relation between PML betaine and Δ PML tHcy was weakened in the whole study group and was abolished in the subgroups administered folic acid. This indicates that enhancement of folate-dependent remethylation catalyzed by methionine synthase downregulates the BHMT reactions and suggests a cross-talk between these two pathways.

The significance of betaine in homocysteine homeostasis is not clear, but the view prevails that the BHMT mainly functions to conserve homocysteine under conditions of methionine deficiency.⁵ This assumption is based on animal experiments demonstrating a dramatic induction of BHMT during methionine deficiency in combination with excess dietary choline.^{5,30,37–39}

Our observation of an inverse relation between betaine and Δ PML tHcy indicates an additional function of the BHMT pathway, ie, reduction of the homocysteine increase after methionine intake. This idea is difficult to reconcile with the fact that BHMT is inhibited by S-adenosylmethionine⁵ but gains some support from experiments in rats, demonstrating a moderate net increase in BHMT activity during methionine excess.⁵ Suppression of high homocysteine under conditions of excess methionine is thus obtained together with salvage of homocysteine carbon backbone. This metabolic effect contrasts to the result of the vitamin B6-dependent cystathionine- β -synthase reaction, which irreversibly directs superfluous homocysteine into the transsulfuration pathway.

Implications and Conclusion

The data presented here indicate that betaine through the BHMT reaction buffers and tends to reduce homocysteine during methionine excess. This reaction, which conserves the homocysteine carbon backbone, may be beneficial under conditions of limiting supply of folate required for enhancement of the methionine synthase reaction. These findings emphasize the complementary relationship between betaine and folate metabolism and motivate population-based studies

on betaine status in pathologies related to folate deficiency and homocysteine status.² Furthermore, because PML tHcy confers increased risk for occlusive vascular disease independent of fasting tHcy,¹¹ betaine and related metabolites should be included in future studies of homocysteine and cardiovascular risk. Because basal betaine showed a significant relation to Δ PML tHcy as well, the role of betaine could be investigated in epidemiological studies based on biobanks, where PML samples may not be available.

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References

- Clarke R, Collins R, Lewington S, Donald A, Alftan G, Tuomilehto J. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*. 2002;288:2015–2022.
- Carmel R, Jacobsen DW. *Homocysteine Health and Disease*. Cambridge, New York, Melbourne, Madrid, Cape Town: Cambridge University Press; 2001.
- Ueland PM, Refsum H, Schneede J. Determinants of plasma homocysteine. In: Robinson K, ed. *Homocysteine and Vascular Disease*. Dordrecht, Boston, London: Kluwer Academic Publishers; 2000.
- Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. *Am J Clin Nutr*. 2001;73:613–621.
- Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem*. 1990;1:228–237.
- Franken DG, Boers GH, Blom HJ, Trijbels JM. Effect of various regimens of vitamin B6 and folic acid on mild hyperhomocystinemia in vascular patients. *J Inherit Metab Dis*. 1994;17:159–162.
- Dierkes J, Kroesen M, Pietrzik K. Folic acid and Vitamin B6 supplementation and plasma homocysteine concentrations in healthy young women. *Int J Vitam Nutr Res*. 1998;68:98–103.
- McKinley MC, McNulty H, McPartlin J, Strain JJ, Pentieva K, Ward M, Weir DG, Scott JM. Low-dose vitamin B-6 effectively lowers fasting plasma homocysteine in healthy elderly persons who are folate and riboflavin replete. *Am J Clin Nutr*. 2001;73:759–764.
- de Jong SC, Stehouwer CD, van den Berg M, Kostense PJ, Alders D, Jakobs C, Pals G, Rauwerda JA. Determinants of fasting and post-methionine homocysteine levels in families predisposed to hyperhomocystinemia and premature vascular disease. *Arterioscler Thromb Vasc Biol*. 1999;19:1316–1324.
- Ubbink JB, Becker PJ, Delport R, Bester M, Riezler R, Vermaak WJ. Variability of post-methionine load plasma homocysteine assays. *Clin Chim Acta*. 2003;330:111–119.
- Graham IM, Daly LE, Refsum H, Robinson K, Brattström LE, Ueland PM, Palma-Reis RJ, Boers GHJ, Sheahan RG, Israelsson B, Uterwaal CS, Meleady R, McMaster D, Verhoef P, Wittman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garçon D, Andria G, et al. Plasma homocysteine as a risk factor for vascular disease. The European concerted action project. *JAMA*. 1997;277:1775–1781.
- Miller JW, Nadeau MR, Smith D, Selhub J. Vitamin B-6 deficiency vs folate deficiency: comparison of responses to methionine loading in rats. *Am J Clin Nutr*. 1994;59:1033–1039.
- Zeisel SH. Choline: An essential nutrient for humans. *Nutrition*. 2000;16:669–671.
- Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inherit Metab Dis*. 1997;20:295–300.
- Brouwer IA, Verhoef P, Urgert R. Betaine supplementation and plasma homocysteine in healthy volunteers. *Arch Intern Med* 2000;160:2546–2547.
- Steenge GR, Verhoef P, Olthof MR, Katan MB. Effect of betaine on plasma concentrations of fasting and post-methionine loading homo-

- cysteine in healthy volunteers. Abstract 257. 3rd International Conference on Homocysteine Metabolism, July 1–5, Sorrento, Naples. 2001.
17. Schwab U, Torronen A, Toppinen L, Alfthan G, Saarinen M, Aro A, Uusitupa M. Betaine supplementation decreases plasma homocysteine concentrations but does not affect body weight, body composition, or resting energy expenditure in human subjects. *Am J Clin Nutr.* 2002;76:961–967.
 18. Steenge GR, Verhoef P, Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. *J Nutr.* 2003;133:1291–1295.
 19. Dudman NP, Wilcken DE, Wang J, Lynch JF, Macey D, Lundberg P. Disordered methionine/homocysteine metabolism in premature vascular disease. Its occurrence, cofactor therapy, and enzymology. *Arterioscler Thromb.* 1993;13:1253–1260.
 20. McGregor DO, Dellow WJ, Robson RA, Lever M, George PM, Chambers ST. Betaine supplementation decreases post-methionine hyperhomocystinemia in chronic renal failure. *Kidney Int.* 2002;61:1040–1046.
 21. Schwahn BC, Chen Z, Laryea MD, Wendel U, Lussier-Cacan S, Genest J, Jr., Mar MH, Zeisel SH, Castro C, Garrow T, Rozen R. Homocysteine-betaine interactions in a murine model of 5, 10-methylenetetrahydrofolate reductase deficiency. *FASEB J.* 2003;17:512–514.
 22. Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-tandem mass spectrometry. *Clin Chem.* 2003;49:286–294.
 23. Husek P. Chloroformates in gas chromatography as general purpose derivatizing agents. *J Chromatogr B Biomed Sci Appl.* 1998;717:57–91.
 24. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. *J Clin Pathol.* 1991;44:592–595.
 25. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. *J Clin Pathol.* 1992;45:344–347.
 26. Bor MV, Refsum H, Bisp MR, Bleie O, Schneede J, Nordrehaug JE, Ueland PM, Nygard OK, Nexo E. Plasma vitamin B6 vitamers before and after oral vitamin B6 treatment: a randomized placebo-controlled study. *Clin Chem.* 2003;49:155–161.
 27. Nexo E, Hvas AM, Bleie O, Refsum H, Fedosov SN, Vollset SE, Schneede J, Nordrehaug JE, Ueland PM, Nygard OK. Holotranscobalamin is an early marker of changes in cobalamin homeostasis. A randomized placebo-controlled study. *Clin Chem.* 2002;48:1768–1771.
 28. Allen RH, Stabler SP, Lindenbaum J. Serum betaine, N,N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. *Metabolism.* 1993;42:1448–1460.
 29. Lever M, Sizeland PC, Bason LM, Hayman CM, Robson RA, Chambers ST. Abnormal glycine betaine content of the blood and urine of diabetic and renal patients. *Clin Chim Acta.* 1994;230:69–79.
 30. Park EI, Garrow TA. Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J Biol Chem.* 1999;274:7816–7824.
 31. Eckernas SA, Aquilonius SM. Free choline in human plasma analysed by simple radio-enzymatic procedure: age distribution and effect of a meal. *Scand J Clin Lab Invest.* 1977;37:183–187.
 32. Savendahl L, Mar MH, Underwood LE, Zeisel SH. Prolonged fasting in humans results in diminished plasma choline concentrations but does not cause liver dysfunction. *Am J Clin Nutr.* 1997;66:622–625.
 33. McGregor DO, Dellow WJ, Lever M, George PM, Robson RA, Chambers ST. Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations. *Kidney Int.* 2001;59:2267–2272.
 34. Wilcken DEL, Wilcken B, Dudman NPB, Tyrrell P, A. Homocystinuria: the effects of betaine in the treatment of patients not responsive to pyridoxine. *N Engl J Med.* 1983;309:448–453.
 35. Wendel U, Bremer HJ. Betaine in the treatment of homocystinuria due to 5, 10-methylenetetrahydrofolate reductase deficiency. *Eur J Pediatr.* 1984;142:147–150.
 36. Holme E, Kjellman B, Ronge E. Betaine for treatment of homocystinuria caused by methylenetetrahydrofolate reductase deficiency. *Arch Dis Child.* 1989;64:1061–1064.
 37. Emmert JL, Garrow TA, Baker DH. Hepatic betaine-homocysteine methyltransferase activity in the chicken is influenced by dietary intake of sulfur amino acids, choline and betaine. *J Nutr.* 1996;126:2050–2058.
 38. Park EI, Renduchintala MS, Garrow TA. Diet-induced changes in hepatic betaine-homocysteine methyltransferase activity are mediated by changes in the steady-state level of its mRNA. *J Nutr Biochem.* 1997;8:541–545.
 39. Emmert JL, Webel DM, Biehl RR, Griffiths MA, Garrow LS, Garrow TA, Baker DH. Hepatic and renal betaine-homocysteine methyltransferase activity in pigs as affected by dietary intakes of sulfur amino acids, choline, and betaine. *J Animal Sci.* 1998;76:606–610.

