Low folate status enhances pregnancy changes in plasma betaine and dimethylglycine concentrations and the association between betaine and homocysteine[1–3]

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ABSTRACT

Background: Folate, choline, and betaine participate in homocysteine metabolism. It is not known whether they interact during pregnancy.

Objective: The objective was to investigate how folate status affects choline, betaine, and dimethylglycine during pregnancy.

Design: Fasting plasma folate, cobalamin, free choline, betaine, dimethylglycine, and total homocysteine (tHcy) were measured longitudinally at <12, 15, 24–27, and 34 gestational weeks (GW); at labor (nonfasting); and in the cord in participants (n = 522) from the Reus-Tarragona Birth Cohort (NUTrició i Creixement Intrauterí Retardat phase). Timing, dose, and duration of folic acid supplement use were recorded. Folate status was classified as below (low) or above (high) median plasma folate at baseline (27.6 nmol/L) and at 24–27 GW (11.4 nmol/L). Associations between folate or betaine with tHcy were investigated by using multiple linear regression analysis.

Results: Plasma betaine decreased by 34.8% (1.0%) throughout pregnancy, and dimethylglycine increased by 39.7% (2.7%) between 24–27 GW and labor (all $P < 0.001$). Compared with high folate status, low status was associated with a higher dimethylglycine/betaine ratio from 15 GW and with lower plasma betaine and higher dimethylglycine from 24 to 27 GW, for the rest of pregnancy. Regression analysis showed that by 24–27 GW, both plasma folate and betaine were inversely associated with tHcy when folate status was low and that the association between betaine and tHcy depended on folate status at 24–27 and 34 GW (interaction terms: $P < 0.001$ and $P < 0.01$). Betaine was inversely associated with tHcy at labor regardless of folate status.

Conclusion: Low folate status enhances the reduction in betaine and the increase in dimethylglycine during pregnancy and strengthens the association between betaine and tHcy. This trial was registered at clinicaltrials.gov as NCT01778205. *Am J Clin Nutr* 2013;97:1252–9.

INTRODUCTION

Choline is an essential nutrient that plays a role in cell structure and signaling (1), lipid transport (2, 3), and neurotransmission (4). It is either supplied by the diet or synthesized de novo from phosphatidylethanolamine (5). Choline oxidation leads to the formation of betaine (6), which acts as a methyl donor in homocysteine remethylation in the liver and kidneys, catalyzed by betaine homocysteine methyltransferase (BHMT)[4] (7). Dimethylglycine is the other product of this reaction (8). In most tissues, homocysteine remethylation to methionine is catalyzed by ubiquitous methionine synthase, with cobalamin as a cofactor and 5-methyltetrahydrofolate as the methyl donor (7). Homocysteine remethylation by BHMT may be relatively minor compared with methionine synthase. It appears to be more important when folate status (9) or intake (10) is low.

Choline and related one-carbon donors affect pregnancy outcome, fetal growth, and development. Diets rich in choline, betaine, and methionine reduce the risk of neural tube defects in humans (11), and choline is essential for neural tube closure (12) and brain development in experimental animals (13). The recommended intake of 450 mg choline/d for pregnant women (14) may not cover maternal and fetal requirements to support one-carbon metabolism during pregnancy (15). Estrogen production is increased during pregnancy (16), and this may enhance endogenous choline synthesis (17).

The increase in plasma free choline during pregnancy (18) and its positive association with total homocysteine (tHcy) during late pregnancy (19) may reflect increased production via the phos-

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[4] Abbreviations used: BHMT, betaine homocysteine methyltransferase; GW, gestational weeks; tHcy, total homocysteine.

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phatidylethanolamine *N*-methyltransferase pathway. The considerably higher choline concentrations in the fetal than in the maternal compartment (19) suggest transport across the placenta to meet fetal requirements.

There is also an increased demand on folate during pregnancy, which may lead to the depletion of folate maternal reserves (20). Betaine has been reported to be inversely associated with tHcy in late pregnancy (21), which may reflect its role in homocysteine remethylation when folate availability becomes limited.

There is little information on how folate status affects choline, betaine, and dimethylglycine during pregnancy. The aim of this longitudinal study from early pregnancy [<12 gestational weeks (GW)] to labor in 522 women from Reus and Tarragona, Spain, was to investigate how folate status affects plasma choline, betaine, and dimethylglycine and the association between betaine and tHcy throughout pregnancy.

SUBJECTS AND METHODS

Participants

The participants were from the ongoing NUTCIR phase of the Reus-Tarragona Birth Cohort—a longitudinal study of maternal nutritional status and pregnancy that is being carried out in the Area of Preventive Medicine and Public Health of the Universitat Rovira i Virgili, in collaboration with the Areas of Obstetrics and Gynecology of the University Hospitals: Sant Joan, Reus and Joan XXIII, Tarragona. All participants that completed their pregnancies before December 2011 were included in this analysis. The study was carried out in accordance with the Declaration of Helsinki, was approved by the Ethics Committees of both hospitals, and signed informed consent was obtained from all participants. Women that had their first antenatal check-up before 12 GW, and with viable singleton pregnancies confirmed by ultrasonography, were eligible to participate in the study. Exclusion criteria were chronic diseases or surgical interventions that affect nutritional status or habits or treatment with medication that affect folate metabolism.

All women were prescribed daily supplements containing 400 μg folic acid to be taken until the end of the first trimester of pregnancy, in keeping with recommendations by the Spanish Obstetrics and Gynecology Society (22). The prescribed supplements also contained 2 μg cyanocobalamin.

Of 1451 pregnant women who had their first antenatal check-ups between January 2005 and May 2011, 839 were not eligible to participate in the study (mainly because their pregnancy was more advanced than 12 GW). Of the remaining 612 eligible women who were invited to participate, 29 declined the invitation and 9 did not turn up for their next appointment. A total of 574 women (94% of those that were eligible) entered the study.

Blood samples, biochemical analysis, and data collection

Fasting blood samples [before 12 (baseline), 15, 24–27, and 34 GW], a sample on admission to the hospital with confirmed labor, and one from the cord were collected into tubes containing EDTA-K_3. The time of blood collection was recorded, and the samples were maintained at 4°C for <1 h before processing to separate the different blood fractions for red blood cell folate and for plasma and genotype determinations. Time of sample processing was also recorded. Samples were kept in aliquots at −80°C at the Institut d'Investigació Sanitària Pere Virgili biobank until they were analyzed within <18 mo of collection. It was ensured that all samples from the same pregnancy were analyzed in the same batch. Plasma choline, betaine, dimethylglycine, and tHcy were measured by liquid chromatography–tandem mass spectrometry (23, 24) and plasma creatinine by Jaffé reaction (Química Clínica Aplicada SA). Plasma and red blood cell folate were determined by microbiological assay with *Lactobacillus casei* (25) and plasma cobalamin by microbiological assay with *Lactobacillus leichmannii* (26). Plasma cotinine, indicative of recent nicotine exposure, was determined in the maternal samples at baseline and 24–27 GW and in the cord samples by liquid chromatography–tandem mass spectrometry (27). The *MTHFR* 677C>T genotype was determined in the mothers by using matrix-assisted laser-desorption/ionization-time-of-flight mass spectrometry (28).

Maternal age and BMI at the first antenatal check-up were recorded. Participants completed detailed questionnaires on supplement use (including folic acid, cobalamin, multivitamins, and iron), lifestyle, and habits with the help of a member of the study team at 20 GW and 32 GW. They were questioned in detail on the use of supplements during the 3 mo before conception and throughout pregnancy. The commercial name and frequency of use both during preconception and during each month of pregnancy (daily, 4–6 d/wk, irregular or never, and number of weeks) were recorded. On the basis of this information, the approximate total quantity of folic acid consumed (μg during the first trimester) was calculated. This information was used to classify participants into 3 different folic acid–supplement groups during the first trimester: 400 μg/d, ≤4 times/wk; 400 μg/d, 5–7 times/wk; or >400 μg/d, ≥4 times/wk. Data regarding smoking habits, alcohol consumption, or illegal drug use during the year before pregnancy, during the first trimester, and throughout the remainder of pregnancy were also recorded. Women with plasma cotinine >10 ng/mL or that confirmed smoking during pregnancy in the lifestyle and habits questionnaires were classified as smokers. This enabled us to identify women that never smoked, who gave up smoking during early pregnancy (before their first antenatal check up), or who gave up smoking at a later stage of pregnancy. Level of education and occupation of both parents and their combined income category were recorded. These data were used to classify the socioeconomic level of the participants as high, medium, or low (29). The participants were also asked whether they had planned their pregnancy.

Statistical analysis

Normality of the distributions of all variables was checked by using the Kolmogorov-Smirnov test. None of the plasma variables had a normal distribution and thus were natural log transformed. Continuous variables are reported as arithmetic or geometric means (95% CIs) and categorical variables as absolute frequencies and percentages.

Participants were classified as below or above the median plasma folate concentration at baseline and at 24–27 GW. They are referred to here as the low- and high-folate status groups. For longitudinal analyses, baseline folate status was applied to first-trimester analyses and 24–27 GW folate status for all analyses from this point throughout the rest of pregnancy. Longitudinal changes in plasma nutrient or metabolite concentrations according

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to folate status were investigated by using 2-factor ANOVA for repeated measures (intrasubject factor: gestational age; intersubject factor: folate status) with post hoc Bonferroni correction for multiple comparisons. When the interaction term (gestational age × folate status) was significant, we analyzed the effect of gestational age on plasma metabolite concentrations within each folate status group. Metabolite plasma concentrations at 15 GW were compared with <12 GW in a model using baseline folate status and at 34 GW and labor were compared with 24–27 GW in another model. Cord plasma concentrations were compared with maternal (at labor) concentrations by using Student's paired *t* test. Concentrations between the low- and high-folate status groups during pregnancy or in the cord were compared by using Student's *t* test. Mean percentage differences in plasma choline, betaine, and dimethylglycine between baseline and each of 24–27 GW, 34 GW, and labor, were compared between participants who stopped folic acid supplement use at the end of the first trimester and those who continued throughout pregnancy by using Student's *t* test. Mean dimethylglycine/betaine ratios were compared between low- and high-folate status groups (below or above the median plasma folate concentrations at each time point) by using Student's *t* test. Multiple linear regression models were fitted to estimate the associations between plasma folate and betaine with tHcy according to plasma folate status. Models at each time point were fitted for all of the participants together and then separate models according to the corresponding folate status category at each time point. All models were adjusted for physiologic, environmental, and lifestyle factors known to affect tHcy: maternal age, study center, smoking, gestational age at the time of sample collection, plasma creatinine, plasma cobalamin, and the *MTHFR* 677C>T polymorphism. β-standardized regression coefficients are reported to allow comparison of the relative importance of plasma folate and betaine in the models. The association between plasma choline and tHcy at each of the studied time points of pregnancy was assessed by Pearson's correlation. SPSS (SAS Institute Inc) for Windows, version 19.0, was used for all statistical analyses. The significance level was set at $P < 0.05$ for bilateral contrasts.

RESULTS

Characteristics of the study population

Of the women that entered the study, 522 (91%) went on to have a live birth. Of the remaining 52, 2 were excluded for twin pregnancies, 6 abandoned the study, 2 had stillbirths, 8 had their pregnancy terminated because of congenital malformations, and 34 miscarried. Baseline characteristics of the participant on entering the study are summarized in **Table 1**. Sixty percent of the participants regularly took the prescribed daily prenatal folic acid supplements (which contained 400 μg during the first trimester); 28.2% exceeded this dose during the first trimester, and 55.1% continued to take prenatal supplements containing ≤400 μg folic acid throughout the remainder of pregnancy.

TABLE 1
Descriptive baseline characteristics of the participants who completed their pregnancy with a live birth

	No. of subjects	Value (95% CI)
Age (y)	522	31.8[1] (31.4, 32.2)
BMI at first prenatal visit (kg/m^2)[2]	490	23.7[1] (23.3, 24.1)
Socioeconomic status (%)[3]		
Low	33	6.4 (4.3, 8.5)
Middle	254	49.0 (44.7, 53.4)
High	231	44.6 (40.3, 48.9)
Smoking (%)		
Periconception	165	31.7 (27.9, 35.9)
Throughout pregnancy	95	18.3 (15.2, 21.8)
Alcohol consumption (%)		
Periconception	29	5.8 (4.1, 8.2)
Throughout pregnancy	1	0.2 (0.0, 1.1)
Illegal drug use (%)		
Periconception	15	3.0 (1.8, 4.9)
Throughout pregnancy	4	0.8 (0.3, 2.0)
Folic acid supplement use (%)		
Periconception	163	34.7 (30.5, 39.1)
First trimester[4]	490	93.9 (91.5, 95.6)
Never	26	5.5 (3.8, 8.0)
400 μg/d, ≤4 times/wk	58	11.8 (9.3, 15.0)
400 μg/d, 5–7 times/wk	294	60.0 (55.6, 64.2)
>400 μg/d	138	28.2 (24.4, 32.3)
Throughout pregnancy	272	55.1 (50.7, 59.4)
MTHFR 667 C>T (%)[5]		
CC	165	34.7 (30.5, 39.0)
CT	228	47.9 (43.4, 52.4)
TT	83	17.4 (14.3, 21.1)

[1] Values are means.
[2] BMI was not recorded for 32 participants.
[3] Socioeconomic information was not provided by 4 participants.
[4] Folic acid supplement use information was incomplete for 6 participants.
[5] DNA was not available for 46 participants.

Fluctuation of B vitamin concentrations during pregnancy and according to folate status

Change in plasma folate, red blood cell folate, and plasma cobalamin concentrations according to folate status at baseline or at 24–27 GW are summarized in **Table 2**. Plasma and red blood cell folate were higher at 15 GW than at baseline in both folate-status groups. Both indexes then decreased after 15 GW, independently of folate status. The mean reductions in plasma folate were 50% between 15 GW and 24–27 GW in the women who stopped regular folic acid supplement use at the end of the first trimester and 30% in the women who regularly used multivitamins throughout pregnancy (data not shown). Given that variations in the timing, dose, and duration of supplement use affected plasma folate, 34% of the participants who were in the low-folate status group at baseline had moved to the high-status group by 24–27 GW. Similarly, 38% of the participants moved from the high-folate status group at baseline to the low-folate status group at 24–27 GW. Plasma cobalamin concentrations decreased gradually as pregnancy progressed in both folate-status groups. All vitamin concentrations in the cord were higher than in the mother at labor. Cord folate and cobalamin were higher in the high-folate than in the low-folate status group.

Fluctuation of choline and its oxidation pathway components and tHcy during pregnancy according to folate status

Fluctuations in plasma choline, betaine, dimethylglycine, and tHcy in participants with low- or high-folate status at baseline and

TABLE 2
Plasma folate, red blood cell folate, and plasma cobalamin concentrations throughout pregnancy according to folate status[1]

	Time of pregnancy					
	Gestational weeks					
Folate status	<12	15	24–27	34	Labor	Cord
Plasma folate (nmol/L)						
Low						
Geometric mean (95% CI)	14.1 (13.3, 14.9)[a]	17.0 (15.7, 18.4)[a,b]	7.2 (7.0, 7.5)[a]	6.4 (6.1, 6.8)[a,c]	6.3 (5.9, 6.6)[a,c]	18.9 (17.7, 20.1)[d,e]
P_{10}, median, P_{90}	7.1, 15.0, 24.9	7.1, 18.1, 31.9	7.4, 4.8, 10.4	4.1, 6.4, 11.1	3.9, 6.3, 10.2	10.4, 18.8, 33.0
High						
Geometric mean (95% CI)	45.6 (43.4, 48.0)	34.8 (32.2, 37.6)[b]	22.4 (21.1, 23.9)	18.0 (16.5, 19.8)[c]	18.0 (16.2, 20.0)[c]	30.5 (28.3, 32.8)[e]
P_{10}, median, P_{90}	30.7, 41.5, 87.1	17.2, 34.8, 70.2	12.6, 20.4, 44.8	8.0, 17.0, 49.0	7.0, 17.8, 47.6	15.3, 30.0, 58.5
Red blood cell folate (nmol/L)						
Low						
Geometric mean (95% CI)	712 (668, 759)[a]	959 (890, 1033)[a,b]	821 (779, 866)[a]	637 (602, 675)[a,c]	NA	NA
P_{10}, median, P_{90}	399, 685, 1406	535, 931, 1561	450, 843, 1302	373, 625, 1074	NA	NA
High						
Geometric mean (95% CI)	1331 (1256, 1412)	1685 (1579, 1799)[b]	1465 (1385, 1550)	1271 (1188, 1359)[c]	NA	NA
P_{10}, median, P_{90}	758, 1342, 2218	997, 1631, 2730	878, 1428, 2468	705, 1230, 2483	NA	NA
Cobalamin (pmol/L)						
Low						
Geometric mean (95% CI)	349 (334, 365)[f]	314 (298, 330)[b]	251 (241, 262)[a]	228 (218, 238)[a,c]	214 (203, 225)[a,c]	291 (269, 314)[d,e]
P_{10}, median, P_{90}	231, 348, 514	199, 319, 461	161, 256, 383	145, 231, 350	138, 222, 334	151, 309, 541
High						
Geometric mean (95% CI)	374 (359, 390)	326 (313, 340)[b]	295 (283, 308)	266 (253, 279)[c]	251 (237, 265)[c]	366 (333, 401)[e]
P_{10}, median, P_{90}	199, 319, 461	220, 338, 465	197, 295, 447	170, 270, 406	158, 251, 397	137, 400, 717

[1] Participants were classified into low- or high-folate status groups based on plasma folate below or above the median at <12 GW (baseline) (27.6 nmol/L) and at 24–27 GW (11.4 nmol/L). Data reported at <12 GW and 15 GW are according to baseline folate status. Data reported throughout the rest of pregnancy and in the cord are according to folate status at 24–27 GW. Plasma folate and cobalamin in folate status groups: <12 GW (n = 257 in each group), 15 GW (low: n = 202; high: n = 218), 24–27 GW (low: n = 238; high: n = 239), 34 GW (low: n = 215; high: n = 221), labor (low: n = 203; high folate: n = 210; high cobalamin n = 208), cord (low: n = 197; high: n = 203). Red blood cell folate in folate status groups: <12 GW (low: n = 249; high: n = 250), 15 GW (low: n = 193; high: n = 211), 24–27 GW (n = 233 in each group), 34 GW (low: n = 209; high: n = 218). Concentrations between the different time points were compared by 2-factor repeated-measures ANOVA (intrasubject factor: gestational age; intersubject factor: plasma folate status) followed by post hoc Bonferroni correction for multiple comparison in one model that compared between <12 and 15 GW and another that compared between 24–27 GW and 34 GW or labor: [b]$P < 0.001$ compared with <12 GW, [c]$P < 0.001$ compared with 24–27 GW. Interaction terms between gestational age and folate status: $P < 0.001$ for plasma folate from <12–15 GW and for red blood cell folate from 24–27 to 34 GW; between folate status at 24–27 GW and cord plasma folate: $P < 0.001$. Student's t test compared with high folate: [a]$P < 0.001$, [d]$P < 0.001$, [f]$P < 0.05$. Student's paired t test: [e]$P < 0.001$ compared with labor. GW, gestational weeks; NA, not available; P_{10}, 10th percentile; P_{90}, 90th percentile.

at 24–27 GW are illustrated in **Figure 1**. The patterns of change during pregnancy in all metabolites were similar in both folate-status groups. Globally, plasma choline increased as pregnancy progressed. Choline did not differ between the different folate-status groups or between participants with different first-trimester folic acid supplementation patterns at any stage of pregnancy. Globally, plasma betaine decreased until 24–27 GW, at which point it plateaued. Participants with low-folate status had lower plasma betaine at 24–27 GW than did those with high-folate status. This was also confirmed by the higher betaine concentrations at 15 and 24–27 GW in women who consumed folic acid from supplements in excess of 400 μg/d (data not shown). Globally, plasma dimethylglycine increased progressively from 24 to 27 GW. From 24 to 27 GW throughout the remainder of pregnancy, dimethylglycine was higher in women with low folate status than in the rest of the women. tHcy was higher throughout pregnancy in the women with low folate status.

All plasma metabolites in the cord were higher than in the mother at labor except for tHcy, which was lower. Cord choline and betaine did not differ between the different folate-status groups. However, cord dimethylglycine and tHcy were higher when the mothers had low folate status at 24–27 GW.

Mean percentage differences from baseline in plasma betaine and dimethylglycine at 24–27 GW, at 34 GW, and at labor according to continued regular folic acid use after the first trimester are shown in **Figure 2**. The reduction in plasma betaine as pregnancy progressed was greater in women who did not regularly take folic acid after the first trimester than in women who did. By 34 GW, a mean increase in plasma dimethylglycine of 10% was observed in women who had stopped taking folic acid supplements compared with no change in women who continued taking them. By the end of pregnancy, plasma dimethylglycine had increased in all participants but the increase tended to be higher in nonusers of supplements than in users.

Comparison of dimethylglycine/betaine ratios between low- and high-folate status groups

Mean dimethylglycine/betaine ratios according to folate status are reported in **Table 3**. The ratio was higher in the low-folate status group from 15 GW throughout the rest of pregnancy.

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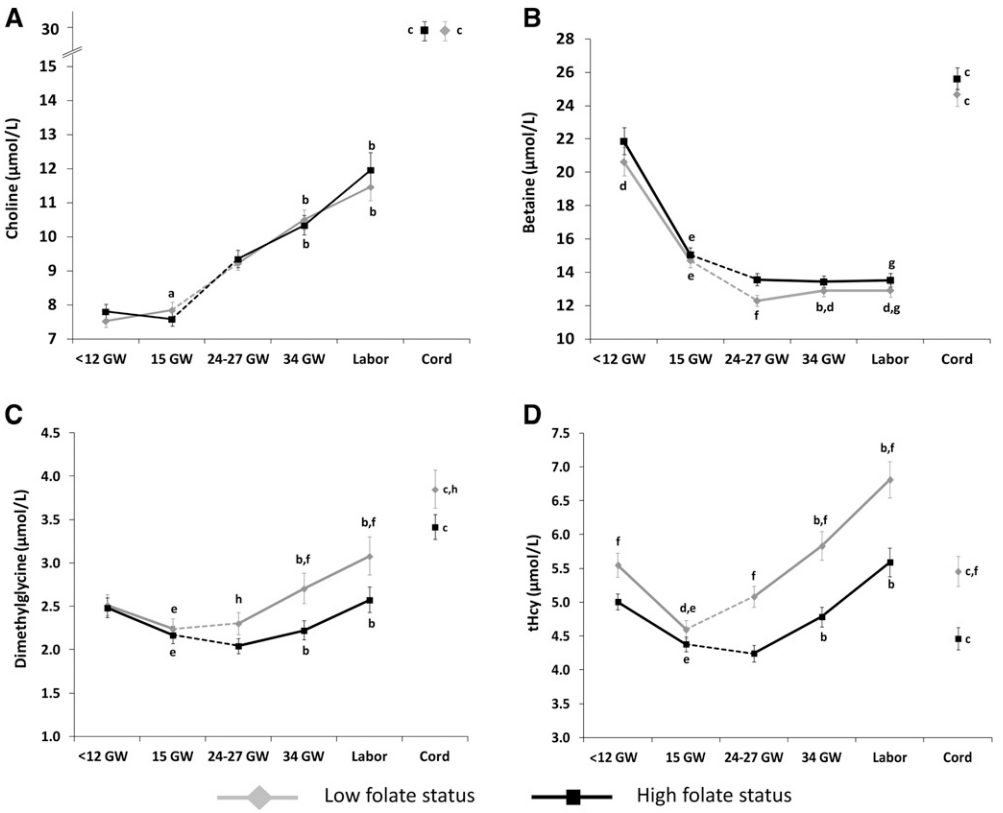

FIGURE 1. Geometric mean (95% CI) plasma choline (A), betaine (B), dimethylglycine (C), and tHcy (D) according to folate status. Folate status at baseline (data plotted before the dashed lines) and at 24–27 GW (data plotted after the dashed lines) was classified as below (low) or above (high) median plasma folate (27.6 nmol/L at baseline; 11.4 nmol/L at 24–27 GW). Low-/high-status groups: <12 GW (n = 256/n = 257), 15 GW (n = 202/n = 218), 24–27 GW (n = 238/n = 239), and 34 GW (n = 215/n = 221); labor (n = 211 in each group); and cord (n = 206/n = 208). Metabolite concentrations between the different time points were compared by 2-factor repeated-measures ANOVA (intrasubject factor: gestational age; intersubject factor: folate status) with post hoc Bonferroni correction for multiple comparison in one model that compared between <12 and 15 GW and another that compared between 24–27 GW and 34 GW or labor: $^{a}P < 0.01$ compared with <12 GW, $^{b}P < 0.001$ compared with 24–27 GW, $^{e}P < 0.001$ compared with <12 GW, $^{g}P < 0.05$ compared with 24–27 GW. Interaction terms between gestational age and folate status: $P < 0.001$ for choline from <12 to 15 GW and for betaine from 24–27 to 34 GW, $P < 0.01$ for dimethylglycine from 24–27 to 34 GW, and $P < 0.01$ for tHcy from <12 to 15 GW. Student's t test was used to compare low-folate with high-folate status: $^{d}P < 0.05$, $^{f}P < 0.001$, $^{h}P < 0.01$. Student's paired t test was used to compare cord with labor concentrations: $^{c}P < 0.001$. GW, gestational weeks; tHcy, total homocysteine.

Associations between choline and tHcy according to plasma folate status

Pearson linear correlation coefficients between choline and tHcy on log-transformed data showed weak correlations between choline and tHcy in the low-folate status groups at <12 GW (r = 0.17, $P < 0.01$), at 24–27 GW (r = 0.15, $P < 0.05$), and at labor (r = 0.22, $P < 0.01$). In the high-folate status groups, choline and tHcy were correlated at 15 GW (r = 0.18, $P < 0.01$), at 24–27 GW (r = 0.13, P = 0.05), at 34 GW (r = 0.31, $P < 0.001$), and at labor (r = 0.34, $P < 0.001$).

Associations of plasma folate or betaine with tHcy according to plasma folate status

Whether the associations between folate and betaine with tHcy vary depending on folate status was investigated by using multiple linear regression analysis. In models (not shown) including all of the participants, neither plasma folate nor betaine were associated with tHcy during the first half of pregnancy. Significant interactions between plasma folate and betaine were observed at 24–27 GW (P-interaction <0.001) and 34 GW (P-interaction <0.01). No interaction was observed at labor.

Separate models, fitted at each time point of pregnancy according to folate status, are shown in **Figure 3**. Plasma folate was inversely associated with tHcy throughout pregnancy in women with low folate status but only from 24 to 27 GW in those with high folate status. In the low-folate status group, betaine was not associated with tHcy until 24–27 GW. From then onward, an inverse association was observed. Betaine was not significantly associated with tHcy until labor in the high-folate status group.

DISCUSSION

Principal findings

This large longitudinal study tracked the effect of folate status on components of the choline oxidation pathway from early pregnancy until labor. There was a progressive increase in plasma choline and decrease in betaine. Dimethylglycine increased during the second half of pregnancy. Women with low folate status had lower betaine throughout pregnancy, higher dimethylglycine during the second half of pregnancy, and a higher dimethylglycine/betaine ratio from 15 GW throughout the rest of pregnancy compared with women with high status. Betaine was inversely associated

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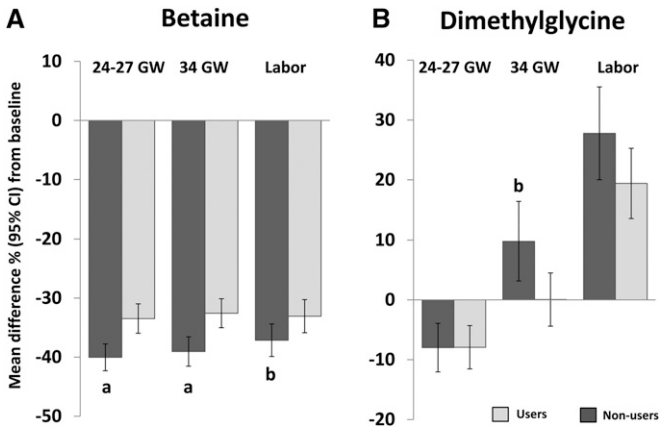

FIGURE 2. Mean percentage differences from baseline (<12 GW) in plasma betaine (A) and dimethylglycine (B) according to continued folic acid supplement use during months 4–7 of pregnancy. Nonusers: 24–27 GW (n = 206), 34 GW (n = 199), labor (n = 201). Users: 24–27 GW (n = 251), 34 GW (n = 246), labor (n = 227). Differences were compared between nonusers and users of folic acid supplements by using Student's t test: [a]$P < 0.001$, [b]$P < 0.05$. GW, gestational weeks.

with tHcy from midpregnancy in the low-folate status group but not until the end of pregnancy in the high-folate status group.

Strengths and limitations

The absence of mandatory fortification with folic acid prevented the possible masking of physiologic changes in pregnancy folate and of the effect of folate status on choline, its metabolites and the association between betaine and tHcy. Data on folic acid and multivitamin supplement use from preconception throughout pregnancy was corroborated with red blood cell folate determinations (data not shown). Blood sample processing protocols to prevent time and temperature-dependent artifacts in plasma choline and tHcy (30) were implemented.

Some participants did not attend all of their appointments (especially at 15 GW), were not identified in the labor ward as study participants, or gave birth in other centers. Elapsed time since the last meal may have affected plasma choline and betaine determinations only in some labor samples that were nonfasting (49%).

Main observations

Both folic acid supplement use and folate status affected the components of the choline oxidation pathway. Cessation of folic acid supplement use at the end of the first trimester, was associated with a greater reduction in plasma betaine throughout the rest of pregnancy compared with continued use and with a 10% increase in dimethylglycine by 34 GW that was not observed until labor in supplement users. In agreement with an effect of folate status on the choline oxidation pathway, plasma betaine was lower and dimethylglycine was higher during the second half of pregnancy in participants with low folate status than in those with high folate status. It is unlikely that this was due to lower betaine intake because plasma betaine did not vary between the folate-status groups before midpregnancy.

Cord dimethylglycine was higher in the low- than in the high-folate status group despite no differences in cord choline and betaine. Metabolites in cord blood reflect fetal metabolism and transport across the placenta.

Comparison with previous studies

Folate status in our study population was lower than reported in previous studies of choline metabolism during pregnancy (18, 19, 31). First- and second-trimester red blood cell folate concentrations in the high-folate status group were similar to those of supplement users in a recent US study (32). However, red blood cell folate was lower compared with the US study throughout pregnancy in the low-folate status group and in the last trimester in both groups. tHcy fluctuation patterns in pregnancy were similar to those previously reported (18, 33–35), despite slightly higher concentrations than in some reports (35, 36) and lower concentrations in another (18). Differences in plasma folate and tHcy between studies are likely to be due to differences in exposure to folic acid from supplements and/or fortified foods.

Two smaller studies (18, 31) reported choline concentrations throughout pregnancy similar to those observed in the current study, although concentrations in late pregnancy were slightly lower in one study (31). A study of 404 pregnant (primarily black) women in Alabama reported that free choline does not fluctuate during pregnancy (37). The timing of blood sample collection did not cover the beginning and the end of pregnancy and therefore

TABLE 3
Dimethylglycine/betaine ratio throughout pregnancy according to folate status[1]

	Dimethylglycine/betaine ratio				
	Gestational weeks				
Folate status	<12	15	24–27	34	Labor
Low					
n	256	206	238	234	222
Mean (95% CI)	0.13 (0.12, 0.14)	0.17 (0.16, 0.18)[a]	0.21 (0.19, 0.23)[b]	0.23 (0.21, 0.26)[b]	0.28 (0.25, 0.31)[b]
High					
n	257	218	239	234	222
Mean (95% CI)	0.13 (0.12, 0.14)	0.15 (0.14, 0.16)	0.16 (0.15, 0.17)	0.18 (0.17, 0.19)	0.21 (0.19, 0.22)

[1] Participants were classified into low- or high-folate status groups based on plasma folate below or above the median at baseline (27.6 nmol/L), 15 GW (26.3 nmol/L), 24–27 GW (11.4 nmol/L), 34 GW (8.9 nmol/L), and labor (8.4 nmol/L). The mean ratios were compared between the low- and high-folate status groups by using Student's t test: [a]$P < 0.01$, [b]$P < 0.001$.

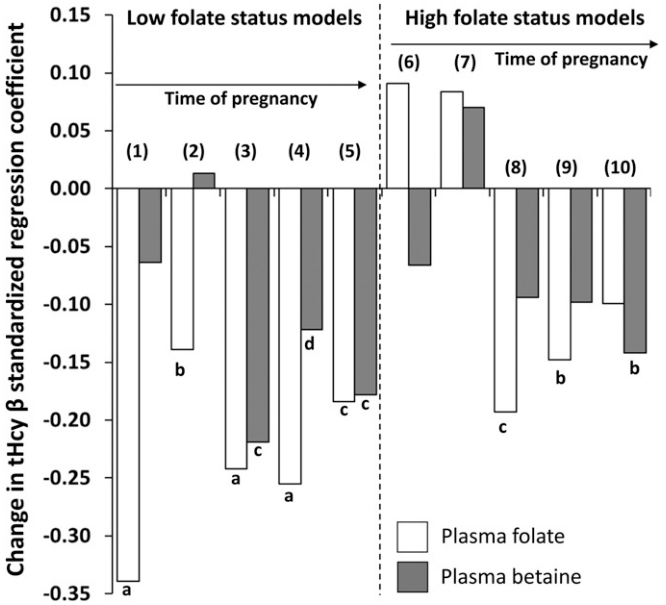

FIGURE 3. Multiple linear regression models that were fitted to investigate the associations of plasma folate and betaine with tHcy (dependent variable) according to folate status and time of pregnancy. Participants were classified into low- or high-folate status groups based on plasma folate below or above the median at baseline (27.6 nmol/L), 15 GW (26.3 nmol/L), 24–27 GW (11.4 nmol/L), 34 GW (8.9 nmol/L), and labor (8.4 nmol/L). All models were adjusted for maternal age, study center, smoking status, gestational age at the time of sample collection, plasma creatinine, plasma cobalamin, and *MTHFR* 677C>T polymorphism. All models were significant ($P < 0.001$). R^2 for the models: (1) <12 GW, 0.28 ($n = 232$); (2) 15 GW, 0.27 ($n = 187$); (3) 24–27 GW, 0.19 ($n = 219$); (4) 34 GW, 0.26 ($n = 200$); (5) labor, 0.27, ($n = 189$); (6) <12 GW, 0.21 ($n = 239$); (7) 15 GW, 0.17 ($n = 206$); (8) 24–27 GW, 0.18 ($n = 215$); (9) 34 GW, 0.18 ($n = 199$); and (10) labor, 0.22 ($n = 186$). $^{a}P < 0.001$, $^{b}P < 0.05$, $^{c}P < 0.01$, $^{d}P = 0.055$. GW, gestational weeks; tHcy, total homocysteine.

could not detect the increases in choline between early and midpregnancy or at the end.

We observed that plasma betaine decreased between the first trimester and weeks 24–27, as previously reported (18, 21). However, observed concentrations were higher during the first trimester and midpregnancy compared with previous reports in Curaçao (18) and in the Seychelles (21). Betaine status might vary between studies because of differences in diet, lifestyle, and prenatal supplement use.

The reduction in plasma dimethylglycine between early to midpregnancy has been reported before (18), but the current study also observed an increase in dimethylglycine in late pregnancy and that the dimethylglycine/betaine ratio was higher when folate status was lower. Two studies that reported higher folate status also reported lower dimethylglycine concentrations than in the current study at 16 GW (31) compared with 15 GW and 32 GW (18) compared with 34 GW.

Interpretation

Choline did not differ with folate status, probably because endogenous choline production prevailed during pregnancy (18). The positive associations observed between choline and tHcy from 15 GW throughout pregnancy in the high-folate status group and, at most points of pregnancy in the low-folate status group, may reflect homocysteine production during synthesis of phosphatidylcholine, which supports Molloy et al's hypothesis (19). Elevated plasma choline (despite the presence of physiologic factors such as hemodilution and increased glomerular filtration rate) indicates that its demand was substantial, as shown in rats (38). Depletion of choline-derived methyl donors during pregnancy have been reported, even in folate-replete mothers supplemented with choline (15).

Both the decrease in plasma betaine and increase in dimethylglycine as pregnancy progressed were potentiated by low folate status and cessation of folic acid supplement use after the first trimester. We hypothesize that these divergent changes in betaine and dimethylglycine, a substrate-product pair in the BHMT reaction, are due to upregulation of the enzyme's activity. This is supported by the greater dimethylglycine/betaine ratio in the low-folate status group than in the high-status group and the regression models that tested the effects of folate and betaine on tHcy. Folate and betaine interacted in the models that were not stratified by folate status (data not shown). Stratification by folate status showed that the effect size of betaine on tHcy was similar to folate in mid- and late pregnancy in the low-folate status group. Unlike folate, betaine was not significantly associated with tHcy in the early-pregnancy models. Thus, betaine appeared to be spared when folate status was replete as previously reported in adults (39) and in the elderly (40). From midpregnancy throughout the remainder of pregnancy, both folate and betaine were inversely associated with tHcy. In the high-folate status group, the inverse association between betaine and tHcy was only significant at labor when folate status in this group was at its lowest. These results show that both folate and betaine are sources of fungible C1 units during pregnancy. They suggest that homocysteine remethylation was mainly by methionine synthase when folate status was replete in the first half of pregnancy. However, reduced betaine and increased dimethylglycine when folate status was low or as it worsened suggest increased BHMT activity and a greater role of BHMT in homocysteine remethylation in this situation. This hypothesis should be tested in future studies. We conclude that folate status affects plasma betaine and dimethylglycine concentrations and the association between betaine and tHcy during pregnancy.

The authors' responsibilities were as follows—MMM, JDF-B, and SF-R: designed the research; SF-R, PC-B, MB, MIB-Z, and JS-B: conducted the research; PC-B, MB, and PMU: provided essential reagents or essential materials; SF-R, MMM, and JDF-B: analyzed the data or performed the statistical analysis; SF-R, MMM, JDF-B, and PMU: wrote the manuscript; and MMM: had primary responsibility for the final content. None of the authors declared a conflict of interest.

REFERENCES

1. Zeisel SH. Choline phospholipids: signal transduction and carcinogenesis. FASEB J 1993;7:551–7.
2. Noga AA, Vance DE. A gender-specific role for phosphatidylethanolamine N-methyltransferase-derived phosphatidylcholine in the regulation of plasma high density and very low density lipoproteins in mice. J Biol Chem 2003;278:21851–9.
3. Li Z, Agellon LB, Vance DE. Phosphatidylcholine homeostasis and liver failure. J Biol Chem 2005;280:37798–802.
4. Blusztajn JK, Wurtman RJ. Choline and cholinergic neurons. Science 1983;221:614–20.
5. Vance DE, Walkey C, Cui Z. Phosphatidylethanolamine N-methyltransferase from liver. Biochim Biophys Acta 1997;1348:142–50.
6. McKeever MP, Weir DG, Molloy A, Scott JM. Betaine-homocysteine methyltransferase: organ distribution in man, pig and rat and subcellular distribution in the rat. Clin Sci (Lond) 1991;81:551–6.

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7. Garrow TA. Purification, kinetic properties, and cDNA cloning of mammalian betaine-homocysteine methyltransferase. J Biol Chem 1996;271:22831–8.
8. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost 2000;26:219–25.
9. Holm PI, Ueland PM, Vollset SE, Midttun O, Blom HJ, Keijzer MB, den Heijer M. Betaine and folate status as cooperative determinants of plasma homocysteine in humans. Arterioscler Thromb Vasc Biol 2005;25:379–85.
10. Lee JE, Jacques PF, Dougherty L, Selhub J, Giovannucci E, Zeisel SH, Cho E. Are dietary choline and betaine intakes determinants of total homocysteine concentration? Am J Clin Nutr 2010;91:1303–10.
11. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. Am J Epidemiol 2004;160:102–9.
12. Fisher MC, Zeisel SH, Mar MH, Sadler TW. Perturbations in choline metabolism cause neural tube defects in mouse embryos in vitro. FASEB J 2002;16:619–21.
13. Montoya DA, White AM, Williams CL, Blusztajn JK, Meck WH, Swartzwelder HS. Prenatal choline exposure alters hippocampal responsiveness to cholinergic stimulation in adulthood. Brain Res Dev Brain Res 2000;123:25–32.
14. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: The National Academy Press, 1998.
15. Yan J, Jiang X, West AA, Perry CA, Malysheva OV, Devapatla S, Pressman E, Vermeylen F, Stabler SP, Allen RH, et al. Maternal choline intake modulates maternal and fetal biomarkers of choline metabolism in humans. Am J Clin Nutr 2012;95:1060–71.
16. O'Leary P, Boyne P, Flett P, Beilby J, James I. Longitudinal assessment of changes in reproductive hormones during normal pregnancy. Clin Chem 1991;37:667–72.
17. Resseguie M, Song J, Niculescu MD, da Costa KA, Randall TA, Zeisel SH. Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. FASEB J 2007;21:2622–32.
18. Velzing-Aarts FV, Holm PI, Fokkema MR, van der Dijs FP, Ueland PM, Muskiet FA. Plasma choline and betaine and their relation to plasma homocysteine in normal pregnancy. Am J Clin Nutr 2005;81:1383–9.
19. Molloy AM, Mills JL, Cox C, Daly SF, Conley M, Brody LC, Kirke PN, Scott JM, Ueland PM. Choline and homocysteine interrelations in umbilical cord and maternal plasma at delivery. Am J Clin Nutr 2005; 82:836–42.
20. Fernández-Ballart JD, Murphy MM. Preventive nutritional supplementation throughout the reproductive life cycle. Public Health Nutr 2001;4:1363–6.
21. Wallace JM, Bonham MP, Strain J, Duffy EM, Robson PJ, Ward M, McNulty H, Davidson PW, Myers GJ, Shamlaye CF, et al. Homocysteine concentration, related B vitamins, and betaine in pregnant women recruited to the Seychelles Child Development Study. Am J Clin Nutr 2008;87:391–7.
22. General de Salut Pública D, Ministerio de Sanidad y Consumo. Recomendaciones sobre suplementación con ácido fólico para la prevención de defectos del tubo neural. [Folic acid supplementation recommendations for the prevention of neural tube defects.] Inf Ter Sist Nac Salud 2001;25: 66–7 (in Spanish).
23. 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;49:286–94.
24. Ueland PM, Midttun O, Windelberg A, Svardal A, Skalevik R, Hustad S. Quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. Clin Chem Lab Med 2007;45:1737–45.
25. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. Methods Enzymol 1997;281:43–53.
26. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. J Clin Pathol 1991;44:592–5.
27. Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23:1371–9.
28. Meyer K, Fredriksen A, Ueland PM. High-level multiplex genotyping of polymorphisms involved in folate or homocysteine metabolism by matrix-assisted laser desorption/ionization mass spectrometry. Clin Chem 2004;50:391–402.
29. Grupo de Trabajo de la Sociedad Española de Epidemiología. La medición de la clase social en Ciencias de la Salud. [Measurement of social class in health sciences.] Barcelona, Spain: SG Editores, 1995 (in Spanish).
30. Hustad S, Eussen S, Midttun O, Ulvik A, van de Kant PM, Mørkrid L, Gislefoss R, Ueland PM. Kinetic modeling of storage effects on biomarkers related to B vitamin status and one-carbon metabolism. Clin Chem 2012;58:402–10.
31. Wu BT, Dyer RA, King DJ, Richardson KJ, Innis SM. Early second trimester maternal plasma choline and betaine are related to measures of early cognitive development in term infants. PLoS ONE 2012;7: e43448.
32. Branum AM, Bailey R, Singer BJ. Dietary supplement use and folate status during pregnancy in the United States. J Nutr (Epub ahead of print 30 January 2013).
33. Murphy MM, Scott JM, McPartlin JM, Fernandez-Ballart JD. The pregnancy-related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study. Am J Clin Nutr 2002;76:614–9.
34. Murphy MM, Scott JM, Arija V, Molloy AM, Fernandez-Ballart JD. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. Clin Chem 2004;50: 1406–12.
35. Ubeda N, Reyes L, Gonzalez-Medina A, Alonso-Aperte E, Varela-Moreiras G. Physiologic changes in homocysteine metabolism in pregnancy: a longitudinal study in Spain. Nutrition 2011;27:925–30.
36. Dodds L, Fell DB, Dooley KC, Armson BA, Allen AC, Nassar BA, Perkins S, Joseph KS. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clin Chem 2008;54:326–34.
37. Signore C, Ueland PM, Troendle J, Mills JL. Choline concentrations in human maternal and cord blood and intelligence at 5 y of age. Am J Clin Nutr 2008;87:896–902.
38. Gwee MC, Sim MK. Free choline concentration and cephalin-N-methyltransferase activity in the maternal and foetal liver and placenta of pregnant rats. Clin Exp Pharmacol Physiol 1978;5:649–53.
39. Melse-Boonstra A, Holm PI, Ueland PM, Olthof M, Clarke R, Verhoef P. Betaine concentration as a determinant of fasting total homocysteine concentrations and the effect of folic acid supplementation on betaine concentrations. Am J Clin Nutr 2005;81:1378–82.
40. Eussen SJ, Ueland PM, Clarke R, Blom HJ, Hoefnagels WH, van Staveren WA, de Groot LC. The association of betaine, homocysteine and related metabolites with cognitive function in Dutch elderly people. Br J Nutr 2007;98:960–8.

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