Modulation of the Homocysteine-Betaine Relationship by Methylenetetrahydrofolate Reductase 677 C->T Genotypes and B-Vitamin Status in a Large-Scale Epidemiological Study

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Context: Betaine is formed from the essential nutrient choline or is supplied from the diet. It serves as a substrate in the betaine-homocysteine methyltransferase reaction and thereby provides methyl groups for the homocysteine-methionine cycle, which is regulated by enzymes dependent on folate, vitamin B12, riboflavin (vitamin B2), or vitamin B6.

Objective: We investigated how betaine affected total homocysteine (tHcy) concentration within the frame of variable B-vitamin status and according to the methylenetetrahydrofolate reductase (MTHFR) 677C->T genotype.

Design/Setting/Patients: This is a population-based study with a cross-sectional design. It includes 10,601 healthy men and women aged 50–64 yr.

Outcome Measures: Plasma samples were analyzed for tHcy, betaine, choline, dimethylglycine, riboflavin, and vitamin B6, whereas folate and vitamin B12 were analyzed in serum.

Results: Betaine was a strong determinant of plasma tHcy in subjects with low serum folate and the MTHFR TT genotype. The association was further strengthened at low levels in the circulation of the other B-vitamins (B2, B6, and B12). Thus, in subjects with the combination of serum folate in the lowest quartile, low vitamin B2, B6, and B12 status, and the MTHFR TT genotype, the difference in tHcy (mean, 95% confidence interval) across extreme plasma betaine quartiles was 8.8 (1.3–16.2) μmol/liter.

Conclusion: Betaine may thus be an important one-carbon source, particularly in MTHFR 677 TT subjects with inadequate B-vitamin status. (*J Clin Endocrinol Metab* 92: 1535–1541, 2007)

HOMOCYSTEINE (Hcy) is a sulfhydryl amino acid, which is generated from methionine in transmethylation reactions. The plasma concentration of reduced and oxidized forms of Hcy, collectively termed total Hcy (tHcy), is affected by lifestyle and nutritional factors (1). Moderately elevated levels of tHcy are related to an increased risk of cardiovascular disease, impaired cognition, and other conditions (2, 3). Severe hyperhomocysteinemia is found in patients with homocystinuria, who have a high risk of occlusive vascular disease (4).

Betaine, at doses up to 9 g/d, has for years been used as a tHcy-lowering agent in patients with homocystinuria (4). It also reduces fasting tHcy (5–10) and reduces the increase in tHcy induced by high doses of methionine, *i.e.* postmethionine load (PML) tHcy in healthy subjects (7, 8). We recently demonstrated that plasma betaine is a strong predictor

of PML tHcy (11). Betaine was a weaker predictor of fasting tHcy, but the association was strengthened under conditions of low serum folate (11), suggesting that betaine is an alternative source of one-carbon units in folate-depleted subjects (12). Betaine provides one-carbon units by serving as a substrate for betaine-Hcy methyltransferase (BHMT), which catalyzes the remethylation of Hcy to methionine in the liver and kidney (13).

In most studies of healthy subjects, folate status is a more powerful predictor of plasma tHcy than vitamin B12, but overt B12 deficiency is associated with severe hyperhomocysteinemia (1). Vitamin B6 has minor (14) or no (15) effect on fasting tHcy, but may exert an effect on PML tHcy (16). Recent studies have added riboflavin (vitamin B2) to the spectrum of B-vitamins affecting the tHcy concentration (17). The relationship between B-vitamins and plasma concentration of tHcy is probably explained by their roles as substrates or cofactors for enzymes involved in Hcy remethylation or transsulfuration (18). Methylenetetrahydrofolate reductase (MTHFR) catalyzes the formation of 5-methyltetrahydrofolate (5-MTHF), which is the prevailing folate form in plasma (19) and the form involved in the conversion of Hcy to methionine (20). The homozygous TT genotype of the common MTHFR 677 C->T polymorphism, which has a prevalence of approximately 10% in most Caucasian populations (21), is

associated with impaired catalytic properties of the enzyme (22, 23), and is an important genetic determinant of plasma tHcy, particularly under conditions of low folate (24) or riboflavin (17) status.

Plasma tHcy is a responsive indicator of B-vitamin and MTHFR status. This is explained by metabolic reactions dependent on folate, vitamin B12, riboflavin, or vitamin B6, which converge on Hcy. In this study we used plasma tHcy as a probe to investigate how betaine affects one-carbon metabolism within the frame of variable B-vitamin status. We investigated a large population of 10,601 subjects. The study had sufficient statistical power to obtain novel data on the influence of betaine on one-carbon metabolism, with emphasis on the effect modification by B-vitamin status and the MTHFR 677 C->T polymorphism.

Subjects and Methods

Subjects and protocol

A total of 10,601 samples from subjects enrolled in the Norwegian Colorectal Cancer Prevention (NORCCAP) study were analyzed. NORCCAP is a prospective randomized study investigating sigmoid-oscopy combined with fecal occult blood testing as screening modalities for colorectal cancer in a middle aged (range, 50–64 yr) population of both genders (25). Inclusion in the study took place from 1999–2001 at three hospitals in the city of Oslo and in Telemark County in southern Norway. A number of lifestyle variables were recorded, and blood samples were collected (26). The study was approved by the Regional Ethics Committee, and The Data Inspectorate approved the study protocol. Written informed consent was obtained from all participants.

Blood collection and biochemical analysis

At inclusion, blood samples were collected into EDTA Vacutainer tubes and tubes without additive. Serum was allowed to clot for 1 h at room temperature, whereas EDTA samples were immediately put on ice. Samples were centrifuged at $1100 \times g$ for 10 min; serum and plasma were then separated and frozen at -20 C and stored at -80 C until analysis. Folate (27) and vitamin B12 (28) were determined in serum, and tHcy (29), vitamin B6 (30), riboflavin (the nonphosphorylated form) (30), choline, betaine, and dimethylglycine (DMG) (31) were determined in plasma by published methods. Vitamin B6 exists in three main forms in plasma, *i.e.* pyridoxal 5'-phosphate (PLP), pyridoxal, and pyridoxic acid. PLP is the cofactor form and is measured in the present study (30). The vitamins and metabolites were analyzed in different matrices (serum *vs.* EDTA plasma) because EDTA interferes with the folate assay (by inhibiting the growth of *Lactobacillus casei*) and decreases the preanalytical variability for some metabolites, particularly for choline. MTHFR 677 C->T genotyping was performed by using real-time PCR with 5V exonuclease (Taqman) probes (32).

Statistics

Summary measures include medians with 5th to 95th percentiles. Partial Spearman rank coefficients, adjusted for age, sex, and study center, were used to evaluate associations between variables. The relationship between betaine and tHcy was studied according to folate levels and MTHFR 677 genotype by a Gaussian generalized additive regression model (GAM) (33), as implemented in R (34). The relationships between plasma tHcy and various predictors were also assessed in multiple linear regression models, which were adjusted for age, sex, creatinine, study center, betaine, folate, riboflavin, vitamin B6, vitamin B12, and MTHFR 677 C->T genotype. The vitamin levels were categorized into quartiles for the whole study population. Biochemical variables were represented in the regression models as indicator variables denoting membership to a quartile. The regression coefficient thus estimated the difference in mean tHcy between a reference quartile and the other quartiles. The upper quartile was chosen as reference quartile for betaine, folate, riboflavin, vitamin B6, and vitamin B12, whereas the lower quartile was chosen as the reference for creatinine. Mean tHcy levels across categories for each factor were tested for linear trend. We estimated the strength of the betaine-tHcy relationship in separate groups according to levels of folate (high quartile, Q4; intermediate, Q2 + Q3; low, Q1), MTHFR 677 C->T genotype, and vitamin B2–6-12 status (high, intermediate, or low). Vitamin B2–6-12 status was based on a vitamin B2–6-12 index score. This index was calculated as the sum of quartile scores (0–3) for riboflavin, vitamin B12, and vitamin B6. An index of 0–2 was designated low, 3–5 was intermediate, and 6–9 was high vitamin B2–6-12 status. In linear regression analyses with tHcy as the dependent variable, test for trends in the metabolite regression coefficients across the MTHFR 677 C->T genotype (three levels) or folate status (three levels) or the combined B2-B6-B12 status variable (three levels) were obtained from appropriate product terms in the regression model with main effects retained in the model. In these models, we used a linear categorized representation (equally spaced integer scores) of the metabolites. For example, a test for trend in the betaine coefficients across MTHFR genotypes was obtained from the product term in a model with betaine (linear; four levels) + MTHFR (linear; three levels) + betaine*MTHFR + adjustment variables.

SPSS (SPSS Inc., Chicago, IL.) version 11.0 was used for all analyses except the GAM regression analyses, for which we used the statistical package R (34).

Results

Characteristics of the study population

Plasma and serum samples from 10,601 healthy subjects (age range, 50–64 yr; 50.8% female) were analyzed. Sex and age distributions were similar within the CC (51.4%), CT (40.6%), and TT (8.0%) MTHFR genotypes. Men had higher levels of tHcy, betaine, choline, DMG, and creatinine, but lower levels of vitamin B12, folate, and riboflavin than women ($P < 0.001$, data not shown). Subjects with the TT

TABLE 1. Study population characteristics by MTHFR 677 C->T genotype[a]

	All (n = 10,601)	CC (n = 5,452)	CT (n = 4,299)	TT (n = 850)	P for trend
tHcy (µmol/liter)	10.2 (6.8–16.4)	9.9 (6.7–15.3)	10.4 (6.8–16.4)	11.2 (7.0–27.0)	<0.001
Betaine (µmol/liter)	35.4 (19.1–58.3)	35.8 (19.3–59.5)	35.3 (19.2–57.6)	32.5 (17.5–54.6)	<0.001
Choline (µmol/liter)	8.6 (5.8–12.9)	8.6 (5.8–13.0)	8.6 (5.8–12.9)	8.5 (5.7–12.2)	0.09
DMG (µmol/liter)	3.7 (2.4–5.9)	3.8 (2.5–6.0)	3.7 (2.5–5.9)	3.4 (2.2–5.5)	<0.001
Folate (nmol/liter)	13.7 (6.6–39.4)	14.5 (7.3–40.3)	13.4 (6.6–39.0)	10.5 (5.0–32.1)	<0.001
Vitamin B12 (pmol/liter)	307 (172–536)	308 (175–542)	308 (170–532)	300 (157–516)	0.19
Vitamin B6 (nmol/liter)	48.0 (18.7–152.4)	47.9 (19.1–153.4)	49.0 (18.8–153.2)	44.4 (15.6–143.1)	0.34
Riboflavin (nmol/liter)	10.4 (4.1–55.9)	10.4 (4.1–57.6)	10.4 (4.1–54.8)	10.7 (3.9–46.5)	0.06
Creatinine (µmol/liter)	69 (51–92)	69 (50–92)	70 (51–93)	66 (50–89)	0.01
Vitamin B2–6-12 index	4.0 (1.0–8.0)	4.0 (1.0–8.0)	5.0 (1.0–8.4)	4.0 (1.0–8.0)	0.42
Age (yr)	55 (51–63)	55 (51–63)	55 (51–63)	55 (51–63)	0.11
Males (%)	49	48	50	51	

[a] Median (5th to 95th) percentiles.

TABLE 2. Partial Spearman correlation coefficients[a]

	Choline	DMG	Folate	Vitamin B6	Riboflavin	Vitamin B12	tHcy
Betaine	0.38[c]	0.31[c]	0.19[c]	0.16[c]	0.04[c]	0.07[c]	−0.22[c]
Choline		0.33[c]	0.11[c]	0.08[c]	0.05[c]	0.00	−0.06[c]
DMG			0.04[c]	0.04[c]	0.02[b]	0.02[b]	0.02[b]
Folate				0.39[c]	0.26[c]	0.16[c]	−0.44[c]
Vitamin B6					0.36[c]	0.18[c]	−0.24[c]
Riboflavin						0.20[c]	−0.18[c]
Vitamin B12							−0.24[c]

[a] Ajusted for age, sex, and study center.
[b] $P < 0.05$.
[c] $P < 0.001$.

genotype had approximately 10% lower betaine levels, 30% lower folate levels, and 15% higher levels of tHcy, compared with CC subjects (Table 1).

Bivariate correlations

Adjusted Spearman correlation coefficients between concentrations of betaine, choline, DMG, and B-vitamins are shown in Table 2. Betaine, choline, and DMG correlated strongly, but strong positive relations were also found between several B-vitamins, as well as between betaine and B-vitamins.

Betaine determinants

Determinants of plasma betaine were assessed in a multiple regression model including study center, age, sex, creatinine, choline, folate, riboflavin, vitamins B6 and B12, and MTHFR 677 C->T genotype. Plasma choline (standardized β-coefficient = 0.37), sex (β = 0.36), and serum folate (β = 0.12) were the strongest predictors of betaine level ($P < 0.001$, adjusted R^2 = 0.28). Subgroup analyses showed that these three variables were also the strongest determinants of plasma betaine in each of the three MTHFR genotypes (data not shown).

Determinants of plasma tHcy

The relationships between plasma tHcy and concentrations of various B-vitamins and creatinine were assessed in a multiple regression model, which also included age, sex, and study center (Table 3). Betaine, all B-vitamins, and creatinine were significant predictors of tHcy, and the associations with betaine, folate, and riboflavin in particular were

strongest in the TT group (Table 3). The estimated difference in mean plasma tHcy between subjects in the lowest compared with the highest quartile of betaine concentrations were 3.2 μmol/liter in the TT, 1.2 μmol/liter in the CT, and 1.1 μmol/liter in the CC groups.

Dose-response relationship between betaine and tHcy

We constructed dose-response curves for the relationship between plasma betaine and tHcy in strata defined by quartiles of serum folate and by MTHFR 677 C->T genotypes. The curves were obtained using a Gaussian generalized additive model (GAM) (33) and were adjusted for age, sex, creatinine, and study center (Fig. 1). An inverse dose-response relationship between betaine and tHcy was found, which was strong in subjects with the TT genotype and folate in the lowest quartile. A moderate negative betaine-tHcy association was observed in subjects in the lowest folate quartile combined with the CT or CC genotypes, whereas in subgroups with serum folate in quartiles 2–4, the betaine-tHcy relation was weak or essentially absent, irrespective of genotype.

The combined effect of MTHFR status, folate, and other B-vitamins

To estimate the effect of B-vitamins other than folate, we constructed a B2–6-12 index score, which is the sum of quartile scores (0–3) for concentrations of riboflavin, vitamin B12, and vitamin B6. A B2–6-12 index score was used to avoid small subgroups defined by combinations with each of the B-vitamins. In a multiple regression analyses (adjusting for age, sex, study center, and creatinine), this index score was a stronger predictor of plasma tHcy (β = −0.20) than quar-

TABLE 3. Relative strength of plasma tHcy determinants according to MTHFR 677 C->T genotype

Determinant	Genotype								P^a
	All (n = 10,576)		CC (n = 5,439)		CT (n = 4,288)		TT (n = 849)		
	μmol/liter	P^b	μmol/liter	P^b	μmol/liter	P^b	μmol/liter	P^b	
Betaine	1.46 (1.26−1.65)	<0.001	1.11 (0.91−1.31)	<0.001	1.17 (0.89−1.46)	<0.001	3.21 (1.83−4.59)	<0.001	<0.001
Folate	3.04 (2.84−3.23)	<0.001	2.17 (1.96−2.37)	<0.001	2.88 (2.60−3.16)	<0.001	4.97 (3.58−6.36)	<0.001	<0.001
Vitamin B12	1.42 (1.24−1.60)	<0.001	1.14 (0.95−1.33)	<0.001	1.46 (1.20−1.73)	<0.001	2.78 (1.54−4.01)	<0.001	<0.001
Riboflavin	0.82 (0.63−1.01)	<0.001	0.50 (0.30−0.70)	<0.001	0.72 (0.44−0.99)	<0.001	3.92 (2.57−5.26)	<0.001	<0.001
Vitamin B6	0.55 (0.33−0.76)	<0.001	0.42 (0.20−0.64)	<0.001	0.36 (0.05−0.68)	0.08	1.98 (0.45−3.51)	0.028	0.014
Creatinine	1.62 (1.42−1.83)	<0.001	1.81 (1.60−2.03)	<0.001	1.49 (1.20−1.79)	<0.001	2.76 (1.29−4.23)	0.001	0.27

Data are expressed as difference in mean tHcy (95% confidence interval) between extreme quartiles of each determinant. Data are obtained by multiple regression with tHcy as the dependent variable and all variables in the table as covariates. The model is adjusted for age, sex, and study center.
[a] P for test of trend in the regression coefficient of each determinant across MTHFR 677 C->T genotypes.
[b] P for trend across quartiles of vitamins and metabolites.
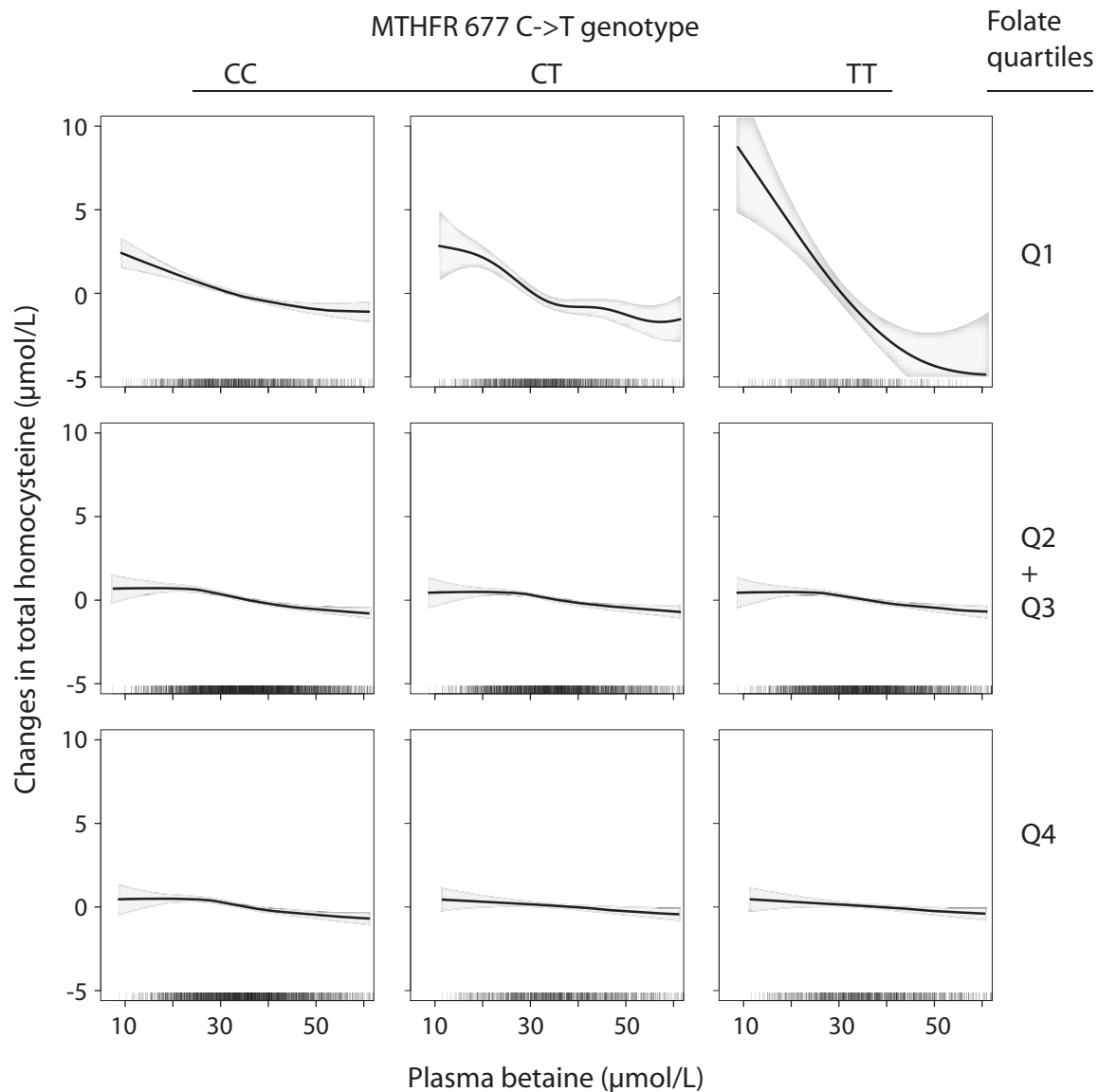
FIG. 1. Dose-response relationship between plasma betaine and tHcy according to MTHFR 677 C->T genotype and folate status. The *upper panels* show the relationship when serum folate is in the lowest quartile (Q1, <10.1 nmol/liter), the *middle panels* depict the relationship at intermediate levels of folate (Q2 + Q3, 10.1–20.2 nmol/liter), and the *lower panels* show the relationship when folate is in the highest quartile (Q4, >20.2 nmol/liter). The *solid lines* indicate the estimate dose-response curves, and the *shaded areas* indicate 95% confidence intervals.

tiles of each B-vitamin or summary scores of each combination of two B-vitamins ($\beta < -0.18$) (result not shown).

We estimated the simultaneous effect of MTHFR genotype, folate, and other B-vitamins (*i.e.* B2–6-12 index score) on the betaine-tHcy relationship by multiple linear regression. The model contained these three variables and was additionally adjusted for age, sex, creatinine, and study center. The effect parameter was the difference in mean tHcy between the highest and lowest betaine quartiles (Fig. 2 and Table 4).

In subjects with both low serum folate (Q1) and low vitamin B2–6-12 status, the difference in tHcy (mean, 95% confidence interval) between those with low (Q1) *vs.* high-plasma betaine (Q4) was 8.79 μmol/liter in TT subjects, compared with 4.11 μmol/liter in CT and 2.40 μmol/liter in CC subjects. In subjects with low serum folate (Q1) and high vi-

vitamin B2–6-12 status, these tHcy differences were 4.68, 1.90, and 0.68 μmol/liter in TT, CT, and CC subjects, respectively. The differences in tHcy between extreme betaine quartiles in the subgroups with the combination of high folate and TT genotype were small and not significantly different from zero, but the estimates were imprecise due to the low subject number (10–63) in these subgroups (Table 4).

Discussion

This is a cross-sectional study on plasma betaine as a determinant of tHcy in a population of 10,601 subjects. We confirmed previous observations (11, 35) that plasma betaine is inversely related to tHcy. A novel observation is that betaine is a particularly strong determinant of tHcy in subjects

FIG. 2. Betaine as a determinant of tHcy, according to folate status, vitamin B2–6-12 status, and MTHFR 677 C->T genotype. *Bars* show the difference in mean plasma tHcy (μmol/liter) between the lowest plasma betaine quartile (<28.2 μmol/liter) compared with the highest betaine quartile (>43.5 μmol/liter). Data are obtained by multiple regression analysis adjusted for age, sex, serum creatinine, and study center in 27 subgroups, each defined by MTHFR 677 C->T genotype (CC, CT, or TT), level of folate (low, Q1; intermediate, Q2 + 3; or high, Q4) and vitamin B2–6-12 status (low, intermediate, or high).
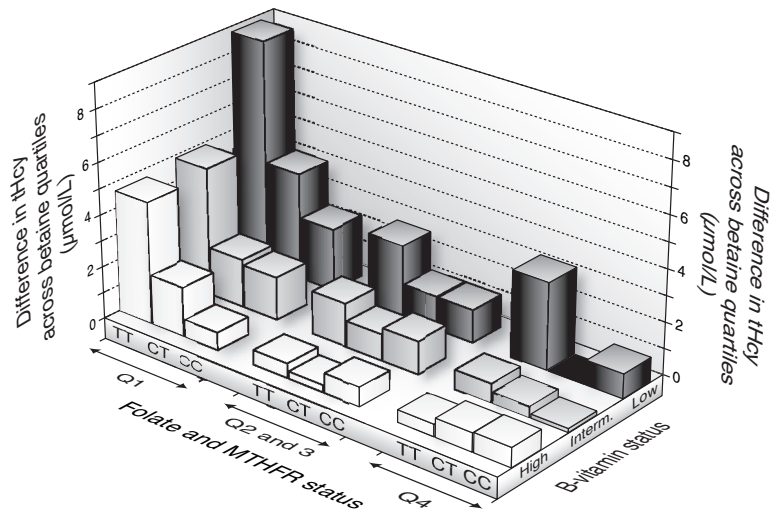
with the MTHFR 677 TT genotype in combination with low levels of folate and other B-vitamins.

Study design

The large study population allowed precise estimates of betaine-tHcy associations in the entire population, as well as in subgroups. For example, of the 850 subjects with the TT genotype, 397 had low serum folate and 144 individuals (1.4% of the study population) had both low serum folate and low vitamin B2–6-12 status. Furthermore, age and ethnicity can influence tHcy levels, and the homogeneity of the study population reduces confounding from these variables.

Concentrations of tHcy, betaine, and folate

The median concentration of plasma betaine was 35.4 μmol/liter, which is in agreement with previous reports from studies of healthy individuals (11, 31, 36). Median tHcy was approximately 15% higher and folate was 30% lower in subjects with the TT genotype, compared with the CC genotype, as has consistently been demonstrated in other studies (24, 37). Betaine concentrations were related to MTHFR genotype and showed a small decrease according to the number of T alleles. This may reflect that betaine and 5-MTHF are fungible sources of methyl groups (38), and impaired formation of 5-MTHF in subjects with the MTHFR 677 TT genotype may lead to increased betaine consumption through the BHMT pathway.

Folate and other B-vitamins

B-vitamins, including folate, vitamin B12, riboflavin, and vitamin B6, serve as cofactors in several pathways of one-carbon metabolism (18). In addition, the metabolic functions of these B-vitamins are interconnected, because some enzymes involved in their activation require another B-vitamin as a cofactor (39). Such a functional network, and the fact that intakes of different B-vitamins correlate (40), may explain the positive relationships between the concentrations of B-vitamins reported in the present study (Table 2) and by others (40). This is the background for constructing a composite

variable for the concentrations of riboflavin, vitamin B6 and vitamin B12, *i.e.* the vitamin B2–6-12 index.

We observed an inverse relationship between plasma betaine and tHcy, which extended across the whole concentration range of betaine (Fig. 1). The tHcy difference between subjects in extreme betaine quartiles was strongest at low serum folate and was particularly pronounced in the subgroup with a low vitamin B2–6-12 status (Fig. 2 and Table 4). These observations agree with the idea that Hcy is directed to the BHMT pathway under conditions of impaired folate-dependent remethylation (41). Impaired remethylation may occur if methionine synthase (MS) function is inhibited directly, by insufficient dietary supply of vitamin B12 or folate, or indirectly, by low MTHFR activity. Conceivably, high concentrations of the cofactors (methylcobalamin for MS or flavin adenine dinucleotide for MTHFR) may boost low enzyme activities caused by insufficient concentrations of substrates (5-MTHF and 5,10-methylenetetrahydrofolate, respectively) and vice versa. Such cooperative mechanisms may explain the interaction between folate and other B-vitamins observed in the present study.

MTHFR 677 C->T genotype

The strong association between tHcy and betaine in subjects with the combination of low B-vitamin status and the MTHFR TT genotype may reflect up-regulation of the betaine-dependent Hcy remethylation under conditions of impaired activity of 5-MTHF-dependent MS. 5-MTHF formation is inhibited when MTHFR activity is low (20), and *in vitro* studies on recombinant human MTHFR show that the variant associated with the 677 C->T transition has a propensity to lose its cofactor, flavin adenine dinucleotide, particularly at low folate (42). This might explain why riboflavin, folate, and MTHFR genotype are cooperative determinants of the tHcy-betaine relationship.

Conclusion

Plasma betaine is a strong determinant of plasma tHcy, predominantly in subjects with low levels of folate and other

TABLE 4. Difference in tHcy across extreme betaine quartiles in subgroups defined according to folate/B-vitamin status and MTHFR 677C->T genotype[a]

| | Folate status | | | | | | | | | P for int[d] |
| | Q1 | | | Q2 + Q3 | | | Q4 | | | |
	n	μmol/liter	P	n	μmol/liter	P	n	μmol/liter	P	
Low B-vitamin										
All genotypes	886	4.21 (2.74–5.69)	<0.001[b]	1195	1.29 (0.80–1.78)	<0.001[b]	257	0.37 (−0.69–1.44)	0.53[b]	<0.001
CC	357	2.40 (1.20–3.59)		674	1.25 (0.62–1.89)		162	0.94 (−0.13–2.02)		
CT	385	4.11 (2.07–6.14)	0.011[c]	463	1.26 (0.49–2.03)	0.56[c]	85	−0.95 (−3.46–1.57)	0.20[c]	
TT	144	8.79 (1.38–16.20)		58	2.74 (−1.05–6.53)		10	3.33 (−6.00–12.66)		
Intermediate B-vitamin										
All genotypes	1497	2.26 (1.65–2.86)	<0.001[b]	3148	1.18 (0.92–1.43)	<0.001[b]	1304	0.26 (−0.10–0.62)	0.047[b]	<0.001
CC	631	1.65 (1.01–2.29)		1736	1.25 (0.92–1.58)		724	0.16 (−0.34–0.65)		
CT	643	1.84 (1.00–2.68)	0.001[c]	1219	1.00 (0.57–1.43)	0.16[c]	517	0.43 (−0.12–0.99)	0.43[c]	
TT	223	4.90 (1.98–7.82)		193	1.65 (0.49–2.80)		63	0.74 (−1.32–2.80)		
High B-vitamin										
All genotypes	264	1.67 (0.69–2.66)	<0.001[b]	945	0.58 (0.17–0.99)	0.002[b]	1082	0.73 (0.39–1.06)	<0.001[b]	0.014
CC	114	0.68 (−0.54–1.90)		471	0.75 (0.16–1.34)		571	0.75 (0.28–1.22)		
CT	120	1.90 (0.23–3.56)	0.027[c]	404	0.28 (−0.35–0.90)	0.54[c]	453	0.72 (0.20–1.24)	0.91[c]	
TT	30	4.68 (0.41–8.94)		70	0.56 (−1.10–2.22)		58	0.47 (−0.95–1.89)		
P for int.[e]			0.009			0.077			0.11	

[a] Data are given as mean (95% confidence interval). Data are obtained by multiple regression with tHcy as the dependent variable. The model is adjusted for age, sex, study center, and creatinine (and MTHFR 677C->T genotype when all genotypes are combined).
[b] P for test of betaine regression coefficient.
[c] P for test of trend in the betaine regression coefficients across MTHFR 677 C->T genotypes.
[d] P for test of trend in the betaine regression coefficients across folate status.
[e] P for test of trend in the betaine regression coefficients across B-vitamin status.

B-vitamins and the MTHFR 677TT genotype. This observation strongly supports the idea that betaine is an important source of one-carbon units, in particular when folate status is compromised, whether caused by inadequate nutrition or genetic factors. This points to the role of betaine as a one-carbon source but also highlights the importance of betaine measurement in studies on folate and Hcy status in relation to chronic diseases.

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