

# Plasma choline and betaine and their relation to plasma homocysteine in normal pregnancy<sup>1-3</sup>

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## ABSTRACT

**Background:** Plasma concentrations of total homocysteine (tHcy) decrease during pregnancy. This reduction has been investigated in relation to folate status, but no study has addressed the possible role of betaine and its precursor choline.

**Objective:** We investigated the courses of plasma choline and betaine during normal human pregnancy and their relations to plasma tHcy.

**Design:** Blood samples were obtained monthly; the initial samples were taken at gestational week (GW) 9, and the last samples were taken  $\approx$ 3 mo postpartum. The study population comprised 50 women of West African descent. Most of the subjects took folic acid irregularly.

**Results:** Plasma choline (geometric  $\bar{x}$ ; 95% reference interval) increased continuously during pregnancy, from 6.6 (4.5, 9.7)  $\mu\text{mol/L}$  at GW 9 to 10.8 (7.4, 15.6)  $\mu\text{mol/L}$  at GW 36. Plasma betaine decreased in the first half of pregnancy, from 16.3 (8.6, 30.8)  $\mu\text{mol/L}$  at GW 9 to 10.3 (6.6, 16.2)  $\mu\text{mol/L}$  at GW 20 and remained constant thereafter. We confirmed a reduction in plasma tHcy, and the lowest concentration was found in the second trimester. From GW 16 onward, an inverse relation between plasma tHcy and betaine was observed. Multiple regression analysis showed that plasma betaine was a strong predictor of plasma tHcy from GW 20 onward.

**Conclusions:** The steady increase in choline throughout gestation may ensure choline availability for placental transfer with subsequent use by the growing fetus. Betaine becomes a strong predictor of tHcy during the course of pregnancy. Both of these findings emphasize the importance of choline and betaine status during normal human pregnancy. *Am J Clin Nutr* 2005;81:1383-9.

**KEY WORDS** Homocysteine, folate, choline, betaine, pregnancy

## INTRODUCTION

Choline is considered to be an essential nutrient because the de novo synthesis via sequential methylation of phosphatidylethanolamine (PE) (**Figure 1**) is not sufficient to meet the metabolic demands when humans are deprived of dietary choline (1). Choline is needed for the normal function of cells (2). It is incorporated into membrane phospholipids and plays a crucial role in membrane integrity and signal transduction. It is converted to the neurotransmitter acetylcholine or oxidized to betaine and provides a source of methyl groups (2).

Human diets are rich in choline, particularly phosphatidylcholine (PC), but under conditions of high nutritional requirements,

eg, pregnancy, dietary intake could be a limiting factor (3). Experimental studies show that pregnant rats are more vulnerable to dietary choline deficiency than are nonpregnant female rats (4). Large amounts of choline are transported to the embryo (5), which may deplete maternal choline stores (1). The 60% lower hepatic choline content in late-pregnant rats than in nonpregnant rats is in line with this notion (6). The insight that prenatal choline availability is crucial for rat brain development with lifelong lasting effects (7) warrants attention. The Institute of Medicine (IOM) recommends a higher intake of choline for pregnant than for nonpregnant women (450 and 400 mg/d, respectively) (8). The IOM cautions, however, that insufficient data are available to establish estimated average requirements for different populations. Two cross-sectional studies of free choline concentrations during human pregnancy have shown conflicting results (9, 10). Longitudinal data are not available.

Betaine, which is mostly generated via the hepatic oxidation of choline, donates its methyl group directly to homocysteine (**Figure 1**). Betaine:homocysteine methyltransferase (BHMT) catalyzes this reaction and resides at the intersection of choline, methionine, and folate metabolism. The sole alternate route of homocysteine remethylation uses 5-methyltetrahydrofolate as a methyl donor and is catalyzed by methionine synthase. In this reaction, vitamin B-12 serves as a cofactor (11). Folate-dependent methionine synthase is present in most tissues, whereas betaine-dependent BHMT is confined to human liver and kidney. Under normal conditions, plasma folate is the main determinant of plasma total homocysteine (tHcy) concentrations (12). Plasma betaine is related to fasting tHcy concentrations in healthy subjects (13) and in patients with cardiovascular disease (14).

Cross-sectional (15-17) and longitudinal (18-23) studies have investigated plasma tHcy during pregnancy. Plasma tHcy is

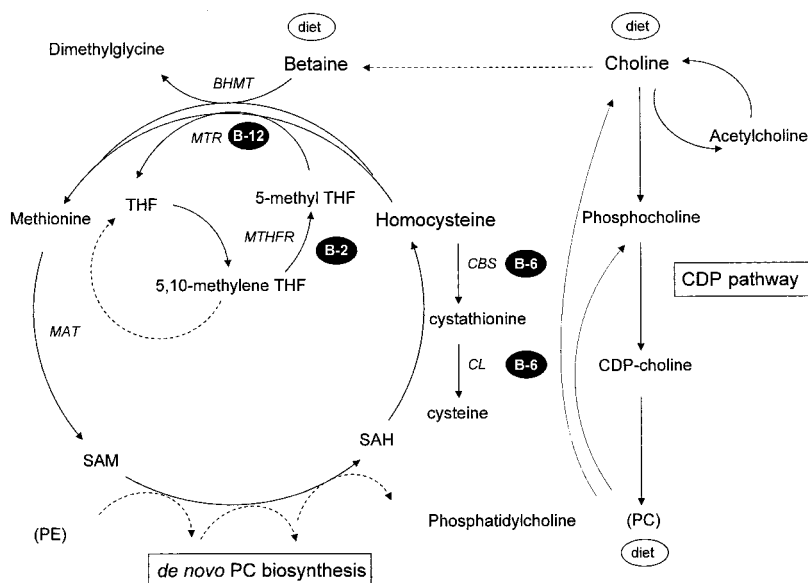
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**FIGURE 1.** Choline, betaine, and one-carbon metabolism. Choline is provided by food or can be formed de novo via sequential *S*-adenosylmethionine (SAM)-dependent methylation of phosphatidylethanolamine (PE) to form phosphatidylcholine (PC), which in turn can be converted back to choline. Choline serves as a precursor in the synthesis of the neurotransmitter acetylcholine or is oxidized to betaine. The latter compound serves as a methyl donor in the betaine:homocysteine methyltransferase (BHMT) reaction, which converts homocysteine to methionine. This conversion is also catalyzed by methionine synthase (MTR), which requires 5-methyltetrahydrofolate (THF) as methyl donor and couples the choline-betaine pathway to folate metabolism. The solid arrows represent single enzymatic reactions; the dashed arrows represent multiple enzyme reactions. CDP, cytidine 5'-diphosphate; MTHFR, 5,10-methylenetetrahydrofolate reductase; MAT, methionine adenosyltransferase; SAH, *S*-adenosylhomocysteine; CBS, cystathionine- $\beta$ -synthase; CL,  $\gamma$ -cystathioninase.

30–60% lower in pregnant women than in nonpregnant women, and the lowest tHcy concentrations are observed in the second trimester. Malinow et al (24) noted that low folate in pregnant women does not result in high plasma tHcy, as is observed in nonpregnant subjects. This observation suggests the presence of an effective tHcy-lowering mechanism in pregnant women. Nutritional and hormonal factors have been shown to affect betaine-dependent homocysteine remethylation in experimental animals (25, 26). Conceivably, the higher nutritional demands and alterations in hormonal environment during pregnancy may have similar effects in humans.

We investigated the changes in plasma choline and plasma betaine during normal human pregnancy and their relations to plasma tHcy. Plasma choline, plasma betaine, and plasma dimethylglycine (DMG; the demethylated product of betaine) together with plasma tHcy, plasma folate, and plasma vitamin B-12 were measured in 50 women with uneventful pregnancies. Samples were obtained monthly during pregnancy. The initial sampling was at gestational week (GW) 9 and the last sampling at 14.5 wk postpartum.

## SUBJECTS AND METHODS

### Study population and protocol

The study participants were recruited from a larger study on clinical chemical reference values of pregnant women in the island of Curaçao, Netherlands Antilles. Curaçao is predominantly inhabited by a population of West African descent with ample Caucasian mixture. The dietary habits of this population

are essentially Western. Women were asked to participate at their first prenatal visit. On agreement, singleton pregnancy was confirmed by ultrasonic examination with assessment of gestational age (all before 20 wk of gestation). Thereafter, the visits were monthly. At each visit, blood samples were taken for immediate determination of routine clinical chemical indexes, whereas aliquots of plasma were stored. In addition, women were asked about their use of prescribed medication and over-the-counter prenatal vitamin supplements. All participants gave their written consent. The study followed local ethical standards and the Helsinki Declaration of 1975, as revised in 1989.

Of the 194 women who entered the original study, 108 experienced uneventful pregnancies and delivered healthy term babies weighing >2500 g. Fifty of these women were randomly selected to have B vitamins and metabolites measured in their stored plasma samples. The mean and median (range) gestational ages at the initial visit were 9 and 9.5 (10.2) wk, respectively. This first sampling date is thus referred to as GW 9. Postpartum samples were available for 40 women. The postpartum samples were collected on average 14.5 wk after delivery.

Most women reported taking folic acid irregularly, whereas 7 women reported taking no supplements containing folic acid during pregnancy. Of the 43 women who reported using supplements containing folic acid, 15 used Fero-Folic (800  $\mu$ g folic acid/d; Abbott Diagnosis, Abbott Park, IL), 10 used prenatal vitamin supplements (1000  $\mu$ g folic acid/d) such as Materna (Lederle Laboratories, Pearl River, NY) and Natalins (Mead Johnson, Evansville, IL), and 17 women reported using both. At the postpartum sampling, none of the 40 women reported using folic acid.

### Blood sampling and analysis

Serial venous blood samples were collected at GW 9, 16, 20, 24, 28, 32, and 36 and postpartum. Women were instructed not to consume breakfast before sampling, which took place at  $\approx 0800$ .

Samples were taken by venipuncture. EDTA plasma was prepared by centrifugation (2500 rpm, 10 min,  $-4^{\circ}\text{C}$ ) within 1 h, and aliquots of plasma were stored at  $-70^{\circ}\text{C}$  until analyzed. The storage time was 4–9 y. Plasma tHcy, plasma folate, and plasma vitamin B-12 were analyzed by competitive protein binding assays with the use of an immunochemistry analyzer (IMX; Abbott Laboratories, Chicago, IL) in the Analytic Diagnostic Center, Curaçao, Netherlands Antilles. Plasma choline, plasma betaine, and plasma DMG were analyzed at the Institute of Pharmacology, University of Bergen, Bergen, Norway, by using a method based on normal-phase chromatography tandem mass spectrometry (27). Creatinine and hematocrit were measured immediately after sampling with the use of a Vitros 9550 (Johnson and Johnson, New Brunswick, NJ) and a CellDyne 3200 (Abbott Diagnostics), respectively. Samples from individual subjects were analyzed in a single run to minimize the effect of analytic variance.

### Statistical analysis

Logarithmic transformation was performed to approximate normal distribution for plasma tHcy, folate, vitamin B-12, choline, betaine, and DMG. Geometric means were used when appropriate. Changes in variables throughout gestation were analyzed with repeated-measures analysis of variance. Post hoc analyses were performed with the Student's *t* test for paired samples. Data at each sampling were compared with those of the previous sampling; postpartum data were compared with those of GW 36 and GW 9. The results were corrected for multiple testing by using the Bonferroni method to minimize type I errors. Correlations were investigated by using Spearman's rank-test. Longitudinal values of 5th, 50th, and 95th percentiles for plasma choline and betaine throughout pregnancy were estimated with a linear mixed model for repeated measurements (19). Multiple regression analysis was used to study predictors of plasma tHcy at each sampling point during the course of gestation and postpartum. Most statistical analyses were performed by using SPSS version 10 (SPSS Inc, Chicago, IL). The linear mixed model for repeated measurements was performed by using SAS version 8.2 (SAS Institute Inc, Cary, NC).

## RESULTS

### Characteristics of the study population

The mean ( $\pm$ SD) age of the study population was  $29 \pm 5.4$  y, 30% were nullipara, and the women delivered at a mean ( $\pm$ SD) gestational age of  $39.6 \pm 1.0$  wk. The mean ( $\pm$ SD) birth weight of their babies was  $3260 \pm 297$  g. The percentage of women who reported using folic acid increased with advancing gestation; only 20.9% of the women reported using folic acid at GW 9, whereas 69.8% and 62.8% of them reported using folic acid at GW 32 and GW 36, respectively.

### Changes in blood indexes during pregnancy

The geometric means and 95% reference intervals for plasma tHcy, folate, vitamin B-12, choline, betaine, and DMG at GW 9,

16, 20, 24, 28, 32, and 36 and postpartum are presented in **Table 1**. All of these variables changed significantly (by repeated-measures analysis of variance) during gestation:  $P < 0.0001$  for plasma tHcy, vitamin B-12, choline, and betaine;  $P = 0.01$  for plasma folate; and  $P = 0.02$  for plasma DMG.

A post hoc analysis showed that plasma tHcy decreased in early pregnancy (from GW 9 to GW 16;  $P < 0.0001$ ). Although plasma tHcy tended to decrease until GW 28, these decrements were not significant. Plasma folate increased from GW 9 to GW 16 ( $P = 0.01$ ). Plasma vitamin B-12 decreased from GW 9 to GW 16, from GW 16 to GW 20 ( $P < 0.0001$  for both), and from GW 28 to GW 32 ( $P = 0.009$ ). Plasma choline increased from GW 9 to GW 16 ( $P = 0.03$ ), from GW 16 to GW 20 ( $P = 0.02$ ), from GW 20 to GW 24 ( $P < 0.0001$ ), and from GW 32 to GW 36 ( $P = 0.002$ ). Plasma betaine decreased from GW 9 to GW 16 and from GW 16 to GW 20 ( $P < 0.0001$  for both). Plasma DMG decreased from GW 9 to GW 16 ( $P < 0.0001$ ).

The 5th, 50th, and 95th percentiles of longitudinal values of plasma choline and plasma betaine throughout pregnancy, estimated by using a linear fixed model for repeated measures, are shown in **Figures 2** and **3**, respectively.

### Postpartum blood indexes

Three months after delivery, plasma tHcy, vitamin B-12, betaine, and DMG were higher, whereas plasma folate and choline were lower than the concentrations at GW 36 ( $P < 0.0001$  for all except folate;  $P = 0.004$ ). Postpartum concentrations of plasma betaine and DMG were also higher than the concentrations at GW 9 ( $P < 0.0001$  for both). On an individual basis, 35 of 40 women had a lower plasma choline concentration postpartum and 40 of 40 had a higher plasma betaine concentration postpartum than at late pregnancy (GW 36).

### Univariate correlations

Spearman coefficients for the correlations of plasma tHcy with plasma folate, vitamin B-12, and betaine are listed in **Table 2**. Plasma tHcy was significantly and inversely related to plasma folate throughout gestation and postpartum and with plasma vitamin B-12 between GW 16 and GW 28. Plasma tHcy became inversely related to plasma betaine at GW 16, and this relation was maintained throughout gestation (except at GW 32) and postpartum. Plasma tHcy was unrelated to plasma choline, DMG, or creatinine (data not shown).

Plasma betaine was positively related to plasma choline at each sampling point during pregnancy and postpartum. The weakest correlation was found at GW 28 ( $r = 0.35$ ,  $P = 0.02$ ), whereas the strongest correlation was found at GW 20 ( $r = 0.56$ ,  $P < 0.0001$ ). The relation between plasma betaine and plasma DMG during pregnancy was significant at GW 9 ( $r = 0.38$ ,  $P = 0.006$ ), GW 16 ( $r = 0.48$ ,  $P = < 0.0001$ ), GW 28 ( $r = 0.41$ ,  $P = 0.004$ ), GW 36 ( $r = 0.33$ ,  $P = 0.02$ ), and postpartum ( $r = 0.45$ ,  $P = 0.004$ ). Plasma betaine was related to plasma folate at GW 16 ( $r = 0.40$ ,  $P = 0.004$ ) and GW 20 ( $r = 0.31$ ,  $P = 0.03$ ).

Hematocrit was not significantly related to plasma tHcy or betaine at any time during pregnancy, except at GW 36. At this time point, an inverse relation with hematocrit and plasma tHcy was observed ( $r = -0.36$ ,  $P = 0.01$ ).

**TABLE 1**

Plasma concentrations of total homocysteine (tHcy), folate, vitamin B-12, choline, betaine, and dimethylglycine (DMG) throughout gestation and postpartum (PP)<sup>1</sup>

	GW 9	GW 16	GW 20	GW 24	GW 28	GW 32	GW 36	PP
tHcy ( $\mu\text{mol/L}$ )								
Geometric $\bar{x}$	9.42	7.28 <sup>2</sup>	7.33	7.11	6.89	7.17	7.60	10.15 <sup>2</sup>
95% RI	5.50, 16.12	4.28, 12.40	4.25, 12.64	4.03, 12.55	3.93, 12.06	4.38, 11.73	4.46, 12.96	5.51, 18.70
Folate (nmol/L)								
Geometric $\bar{x}$	16.38	19.61 <sup>3</sup>	18.46	17.31	19.98	20.04	20.46	14.93 <sup>4</sup>
95% RI	7.92, 33.85	9.05, 42.52	8.44, 40.39	6.52, 45.91	8.13, 49.13	7.14, 56.25	7.35, 56.91	6.43, 34.67
Vitamin B-12 (pmol/L)								
Geometric $\bar{x}$	293.2	221.2 <sup>2</sup>	195.4 <sup>2</sup>	185.5	179.3	164.4 <sup>4</sup>	164.1	276.7 <sup>2</sup>
95% RI	122.7, 700.7	102.5, 477.4	89.4, 427.1	81.9, 420.0	80.9, 397.3	73.8, 366.3	72.7, 370.2	118.8, 644.4
Choline ( $\mu\text{mol/L}$ )								
Geometric $\bar{x}$	6.62	7.32 <sup>3</sup>	7.94 <sup>3</sup>	8.87 <sup>2</sup>	9.36	9.78	10.77 <sup>4</sup>	7.92 <sup>2</sup>
95% RI	4.51, 9.70	4.89, 10.97	5.62, 11.20	6.38, 12.33	6.61, 13.26	7.12, 13.41	7.45, 15.58	5.44, 11.52
Betaine ( $\mu\text{mol/L}$ )								
Geometric $\bar{x}$	16.27	11.47 <sup>2</sup>	10.29 <sup>2</sup>	10.29	10.73	10.76	10.93	27.45 <sup>2,5</sup>
95% RI	8.59, 30.78	6.77, 19.45	6.56, 16.15	6.96, 15.20	7.41, 15.53	7.18, 16.13	7.13, 16.75	12.63, 59.70
DMG ( $\mu\text{mol/L}$ )								
Geometric $\bar{x}$	1.96	1.54 <sup>2</sup>	1.57	1.61	1.74	1.71	1.76	2.66 <sup>2,5</sup>
95% RI	0.89, 4.28	0.74, 3.19	0.73, 3.39	0.67, 3.92	0.59, 5.15	0.85, 3.44	0.84, 3.68	1.43, 4.96

<sup>1</sup> Blood samples were collected from 50 women with uncomplicated pregnancies at their initial visit [gestational week (GW) 9] and subsequently at GW 16, 20, 24, 28, 32, and 36. PP blood samples were obtained from 40 women approximately 14.5 wk after delivery. The values were logarithmically transformed to approximate normality. RI, reference interval. All variables were analyzed with repeated-measures ANOVA. All variables changed during gestation:  $P < 0.001$  for tHcy, vitamin B-12, choline, and betaine;  $P = 0.01$  for folate; and  $P = 0.02$  for DMG. Data at each time point were compared with data for the preceding time point by Student's  $t$  test for paired samples. The results were corrected for multiple testing by using the Bonferroni method to minimize type 1 errors.

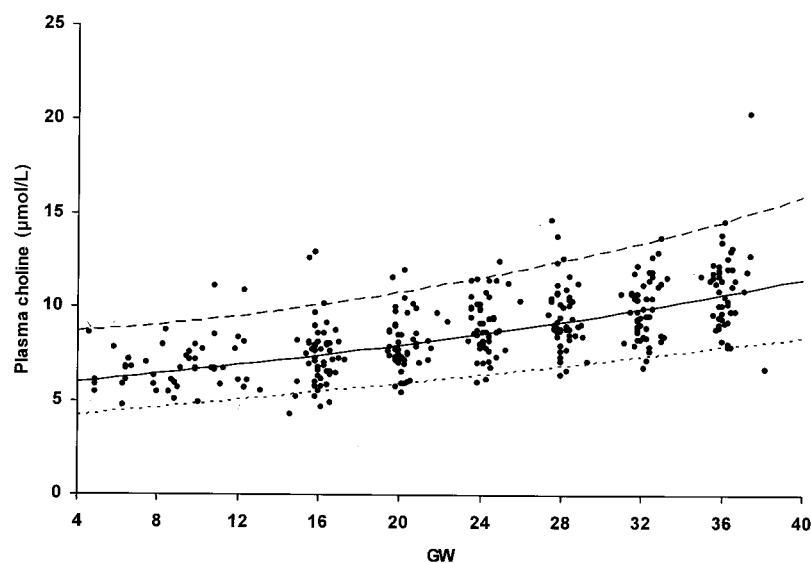
<sup>2-4</sup> Significantly different from previous time point (Student's  $t$  test): <sup>2</sup> $P < 0.001$ , <sup>3</sup> $P < 0.05$ , <sup>4</sup> $P < 0.01$ .

<sup>5</sup> Significantly different from GW 9,  $P < 0.001$  (Student's  $t$  test).

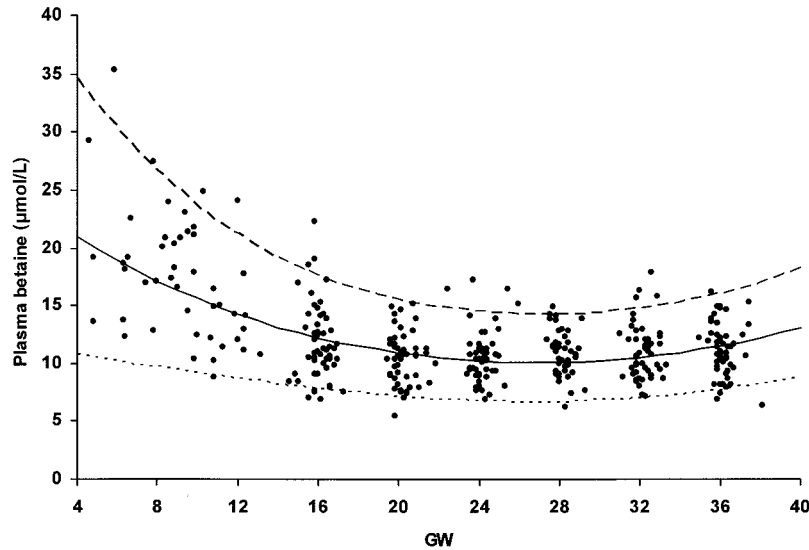
### Multiple regression analysis

Predictors of plasma tHcy in all 50 women were determined by multiple regression. The model included maternal age, folate, vitamin B-12, choline, betaine, DMG, creatinine, and hematocrit as independent variables. Plasma folate, vitamin B-12, betaine, and hematocrit were significantly associated with plasma tHcy. The results for plasma folate, vitamin B-12, and betaine are listed

in **Table 3**. Plasma folate was the strongest predictor of tHcy at GW 9 ( $P = 0.01$ ) and postpartum ( $P < 0.0001$ ), whereas plasma betaine was the strongest predictor at GW 20 ( $P = 0.02$ ), GW 24 ( $P = 0.04$ ), GW 28 ( $P = 0.003$ ), and GW 36 ( $P < 0.0001$ ). Plasma vitamin B-12 was related to tHcy only at GW 16 ( $P = 0.04$ ; Table 3). Hematocrit was related to tHcy only at GW 36 ( $P = 0.03$ ). A repeat of the multiple regression analyses after the



**FIGURE 2.** Plasma choline throughout normal human pregnancy. Blood samples were taken from 50 women at the initial visit [gestational week (GW) 9] and subsequently at GW 16, 20, 24, 28, 32, and 36. Data are presented as individual values (●) and the 5th (lower, - - -), 50th (—), and 95th (upper, - - -) percentiles, as estimated with a linear mixed model for repeated measurements (19).



**FIGURE 3.** Plasma betaine throughout normal human pregnancy. Blood samples were taken from 50 women at the initial visit [gestational week (GW) 9] and subsequently at GW 16, 20, 24, 28, 32, and 36. Data are presented as individual values (●) and the 5th (lower, - - -), 50th (—), and 95th (upper, - - -) percentiles, as estimated with a linear mixed model for repeated measurements (19).

exclusion of 7 women who reported not taking folic acid supplements gave essentially the same results (data not shown).

**DISCUSSION**

In the present longitudinal study, we measured metabolites and vitamins involved in one-carbon metabolism at 7 time points during the course of uncomplicated pregnancies and once postpartum in 50 women.

Plasma tHcy, betaine, DMG, and vitamin B-12 decreased during pregnancy, whereas folate increased. The decrease in plasma tHcy reached a nadir in the early third trimester (GW 28), which is in general agreement with previous reports (15–23). The observed increase in plasma folate concentrations during pregnancy may reflect the reported folic acid use of the women. The steady reduction in plasma vitamin B-12 was in agreement with

the findings of previous studies (28, 29). The novel and most important findings are a steady increase in plasma choline throughout gestation and an inverse relation between plasma betaine and tHcy with advancing gestation. There seems to be a concurrent attenuation of the folate-tHcy relation, especially in midtrimester.

Animal experiments have shown that tHcy in mice with low 5,10-methylenetetrahydrofolate reductase (MTHFR) activity was more responsive to betaine than was tHcy in wild-type animals (14). However, we did not determine the *MTHFR* 677C→T genotype in this study, but a previous study of 178 women from the same population showed a homozygous *MTHFR* 677C→T genotype frequency of only 1.7% (FV Velzing-Aarts et al, unpublished observations, 2000). Therefore, it is unlikely that the tHcy-folate and tHcy-betaine relations observed by us were significantly influenced by the *MTHFR* 677C→T polymorphism.

Two cross-sectional studies have addressed free choline concentrations in pregnancy (9, 10). Lower plasma choline concentrations were found in women at the time they gave birth than in nonpregnant women in an early study (9), and a more recent study showed higher serum free choline concentrations in the second and third trimesters of pregnant women than in nonpregnant women (10). These latter findings essentially agree with our finding of a progressive increase in choline throughout gestation (Figure 2). In the same studies, choline concentrations in the amniotic fluid (10) and in newborns (9, 10) were almost 2-fold those of maternal concentrations. Conceivably, the observed increase in maternal plasma choline ensures choline availability for (active) placental transfer (30) to meet fetal choline requirements (3).

In animals, maternal hepatic choline is mobilized to supply the placenta and fetus (6). Compared with nonpregnant rats, pregnant rats have a 24% higher hepatic activity of phosphatidylethanolamine-*N*-methyltransferase, which is the enzyme responsible for de novo choline synthesis (6). This increased de novo choline synthesis cannot compensate for the

**TABLE 2**

Univariate correlations (*r*) of plasma total homocysteine (tHcy) with plasma folate, vitamin B-12, and betaine throughout gestation and postpartum (PP)<sup>1</sup>

	tHcy and folate	tHcy and vitamin B-12	tHcy and betaine
GW 9	-0.351 <sup>2</sup>	-0.088	-0.077
GW 16	-0.352 <sup>2</sup>	-0.454 <sup>3</sup>	-0.440 <sup>3</sup>
GW 20	-0.470 <sup>3</sup>	-0.430 <sup>3</sup>	-0.322 <sup>2</sup>
GW 24	-0.306 <sup>2</sup>	-0.393 <sup>3</sup>	-0.456 <sup>3</sup>
GW 28	-0.289 <sup>2</sup>	-0.353 <sup>2</sup>	-0.379 <sup>3</sup>
GW 32	-0.311 <sup>2</sup>	-0.272	-0.258
GW 36	-0.377 <sup>3</sup>	-0.202	-0.472 <sup>3</sup>
PP	-0.534 <sup>4</sup>	-0.169	-0.378 <sup>2</sup>

<sup>1</sup> Blood samples were collected from 50 women with uncomplicated pregnancies at their initial visit [gestational week (GW) 9] and subsequently at GW 16, 20, 24, 28, 32, and 36. PP blood samples were obtained from 40 women approximately 14.5 wk after delivery. Univariate analysis was performed with Spearman's rank-sum test.

<sup>2</sup> *P* < 0.05.

<sup>3</sup> *P* < 0.01.

<sup>4</sup> *P* < 0.001.

TABLE 3

Predictors of plasma total homocysteine (tHcy) throughout gestation and postpartum (PP) by multiple regression analysis<sup>1</sup>

	$\beta$			B			$R^2$
	Folate	Vitamin B-12	Betaine	Folate	Vitamin B-12	Betaine	
GW 9	-0.405 <sup>2</sup>	-0.011	0.068	-0.297	-0.007	0.059	0.186
GW 16	-0.209	-0.296 <sup>2</sup>	-0.326	-0.144	-0.205	-0.332	0.396
GW 20	-0.253	-0.233	-0.402 <sup>2</sup>	-0.176	-0.163	-0.486	0.394
GW 24	-0.232	-0.222	-0.337 <sup>2</sup>	-0.134	-0.153	-0.486	0.421
GW 28	-0.067	-0.174	-0.507 <sup>3</sup>	-0.043	-0.122	-0.788	0.406
GW 32	-0.163	-0.173	-0.259	-0.076	-0.111	-0.327	0.222
GW 36	-0.276 <sup>2</sup>	0.065	-0.643 <sup>4</sup>	-0.139	0.045	-0.774	0.596
PP	-0.677 <sup>4</sup>	0.061	-0.117	-0.511	0.045	-0.097	0.608

<sup>1</sup> Blood samples were collected from 50 women with uncomplicated pregnancies at their initial visit [gestational week (GW) 9] and subsequently at GW 16, 20, 24, 28, 32, and 36. PP blood samples were obtained from 40 women approximately 14.5 wk after delivery.  $\beta$ , standardized coefficient; B, unstandardized coefficient. Multiple regression analysis was performed with plasma tHcy as a dependent variable and maternal age, plasma folate, vitamin B-12, betaine, choline, dimethylglycine, creatinine, and hematocrit as independent variables. Of the other independent variables, only hematocrit was related to plasma tHcy (at GW 36:  $\beta = -0.256$ ,  $P = 0.03$ ; data not shown).

<sup>2</sup>  $P < 0.05$ .

<sup>3</sup>  $P < 0.01$ .

<sup>4</sup>  $P < 0.0001$ .


mobilization of choline stores, as indicated by the depletion of maternal hepatic choline stores at the end of pregnancy (6). Thus, both mobilization of maternal choline stores and enhanced de novo choline synthesis may cause maternal plasma choline concentrations to rise. However, the net effect may be the diminution of maternal choline reserves. This emphasizes the importance of a higher dietary intake of choline during pregnancy.

A notable finding in this study was the inverse relation between plasma betaine and tHcy with advancing gestation. In nonpregnant subjects, plasma betaine is strongly related to the increase in tHcy after methionine loading, particularly in subjects who did not take B vitamin supplements (31). Plasma betaine is only weakly related to fasting tHcy in healthy subjects (13) and in patients with cardiovascular disease (14). The relation between fasting tHcy and betaine becomes more pronounced in subjects with low folate concentrations (13), but still does not reach the strength that we observed during pregnancy. Thus, the reported irregular folic acid use in our study population is expected to attenuate the association between tHcy and folate (31) and cannot explain the enforcement of the tHcy-betaine relation. The tHcy-betaine relation observed in the present study emphasizes the importance of betaine in one-carbon metabolism during pregnancy. The exact role of betaine is not known, but conserving methionine via a folate-independent route may be beneficial during pregnancy.

The hormonal environment of pregnancy, characterized by increased concentrations of estrogens (32) and cortisol (33), may affect BHMT-catalyzed homocysteine remethylation. The recently cloned human BHMT gene contains consensus sites for steroid hormone receptors, including estrogens and glucocorticoids (26). Cortisol is reported to increase BHMT gene expression (26), whereas cortisol-treated animals exert a 300% increase in liver BHMT activity (25). Estrogens are well-established tHcy-lowering agents in humans and may contribute to pregnancy-associated decreases in tHcy (15, 18, 20, 24).

Both cortisol (34) and estrogens (32, 35) increase continuously throughout gestation, which parallels our finding of a stronger betaine-tHcy relation with advancing gestation. Plasma betaine concentrations are nearly 50% lower during pregnancy

than at postpartum (Table 1) or in nonpregnant women (31). Hemodilution cannot explain this reduction, because plasma betaine concentrations were unrelated to hematocrit. Increased consumption through the BHMT pathway, distribution to intracellular compartments, placental transfer, and reduced synthesis from choline may account for the reduction. The latter may have a choline-sparing effect but may also reflect a suboptimal choline status.

In conclusion, the second half of pregnancy in humans is characterized by a progressive increase in plasma choline and a strong inverse relation between plasma betaine and tHcy. The steady increase in plasma choline throughout gestation may ensure choline availability for placental transfer, with subsequent use by the growing fetus. The inverse relation between plasma betaine and tHcy emphasizes the role of betaine during normal pregnancy. This relation also points to the possibility that a low betaine status may predispose to pregnancy complications associated with high tHcy (36). Our results emphasize the importance of choline and betaine status during normal human pregnancy and encourage further studies, including intervention trials. 

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FVV-A designed the study, participated in the organization of data collection, and wrote the manuscript. PIH and PMU performed the data analysis and contributed to the writing of the manuscript. MRF performed the statistical analysis and contributed to the writing of the manuscript. FPvdD participated in the organization of data collection. FAM contributed to the writing of the manuscript. No conflicts of interest were declared.

## REFERENCES

1. Zeisel SH. Choline: an essential nutrient for humans. *Nutrition* 2000; 16:669–71.
2. Zeisel SH. Choline and phosphatidylcholine. In: Shills ME, Olson JA, Shike M, Ross AC, eds. *Modern nutrition in health and disease*. Baltimore, MD: Lippincott Williams & Wilkins, 1998:513–23.
3. Zeisel SH, Blusztajn JK. Choline and human nutrition. *Annu Rev Nutr* 1994;14:269–96.
4. Zeisel SH, Mar MH, Zhou Z, da Costa KA. Pregnancy and lactation are

- associated with diminished concentrations of choline and its metabolites in rat liver. *J Nutr* 1995;125:3049–54.
5. Welsch F. Studies on accumulation and metabolic fate of (N-Me3H) choline in human term placenta fragments. *Biochem Pharmacol* 1976; 25:1021–30.
  6. 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.
  7. Zeisel SH. Choline: needed for normal development of memory. *J Am Coll Nutr* 2000;19:528S–31S.
  8. Institute of Medicine, National Academy Of Sciences USA. Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. Washington, DC: National Academy Press, 1998:390–422.
  9. McMahon KE, Farrell PM. Measurement of free choline concentrations in maternal and neonatal blood by micropyrolysis gas chromatography. *Clin Chim Acta* 1985;149:1–12.
  10. Ozarda Ilcol Y, Uncu G, Ulus IH. Free and phospholipid-bound choline concentrations in serum during pregnancy, after delivery and in newborns. *Arch Physiol Biochem* 2002;110:393–9.
  11. Selhub J. Homocysteine metabolism. *Annu Rev Nutr* 1999;19:217–46.
  12. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1998;49:31–62.
  13. Holm PI, Ueland PM, Vollset SE, et al. Betaine and folate status as cooperative determinants of plasma homocysteine in humans. *Arterioscler Thromb Vasc Biol* 2005;25:379–85.
  14. Schwahn BC, Chen Z, Laryea MD, et al. Homocysteine-betaine interactions in a murine model of 5,10-methylenetetrahydrofolate reductase deficiency. *FASEB J* 2003;17:512–4.
  15. Kang S-S, Wong PWK, Zhou J, Cook HY. Total homocyst(e)ine in plasma and amniotic fluid of pregnant women. *Metabolism* 1986;35: 889–91.
  16. Andersson A, Hultberg B, Brattström L, Isaksson A. Decreased serum homocysteine in pregnancy. *Eur J Clin Chem Clin Biochem* 1992;30: 377–9.
  17. Walker MC, Smith GN, Perkins SL, Keeley EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. *Am J Obstet Gynecol* 1999;180:660–4.
  18. Bonnette RE, Caudill MA, Boddie AM, Hutson AD, Kauwell GPA, Bailey LB. Plasma homocyst(e)ine concentrations in pregnant and non-pregnant women with controlled folate intake. *Obstet Gynecol* 1998;92: 167–70.
  19. Cikot RJLM, Steegers-Theunissen RPM, Thomas CMG, de Boo TM, Merkus HMWM, Steegers EAP. Longitudinal vitamin and homocysteine levels in normal pregnancy. *Br J Nutr* 2001;85:49–58.
  20. 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.
  21. López-Quesada E, Vilaseca M-A, Lailla JM. Plasma total homocysteine in uncomplicated pregnancy and in preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2003;108:45–9.
  22. López-Quesada E, Vilaseca M-A, Artuch R, Gómez E, Lailla JM. Homocysteine and other plasma amino acids in preeclampsia and in pregnancies without complications. *Clin Biochem* 2003;36:185–92.
  23. Powers RW, Majors AK, Kerchner LJ, Conrad KP. Renal handling of homocysteine during normal pregnancy and preeclampsia. *J Soc Gynecol Invest* 2004;11:45–50.
  24. Malinow MR, Rajkovic A, Duell PB, Hess DL, Upson BM. The relationship between maternal and neonatal umbilical cord plasma homocyst(e)ine suggests a potential role for maternal homocyst(e)ine in fetal metabolism. *Am J Obstet Gynecol* 1998;178:228–33.
  25. Finkelstein JD, Kyle W, Harris BJ. Methionine metabolism in mammals. Regulation of homocysteine methyltransferases in rat tissue. *Arch Biochem Biophys* 1971;146:84–92.
  26. Park EI, Garrow TA. Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J Biol Chem* 1999;274: 7816–24.
  27. 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.
  28. Koebnick C, Heins UA, Dagnelie PC, et al. Longitudinal concentrations of vitamin B(12) and vitamin B(12)-binding proteins during uncomplicated pregnancy. *Clin Chem* 2002;48(6):928–33.
  29. Carretti N, Eremita GA, Porcelli B, Paternoster D, Grella P. Pattern of vitamin B12 and folic acid during pregnancy. *Gynecol Obstet Invest* 1994;38:78–81.
  30. Sweiry JH, Page KR, Dacke CG, Abramovich DR, Yudilevich DL. Evidence of saturable uptake mechanisms at maternal and fetal sides of the perfused human placenta by rapid paired-tracer dilution: studies with calcium and choline. *J Dev Physiol* 1986;8:435–45.
  31. Holm PI, Bleie O, Ueland PM, et al. Betaine as a determinant of post-methionine load total plasma homocysteine before and after B-vitamin supplementation. *Arterioscler Thromb Vasc Biol* 2004;24:301–7.
  32. Lauritzen C, Kloppen A. Estrogens and androgens. In: Fuchs F, ed. *Endocrinology in pregnancy*. Philadelphia, PA: Harper & Row, 1983: 73–89.
  33. Ramsay ID, Davison RM. The adrenal gland. In: Chamberlain G, Pipkin FB, eds. *Clinical physiology in obstetrics*. Oxford, United Kingdom: Blackwell Science Ltd, 1998:385–95.
  34. Sasaki A, Shinkawa O, Yoshinaga K. Placental corticotropin-releasing hormone may be a stimulator of maternal pituitary adrenocorticotrophic hormone secretion in humans. *J Clin Invest* 1989;84:1997–2001.
  35. 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.
  36. 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.