

Choline and homocysteine interrelations in umbilical cord and maternal plasma at delivery¹⁻³

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ABSTRACT

Background: Little is known about the interactions between choline and folate and homocysteine metabolism during pregnancy despite the facts that pregnancy places considerable stress on maternal folate and choline stores and that choline is a critical nutrient for the fetus. Choline, via betaine, is an important folate-independent source of methyl groups for remethylating homocysteine in liver.

Objectives: Our aims were to examine the intermediates of choline oxidation in maternal and umbilical cord plasma and to determine the relations between this pathway and folate-dependent homocysteine remethylation.

Design: Blood samples were taken from 201 pregnant women and, at delivery, from the umbilical cord veins of their healthy, full-term infants. The blood samples were analyzed for plasma free choline, betaine, dimethylglycine, folate, vitamin B-12, total homocysteine (tHcy), and creatinine concentrations.

Results: Choline concentrations in umbilical cord plasma were ≈ 3 times those in maternal plasma (geometric \bar{x} : 36.6 and 12.3 $\mu\text{mol/L}$, respectively; $P < 0.0001$). Betaine and dimethylglycine concentrations were also significantly higher in umbilical cord than in maternal plasma. Choline was positively associated with tHcy ($r = 0.34$, $P < 0.0001$), betaine ($r = 0.58$, $P < 0.0001$), and dimethylglycine ($r = 0.30$, $P < 0.0001$) in maternal blood. Much weaker relations were seen in the fetal circulation. In a multiple regression model, choline was a positive predictor of maternal tHcy, whereas vitamin B-12 and betaine were negative predictors.

Conclusions: The positive association between maternal choline and tHcy during pregnancy suggests that the high fetal demand for choline stimulates de novo synthesis of choline in maternal liver, with a resultant increase in tHcy concentrations. If this is confirmed, it may be appropriate to provide choline supplements during pregnancy to prevent elevated tHcy concentrations. *Am J Clin Nutr* 2005;82:836-42.

KEY WORDS Choline, betaine, dimethylglycine, homocysteine, umbilical cord, neonate, pregnancy, folate, vitamin B-12

INTRODUCTION

Choline is a key nutrient with various metabolic roles ranging from an involvement in lipid metabolism and cell membrane structure and function to aspects of brain development and neurotransmission (1). Animal models have provided convincing evidence that choline is critical during fetal and neonatal life to ensure optimal brain and cognitive development (2-5). Consequently, the requirements of the fetus place a heavy burden on

maternal stores of choline (6). In the liver and kidney, the oxidation of choline is an important source of methyl groups for *S*-adenosylmethionine (SAM)-dependent methylation reactions, which are crucial for regulation of DNA expression, protein functions, and intermediary metabolism. This pathway intersects with the folate and vitamin B-12 metabolism pathway (Figure 1) in that either 5-methyltetrahydrofolate or betaine, the primary oxidative product of choline, can provide the methyl group for conversion of homocysteine to methionine, which is the precursor of SAM. One of the coproducts of the betaine homocysteine methyltransferase (BHMT) reaction, along with methionine, is dimethylglycine. Additional metabolism of dimethylglycine generates two more 1-carbon units, thereby recovering all carbons that originated as choline methyl groups into the folate cofactor pool (7, 8).

Although choline is principally provided from the diet, hepatic de novo synthesis as phosphatidylcholine is an important endogenous source in specific circumstances, such as during pregnancy (9-12). The de novo pathway is related to folate in that it involves three SAM-dependent methylation steps to convert phosphatidylethanolamine to phosphatidylcholine via the enzyme phosphatidylethanolamine *N*-methyltransferase (PEMT) (Figure 1). Evidence for a mutually supportive role for choline and folate is available from a variety of animal and human studies. These studies have shown that folate metabolism is disturbed during experimental choline deficiency (13-15), that choline becomes a limiting nutrient during folate deficiency or treatment

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² Supported by the National Institute of Child Health and Human Development, contract number NOI-HD-3-3348, in association with the Health Research Board of Ireland and supported by the Foundation to promote research into functional vitamin B12-deficiency. EU demonstration project BMH 4983549 and Abbott GmbH, Weisbaden-Delkenheim, Germany, contributed to homocysteine measurements.

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Received March 8, 2005.

Accepted for publication April 22, 2005.

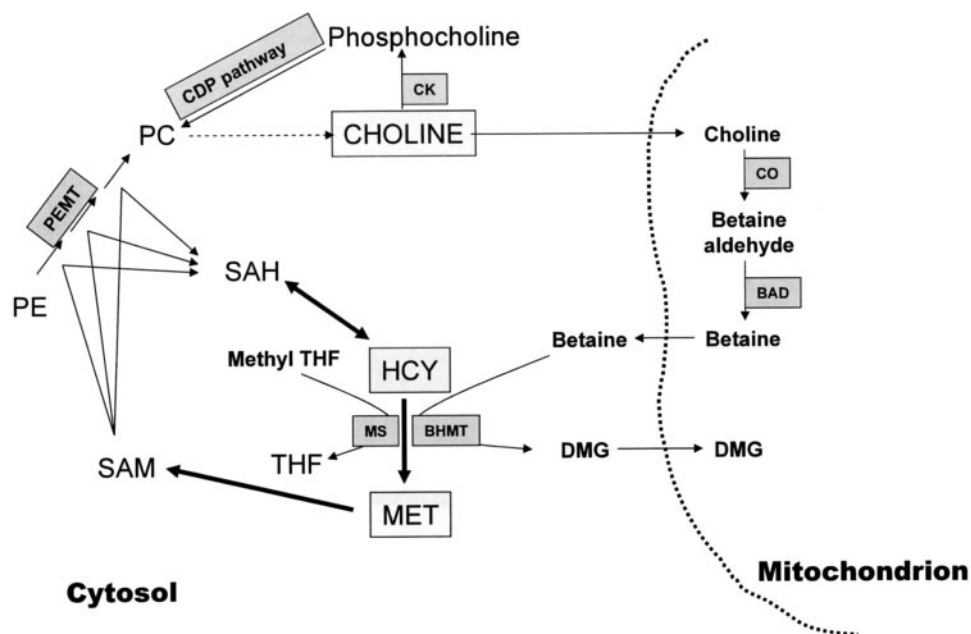


FIGURE 1. Choline and homocysteine interrelations in the liver. BAD, betaine aldehyde dehydrogenase; BHMT, betaine homocysteine methyltransferase; CDP, cytidine diphosphocholine; CK, choline kinase; CO, choline oxidase; DMG, dimethylglycine; HCY, homocysteine; MET, methionine; MS, methionine synthase; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; PC, phosphatidylcholine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

with the antifolate drug methotrexate (14–17), and that folate supplementation can ameliorate some of the adverse effects caused by low plasma choline status (18). Because of this, the United States Institute of Medicine has classified choline as an essential nutrient for humans and has made recommendations for adequate dietary intakes (19, 20).

Few population studies have examined the intermediates of choline oxidation under different life conditions, partly because of analytic difficulties, although serum choline concentrations have been described in various reports, and changes in choline resulting from diet, exercise, pregnancy, trauma, and renal disease have been noted (18, 21–30). Little information is available on blood betaine or dimethylglycine concentrations in health and disease (21, 31–35). Determination of the relative significance of this pathway during pregnancy is of particular interest in view of the important, but separate, roles of folate and choline in fetal development. The objective of the present study was thus to explore the relative importance of folate, vitamin B-12, and key intermediates of choline oxidation as predictors of total homocysteine (tHcy) concentrations in maternal and umbilical cord plasma.

SUBJECTS AND METHODS

Women who went to the Coombe Women's Hospital in Dublin, Ireland, for labor and delivery with uncomplicated pregnancies of 37–41 wk gestation were sequentially invited to participate in this cross-sectional study. During an approximate 3-month period, 201 women in whom there were no suspected fetal abnormalities, no suspicion of intrauterine growth retardation, and no maternal history of gestational diabetes, preeclampsia, or pregnancy-induced hypertension agreed to participate in the study. The study was approved by the Research Ethics Committee of the Coombe Women's Hospital, and written consent for the

collection of blood from both the mother and from the umbilical cord vein after delivery of her infant was obtained from all women. Because the study was conducted in the labor ward, the women were not requested to complete demographic questionnaires. Blood samples were made anonymous as soon as they were collected with the use of a labeled number for each mother and umbilical cord pair. Although it was not possible to abstract demographic or nutritional data from the women's hospital charts, we were aware that supplements containing iron with 400 μg folic acid were commonly prescribed to women during their pregnancies, with variable compliance; but no vitamin B-12 or other supplement was used by pregnant women in Ireland at that time. A 10-mL sample of maternal blood was collected into K-EDTA-coated tubes. After delivery, an umbilical vein blood sample was also taken into a 10-mL K-EDTA-coated tube. Maternal and cord blood samples were linked and numbered from 1 to 201 in chronological order of recruitment. The research midwife who recruited the participants for the study immediately placed the blood samples in containers at 4 °C in the labor ward and then processed them within 2 h of collection by centrifugation at $1200 \times g$ at ambient temperature for 15 min. Plasma samples were drawn off into separate tubes and were stored frozen at –20 °C.

Plasma free choline, betaine, and dimethylglycine concentrations were measured with the use of normal-phase liquid chromatography linked to tandem mass spectrometry (21). Recovery of all 3 compounds from the plasma matrix was 87–105%. The within- and between-day imprecision CVs for all 3 metabolites were between 2.1% and 8.8%. Free choline appears to be stable in EDTA-containing plasma, whereas in serum a linear increase in concentration of choline over time is observed at room temperature. The increase in choline in serum is presumably due to the hydrolysis of phosphatidylcholine, because minimal other

TABLE 1
Concentrations of the biochemical variables in maternal and umbilical cord blood

	Mothers	Umbilical cord	r^1
Choline ($\mu\text{mol/L}$)	12.3 (11.9, 12.8) ²	36.6 (34.9, 38.4) ³	0.06
Betaine ($\mu\text{mol/L}$)	9.52 (9.09, 9.96)	21.1 (20.4, 21.8) ³	0.40 ⁴
Dimethylglycine ($\mu\text{mol/L}$)	1.81 (1.71, 1.92)	2.45 (2.34, 2.56) ³	0.58 ⁴
Total homocysteine ($\mu\text{mol/L}$)	7.88 (7.51, 8.27)	7.40 (7.04, 7.78) ⁵	0.66 ⁴
Folate (nmol/L)	24.8 (22.3, 27.5)	44.8 (42.1, 47.6) ³	0.58 ⁴
Vitamin B-12 (pmol/L)	127 (120, 134)	216 (203, 230) ³	0.58 ⁴
Creatinine ($\mu\text{mol/L}$)	45.3 (43.9, 46.7)	44.7 (43.6, 45.8)	0.69 ⁴

¹ Pearson correlation coefficient on transformed data. Test for correlations between maternal and umbilical cord variables.

² Geometric \bar{x} ; 95% CI in parentheses (all such values).

^{3,5} Significantly different from mothers (paired Student's t test): ³ $P < 0.0001$, ⁵ $P < 0.01$.

⁴ $P < 0.0001$.

choline compounds exist in serum (21). Betaine and dimethylglycine are relatively stable both in EDTA-containing plasma and in serum. Plasma folate and vitamin B-12 were measured with the use of microbiological methods as previously described (36, 37). Inter- and intraassay CVs were $<11\%$ for plasma folate and $<12\%$ for vitamin B-12. Plasma tHcy concentrations were measured with an IMX assay (38). The inter- and intraassay CVs were $<5.0\%$.

Data analysis

Data were not normally distributed and were converted to ln values for analysis. For presentation of the data in all tables, variables are summarized as geometric means (95% CIs). We assessed correlations between maternal and umbilical cord variables by using the Pearson correlation coefficient on transformed data. For comparison of differences between maternal and umbilical cord concentrations, we used paired Student's t tests on the transformed data. To examine simultaneous relations between the choline oxidation metabolites and folate-related variables as predictors of tHcy concentrations in mothers and fetuses, we used 2 multiple linear regression models. Each regression analysis included an examination of residuals as a check on the required assumptions of normally distributed errors with constant variance. To check for outliers, we computed the SD of the residuals for each variable and then standardized these values. We defined an outlier as any value that had a standardized residual >3 or <-3 . Variance inflation factors [calculated as $1/(1-R^2)$] were used to measure the effect of multicollinearity as a confounding factor in the models, with values <2.5 considered to be acceptable for each variable.

In the maternal model, the maternal plasma tHcy concentration was the dependent variable and independent variables were the transformed maternal plasma folate, plasma vitamin B-12, plasma choline, and plasma betaine concentrations. Analyses were performed with and without ln plasma creatinine concentrations in the model because of reported associations of these metabolites with renal function (29, 31, 34). For the fetal model, the umbilical cord plasma tHcy concentration was the dependent variable and independent variables were the transformed values for umbilical cord plasma folate, umbilical cord vitamin B-12, umbilical cord choline, umbilical cord betaine, maternal tHcy, maternal plasma folate, maternal vitamin B-12, maternal choline, and maternal betaine concentrations. The model was analyzed with and without umbilical cord ln plasma creatinine concentrations. All statistical analyses were carried out with the use

of SAS software version 9.1 (SAS Institute Inc, Cary, NC). Significance was set at $P < 0.05$.

RESULTS

In preliminary scatter plot examinations of the raw data, the data for one subject for several of the relations were clear outliers and were removed before analysis. The data therefore relate to 200 mother–umbilical cord pairs. Values for 4 umbilical cord plasma folate concentrations are missing. In previous work on plasma folate, vitamin B-12, tHcy, and the 5,10-methylenetetrahydrofolate reductase 677C \rightarrow T genotype, we showed that the *TT* genotype did not predict umbilical cord tHcy concentrations in this cohort (39); therefore, we did not consider *TT* homozygotes separately in the present study. The data in the present study refer primarily to metabolites of the choline oxidation pathway and the inclusion of these metabolites, along with folate and vitamin B-12, as predictors of plasma tHcy concentrations.

Choline oxidation intermediates in maternal and umbilical cord plasma

Maternal and umbilical cord plasma choline, betaine, dimethylglycine, tHcy, folate, vitamin B-12, and creatinine concentrations are reported in **Table 1**. In the fetal circulation, choline concentrations were ≈ 3 times those in the maternal circulation ($P < 0.0001$); however, we did not observe any significant correlation between the choline concentrations in the 2 compartments ($r = 0.06$). Umbilical cord plasma betaine concentrations were more than twice the amount in maternal plasma ($P < 0.0001$), and dimethylglycine concentrations were also significantly higher in the fetal circulation than in maternal circulation ($P < 0.0001$), but the difference was not as dramatic. In contrast with choline, moderately strong correlations were observed between the maternal and fetal compartments for both betaine concentrations ($r = 0.40$) and dimethylglycine concentrations ($r = 0.58$). As previously reported (39), folate and vitamin B-12 concentrations were also higher in umbilical cord plasma than in maternal plasma, whereas tHcy concentrations were lower in umbilical cord than in maternal plasma.

Relations between choline and folate metabolism in maternal blood

Pearson's correlation coefficients between tHcy, related B vitamins, and metabolites of choline oxidation in maternal blood

TABLE 2

Pearson correlation coefficients between choline- and folate-related metabolites in 200 maternal blood samples¹

	Choline	Betaine	Dimethylglycine	Folate	Vitamin B-12
Total homocysteine	0.34 ²	-0.02	0.23 ³	-0.22 ³	-0.17 ⁴
Choline		0.58 ²	0.30 ²	-0.03	0.11
Betaine			0.34 ²	0.26 ³	0.18 ⁵
Dimethylglycine				-0.19 ⁵	-0.003
Folate					0.06

¹ Correlations were assessed on log-transformed data.

² $P \leq 0.0001$.

³ $P \leq 0.001$.

⁴ $P \leq 0.05$.

⁵ $P \leq 0.01$.

are presented in **Table 2**. A strong positive correlation was observed between choline and betaine in maternal plasma ($r = 0.58$). Dimethylglycine exhibited weaker positive associations with both choline ($r = 0.30$) and betaine ($r = 0.34$). A moderately strong positive association was observed between plasma choline and plasma tHcy ($r = 0.34$), but no significant correlations were observed between choline and either plasma folate or plasma vitamin B-12; however, both plasma folate and vitamin B-12 were positively associated with betaine in maternal blood. As expected, plasma tHcy showed significant negative correlations with both plasma folate and vitamin B-12. Creatinine was positively associated with all variables except plasma folate (data not shown).

We then estimated the predictors of tHcy in the maternal multiple linear regression model (**Table 3**). The model explained $\approx 28\%$ of the variance in maternal tHcy concentrations, and the influence of the nutrients was not substantially different when creatinine was removed ($R^2 = 0.24$). In this model, choline concentrations were a highly significant positive predictor of maternal plasma tHcy concentrations ($P < 0.0001$). As expected, plasma folate and vitamin B-12 were negative predictors of plasma tHcy concentrations ($P = 0.055$ and $P = 0.002$, respectively). Although betaine was not significantly correlated with tHcy when considered alone (Table 2), in the partial regression model, which is designed to take the relation of one variable into account after adjustment for all other variables, betaine also

TABLE 3

Regression analysis of predictors of plasma total homocysteine in mothers¹

Variable	β (95% CI)	P
Intercept	0.810 (-0.068, 1.688)	0.07
ln Folate	-0.060 (-0.121, 0.001)	0.055
ln Vitamin B-12	-0.179 (-0.291, -0.067)	0.002
ln Choline	0.579 (0.364, 0.794)	<0.0001
ln Betaine	-0.281 (-0.451, -0.111)	0.001
Creatinine	0.393 (0.184, 0.602)	0.0003

¹ A multiple regression analysis model was used ($n = 200$). The model accounted for 28% of the observed variability in maternal plasma total homocysteine ($R^2 = 0.28$). Variance inflation factors [determined as $1/(1 - R^2)$] were between 1.13 and 1.73, which indicated that multicollinearity did not have a major influence on the model. The model was not substantially different when analyzed without maternal creatinine ($R^2 = 0.24$).

TABLE 4

Pearson correlation coefficients between choline- and folate-related metabolites in 200 umbilical cord blood samples¹

	Choline	Betaine	Dimethylglycine	Folate ²	Vitamin B-12
Total homocysteine	0.11	-0.12	0.11	-0.25 ³	-0.30 ⁴
Choline		0.23 ³	-0.10	-0.01	0.13
Betaine			0.14 ⁵	0.08	0.04
Dimethylglycine				-0.05	-0.08
Folate					-0.03

¹ Correlations were assessed on log-transformed data.

² Four umbilical cord plasma folate values are missing.

³ $P \leq 0.001$.

⁴ $P \leq 0.0001$.

⁵ $P \leq 0.05$.

emerged as a highly significant negative predictor of tHcy concentrations ($P = 0.001$). Colinearity was not a factor because variance inflation factors were < 1.73 for all variables.

Fetal choline oxidation and maternal-fetal relations

In umbilical cord plasma, relations between the metabolites of the choline oxidation pathway were much weaker than in the maternal circulation (**Table 4**). Choline was significantly associated with betaine ($r = 0.23$, $P = 0.001$) but not with dimethylglycine or tHcy. We previously showed that maternal tHcy is a major predictor of fetal plasma tHcy; therefore, in choosing a model to determine the predictors of umbilical cord tHcy, we included maternal variables as well as umbilical cord variables (**Table 5**). The model accounted for 58% of the total variability in umbilical cord tHcy concentrations. Umbilical cord creatinine concentrations had negligible effects on tHcy concentrations ($R^2 = 0.57$ without creatinine). As previously shown (39), maternal tHcy concentrations were the most important predictor of umbilical cord tHcy concentrations ($P < 0.0001$), with umbilical cord and maternal vitamin B-12 concentrations being significant negative predictors of umbilical cord tHcy concentrations ($P =$

TABLE 5

Regression analysis of predictors of plasma total homocysteine (tHcy) in umbilical cord blood¹

Variable	β (95% CI)	P
Intercept	2.232 (1.378, 3.086)	<0.0001
ln Neonatal folate	-0.022 (-0.119, 0.075)	0.66
ln Neonatal vitamin B-12	-0.135 (-0.234, -0.036)	0.008
ln Neonatal choline	0.099 (-0.006, 0.204)	0.065
ln Neonatal betaine	-0.081 (-0.251, 0.089)	0.36
Neonatal creatinine	0.138 (-0.075, 0.351)	0.20
ln Maternal tHcy	0.635 (0.525, 0.745)	<0.0001
ln Maternal folate	-0.044 (-0.101, 0.013)	0.14
ln Maternal vitamin B-12	-0.138 (-0.248, -0.028)	0.018
ln Maternal choline	-0.245 (-0.425, -0.065)	0.009
ln Maternal betaine	0.044 (-0.100, 0.188)	0.56

¹ A multiple regression analysis model was used ($n = 196$). The model accounted for 58% of the observed variability in umbilical cord plasma tHcy ($R^2 = 0.58$). Variance inflation factors [determined as $1/(1 - R^2)$] were between 1.16 and 2.06, which indicated that multicollinearity did not have a major influence on the model. Removal of umbilical cord creatinine from the model did not significantly change the variables ($R^2 = 0.57$).

0.008 and $P = 0.018$, respectively). In the present study, umbilical cord choline concentrations were marginally significant positive predictors of umbilical cord tHcy concentrations ($P = 0.06$), but maternal choline concentrations were negative predictors of umbilical cord tHcy concentrations ($P = 0.009$).

DISCUSSION

The results of the present study show that during pregnancy, maternal plasma choline has a highly significant positive association with plasma tHcy ($r = 0.34$). This is consistent with findings from a study by Holm et al (31), who found a significant positive association between choline and plasma tHcy ($r = 0.11$, $P < 0.05$) in a cohort of 500 nonpregnant subjects, and with the positive but nonsignificant association ($r = 0.11$, $P = 0.1$) found in the present study between choline and tHcy in umbilical cord plasma. It seems unlikely that homocysteine per se would cause concentrations of choline to rise or that choline would cause the concentration of tHcy to rise. We therefore suggest that this positive association might arise in 2 ways. First, it might indicate an upregulated activity of the PEMT pathway, which would generate both choline and homocysteine to cope with the needs of the fetus and neonate. This pathway produces 3 mol of S-adenosylhomocysteine (and thus homocysteine) per 1 mol of newly synthesized phosphatidylcholine (Figure 1). Increased flux through the PEMT pathway would cause mothers with higher choline concentrations in plasma to also have higher tHcy concentrations, because both are indirect products of this pathway. Recent studies that used a genetic knockout mouse model for the *PEMT* gene showed that flux through this enzyme is an important source of plasma tHcy (40, 41). The mouse model also showed that PEMT function is sex-specific and thereby provides a greater proportion of liver and plasma phosphatidylcholine in females than males (42, 43) and is a crucial source of choline metabolites during pregnancy (10). Because free choline appears to be the major metabolite supplied to the fetus (44–47), it is likely that the PEMT pathway ultimately contributes to the maternal plasma pool of free choline, as reflected in the relation between choline and tHcy seen in the present study. If this is true, then optimal dietary choline intakes during pregnancy, as currently recommended (20), may help to meet the maternal demands on this pathway, prevent elevated maternal tHcy concentrations, and ensure adequate fetal and neonatal choline status.

Alternatively, our finding might be a direct consequence of depletion of the maternal hepatic choline stores. If hepatic choline is being mobilized and released into the circulation for delivery to the fetus (6, 23), the resultant low hepatic choline concentrations could cause reduced remethylation of homocysteine via BHMT. Several studies in humans have shown that depleted choline status is associated with elevated tHcy (13, 18). However, the strong correlation between maternal choline and betaine concentrations in this study ($r = 0.58$) argues against this hypothesis and indicates that maternal choline oxidation remains functional during pregnancy, as was suggested in animal studies (6, 44, 45).

Our study shows that choline oxidation remains an essential source of hepatic methyl groups in the mother, despite conflicting demands for choline imposed by the fetus. This is indicated by strong positive correlations in the maternal circulation between the 3 principal components of choline oxidation: free


choline, betaine, and dimethylglycine (Table 2). These correlations were similar to previous findings, which used the same technology, from a mixed group of healthy adults (21). Our data also agree with previous findings that showed betaine to be a predictor of tHcy (31, 32, 48, 49). Furthermore, our finding of a significant association between dimethylglycine, which is a co-product of the BHMT reaction (Figure 1), and tHcy ($r = 0.23$) in maternal plasma is consistent with a strong flux of choline toward the remethylation of homocysteine.

We observed a 3-fold accumulation of choline in the fetal circulation. This is a relatively high concentration gradient, but it agrees with findings of animal studies (50) and of some smaller studies in humans (23–25, 50). Many nutrients are 1- to 2-fold higher in the fetal circulation than in the maternal circulation. Betaine and dimethylglycine appear to fall into this general category, and we previously found similar relations for folate and vitamin B-12 (39). However, the greater gradient for choline probably reflects the importance of the placental active transport system for this compound (46, 47) that is designed to meet the high choline requirements of the developing fetus (3, 19). We found no significant association between the concentrations of choline in maternal and umbilical cord plasma. Because the placenta itself is a major site for choline utilization (47), the concentration of free choline in the fetal circulation is more likely to be related to placental and fetal metabolism and distribution than to the original maternal source of the nutrient. One previous study, which incorporated 25 mother and neonate pairs within a larger cohort of neonates and infants, also found no significant association between choline concentrations in maternal blood and umbilical cord venous blood, although a strong correlation in choline concentrations between maternal blood and umbilical cord arterial blood was found (24).

The concentrations of free choline in umbilical cord and maternal blood found in our study were similar to those found in previous studies of pregnant women (23–25). In the present observational study, we did not include a nonpregnant cohort. However, the median choline concentration in our maternal plasma samples at delivery was $\approx 20\%$ higher than the nonfasting median value for an unselected adult cohort in a study that used the same technology (21). This agrees with a previous study that also showed higher concentrations of free choline in pregnant women than in nonpregnant women (23). Interestingly, our findings showed that the concentrations of two products of choline oxidation, betaine and dimethylglycine, were lower in the pregnant women than in the nonpregnant women in the study by Holm et al (21). In light of the decreases in the concentrations of many maternal blood components, the moderate increase in maternal plasma free choline concentrations during pregnancy is unusual and may reflect a mobilization of free choline to meet the high fetal demands for this nutrient (23).

In contrast, we found no convincing evidence for an important contribution of the choline oxidation pathway to homocysteine remethylation in the fetal circulation. In umbilical cord plasma, associations between these metabolites were weak and could have been residual from the maternal blood as a result of diffusion from the maternal to the fetal circulation. The strong correlations between maternal and umbilical cord betaine concentrations ($r = 0.40$) and between maternal and umbilical cord dimethylglycine concentrations ($r = 0.58$) are consistent with these metabolites being derived from the maternal circulation.

The lack of association between dimethylglycine and homocysteine also suggests low fetal BHMT activity and agrees with previous studies that showed that high concentrations of betaine are excreted by the neonate (51, 52).

In conclusion, the present study has identified important links between choline metabolism and homocysteine production during normal pregnancies. Our results suggest that one source of elevated tHcy concentrations during pregnancy may be the maternal requirement to synthesize new choline, because fetal demands for choline exceed dietary intake. Elevated maternal tHcy concentrations are a risk factor for several adverse pregnancy events (53); therefore, our hypothesis needs urgent confirmation followed by intervention to ensure optimal choline intakes during pregnancy. 

We thank the Master and staff of the Coombe Women's Hospital, Dublin, Ireland, for their assistance in subject recruitment. None of the authors had any conflicts of interest.

AMM was involved in hypothesis generation, sample analysis, data interpretation, and manuscript preparation. CC was responsible for statistical analysis and data interpretation. JLM contributed to hypothesis generation, data interpretation, and manuscript preparation. JMS contributed to hypothesis generation, study design, and data analysis and interpretation. LCB contributed to hypothesis generation and data interpretation. MC contributed to data handling and analysis. PMU was responsible for sample analysis, data interpretation, and manuscript preparation. PNK contributed to study design and data interpretation. SFD was responsible for subject recruitment and sample collection.

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