Pregnancy alters choline dynamics: results of a randomized trial using stable isotope methodology in pregnant and nonpregnant women

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ABSTRACT

Background: Although biomarkers of choline metabolism are altered by pregnancy, little is known about the influence of human pregnancy on the dynamics of choline-related metabolic processes.

Objective: This study used stable isotope methodology to examine the effects of pregnancy on choline partitioning and the metabolic activity of choline-related pathways.

Design: Healthy third-trimester pregnant (n = 26; initially week 27 of gestation) and nonpregnant (n = 21) women consumed 22% of their total choline intake (480 or 930 mg/d) as methyl-d9-choline for the final 6 wk of a 12-wk feeding study.

Results: Plasma d9-betaine:d9-phosphatidylcholine (PC) was lower (P ≤ 0.04) in pregnant than in nonpregnant women, suggesting greater partitioning of choline into the cytidine diphosphate-choline (CDP-choline) PC biosynthetic pathway relative to betaine synthesis during pregnancy. Pregnant women also used more choline-derived methyl groups for PC synthesis via phosphatidylethanolamine N-methyltransferase (PEMT) as indicated by comparable increases in PEMT-PC enrichment in pregnant and nonpregnant women despite unequal (pregnant > nonpregnant; P < 0.001) PC pool sizes. Pregnancy enhanced the hydrolysis of PEMT-PC to free choline as shown by greater (P < 0.001) plasma d3-choline:d3-PC. Notably, d3-PC enrichment increased (P ≤ 0.011) incrementally from maternal to placental to fetal compartments, signifying the selective transfer of PEMT-PC to the fetus.

Conclusions: The enhanced use of choline for PC production via both the CDP-choline and PEMT pathways shows the substantial demand for choline during late pregnancy. Selective partitioning of PEMT-PC to the fetal compartment may imply a unique requirement of PEMT-PC by the developing fetus. This trial was registered at clinicaltrials.gov as NCT01127022.

INTRODUCTION

Choline is an essential nutrient (1) that serves as a precursor molecule for the synthesis of phospholipids and the methyl donor betaine. On entering the cytidine diphosphate-choline (CDP-choline) pathway, choline can be used to produce phosphatidylcholine (PC), a constituent of all cellular membranes and a main component of circulating lipoproteins. Alternatively, choline can be oxidized to betaine, which serves as a source of methyl groups for the synthesis of methionine and S-adenosylmethionine (SAM), the principal methylating agent in mammalian cells. As a methyl group source for SAM, choline is also used to generate PC via the phosphatidylethanolamine N-methyltransferase (PEMT) pathway (2), which catalyzes the SAM-dependent sequential trimethylation of phosphatidylethanolamine.

The demand for choline is high during pregnancy (3, 4) because large amounts of PC are needed for cellular division, tissue expansion, and lipoprotein synthesis. In a 12-wk feeding study examining the impact of pregnancy on biomarkers of choline metabolism, we observed alterations in the concentrations of several choline metabolites (primary endpoint variables) in pregnant (compared with nonpregnant) women including plasma depletion of choline-derived methyl donors and elevations in plasma choline (5). By using stable isotope methodology performed after our initial report (5), the current study sought to provide mechanistic insights into the underlying causes of the pregnancy-induced alterations in choline metabolism. Specifically, we measured the isotopic enrichment of choline-related metabolites in the blood and urine (primary endpoint variables) of our study participants who had consumed methyl-d9-choline during the last half of the 12-wk feeding study (5). As shown in Figure 1, methyl-d9-choline is labeled with deuterium on all 3 methyl groups, which enables assessment of the partitioning of choline (d9-choline metabolite) and its methyl groups (d3-metabolites) among the various choline-related metabolic pathways (2) as well as evaluations of PC biosynthesis via the PEMT pathway (6, 7).

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4 Abbreviations used: CDP-choline, cytidine diphosphate-choline; DMG, dimethylglycine; LC-MS/MS, liquid chromatography–tandem mass spectrometry; PC, phosphatidylcholine; PEMT, phosphatidylethanolamine N-methyltransferase; SAM, S-adenosylmethionine; SM, sphingomyelin.

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Deuterium-labeled methyl-d9-choline traces the distribution of orally consumed choline and its methyl groups. Black circles indicate deuterium-labeled methyl groups; white circles indicate unlabeled methyl groups. The administered methyl-d9-choline can enter the CDP-choline pathway to produce d9-PC or it can be oxidized to d9-betaine, which donates a methyl group to homocysteine, producing d6-DMG and d3-methionine. d3-Methionine serves as a precursor to d3-SAM, which can be used by PEMT to sequentially methylate PE, forming d3-PC as well as smaller amounts of d6-PC (4). Hydrolysis of d3-PC will generate d3-choline as well as d3-betaine, d3-methinone, d3-DMG, and d3-sarcosine when d3-choline is used as a methyl donor. CDP-choline, cytidine diphosphate-choline; DMG, dimethylglycine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SAR, sarcosine; SM, sphingomyelin.

SUBJECTS AND METHODS

Study participants

Twenty-six healthy, third-trimester, singleton pregnant women (aged 22–34 y; initially 27–29 wk of gestation) and 21 nonpregnant women (aged 21–40 y) participated in this study between January 2009 and December 2010 (5). The sample size of the parent study (5) was based on a power calculation that predicted differences of 20% in biomarkers of choline metabolism (ie, choline and betaine) with a power of 80% at an \( \alpha \) of 0.05 between choline intake (ie, 480 compared with 930 mg/d) and reproductive (ie, pregnant compared with nonpregnant women) groups. Entry into this study was contingent on good health status as assessed by a blood chemistry profile, complete blood count, and health history questionnaire. Additional inclusion criteria included the following: no tobacco or alcohol product use, no history of chronic disease, normal kidney and liver function, and a willingness to comply with the study protocol. Screening participants were excluded if they had pregnancy-associated complications or were taking prescription medications known to affect liver function. A detailed flow of study participants throughout the screening and intervention phases has been reported previously (5). The study protocol was approved by the Institutional Review Board for Human Study Participant Use at Cornell University and at Cayuga Medical Center (the hospital where pregnant participants delivered their infants; Ithaca, NY). Written informed consent was obtained from each participant.

Study design

This was a controlled feeding study in which third-trimester pregnant and nonpregnant women were randomly assigned to receive either 480 (approximating the choline Adequate Intake for pregnant women) or 930 mg choline/d for 12 wk as previously detailed (5). Parallel random assignment of pregnant or nonpregnant women into different choline intake groups was conducted by simple random assignment and performed by the study’s principal investigator. Participants and research staff responsible for data collection were blinded to participant treatment allocation.

Choline was obtained from the diet (380 mg/d) and from supplemental choline chloride. During the first 6 wk of the study, unlabeled choline (Balchem), either 100 or 550 mg/d, was consumed by the 480- or 930-mg choline/d intake group, respectively. During the final 6 wk of the study, 22% of the total choline intake was consumed as methyl-d9-choline chloride (Cambridge Isotope Laboratories Inc). During this period, women in the 480-mg choline/d group consumed 100 mg/d as methyl-d9-choline (Cambridge Isotope Laboratories Inc.), whereas women in the 930-mg choline/d intake group consumed 200 mg methyl-d9-choline/d (Cambridge) mixed with 350 mg unlabeled choline/d (Balchem). The choline supplements were prepared by study personnel (5) and consumed as a single bolus with a meal. All participants also consumed a prenatal multivitamin (Pregnancy Plus; Fairhaven Health LLC), labeled as providing 600 \( \mu \) g folic acid, 2.6 \( \mu \) g vitamin B-12, and 1.9 mg vitamin B-6. In addition, all participants consumed DHA (200 mg, Neuromins; Nature’s Way Products) and a potassium and magnesium supplement (General Nutrition Corp) (5).

Throughout the 12-wk feeding phase, meals and snacks were prepared in the Human Metabolic Research Unit at Cornell University. Study participants were required to consume at least one meal per day, and their supplements, in the metabolic unit Monday through Friday. All other food items and supplements were provided as takeaways, and the consumption of each item was verified through the use of a daily checklist provided by the investigators (5). For the period between completion of the 12-wk feeding study and delivery, pregnant women continued to consume their assigned choline supplement. Maternal blood and urine were collected throughout the feeding phase of the study; a maternal blood sample, placenta tissue, and cord blood were obtained at delivery (5, 8).

Sample collection and processing

Fasting maternal blood samples were collected into serum separator gel and clot-activator tubes (SST Vacutainer; Becton, Dickinson, and Company) and EDTA-coated tubes (Vacutainer) throughout the 12-wk study. Serum and plasma were obtained and stored at \(-80^\circ\text{C}\) as previously described (5, 9, 10). Twenty-four-hour urine samples were collected throughout the 12-wk study and processed and stored at \(-80^\circ\text{C}\) (5). Maternal delivery blood samples were collected into EDTA-coated tubes at the hospital within 24 h before delivery. Cord venous blood samples were also collected into EDTA-coated...
tubes at the time of delivery. Maternal delivery blood and cord venous blood were processed for plasma and stored at −80°C (5). In addition, the placenta was obtained at delivery, processed at the hospital within 90 min of delivery, and stored at −80°C (5, 8).

**Measurements of plasma, urine, and placental choline metabolites**

PC and sphingomyelin (SM) were extracted from plasma (study weeks 9, 10, and 12), maternal delivery plasma, placental tissue, and venous cord plasma. The PC and SM isotopomers (d0, d3, d6, and d9) were quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) according to the method of Koc et al (11) with modifications including the use of d4-PC rather than d9-PC as an internal standard (2). Ions with an m/z ratio of 184 were monitored for unlabeled metabolites (d0-PC and d0-SM), ions with a m/z ratio of 188 were monitored for d4-PC, and ions with m/z ratios of 187, 190, and 193 were monitored for d3-labeled metabolites (d3-PC and d3-SM), d6-labeled metabolites (d6-PC and d6-SM), and d9-labeled metabolites (d9-PC and d9-SM), respectively. Because the d4-PC internal standard yielded ions with m/z ratios of 188 (the ions monitored for d4-PC) and 190 (the ions monitored for d6-PC), a second run without the addition of d4-PC was conducted for plasma at study weeks 9, 10, and 12. The second run was used to determine enrichments of the PC isotopomers (ie, d3-PC divided by the sum of d0-, d3-, d6-, and d9-PC) as well as the ratios of d3-PC to d0-PC, d6-PC to d0-PC, and d9-PC to d0-PC. These ratios were subsequently used to calculate the concentrations of d3-, d6-, and d9-PC. For example, to calculate d3-PC, we multiplied the d0-PC concentration by the d3-PC:d0-PC ratio. For maternal delivery plasma, placental tissue, and venous cord plasma, a separate run without the addition of d4-PC was not conducted due to limited sample volume. Therefore, we did not include the d6-PC peak area in the denominator when calculating d3-PC and d9-PC enrichments. Because the d6-PC represented ~0.2% of total PC, d3-PC and d9-PC enrichments of maternal delivery plasma, placental tissue, and venous cord plasma (representing ~2% and 10% of total PC, respectively) were slightly overestimated.

Free choline, betaine, and dimethylglycine (DMG) were extracted from plasma (study weeks 9, 10, and 12), maternal delivery plasma, placental tissue, and venous cord plasma. The isotopomers of these metabolites (d0, d3, d6, and d9) were quantified by LC-MS/MS according to the method of Holm et al (12) with modifications including the use of d13-choline, d3-betaine, and d3-DMG, rather than d9-choline, d9-betaine, and d9-DMG, as internal standards. The ions with m/z ratios monitored for each metabolite were as follows: d0-choline, 104→60; d3-choline, 107→63; d6-choline, 110→66; d9-choline, 113→69; d13-choline, 117→69; d0-betaine, 118→59; d3-betaine, 121→62; d6-betaine, 124→65; d9-betaine, 127→68; d0-DMG, 104→58; d3-DMG, 107→61; and d6-DMG, 110→64. As the internal standards, d3-betaine and d3-DMG were also target metabolites for quantification; a separate run without the addition of internal standards was conducted to measure plasma and urine enrichments (as described above). Because of the limited sample volume, separate runs without the addition of d3-betaine and d3-DMG were not conducted for maternal delivery plasma, placental tissue, and venous cord plasma. Therefore, the calculation of d9-betaine and d6-DMG did not include d3-betaine (~2% of total betaine) and d3-DMG (~2% of total DMG), respectively, in the denominators, leading to a slight overestimation of d9-betaine and d6-DMG for maternal delivery plasma, placental tissue, and venous cord plasma.

Sarcosine and methionine were extracted from serum (study week 12) and urine (study week 12). Gas chromatography–mass spectrometry (13, 14) was used to quantify the isotopomers (d0 and d3) of these metabolites.

SAM was extracted from urine (study week 12), and its isotopomers (d0 and d3) were quantified by LC-MS/MS as described by Kim et al (15) with modifications (8).

**Isotopic enrichment and PEMT activity calculations**

Isotopic enrichment [labeled metabolite/(labeled + unlabeled metabolites)] was examined in blood (ie, plasma or serum), urine, and placenta to monitor the flow of dietary choline (d9-choline metabolites) and its methyl groups (d3-choline metabolites) through the choline metabolic pathways. The isotopic enrichment percentage was calculated by using the peak area under the chromatography curve of the labeled metabolite divided by the total area of all isotopomers of the metabolite. For example, d3-PC enrichment = peak area of d3-PC/peak area of (d0+d3+d6+d9)-PC × 100%. The enrichment of d3-PC indicates the proportion of PEMT-derived d3-PC in the total plasma PC pool. Metabolite enrichment ratios were examined as indicators of the flow of orally consumed choline through a specific portion of a pathway (ie, enrichment of product/enrichment of precursor) or its partitioning between pathways (eg, CDP-choline pathway compared with the choline oxidative pathway).

Under the current labeling strategy, the amount of PEMT-derived d9-PC (~0.02% of plasma total PC) was lower than the detection limits (~0.1% of plasma total PC). Therefore, we assumed that all of the measured d9-PC was derived from the CDP-choline pathway.

Based on the principle of mass isotopomer distribution analysis (6, 7), the mass spectra abundances of d0-, d3-, and d6-PC isotopomers were used to delineate the effect of pregnancy and choline intake on the hepatic enrichment of d3-SAM and the activity of PEMT-mediated PC synthesis (which mainly occurs in the liver and is an indicator of the flow of choline-derived methyl groups through the PEMT pathway). Details regarding the calculations and equations are provided in the Supplemental Methods under “Supplemental data” in the online issue.

**Statistical analysis**

Twenty-one nonpregnant and 26 pregnant women completed the 12-wk study. However, study-end (week 12) samples were not available for 3 pregnant women (1 participant in the 480-mg choline/d group and 2 participants in the 930-mg choline/d group) because of early delivery in the final week of study. For these missing data, a multiple imputation procedure (16, 17) was conducted using all available study-end enrichment data to yield 20 imputed complete data sets.

Plots and histograms of the residuals were used to assess normality and variance homogeneity of dependent variables. All dependent variables fit the assumption of the ANOVA model.

To delineate the effect of pregnancy on study-end (week 12) enrichments of the choline metabolites, linear regression models were constructed that included pregnancy, choline intake, and their interaction term (pregnancy × choline intake) as independent variables. If the interaction term was significant at P ≤ 0.05,
data were stratified by choline intake, and the effect of pregnancy on metabolite enrichments was examined for women consuming 480 or 930 mg choline/d separately.

To evaluate the main effect of choline intake on the dependent variables at week 12, linear regression models, which included choline intake as an independent variable, were constructed for pregnant and nonpregnant women separately.

Additional factors considered in the linear regression models included age, serum folate concentrations, ethnicity, and 6 genetic variants in one-carbon metabolic genes (see Supplemental Table S1 under “Supplemental data” in the online issue). BMI was considered as a covariate when assessing the effect of pregnancy. Nonsignificant factors ($P > 0.1$) were progressively removed until final models were derived.

Samples collected at delivery included 24 placentas, 21 cord plasma samples, and 21 maternal plasma samples. For the missing material (version 20; SPSS Inc). Differences were considered significant at $P \leq 0.05$, whereas $P < 0.10$ was indicative of trends.

### RESULTS

**Isotopic enrichment of choline metabolites at study end (week 12)**

At study end, labeled choline metabolites were detected at acceptable signal-to-noise ratios in blood and urine samples. Because blood and urinary enrichments of the same metabolite were highly correlated (Pearson’s correlation coefficient $r \geq 0.7$, $P < 0.001$), urinary enrichments are presented as supplemental material (see Supplemental Tables S2 and S3 under “Supplemental data” in the online issue).

**Main effect of pregnancy on the isotopic enrichment of choline-related metabolites**

At study end, pregnant (compared with nonpregnant) women had lower plasma enrichment of d9-choline ($P = 0.016$), d6-DMG

### TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpregnant women ($n = 21$)</th>
<th>Pregnant women ($n = 26$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood enrichment percentages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d9-Choline</td>
<td>7.9 ± 0.3</td>
<td>7.0 ± 0.3</td>
<td>0.016</td>
</tr>
<tr>
<td>d9-Betaine</td>
<td>9.9 ± 0.4</td>
<td>7.5 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d6-DMG</td>
<td>34 ± 1.0</td>
<td>28 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d3-Choline</td>
<td>2.6 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>0.33</td>
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<tr>
<td>d3-Betaine</td>
<td>1.8 ± 0.08</td>
<td>2.0 ± 0.08</td>
<td>0.085</td>
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<td>d3-Sarcosine</td>
<td>26 ± 0.8</td>
<td>24 ± 0.8</td>
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</tr>
<tr>
<td>d3-Methionine</td>
<td>0.58 ± 0.07</td>
<td>0.75 ± 0.07</td>
<td>0.07</td>
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<tr>
<td>d3-PC</td>
<td>1.27 ± 0.05</td>
<td>1.06 ± 0.06</td>
<td>0.006</td>
</tr>
<tr>
<td>d6-PC</td>
<td>0.31 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.011</td>
</tr>
<tr>
<td>d9-PC</td>
<td>9.4 ± 0.3</td>
<td>8.7 ± 0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>d9-SM</td>
<td>9.6 ± 0.3</td>
<td>8.8 ± 0.3</td>
<td>0.055</td>
</tr>
<tr>
<td>Hepatic d3-SAM$^2$</td>
<td>0.19 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.025</td>
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<tr>
<td>Blood enrichment ratios</td>
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<tr>
<td>d9-Betaine:d9-choline</td>
<td>1.24 ± 0.03</td>
<td>1.08 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d9-Betaine:d9-PC</td>
<td>1.04 ± 0.03</td>
<td>0.87 ± 0.03</td>
<td>&lt;0.001</td>
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<td>d9-PC:d9-choline</td>
<td>1.20 ± 0.05</td>
<td>1.27 ± 0.05</td>
<td>0.28</td>
</tr>
<tr>
<td>d9-SM:d9-PC</td>
<td>1.02 ± 0.02</td>
<td>1.02 ± 0.02</td>
<td>0.94</td>
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<tr>
<td>d6-DMG:d9-betaine</td>
<td>3.49 ± 0.1</td>
<td>3.71 ± 0.1</td>
<td>0.11</td>
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<tr>
<td>d3-Methionine:d3+d9-betaine</td>
<td>0.05 ± 0.006</td>
<td>0.08 ± 0.007</td>
<td>0.001</td>
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<tr>
<td>d3-Sarcosine:d6-DMG</td>
<td>0.77 ± 0.02</td>
<td>0.87 ± 0.02</td>
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<td>d3+d6-PC:d9-PC</td>
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<td>0.15 ± 0.01</td>
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<td>d3-Choline:d3-PC</td>
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<td>2.74 ± 0.12</td>
<td>&lt;0.001</td>
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<tr>
<td>d3-Betaine:d3-choline</td>
<td>0.68 ± 0.03</td>
<td>0.72 ± 0.04</td>
<td>0.43</td>
</tr>
</tbody>
</table>

$^1$Values are pooled estimated marginal means ± SEMs from multiple imputation analyses. Women consumed either 480 or 930 mg choline/d. Choline intake groups were pooled to show differences between reproductive groups. Linear regression models including pregnancy and choline intake as predictors were used to compare the enrichment or enrichment ratio between pregnant and nonpregnant women. Age, ethnicity, serum folate, and genetic variants of one-carbon metabolic genes were included as covariates in the model if they were predictors of the dependent variable. DMG, dimethylglycine; PC, phosphatidylcholine; SAM, S-adenosylmethionine; SM, sphingomyelin.

$^2$Hepatic enrichment of d3-SAM was calculated by using the plasma enrichment of d3-PC and d6-PC according to the principle of mass isotopomer distribution analysis with the following equation: hepatic d3-SAM enrichment = d6-PC enrichment/(d3-PC enrichment + d6-PC enrichment).
pregnant compared with nonpregnant women, these data imply
Table S4 under “Supplemental data” in the online issue) in
pool was
from week 10 to week 12. However, given that the plasma PC
enrichment ratio of d3-methionine:(d3+d9)-betaine (P = 0.07) as well as a higher
enrichment ratio of plasma d3-choline:d3-PC was higher (P < 0.001) in pregnant (compared with nonpregnant) women consuming 930 mg choline/d. Pregnancy also interacted (P = 0.027) with choline intake to affect plasma enrichment of d9-betaine:d9-PC (Figure 2B).
Specifically, the enrichment ratio of d9-betaine:d9-PC was 9% lower (P = 0.044) in pregnant (compared with nonpregnant) women consuming 480 mg choline/d and was 22% lower (P < 0.001) in pregnant (compared with nonpregnant) women consuming 930 mg choline/d. These data collectively show that pregnancy (compared with nonpregnant) women partition more choline to the CDP-choline pathway and oxidize less choline to betaine; a difference that is magnified with the higher choline intake amount. Notably, the enrichment ratio of d9-betaine:d9-PC in pregnant women consuming 930 mg choline/d did not differ (P = 0.75) from that in nonpregnant women consuming 480 mg choline/d (Figure 2B), indicating that a higher choline intake is needed during pregnancy to ensure adequate partitioning of choline toward betaine.

Main effect of choline intake
Consumption of 930 (compared with 480) mg choline/d yielded greater enrichment of almost all of the choline metabolites at study end (Table 2). In all study participants, the plasma enrichment ratios of (d3+d6)-PC:d9-PC were higher (P = 0.009) in the 930-mg choline/d intake group than in the 480-mg choline/d intake group, suggesting that a higher choline intake favors the use of choline-derived methyl groups for PEMT-mediated PC synthesis relative to CDP-choline pathway–mediated PC synthesis.

FIGURE 2. Enrichment of plasma d9-betaine (A) and the enrichment ratio of plasma d9-betaine:d9-PC (B) in study-end (week 12) blood samples obtained from pregnant and nonpregnant women consuming 22% of 480 or 930 mg choline/d as methyl-d9-choline from study week 6 to week 12 (n = 10–13/group).
Linear regression models including pregnancy, choline intake, and their interaction term (pregnancy × choline intake) were constructed to assess the effects of pregnancy on dependent variables. Pregnancy interacted with choline intake to affect the dependent variables (P = 0.05 for plasma betaine enrichment (panel A); P = 0.027 for plasma d9-betaine:d9-PC (panel B)), thus data were stratified by choline intake, and the effects of pregnancy on dependent variables were examined for women consuming 480 or 930 mg choline/d separately. The enrichment ratio of d9-betaine:d9-PC in pregnant women consuming 930 mg choline/d did not differ (P = 0.75) from that in nonpregnant women consuming 480 mg choline/d. Select genetic variants of one-carbon metabolic genes were included as covariates in the model if they were predictors of the dependent variable. Values are presented as pooled estimated marginal means ± SEMs from multiple imputation analyses. NP, nonpregnant women; P, pregnant women; PC, phosphatidylcholine.
TABLE 2
Effect of choline intake on blood enrichment of choline metabolites and their ratios at study end (week 12) in nonpregnant and third-trimester pregnant women consuming 22% of 480 or 930 mg choline/d as methyl-d9-choline from study weeks 6 to 12\(^1\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpregnant women</th>
<th>Third-trimester pregnant women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>480 mg/d (n = 10)</td>
<td>930 mg/d (n = 11)</td>
<td>480 mg/d (n = 13)</td>
</tr>
<tr>
<td>Blood enrichment percentages</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>d9-Choline</td>
<td>7.2 ± 0.3</td>
<td>8.6 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d9-Betaine</td>
<td>7.9 ± 0.3</td>
<td>11.7 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d6-DMG</td>
<td>30 ± 0.8</td>
<td>37 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d3-Choline</td>
<td>2.2 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d3-Betaine</td>
<td>1.5 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d3-Sarcosine</td>
<td>23 ± 0.9</td>
<td>29 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d3-Methionine</td>
<td>0.49 ± 0.07</td>
<td>0.66 ± 0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>d3-PC</td>
<td>1.0 ± 0.06</td>
<td>1.5 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d6-PC</td>
<td>0.18 ± 0.02</td>
<td>0.44 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d9-PC</td>
<td>9.0 ± 0.3</td>
<td>9.9 ± 0.3</td>
<td>0.017</td>
</tr>
<tr>
<td>d9-SM</td>
<td>8.7 ± 0.3</td>
<td>10.4 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatic d3-SAM(^2)</td>
<td>0.15 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\) Values are presented as pooled estimated marginal means ± SEMs from multiple imputation analyses. Linear regression models including choline intake as a predictor were constructed to compare the enrichment or enrichment ratio between choline intake groups (480 compared with 930 mg choline/d) for nonpregnant women or pregnant women separately. Age, ethnicity, serum folate, BMI, and genetic variants of one-carbon metabolic genes were included as covariates in the model if they were predictors of the dependent variable. DMG, dimethylglycine; PC, phosphatidylcholine; SAM, S-adenosylmethionine; SM, sphingomyelin.

\(^2\) Hepatic enrichment of d3-SAM was calculated by using the plasma enrichment of d3-PC and d6-PC according to the principle of mass isotopomer distribution analysis with the following equation: hepatic d3-SAM enrichment = d6-PC enrichment/d3-PC enrichment + d6-PC enrichment.

In nonpregnant women, the consumption of 930 mg choline/d tended to yield a greater (P = 0.074) change in PEMT-PC (d3+d6-PC) enrichment between weeks 10 and 12 compared with 480 mg choline/d (1.8 ± 0.2 compared with 1.1 ± 0.3), suggesting that a higher choline intake increases PC production through the PEMT pathway. Because PC synthesized through the PEMT (compared with the CDP-choline) pathway is enriched with DHA (18), this finding is consistent with our previous observation of greater PC-DHA enrichment in nonpregnant women consuming 930 compared with 480 mg choline/d (19).

It is also interesting to note that in nonpregnant women, the plasma enrichment ratio of d6-DMG:d9-betaine (P = 0.001) and d3-choline:d3-PC (P = 0.014) were lower in the 930-mg choline/d intake group compared with the 480-mg choline/d intake group. These data suggest that although the higher choline intake increased the use of choline as a methyl donor, segments throughout the oxidative pathway were affected to a different extent.

Enrichment of choline metabolites in delivery plasma, placental tissue, and cord plasma

As presented in the Figure 3, the enrichments of d3-choline and d3-PC in the 3 compartments were in the order of maternal delivery plasma < placental tissue < cord plasma (P ≤ 0.011 for any comparisons between 2 compartments). In contrast, the enrichments of d9-PC and d9-SM were in the reverse order of maternal delivery plasma > placental tissue > cord plasma (P < 0.001 for any comparisons between 2 compartments).

DISCUSSION

This study used stable isotope methodology to investigate the effects of human pregnancy on choline dynamics. In addition, the impact of choline intake on these metabolic processes was explored. Three main findings emerged: 1) pregnancy increases the production of PC through both the CDP-choline and PEMT pathways, 2) PEMT-PC is selectively transferred to the fetus, and 3) a choline intake exceeding current recommendations partially restores pregnancy-induced alterations in choline metabolism.

Pregnancy alters choline dynamics

Pregnancy increases the partitioning of orally consumed choline to the CDP-choline pathway at the expense of betaine synthesis

Third-trimester pregnant (compared with nonpregnant) women used more of the orally consumed choline for PC synthesis through the CDP-choline pathway (Figure 4), as indicated by...
a lower enrichment ratio of d9-betaine:d9-PC. The enhanced use of choline for PC production via the CDP-choline pathway is due in part to the hepatic lipid load experienced by women during late pregnancy (20). PC is required for the biosynthesis of VLDLs, which mediate the export of lipid from liver into circulation. Pregnant (compared with nonpregnant) women also exhibited a lower d9-betaine:d9-choline enrichment ratio as well as diminished circulating concentrations of metabolites produced in the oxidative pathway including betaine, DMG, sarcosine, and methionine (5). Thus, in the face of enhanced use of choline for PC production by the CDP-choline pathway, third-trimester pregnant women require substantially more choline than their nonpregnant counterparts to maintain an adequate supply of choline-derived methyl groups for one-carbon metabolism.

Pregnancy increases the use of choline-derived methyl groups for PC synthesis through the PEMT pathway

Despite an enhanced partitioning of orally consumed choline toward the CDP-choline pathway during late pregnancy, the use of choline-derived methyl groups for PC synthesis through the PEMT pathway was elevated in pregnant (compared with nonpregnant) women (Figure 4). Specifically, the incremental labeling of the plasma PC pool with PEMT-PC (d3+d6-PC) through time did not differ \( (P \geq 0.6) \) between pregnant and nonpregnant women (1.4 ± 0.2 compared with 1.4 ± 0.2, respectively, from week 9 to week 10 and 1.4 ± 0.2 compared with 1.5 ± 0.2, respectively, from week 10 to week 12). Because the PC pool size is ~50% greater \( (P < 0.001) \) among pregnant compared with nonpregnant women (see Supplemental Table S4 under “Supplemental data” in the online issue), these data indicate that the flux of choline-derived methyl groups through the PEMT pathway is enhanced during late pregnancy. The high demand for methyl groups by the PEMT pathway during pregnancy is consistent with its upregulation by estrogen (21, 22), a hormone that increases during the second half of gestation (23, 24).

Pregnant (compared with nonpregnant) women also catabolize more PEMT-PC to generate free choline as indicated by the higher enrichment ratio of d3-choline:d3-PC. This enhanced

FIGURE 3. Choline metabolite enrichments in maternal delivery plasma, placental tissue, and fetal cord plasma obtained from pregnant women consuming 22% of 480 and 930 mg choline/d as methyl-d9-choline from study week 6 until delivery \( (n = 24; \text{paired } t \text{ tests}) \). Dissimilar letters (a, b, c) indicate differences between compartments at \( P \leq 0.011 \). A statistical analysis performed separately for each choline intake group yielded similar results. Values are presented as pooled estimated marginal means \( \pm \) SEMs from multiple imputation analyses. DMG, dimethylglycine; PC, phosphatidylcholine; SM, sphingomyelin

FIGURE 4. Proposed mechanisms through which pregnancy alters choline metabolism based on the isotopic enrichment of choline metabolites after oral consumption of d9-choline by pregnant and nonpregnant women. Pregnancy increases the use of orally consumed choline for PC synthesis through the CDP-choline and PEMT pathways. Pregnancy also increases the use of betaine for methionine synthesis and the hydrolysis of PEMT-PC to free choline. The reduced production of betaine from orally consumed choline (due to its increased use by the CDP-choline pathway), along with enhanced use of betaine as a methyl donor, may contribute to its depletion during the last trimester of human pregnancy. The increase in circulating free choline during this timeframe may stem from greater degradation of PEMT-PC. Thin arrows indicate that metabolic flux did not differ or was not compared between pregnant and nonpregnant women. Thick arrows indicate that metabolic flux was enhanced in third-trimester pregnant compared with nonpregnant women. The dashed arrow indicates that metabolic flux was attenuated in third-trimester pregnant compared with nonpregnant women. CDP-choline, cytidine diphosphate-choline; DMG, dimethylglycine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SAR, sarcosine; SM, sphingomyelin.
hydrolysis of PEMT-PC may explain the increase in plasma free choline during the last third of the gestational period (5).

Pregnancy increases the use of choline-derived methyl groups for methionine biosynthesis

Although less betaine was synthesized from orally consumed choline in pregnant compared with nonpregnant women (ie, lower d9-betaine:d9-choline enrichment ratio in pregnant women), more betaine was used for methionine synthesis in pregnant women (Figure 4). Specifically, serum d3-methionine enrichment was elevated among pregnant (compared with nonpregnant) women as was the enrichment ratio of d3-methionine:(d3+d9)-betaine. Thus, the 50% lower plasma betaine concentration observed in late pregnancy likely arises from both reduced betaine synthesis and enhanced utilization of betaine for methionine production.

PEMT-PC is selectively transferred to the fetus

As shown in Figure 3, the stepwise increase in d3-PC enrichment, but not d9-PC enrichment, from the maternal to the fetal compartment implies selective transfer of PEMT-PC to the fetus. This partitioning of PEMT-PC toward the fetus may be a result of its enrichment with DHA, because others have reported selective transfer of triglyceride-DHA to the fetus (25). Compared with the CDP-choline pathway, the PEMT pathway produces a PC molecule that is enriched in DHA (7, 18, 26), a long-chain unsaturated fatty acid that is critical for fetal brain development (27, 28). Once made, the PC-DHA molecule can be incorporated into VLDL, exported into circulation, and made available to peripheral tissues including the placenta and fetus.

Choline intake exceeding current recommendations may be needed to meet the demands of pregnancy

Based on the ratio of d9-betaine:d9-PC, pregnancy alters the partitioning of choline between the CDP-choline pathway and the betaine synthesis pathway. Specifically, pregnant women used more orally consumed choline for the CDP-choline pathway at the expense of betaine synthesis. However, when the pregnant women consumed 930 mg choline/d, partitioning between these 2 pathways did not differ from the nonpregnant state (ie, nonpregnant women receiving 480 mg choline/d), suggesting that a dose exceeding current recommendations was needed to meet the demands of these competing pathways. A choline intake exceeding current recommendations also enhanced the use of choline as a methyl donor for PC synthesis via the PEMT pathway among both pregnant and nonpregnant women as indicated by a higher ratio of (d3+d6)-PC:d9-PC in the 930-mg choline/d intake group compared with the 480-mg choline/d intake group. In addition, this intake amount (930 compared with 480 mg choline/d) stimulated the activity of the PEMT pathway in nonpregnant women (ie, increased the incremental labeling of the plasma PC pool with PEMT-PC), suggesting that extra choline during early pregnancy may enhance PEMT activity, and ultimately PC-DHA mobilization to the developing fetus (19). Finally, we previously showed that consumption of 930 (compared with 480) mg choline/d in pregnant women 1 elevated their plasma betaine concentrations to a level that was closer to that of nonpregnant women (5), 2 increased the use of choline as a methyl donor in maternal and fetus-derived tissues (5, 8), 3 improved biomarkers of placental function (29), and 4 lowered circulating concentrations of the stress hormone cortisol in venous cord plasma (8). Taken together, these data suggest that a choline intake exceeding current recommendations may be needed to support both PC production via the CDP-choline pathway and choline-mediated one-carbon metabolism, with subsequent benefits on maternal and child health outcomes.

Strengths and limitations

To the best of our knowledge, this is the first human study to use stable isotope methodology to examine the effects of late pregnancy and choline intake on the dynamics of choline-related metabolic processes. Our study was highly controlled, and potential confounders were considered, and accounted for, in the statistical analysis. The study findings are generalizable to healthy, third-trimester, singleton pregnant women, but more studies are needed to understand choline dynamics during the first half of gestation.

Conclusions

The enhanced use of choline for PC production via both the CDP-choline and PEMT pathways shows the substantial demand for choline during late pregnancy. Our finding that pregnant women required more choline than currently recommended to meet the competing demands of the CDP-choline pathway and betaine synthesis pathway suggests that current recommendations may be suboptimal for this reproductive group. Finally, the selective partitioning of PEMT-PC to the fetal compartment may imply a unique requirement of PEMT-PC by the developing fetus.

The authors’ responsibilities were as follows—MAC and JY: designed the research with significant input from JFG and JTB; JY, XJ, AAW, CAP, OVM, SPS, and RHA: conducted the research; JY: analyzed the data; JY and MAC: wrote the manuscript with significant input from JTB; and MAC: had primary responsibility for the final content. All authors read and approved the final manuscript. None of the authors had a conflict of interest in relation to this study.

REFERENCES


