Homocysteine concentration, related B vitamins, and betaine in pregnant women recruited to the Seychelles Child Development Study[1–3]

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ABSTRACT

Background: Both folate and betaine are important predictors of total homocysteine (tHcy) during pregnancy. However, studies to date have only been undertaken in populations with Western dietary patterns.

Objective: We investigated the predictors of tHcy in pregnant women recruited in the Seychelles, a population where access to fortified foods is limited and where women habitually consume diets rich in fish, eggs, rice, and fruit.

Design: Pregnant women ($n = 226$) provided blood samples at enrollment, at week 28 of gestation, and at delivery. Cord blood was obtained from a subset of participants ($n = 135$).

Results: As in other studies, maternal tHcy was lower during pregnancy than at delivery, whereas folate and vitamin B-12 status declined significantly to delivery. Despite low maternal folate status at delivery (median: 9.0 nmol/L), with 35% of women in the deficient range (serum folate: <6.8 nmol/L), cord blood folate status (median: 40.2 nmol/L) was similar to concentrations reported in Western populations. Folate was a significant predictor of tHcy at all time points ($P < 0.001$). In contrast with previous studies, betaine was only a significant predictor of maternal tHcy ($P < 0.001$) when the essential amino acid methionine was low.

Conclusions: The current study reports 2 important findings. First, fetal requirements for folate are paramount, such that cord blood folate status is maintained, even when maternal status is low. Second, betaine is a significant predictor of tHcy in pregnant women with low serum folate and low serum methionine concentrations. *Am J Clin Nutr* 2008;87:391–7.

KEY WORDS Homocysteine, folate, betaine, vitamin B-12, pregnancy, maternal status

INTRODUCTION

Homocysteine is a sulfur-containing amino acid that is converted to methionine through the remethylation pathway. Remethylation occurs via 2 enzymes, methionine synthase (MS), which requires folate and vitamin B-12, and betaine-homocysteine methyltransferase (BHMT), which requires betaine. Folate is derived from naturally occurring food folates or from folic acid, whereas betaine is derived directly from the diet and from the oxidation of choline (1).

Homocysteine concentration is determined by both genetic and nutritional factors. The most significant genetic predictor of homocysteine is the common 677C→T polymorphism in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR). Individuals homozygous for the polymorphism have reduced MTHFR activity, which results in elevated homocysteine (2). To date, a large number of studies have reported inverse associations between B vitamin status and circulating homocysteine concentrations (3, 4). Folic acid supplementation is consistently shown to lower homocysteine (3), and additional roles of vitamin B-12 (5, 6), riboflavin (7, 8), and vitamin B-6 (9) have been identified. Serum betaine and choline concentrations are also inversely associated with homocysteine (10–12), whereas choline or betaine supplementation can decrease homocysteine after a methionine load (13). Thus, it would appear that although remethylation via BHMT is not functional in all tissues (14), it is still important in the overall remethylation of homocysteine in the body, particularly under conditions of impaired folate status (15). Indeed, animal studies have suggested that limiting one remethylation pathway increases the activity of the other pathway (16, 17).

In addition to their function in homocysteine metabolism, folate and choline are essential nutrients for fetal development (18). Folate is crucial for DNA and RNA biosynthesis (18, 19), whereas choline has a number of key metabolic roles in lipid metabolism, cell membrane formation, and neurotransmission in the form of acetylcholine (19). Pregnant women, therefore,

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would be expected to have higher requirements for both folate and choline, thereby leading to lower status and higher circulating total homocysteine (tHcy) concentrations. In contrast to expectations, however, tHcy is in fact lower in pregnant than in nonpregnant women (12, 20–23), and the lower concentration is attributed to folic acid supplement use, decreased albumin concentrations, hemodilution, and an increase in glomerular filtration rate (20–22). Pregnancy is also associated with changes in thyroid function and insulin secretion, hormones that alter BHMT and MS activity (24, 25). In addition, there is an increased demand by the fetus for amino acids, including methionine, required for protein synthesis and the production of *S*-adenosyl methionine, the main biological methyl donor (26). Methionine, which, in excess, is catabolized to homocysteine, is essential for fetal development; animal studies, however, suggest adverse effects with excessive intakes, which highlights the importance of tight regulation of methionine metabolism (26).

We assessed the dietary intakes and serum concentrations of tHcy, folate, vitamin B-12, betaine, and methionine and determined the relative importance of folate, vitamin B-12, and betaine status in modulating tHcy concentrations in pregnancy. Maternal and cord blood samples were collected from pregnant women recruited in Seychelles, where the dietary habits of the population, remain traditional, with low intakes of processed and fortified foods.

SUBJECTS AND METHODS

Subjects

Participants were recruited from a larger study on maternal exposure and neonatal outcomes among mother-child pairs recruited in the Republic of Seychelles. Seychelles is an archipelago of >100 islands in the Indian Ocean ≈1500 km off the coast of East Africa. Healthy pregnant women were recruited into the study during their first antenatal visit on the island of Mahé, where ≈90% of the population of Seychelles resides. Patient records were checked in the postnatal period, and women who reported pregnancy complications were excluded from the analysis. Anthropometric data on the pregnant women were obtained at enrollment, whereas data on the neonates were collected after delivery. Supplement use was established by asking subjects at each visit to state whether or not they were using folic acid supplements at the time of sampling. The research protocols were reviewed and approved by the Institutional Review Boards of the University of Rochester and the Ministry of Health in Republic of Seychelles. The procedures followed were in accordance with the Helsinki Declaration, and all participants gave informed consent.

Pregnant woman had nonfasting blood samples collected at enrollment, at 28 wk of gestation, and at delivery. Cord blood samples were also collected in a subset of participants ($n = 135$). Blood samples were collected into plain serum tubes, immediately wrapped in aluminum foil, and stored at 4 °C until centrifugation within 3 h at 1000 × *g* for 15 min. Whole blood was also collected and portioned for subsequent DNA extraction. Serum and whole blood were stored at −70 °C until analyzed. Samples were transported on dry ice and batch analyzed at the end of the study in laboratories in Norway and Ireland.

Serum betaine, tHcy, creatinine, and methionine were analyzed as previously described (27). Microbiological assays were used to measure serum folate and vitamin B-12 concentrations (28, 29). For each participant, DNA was extracted from whole blood samples with a QIAamp DNA Mini Kit (QIAGEN Ltd, West Sussex, United Kingdom). Genotyping for the MTHFR 677C→T polymorphism was performed by polymerase chain reaction and Hinf1 digestion (2).

Detailed information on the steps involved with the establishment of a dietary survey method in Seychelles has been published (30). At 28 wk of gestation, detailed dietary information was collected from each subject by means of a 4-d semiquantitative food diary (2 consecutive weekdays and 2 weekend days). Foods and beverages recorded in the diet diaries were converted to weights for input into a dietary analysis package (WISP version 2.0; Tinuviel Software, Warrington, United Kingdom). The basic dietary analysis package was supplemented with food composition and recipe data for foods consumed in Seychelles.

In addition, the WISP database was customized to add data for the choline and betaine contents of foods obtained from the US Department of Agriculture food-composition database (31). Choline and betaine values were mapped to the most appropriate food codes in the WISP database by a Registered Nutritionist. Estimated choline and betaine intakes from foods and beverages were then generated as per all other nutrients.

Data analysis

Data are presented as means ± SDs or medians with 5th and 95th percentiles as appropriate. Values with a skewed distribution were transformed logarithmically before statistical analyses to approximate normal distribution. Data were analyzed by repeated-measures analysis of variance (ANOVA) with a general linear model. If there was a significant effect of time, specific comparisons between time points were made by using post hoc comparisons with Bonferroni's correction. Correlations between individual variables were investigated by using the Spearman rank correlation on untransformed data. Multiple regression analysis was used to determine the predictors of plasma tHcy at gestational week 28, at delivery, and in cord blood. Regression analysis was also performed as above with adjustment for serum methionine. Statistical analyses were carried out by using SPSS 12.0 for WINDOWS (SPSS Inc, Chicago, IL). Results were considered statistically significant when $P < 0.05$.

RESULTS

Of 300 women with apparently healthy pregnancies on initial recruitment, 4 subjects were not pregnant; 14 subjects had a miscarriage, termination of pregnancy or stillbirth, 1 did not wish to continue with the study; 1 subject delivered twins prematurely; 2 subjects delivered their babies abroad; 1 subject developed preeclampsia; and 1 infant had a diagnosis of trisomy 21. Of the remaining 276 women who delivered healthy infants, 226 (who had full data sets) were included in the analysis. There was no significant difference with respect to age, body mass index (BMI), frequency of smoking, or MTHFR 677C→ T polymorphism between the 276 pregnant women who delivered healthy infants and those ($n = 226$) included in the final analysis. The mean (±SD) gestational age at enrollment of the 226 women included in the final analysis was 13 (4) wk. The characteristics of the women, together with the characteristics of the neonates, are shown in **Table 1**.

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TABLE 1
Characteristics of the mothers at recruitment and of the neonates at delivery[1]

	Value
Mothers (n = 226)	
Age (y)	27.1 ± 6.2[2]
Height (m)	1.60 ± 0.07
Weight (kg)	66.7 ± 16.7
BMI (kg/m^2)	26.1 ± 6.3
Smokers (%)	4
MTHFR 677C→T genotype (%)	
CC	84.5
CT	15
TT	0.4
Neonates (n = 226)	
Weight (g)	3253 ± 484
Length (cm)	50.9 ± 3.1
Gestational age at delivery (wk)	38.7 ± 1.3
Sex	
Male (n)	112
Female (n)	114
MTHFR 677C→T genotype (%)	
CC	66.4
CT	17.6
TT	2.2
Unknown	13.7

[1] MTHFR, methylenetetrahydrofolate reductase.
[2] x ± SD (all such values).

Dietary intake

The median (5th, 95th percentile) energy, protein, fat, and carbohydrate intakes in the study population were 8.82 (4.89, 13.36) MJ/d, 81 (45, 129) g/d, 84 (46, 137) g/d, and 266 (147, 402) g/d respectively (data not shown). The median (5th, 95th percentile) intakes of folate, vitamin B-12, betaine, and choline at 28 wk of gestation were 219 (109, 407) μg/d, 4.7 (2.4, 11.5) μg/d, 115 (35, 244) mg/d, and 204 (86, 378) mg/d, respectively (data not shown). The main foods contributing to folate intake were breads and vegetables, whereas the main contributors to vitamin B-12 intake were meats, fish, and fish products. Breads contributed >75% to betaine intake, whereas the main foods contributing to choline intake were eggs, meat, and fish. Although eggs and fish are rich sources of choline, median choline intake was found to be considerably below the US adequate intake (AI) for pregnant women of 450 mg/d (32). The median (5th, 95th percentile) intakes of riboflavin and vitamin B-6, vitamins also important in homocysteine metabolism, were 1.5 (0.7, 2.7) and 1.8 (0.9, 2.8) mg/d, respectively (data not shown). Median riboflavin intake was above the US recommended dietary allowance (RDA) for pregnant women of 1.4 mg/d and, the median vitamin B-6 intake was below the RDA of 1.9 mg/d (32).

Biochemical changes during pregnancy and comparisons with cord blood

Serum tHcy, methionine, betaine, folate, and vitamin B-12 concentrations in maternal and cord blood are shown in **Table 2**. Repeated-measures ANOVA showed an overall significant effect of gestational time on serum tHcy ($P < 0.001$) and methionine ($P < 0.001$). Concentrations of both variables were found to increase significantly in a stepwise manner from enrollment to week 28 to delivery ($P < 0.001$). Analysis of the betaine data also showed a significant effect of gestational time ($P < 0.001$). Compared with enrollment, serum betaine concentrations were significantly lower at week 28 and at delivery ($P < 0.001$). Both serum folate and serum vitamin B-12 concentrations were found to be significantly lower at delivery than at week 28 ($P < 0.001$).

The serum concentration of tHcy was significantly lower in cord blood than in the maternal delivery sample ($P < 0.001$), whereas significantly higher serum concentrations of methionine ($P < 0.05$), betaine ($P < 0.001$), folate ($P < 0.001$), and vitamin B-12 ($P < 0.001$) were observed in cord blood than in maternal blood collected at delivery.

There was no significant difference in serum folate concentration between those women who reported supplement use at week 28 (72%) and the nonusers (data not shown). At delivery, serum folate was significantly higher ($P < 0.05$) among the 86% of women who reported supplement use than in nonusers; the median (5th, 95th percentile) values were 9.40 (3.02, 30.92) and 6.24 (1.92, 34.0) nmol/L.

Univariate correlations

Associations between maternal and cord blood tHcy, folate, vitamin B-12, and betaine were observed (data not shown). Cord blood tHcy concentration was positively associated with maternal tHcy at enrollment ($r = 0.274$, $P < 0.001$) and at week 28 ($r = 0.187$, $P < 0.05$) and strongly so at delivery ($r = 0.602$, $P < 0.001$). Cord blood vitamin B-12 concentration was associated with maternal vitamin B-12 status at week 28 ($r = 0.527$, $P < 0.001$) and at delivery ($r = 0.440$, $P < 0.001$), whereas cord

TABLE 2
Serum concentrations of total homocysteine (tHcy), folate, vitamin B-12, betaine, and methionine during pregnancy and in cord blood[1]

	Enrollment (n = 226)	Week 28 of gestation (n = 226)	Delivery[2] (n = 226)	Cord blood (n = 135)
tHcy (μmol/L)	5.83 (4.03, 10.38)[a]	6.84 (4.33, 12.93)[b]	12.4 (5.91, 23.17)[c]	10.2 (5.8, 19.3)[3]
Methionine (μmol/L)	18.7 (12.74, 27.10)[a]	23.8 (15.64, 41.37)[b]	40.7 (23.42, 64.31)[c]	44.4 (24.68, 77.90)[3]
Betaine (μmol/L)	14.15 (8.25, 25.35)[a]	10.56 (7.20, 16.63)[b]	11.2 (6.27, 21.30)[b]	21 (13.30, 36.38)[3]
Folate (nmol/L)	—	14.11 (5.52, 38.36)[a]	9.01 (2.81, 30.73)[b]	40.24 (11.99, 88.33)[3]
Vitamin B-12 (pmol/L)	—	250 (128, 493)[a]	221 (107, 507)[b]	413 (174, 1139)[3]

[1] All values are medians: 5th and 95th percentiles are in parentheses. All variables were analyzed with repeated-measures ANOVA. Cord blood samples were compared with maternal delivery samples by using Student's t test. The analysis of folate and vitamin B-12 was not undertaken on the enrollment samples. Values in a row with different superscript letters are significantly different over time, $P < 0.05$.
[2] Defined as 1 d postpartum.
[3] Significantly different from delivery, $P \leq 0.01$.

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TABLE 3
Predictors of serum total homocysteine (tHcy) concentrations at week 28 of gestation, at delivery, and in cord blood[1]

	Week 28 of gestation			Delivery			Cord blood		
Dependent and independent variables	Standardized β	P	Adjusted R^2	Standardized β	P	Adjusted R^2	Standardized β	P	Adjusted R^2
tHcy									
Age	0.023	0.716		0.040	0.493		−0.063	0.390	
Folate	−0.330	0.000		−0.530	0.001		−0.519	0.001	
Vitamin B-12	−0.045	0.479		−0.044	0.471		−0.164	0.023	
Betaine	−0.072	0.256		0.046	0.442		0.265	0.000	
Creatinine	0.137	0.032	0.119	0.064	0.274	0.281	0.061	0.424	0.368
Model repeated with the inclusion of methionine									
tHcy									
Age	−0.042	0.410		0.039	0.415		−0.060	0.389	
Folate	−0.224	0.000		−0.329	0.001		−0.476	0.001	
Vitamin B-12	−0.073	0.156		−0.070	0.158		−0.176	0.011	
Betaine	−0.207	0.001		−0.112	0.031		0.173	0.021	
Creatinine	0.035	0.490		0.081	0.092		0.043	0.556	
Methionine	0.600	0.001	0.440	0.537	0.001	0.512	0.275	0.001	0.430

[1] Blood samples were collected from 226 women with uncomplicated pregnancies at their initial booking visit, at week 28 of gestation, and 1 d postpartum. Cord blood samples were collected from a subset of 135 mothers. Multiple regression analysis was performed with plasma tHcy as the dependent variable and maternal age, folate, vitamin B-12, betaine, and creatinine as independent variables. The model was repeated with the inclusion of methionine as an independent variable.

blood betaine was significantly associated with maternal betaine concentration at enrollment ($r = 0.208$, $P < 0.05$) and at week 28 ($r = 0.284$, $P < 0.001$) but not at delivery ($r = 0.110$, $P = 0.206$).

Expected associations between serum tHcy and the related B vitamins were also observed (data not shown). Maternal tHcy was inversely associated with maternal serum folate at week 28 ($r = -0.278$, $P < 0.001$) and at delivery ($r = -0.528$, $P < 0.001$) and with maternal betaine at week 28 ($r = -0.146$, $P < 0.05$). Likewise, cord blood tHcy was associated with cord blood serum folate ($r = -0.497$, $P < 0.001$), vitamin B-12 ($r = -0.192$, $P < 0.05$), and betaine ($r = 0.394$, $P < 0.001$).

Multiple regression analysis

The predictors of maternal serum tHcy concentration at week 28, at delivery, and in cord blood were estimated by multiple regression (**Table 3**). At each time point, the model included maternal age, serum folate, serum vitamin B-12, betaine, and creatinine concentrations as independent variables. The model explained ≈12% of the variation in tHcy at week 28, ≈28% of the variation in tHcy at delivery, and ≈37% of the variation in tHcy in cord blood.

Serum folate was the strongest negative predictor of maternal tHcy at both week 28 and at delivery ($P \leq 0.001$). Serum creatinine was a significant positive predictor of tHcy at week 28 only ($P < 0.05$). In relation to tHcy in cord blood, serum folate and vitamin B-12 were significant negative predictors ($P < 0.001$ and $P < 0.05$, respectively), whereas betaine was a significant positive predictor ($P < 0.001$).

The multiple regression analyses were repeated with the inclusion of serum methionine in the model. This model explained ≈44% of the variation in tHcy at week 28, ≈51% of the variation in tHcy at delivery, and ≈43% of the total variation in tHcy in cord blood. Serum folate and vitamin B-12 remained significant predictors as in the earlier model. However, betaine also became a significant negative predictor of tHcy at week 28 and at delivery ($P < 0.001$ and $P < 0.05$, respectively). Testing for an interaction between methionine and betaine on tHcy indicated a significant interaction at week 28 only ($P < 0.05$). The data were then split into low and high methionine, on the basis of median methionine concentrations (data not shown), and univariate correlations indicated a significant inverse association between tHcy and betaine in the low methionine group ($r = -0.358$, $P < 0.001$) which was not apparent in the higher methionine group ($r = -0.137$, $P = 0.149$).

DISCUSSION

We analyzed tHcy concentration and B-vitamins and betaine that are central to homocysteine metabolism in women with uncomplicated pregnancies. Maternal folate at delivery was low, with 35% of women classified as having possible or certain folate deficiency, but cord blood folate was high, comparable with that seen in populations with higher maternal folate status (11). Folate was the strongest predictor of tHcy. Betaine was only a significant predictor of maternal tHcy when the amino acid methionine was included in the statistical model. An interaction between betaine and methionine on tHcy was observed such that the relation between betaine and tHcy was only evident at low serum methionine concentrations.

The longitudinal design of the current study, allowed us to assess changes in the predictors of tHcy throughout pregnancy in a study population unique in terms of 2 major predictors of tHcy. ie dietary intake and MTHFR 677C→T genotype. Dietary patterns in Seychelles, although somewhat influenced by Western foods, remain traditional with little access to foods fortified with folic acid that are habitually consumed in the United States and Europe. There was no significant difference in folate status at week 28 of gestation between those women reporting supplement use and nonusers, which suggests that supplement use was, at best, sporadic. Thus, we speculate that the changes in folate

status, and indeed in tHcy, observed in the current study are perhaps more indicative of natural changes that occur during pregnancy in the absence of supplemental folate. Only one mother was identified with the *TT* genotype for the MTHFR 677C→T polymorphism, a key genetic predictor of tHcy, which is relatively rare in populations of African origin (33).

Homocysteine concentration was similar at week 28 of gestation but was higher at delivery than in previous reports (11, 12, 23). This observation perhaps reflects differences in folate status between study populations. The maternal folate concentration was lower than that observed in other studies (11, 12, 23), such that at delivery 35% of the women had a serum folate concentration <6.8nmol/L—a cutoff used to define folate deficiency (34). The lower folate status observed was most likely related to a low dietary intake. Folate intake from food was 232 μg/d, which together with suggested low supplement use and lack of folic acid–fortified foods indicates that the total folate intake was less than recommendations (32, 35). Thus, it is perhaps not surprising that folate status was lower and the tHcy concentration was higher than what has been observed in populations in whom supplement use is common and fortified foods form part of the habitual diet; the latter sources provide the vitamin in the synthetic folic acid form, well known to be more bioavailable than the natural food folate forms (36).

The vitamin B-12 concentration was higher than in other studies of pregnant women, 2-fold higher at week 28 of gestation and 2.5-fold higher in cord blood than previously reported (11, 12). This is probably owing to the habitual consumption of foods rich in vitamin B-12, such as eggs and fish, which results in intakes well above the recommendations (32, 37). However, vitamin B-12 did decrease significantly in the last trimester, as observed previously by others (12), and most likely was related to an increase in fetal requirements for the vitamin (38).

Betaine concentrations were similar to those reported previously (11, 12) and decreased significantly during pregnancy, as in previous reports (12). This was despite a betaine intake considerably lower than intakes reported among Americans (39), most likely owing to a lower intake of cereals, predominantly whole-grain cereals, which are particularly rich in betaine (40). Intakes of choline, the precursor of betaine, were also lower than the current AI for pregnant women, despite habitual consumption of fish and eggs, both of which are rich sources of choline (31, 40). One limitation of the current study was the absence of a biochemical assessment of choline concentration. Blood samples were not collected in the presence of EDTA anticoagulant, which is essential in determinations of choline because EDTA inhibits the release of choline from choline esters (27). Thus, it was not possible to assess whether choline was a predictor of tHcy in pregnancy, an association that was previously reported (11).

Cord blood vitamin B-12 and betaine concentrations were 2-fold higher than maternal status at delivery. Importantly, despite our observation of low maternal folate status at delivery, cord blood status was high, >4-fold higher than maternal status and similar to observations in populations with significantly higher maternal folate status (11). Clearly, fetal requirements for these nutrients are paramount, such that, even when the maternal status is low, fetal status is maintained. Although maternal delivery and cord blood tHcy and vitamin B-12 were significantly correlated, no association was evident with folate or betaine, in contrast with previous studies (11). We speculate that the lack of an association was due to the active transport of these key nutrients across the placenta to maintain fetal status, which attenuated the simple correlations that may be evident in folate replete-populations.

Multiple regression analysis indicated, as expected, the importance of folate as a predictor of tHcy. In contrast with previous research (11), vitamin B-12 was only a significant predictor of tHcy in cord blood and not in maternal samples, a finding possibly explained by the generally higher vitamin B-12 status in the current cohort than in cohorts in other studies in which vitamin B-12 may have been more limiting with respect to tHcy metabolism. In the current population, betaine was a significant predictor of tHcy at week 28 and delivery only when methionine status was included in the regression model. Subsequent analysis indicated that, at week 28, betaine was only a significant predictor of tHcy in individuals with lower methionine status (as defined by a methionine concentration below the study population median). To our knowledge, this relation has not been previously reported. A relation between methionine status and BHMT activity has been reported: methionine restriction has been reported to increase BHMT activity in animal models, albeit only in the presence of adequate dietary choline or betaine (41). However, an excess of methionine also results in a modest increase in hepatic BHMT activity (42), which indicates a biphasic response of BHMT to methionine. Our observation of an interaction between methionine and betaine on tHcy concentrations may only be evident in pregnancy, a time when methionine and protein turnover are elevated (26). However, this relation may also only be evident when the status of folate is low, because in our study population, 35% of pregnant women had a serum folate concentration in the deficient range. We would not predict or expect an interaction between methionine and betaine on tHcy in populations with regular consumption of fortified foods or supplements and, thus, higher folate status. This hypothesis is supported by evidence from a recent population-based cross-sectional study that highlighted that betaine becomes a stronger predictor of plasma tHcy in subjects with low folate status (15). In contrast with earlier research (11), cord blood betaine was a positive predictor of cord blood tHcy, a finding that we believe may reflect active transport of both homocysteine (43) and betaine to the fetus rather than a role of betaine in one-carbon metabolism. Conceivably, placental betaine transport may be enhanced under conditions of low folate status.

In summary, despite the low maternal folate status of pregnant women resident in Seychelles, fetal folate and tHcy concentrations were similar to those reported in populations with habitual intakes of B vitamin–fortified foods. Given the prevalence of low folate status at delivery in this population, advice to pregnant women on consumption of folic acid (through supplements or fortified foods) is needed to improve status. In our population, betaine was a predictor of maternal tHcy only after control for methionine concentrations. This result perhaps provides further evidence of an interaction between dietary methionine and BHMT activity. Clearly, nutritional intake and status in the current study was markedly different from that of previous studies that investigated tHcy and its predictors in pregnancy in Ireland (11) and the Netherlands Antilles (12). Our findings, together with previous research, suggest that the predictors of tHcy during pregnancy, although undoubtedly influenced by pregnancy-related factors, differ among populations according to their nutritional status, such that predictors of tHcy in a folate-replete population will be different from that in populations consuming a diet suboptimal in dietary folate.

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