Relationship of Dimethylglycine, Choline, and Betaine with Oxoproline in Plasma of Pregnant Women and Their Newborn Infants¹,²

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Abstract

Choline and glycine are inter-related through their roles in methyl metabolism. Choline is metabolized to betaine, which donates a methyl group to homocysteine to form methionine, also generating dimethylglycine, which is further metabolized to glycine. Choline is transported across the placenta and is higher in fetal than maternal plasma. Placental glycine transfer, however, is limited and poor glycine status has been suggested in preterm infants. Insufficient glycine for glutathione (GSH) synthesis results in increased metabolism of γ-glutamyl cysteine to 5-oxoproline. We measured plasma 5-oxoproline as a metabolic indicator to address whether choline, via dimethylglycine, contributes physiologically relevant amounts of glycine in pregnancy. Blood was collected from healthy term pregnant women and their newborn infants at delivery (n = 46) and nonpregnant healthy women (n = 19) as a reference group. Plasma choline, betaine, dimethylglycine, homocysteine, methionine, and 5-oxoproline were quantified by HPLC-tandem MS. Plasma choline was 45% higher, but betaine was 63% lower and dimethylglycine was 28% lower in pregnant than nonpregnant women (P < 0.01). Higher white blood cell choline dehydrogenase messenger RNA levels in a random subset of pregnant (n = 8) than nonpregnant women (n = 7) (P < 0.01) suggest increased betaine and dimethylglycine turnover rather than decreased synthesis. Plasma choline, betaine, and dimethylglycine were higher (P < 0.001) in fetal plasma (36.4 ± 13, 29.4 ± 1.0, and 2.44 ± 0.12 μmol/L, respectively) than maternal plasma (15.3 ± 0.42, 14.1 ± 0.6 and 1.81 ± 0.12 μmol/L, respectively). Concentrations of 5-oxoproline and dimethylglycine were inversely (P < 0.05) correlated in maternal (Spearman rho = −0.35) and fetal plasma (Spearman rho = −0.32), suggesting that choline, via dimethylglycine, contributes glycine for GSH synthesis in human development.  J. Nutr. 137: 2641–2646, 2007.

Introduction

Choline is an essential nutrient that functions in multiple physiological pathways that can be broadly divided into 3 categories: 1) as a component of phosphatidylcholine and sphingomyelin, which are critically important in cell membrane lipids, lung surfactant, bile lipids, and plasma lipoproteins; 2) as a part of the neurotransmitter acetylcholine; and 3) as an important factor in methyl metabolism as a source of 1-carbon groups for remethylation of homocysteine to methionine and for the folate pool (1–6). Choline transport across the placenta involves choline transporters (7,8) and fetal plasma choline concentrations are ~3-fold higher than in maternal plasma at term gestation (9). However, plasma choline concentrations vary over 5-fold in infants at birth (10), suggesting the pathways for maternal to fetal choline transfer are not saturated and may be influenced by maternal choline nutrition. In this regard, recent studies show that although the average choline and betaine intakes are ~320 and 210 mg/d, respectively, in the United States, individual intakes vary widely (11,12) and case-control studies have provided evidence that low choline and betaine intakes are associated with increased risk of neural defects in humans (11).

Glycine plays a central role in many metabolic processes, including collagen and elastin synthesis, conjugation of bile acids, synthesis of purines, porphyrins, creatine, and glutathione (GSH)³ (13). GSH, which is formed from cysteine, glutamate, and glycine, is the most abundant intracellular nonprotein molecule and plays vital roles in the protection against oxidative stress and regulation of protein and cell DNA synthesis (14). Although not considered a dietary essential amino acid, endogenous glycine production pathways are incompletely understood. Available estimates suggest that endogenous formation of glycine is 5–10 times higher than the dietary glycine intake (13,15). Furthermore, glycine accumulation is thought to be

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2 Abbreviations used: GSH, glutathione; mRNA, messenger RNA; SHMT, serine hydroxymethyltransferase.
2- to 10-fold higher than for any other amino acid during pregnancy (16). Currently available evidence suggests that maternal to fetal glycine transfer is limited in humans and other species (13,16–18). Fetal plasma glycine concentrations are only 10% that of maternal plasma glycine concentrations (19), suggesting that glycine may be a conditionally essential for preterm infants (15).

Glycine and serine are readily interchangeable via the enzymatic step catalyzed by serine hydroxymethyltransferase (SHMT) (Fig. 1), the enzyme involved in conversion of tetrahydrofolate to 5,10-methyl tetrahydrofolate (4,20,21). Transplacental serine transport and subsequent metabolism by SHMT in the fetal liver or placenta serine uptake and metabolism could provide a source of glycine for the fetus. However, although the sheep placenta produces large amounts of glycine (22), the activity of SHMT in the human placenta is low (21), consistent with the low plasma levels of glycine in the human newborn (16). Relevant to this study, further metabolism of betaine via dimethylglycine generates glycine and also serves to regenerate methionine from homocysteine and transfer methyl groups to the mitochondrial folate pool (Fig. 1) (4,5). The addition of glycine to γ-glutamyl cysteine by glutathione synthetase generates GSH (14,23), but when glycine is limited, further metabolism of γ-glutamyl cysteine by γ-glutamyl cyclotransferase results in increased 5-oxoproline (23–27). In this study, we sought evidence of a relationship between 5-oxoproline and dimethylglycine as a metabolic indicator that choline and betaine may be an important source of glycine for GSH in infant development.

**Subjects and Methods**

**Subjects.** The subjects in this study were a convenience sample of healthy pregnant women (n = 46) admitted to the low-risk delivery unit at the B.C. Women’s Hospital. Eligible participants were 20–40 y of age at 37–41 wk of gestation and expected to deliver a single, term infant with no known maternal or fetal complications and were not taking any fatty acid or phospholipid supplements. A convenience sample of nonpregnant women (n = 19) with no known health problems, 18–45 y of age, consuming a diet with no dietary restrictions, and not taking any fatty acid or phospholipid supplements were enrolled as a reference sample. All of the procedures were reviewed and approved by the University of British Columbia Ethics Committee and the Ethics Board at the British Columbia Children’s Hospital. All participants gave written informed consent before participation.

**Blood collection.** Venous blood samples were collected from all subjects at the B.C. Children’s and Women’s Hospital. Maternal blood was collected on admission, concurrent with the routine collection of blood as part of clinical care. Fetal cord (newborn infant) blood was collected immediately after delivery and clamping of the cord (28). Immediately after collection, the samples were placed on ice, transferred to the nutrition research laboratory, and the plasma and red cells separated by centrifugation within 20 min of collection, then stored at −80°C until analysis as previously described (28).

**Biochemical analysis by LC-MS/MS.** Choline, betaine, dimethylglycine, 5-oxoproline, methionine, and total homocysteine were analyzed by HPLC-MS/MS. The MS/MS is a Quattro Micro tandem MS configured with an electrospray source and operated in positive ion mode, coupled to an Acquity HPLC equipped with a thermostatted autosampler (Waters Corporation). Three analyses were conducted on each sample. For analysis of methionine and homocysteine, 50 µL of plasma was transferred to a 1.5-mL Eppendorf tube containing 10 µL of homocysteine-d₈ (0.05 mmol/L) and methionine-d₄ (0.1 mmol/L) as internal standards, 10 µL of dithiothreitol (300 mmol/L in 0.1 mol/L NaOH) was added, the mixture was vortexed, and the samples kept at room temperature for at least 15 min to allow reduction of the disulfide bonds. Proteins were then precipitated by addition of 100 µL acetonitrile containing 0.2% (v/v) heptfluorobutyric acid, the samples centrifuged 18,000 × g; 5 min at 5°C, and an aliquot of supernatant (20 µL) transferred to an autosampler vial containing 100 µL of the HPLC mobile phase. Chromatographic separation of homocysteine and methionine was achieved using a Zorbax SB Aqua 2.1-× 100-mm column, 3.5-µm particle size packing with a 2.1-× 12.1-mm precolumn, 5-µm particle size packing (Agilent Technologies Canada) with a mobile phase of H₂O with 0.2% heptfluorobutyric acid. The flow rate was 0.25 mL/min, the injection volume was 4 µL, and the column temperature was maintained at 25°C. For analysis of 5-oxoproline, 50 µL of plasma was transferred to a 1.5-mL Eppendorf tube, 10 µL 5-oxoproline-d₃ (0.26 mmol/L) was added, the sample was vortexed, and 100 µL acetonitrile containing 0.1% (v/v) formic acid was added to precipitate protein. The sample was centrifuged at 13,400 rpm × 5 min at 5°C and 20 µL supernatant transferred to an autosampler vial to which 100 µL of the HPLC mobile phase was added. The analysis of

![FIGURE 1 Schematic of the metabolic inter-relations of choline, glycine, and 5-oxoproline.](Image)

- PC: phosphatidylcholine
- PC: phosphatidylcholine
- 4: dimethylglycine dehydrogenase
- 5: methyltetrahydrofolate homocysteine methyltransferase
- 6: serine hydromethyltransferase
- 7: 5,10-methylene tetrahydrofolate reductase
- 8: methionine adenosyltransf erase
- 9: γ-glutamyl cysteine synthetase
- 10: α-glutamyl transpeptidase
- 11: s-adenosylhomocysteine hydrolase
- 12: γ-glutamyl cyclotransferase
5-oxoproline used a Zorbax RX-SIL 2.1-× 150-mm column with 5-μm particle size packing and 2.1-× 12.1-mm precolumn with 5-μm particle size packing with a mobile phase of 10% H2O, 90% acetonitrile, 0.1% formic acid, and 15 mM/ L ammonium formate. We used a column flow rate of 0.40 mL/min, injection volume of 5 μL, and a column temperature of 25°C for the analysis. Choline, betaine, and dimethylglycine were analyzed by HPLC-MS/MS using deuterated internal standards as recently described in detail (29).

Relative quantification of choline dehydrogenase expression by real-time PCR. Because we found higher plasma choline but lower betaine concentrations in pregnant women compared with nonpregnant women, we also sought evidence of a possible decreased conversion of choline to betaine in pregnancy. We used real-time PCR to determine gene expression of choline dehydrogenase, an enzyme that is expressed in white blood cells. Total RNA was extracted from blood cells of a random subset of pregnant women and nonpregnant women using the RNeasy Mini kit (Qiagen) with DNase I treatment to digest contaminating genomic DNA. RNA integrity was assessed by confirming the presence of 18S and 28S ribosomal RNA (rRNA) on an agarose gel. RNA (500 ng) was reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) in a final reaction volume of 40 μL. Gene expression levels were measured by real-time PCR using the comparative Ct method (ΔΔCt) of relative quantification with commercially available primers and TaqMan MGB probes (Applied Biosystems) specific for choline dehydrogenase (Hs00294875_m1) and β-actin as the endogenous control. Samples (2 μL of cDNA) were incubated with 1× TaqMan Universal PCR mix and 1× TaqMan primer/probe mix in a final reaction volume of 20 μL and incubated in a 7500 Real Time PCR system (Applied Biosystems) at 50°C for 2 min, and 95°C for 10 min followed by 50 cycles at 95°C for 15 s, and 60°C for 1 min. Data were analyzed using the 7500 System Sequence Detection software, version 1.2.3 (Applied Biosystems). Each sample was analyzed in duplicate and the entire experiment was repeated 3 times.

Statistical analysis. All data were analyzed using the Statistical Package for Social Sciences (SPSS for Windows, version 15.0, SPSS). A One-Sample Kolmogorov-Smirnov Test was used to test whether plasma choline, betaine, dimethylglycine, 5-oxoproline, methionine, and homocysteine are normally distributed prior to analysis. Nonparametric Mann-Whitney U tests were to detect differences between plasma metabolites in nonpregnant and pregnant women and between pregnant women and their fetuses. Results are presented as medians (25th–75th percentile) with means and SE provided for reference. Spearman’s rho tests were used to detect relationships between metabolites in maternal and fetal plasma. Independent 2-tailed t tests were used to compare choline dehydrogenase expression between pregnant and nonpregnant women. P < 0.05 was considered significant.

Results
The plasma choline concentration was 45% higher in pregnant women at term gestation compared with the reference group of nonpregnant women (P < 0.01) and was 58% higher in fetal plasma than in maternal plasma (P < 0.01) (Table 1). In contrast to higher plasma choline, the plasma concentration of betaine was 63% lower and the concentration of dimethylglycine was 28% lower in pregnant than in nonpregnant women (P < 0.01). Similar to choline, betaine was >50% higher and dimethylglycine was >25% higher in the fetal plasma than in the maternal plasma (P < 0.01). In the nonpregnant women, the plasma concentration of betaine was higher than that of choline or dimethylglycine. In contrast, the major plasma metabolite in women at term gestation was choline, so the relative plasma concentrations of choline, betaine, and dimethylglycine were 1.0:4.3:5.0:3 in nonpregnant women but 1.0:0.9:0.1 in the pregnant women.

The plasma 5-oxoproline concentration was 56% higher in the pregnant women than in the reference group of nonpregnant women and it was also higher in fetal plasma compared with maternal plasma (P < 0.05). We included analysis of plasma methionine and homocysteine because of the important role of betaine as a methyl donor for remethylation of homocysteine to methionine (1,3,6). The median plasma concentration of homocysteine was modestly lower in the pregnant women than in the reference group of nonpregnant women (P < 0.01) (Table 1). We also found a lower plasma homocysteine concentration in fetal plasma than in the maternal plasma (P < 0.01). However, although the plasma methionine concentration did not differ between the pregnant women and the reference group of nonpregnant women, the fetal plasma concentration was higher than in their mothers (P < 0.05) (Table 1). The plasma methionine:homocysteine ratios were 3.3 ± 0.27 in the reference group of nonpregnant women, 3.5 ± 0.11 in pregnant women at term gestation, and 5.7 ± 0.17 in newborn infants (cord plasma).

The higher plasma choline but lower betaine in pregnant women than in nonpregnant women could be explained by decreased metabolism of choline to betaine, or alternatively by increased turnover of betaine and subsequently dimethylglycine to support remethylation of homocysteine and provide methyl groups to the 1-carbon folate pool with the generation of glycine. To address the latter, we determined the expression of choline dehydrogenase, the first step in choline metabolism. The expression of β-actin messenger RNA (mRNA) did not differ between the 2 groups of women (data not shown). The expression of choline dehydrogenase mRNA relative to β-actin was higher, not lower, in pregnant women than in nonpregnant women (Fig. 2) (P < 0.05). An important question is the extent to which the maternal plasma choline, betaine, dimethylglycine, and 5-oxoproline are transferred to and contribute the levels of the same metabolite in nonpregnant women (P < 0.01) and was 58% higher in fetal plasma than in maternal plasma (P < 0.01) (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Nonpregnant (n = 19)</th>
<th>Maternal (n = 46)</th>
<th>Cord (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline</td>
<td>7.8 (7.15–10.1)</td>
<td>14.3 (13.1–17.0)**</td>
<td>15.3 ± 0.42</td>
</tr>
<tr>
<td>Betaine</td>
<td>35.9 (31.4–45.5)</td>
<td>13.9 (10.6–16.3)**</td>
<td>14.1 ± 0.60</td>
</tr>
<tr>
<td>Dimethylglycine</td>
<td>2.3 (1.82–2.11)</td>
<td>1.6 (1.32–2.03)**</td>
<td>1.8 ± 0.12</td>
</tr>
<tr>
<td>5-Oxoproline</td>
<td>13.8 (12.2–15.3)</td>
<td>41.5 (37.0–47.0)**</td>
<td>44.4 ± 1.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>22.6 (20.3–27.5)</td>
<td>21.2 (19.4–25.2)</td>
<td>22.6 ± 0.66</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>6.86 (5.9)</td>
<td>6.35 (5.9–7.1)**</td>
<td>6.8 ± 0.21</td>
</tr>
</tbody>
</table>

Note: Values are medians (25th–75th percentile) and means ± SEM. Symbols indicate significant differences: *vs. nonpregnant women, P < 0.05; **vs. maternal, P < 0.05 (Mann Whitney U).

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fetal plasma. Concentrations of choline, betaine, and dimethylglycine in maternal plasma were all correlated ($P < 0.05$) with concentrations of the same metabolite in the fetal plasma (Table 2). Consistent with the precursor-product relationship, the plasma choline concentration was also related ($P < 0.01$) to that of betaine in both maternal and fetal. However, the relationship between betaine and dimethylglycine was significant only in maternal plasma ($P < 0.05$). The maternal and fetal plasma were not associated. However, the concentration of dimethylglycine was significantly and inversely related to that of 5-oxoproline in both maternal and fetal plasma (Table 2).

### Discussion

To the best of our knowledge, this is the first study to address a possible role for dimethylglycine, which is generated from the metabolism of choline and betaine in providing glycine for synthesis of GSH. Previous studies have shown that plasma choline levels increase early in pregnancy and that plasma choline levels are higher in fetal than in maternal plasma ($5, 9, 30$). Maternal to fetal transfer of glycine, on the other hand, appears to be limited and glycine has been suggested to be a conditionally essential amino acid in preterm infants ($13, 15–18$). The ability to synthesize adequate amounts of nonessential amino acids is determined by the abundance and activity of the appropriate enzyme systems and an adequate supply of the required substrates and cofactors. Methods for direct quantification of glycine requirements during development or to assess the capacity for de novo synthesis of glycine are currently lacking ($15$). GSH is the major nonprotein intracellular thiol and is formed from glycine, glutamate, and cysteine ($14, 23$), but when the supply of glycine is limiting, $\gamma$-glutamyl-cysteine is further metabolized to oxoproline (Fig. 1) ($31, 32$). We therefore used HPLC-MS/MS analysis of plasma 5-oxoproline and dimethylglycine as a metabolic probe of whether dimethylglycine is likely to contribute glycine for the synthesis of GSH. We interpret our results to show a significant inverse relationship between dimethylglycine and 5-oxoproline in the plasma of pregnant women and in their newborn infants as evidence to support our suggestion of an important physiological link between the choline-betaine-dimethylglycine pathway and the supply of glycine for GSH synthesis.

Although choline is known to be transported across the placenta ($7, 8, 33$), we found no published studies to indicate whether or not dimethylglycine or its betaine precursor is transferred across the human placenta or that of other commonly studied animals. The results of our study showed positive associations between the maternal and fetal plasma concentrations of betaine ($rho 0.37$) ($P < 0.05$) and particularly dimethylglycine ($rho 0.73$) ($P < 0.01$) increases the possibility of maternal to fetal transport of both of these metabolites. The absence of any relationship between the maternal and fetal plasma 5-oxoproline ($rho -0.03$), on the other hand, suggests that the inverse relationships between dimethylglycine and 5-oxoproline reflects glycine metabolism in the maternal or fetal compartment rather than maternal to fetal 5-oxoproline transfer. We suggest the need for further, more specific studies to determine whether dimethylglycine is transported across the human placenta and the extent to which choline and betaine nutrition, or nutrient deficiencies, gene polymorphisms, or other factors that alter the methionine-homocysteine cycle also influence dimethylglycine and, thus, 5-oxoproline.

The results of this study show significantly higher plasma 5-oxoproline levels in pregnant women at term gestation than in nonpregnant women are consistent with previous studies

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Choline</th>
<th>Betaine</th>
<th>Dimethylglycine</th>
<th>5-Oxoproline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline</td>
<td>Maternal</td>
<td>0.35**</td>
<td>0.43**</td>
<td>0.37*</td>
</tr>
<tr>
<td>Cord</td>
<td>1</td>
<td>0.19</td>
<td>0.41**</td>
<td>-0.13</td>
</tr>
<tr>
<td>Betaine</td>
<td>Maternal</td>
<td>1</td>
<td>0.46**</td>
<td>0.38**</td>
</tr>
<tr>
<td>Cord</td>
<td>1</td>
<td>-0.06</td>
<td>0.21</td>
<td>-0.28</td>
</tr>
<tr>
<td>Dimethylglycine</td>
<td>Maternal</td>
<td>1</td>
<td>0.73**</td>
<td>0.17</td>
</tr>
<tr>
<td>Cord</td>
<td>1</td>
<td>0.11</td>
<td>-0.32*</td>
<td></td>
</tr>
<tr>
<td>5-Oxoproline</td>
<td>Maternal</td>
<td>1</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>Cord</td>
<td>1</td>
<td></td>
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</tbody>
</table>

$^1$ Values are Spearman rho correlation coefficients for 46 matched maternal-fetal plasma pairs. $^*P < 0.05$; $^{**}P < 0.01$ (two-tailed test).
showing elevated 5-oxoproline levels in the urine of pregnant women (34,35). To our knowledge, we provide the first results to show high plasma 5-oxoproline levels in infants at birth, suggesting glycine may limit GSH synthesis in the fetus and neonate. Several diseases in premature infants, including bronchopulmonary dysplasia, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity, and necrotizing enterocolitis have been linked to oxidative stress, decreased GSH, and free radical-mediated tissue injury (36–40). Dietary choline deficiency is known to increase markers of oxidative stress, with an overaccumulation of reactive oxygen species in rats (41). Recently, we also showed that choline supplementation increased plasma GSH and improved the glutathione redox balance in children with cystic fibrosis with low plasma levels of choline (42). Thus, an important link between choline, betaine, and dimethylglycine and the response to oxidative stress through the provision of glycine for GSH seems plausible. However, more specific studies will be needed that include measures of plasma GSH and GSSG, as well as choline, betaine, and dimethylglycine.

Previous studies have shown that whereas plasma choline concentrations are increased, plasma betaine and dimethylglycine concentrations are decreased in pregnancy (5,9,30). The increased plasma choline but decreased betaine in pregnant women could reflect decreased metabolism of choline to betaine or increased betaine and, subsequently, increased dimethylglycine turnover. We found increased rather than decreased white cell mRNA for choline dehydrogenase in pregnant women, suggesting that decreased conversion of choline to betaine is not a likely explanation for the lower betaine and dimethylglycine in pregnant women. However, we note that the major sites of choline dehydrogenase activity are the liver and kidney and it is not known whether changes in choline dehydrogenase gene expression in white blood cells reflect that in other tissues or whether changes in mRNA for this enzyme are necessarily accompanied by changes in enzyme activity. Regardless, we suggest that the pattern of increased choline and decreased betaine in pregnant women appears most consistent with increased utilization of choline, perhaps in providing methyl groups for the 1-carbon folate pool, for regeneration of methionine from homocysteine and also as a source of glycine. However, folate deficiency results in upregulation of the choline-dependent remethylation of homocysteine (3), which raises the possibility that the women in our study were folate deficient. Fortification of flour became mandatory in Canada in 1998 and has been effective in increasing dietary folate intake and lowering total plasma homocysteine in men, women, and children (43–45). The plasma homocysteine concentrations in the nonpregnant women (7.64 ± 0.40 μmol/L) in this study are lower than those previously reported for men and women in Canada (9.7 μmol/L) and the US (10.5 μmol/L) prior to fortification and are lower than the plasma homocysteine levels reported for men and women in Europe (10.5 μmol/L), where fortification of the food supply with folate is not present (46–49). Further, in our study, the plasma total homocysteine was significantly lower, not higher, in pregnant women than in the reference group of nonpregnant women. Folate insufficiency leading to changes in enzyme activity. Regardless, we suggest that the pattern of increased choline and decreased betaine in pregnant women appears most consistent with increased utilization of choline, perhaps in providing methyl groups for the 1-carbon folate pool, for regeneration of methionine from homocysteine and also as a source of glycine. However, folate deficiency results in upregulation of the choline-dependent remethylation of homocysteine (3), which raises the possibility that the women in our study were folate deficient. Fortification of flour became mandatory in Canada in 1998 and has been effective in increasing dietary folate intake and lowering total plasma homocysteine in men, women, and children (43–45). The plasma homocysteine concentrations in the nonpregnant women (7.64 ± 0.40 μmol/L) in this study are lower than those previously reported for men and women in Canada (9.7 μmol/L) and the US (10.5 μmol/L) prior to fortification and are lower than the plasma homocysteine levels reported for men and women in Europe (10.5 μmol/L), where fortification of the food supply with folate is not present (46–49). Further, in our study, the plasma total homocysteine was significantly lower, not higher, in pregnant women than in the reference group of nonpregnant women. Folate insufficiency leading to increased dependence on betaine for remethylation of homocysteine, therefore, seems an unlikely explanation for the lower betaine in the pregnant women compared with nonpregnant women in this study.

In summary, choline and betaine play important roles as a source of methyl groups for the remethylation of homocysteine and for the mitochondrial folate pool (4,5). This study provides novel data to show a significant inverse relation between dimethylglycine and 5-oxoproline in pregnant women and newborn infants, raising the question of whether dimethylglycine, and its choline and betaine precursors, may play an important role in generating glycine, an amino acid potentially limiting in early human development. The close inter-relationship of the choline-betaine-dimethylglycine pathway with the generation of glycine, and the role of glycine in GSH, could possibly contribute to the link between methyl metabolism and oxidative stress both in preterm infants and in diseases such as nonalcoholic fatty liver disease.

**Literature Cited**


