Choline intake influences phosphatidylcholine DHA enrichment in nonpregnant women but not in pregnant women in the third trimester\textsuperscript{1–3}

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ABSTRACT
Background: Phosphatidylcholine (PC) produced via the S-adenosylmethionine–dependent phosphatidylethanolamine (PE) N-methyltransferase (PEMT) pathway is enriched with docosahexaenoic acid (DHA). DHA plays a critical role in fetal development and is linked to health endpoints in adulthood. It is unknown whether choline, which can serve as a source of S-adenosylmethionine methyl groups, influences PC-DHA or the PC:PE ratio in pregnant and nonpregnant women.

Objective: This study tested whether choline intake affects indicators of choline-related lipid metabolism, including erythrocyte and plasma PC-DHA and PC:PE ratios, in pregnant women in the third trimester and nonpregnant women.

Design: Pregnant (n = 26) and nonpregnant (n = 21) women consumed 480 or 930 mg choline/d and a daily DHA supplement for 12 wk. Blood was collected at baseline and at the midpoint and end of the study. PC-DHA was analyzed as the proportion of total PC fatty acids.

Results: Pregnant women had greater (P = 0.002) PC-DHA concentrations than did nonpregnant women at baseline. The proportion of erythrocyte and plasma PC-DHA increased (P ≤ 0.002) in pregnant and nonpregnant women regardless of choline intake. However, in nonpregnant women, consumption of 930 mg choline/d led to greater (P < 0.001) erythrocyte PC-DHA and a more rapid increase (P < 0.001) in plasma PC-DHA. Lower (P = 0.001–0.024) erythrocyte and plasma PC:PE in pregnant women was not modified by choline intake.

Conclusions: A higher choline intake may increase PEMT activity, resulting in greater PC-DHA enrichment of the PC molecule in nonpregnant women. Increased production of PC-DHA during pregnancy indicates elevated PEMT activity and a higher demand for methyl donors. This trial was registered at clinicaltrials.gov as NCT01127022.

INTRODUCTION
The ubiquitous phospholipid phosphatidylcholine (PC)\textsuperscript{4} consists of 2 fatty acid constituents linked to a glycerophosphocholine backbone. PC produced via the S-adenosylmethionine (SAM)–dependent phosphatidylethanolamine (PE) N-methyltransferase (PEMT) pathway is enriched in long-chain PUFAs such as DHA (22:6n–3) and arachidonic acid (ARA; 20:4n–6) (1–3). In contrast, PC generated by the cytidine diphosphate (CDP)–choline pathway is enriched in di- and monounsaturated fatty acids such as linoleic acid (18:2n–6) and oleic acid (18:1n–9) (1).

Adequate DHA nutrition is essential for health throughout life. The fetus requires DHA for proper development of the brain and immune systems (4, 5). In adults, DHA nutrition is linked to inflammation, brain function, reproductive health, and cardiovascular disease (6). Most hepatic PC-DHA is produced via the PEMT pathway (1), where it is subsequently available for very-low-density lipoprotein incorporation. After the entrance of very-low-density lipoprotein into the circulation, PC-DHA is conveyed to peripheral tissues and becomes available to the fetus in pregnant women.

PEMT substrate concentrations, such as SAM (which is influenced by folate and choline availability), may alter PEMT activity (7), whereas substrate concentrations for the CDP-choline pathway, including choline and diacylglycerol, can influence PC production via this pathway (8). An alteration in activity of either hepatic PC biosynthetic pathway has the potential to affect the fatty acid composition of newly synthesized PC, including the amount of PC-DHA produced. However, the effect of choline intake on the PC-DHA content of plasma and erythrocytes in pregnant and nonpregnant women is unknown. Erythrocyte PC-DHA is of particular interest because erythrocyte DHA content correlates with tissue DHA content including that of liver, brain, retina, and adipose (9, 10).

The ratio of PC to PE in cellular membranes is tightly regulated (11, 12). Changes in the PC:PE ratio can impair membrane functionality, and reduced membrane PC has been found during choline deficiency and in patients with nonalcoholic fatty liver disease—a condition associated with deranged choline metabolism (13, 14). It is unknown whether choline intake affects the

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\textsuperscript{4} Abbreviations used: ARA, arachidonic acid; CDP, cytidine diphosphate; HMRU, Francis A. Johnston and Charlotte M. Young Human Metabolic Research Unit; LMM, linear mixed model; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase; SAM, S-adenosylmethionine.

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PC:PE ratio in healthy pregnant and nonpregnant women or whether the increased requirement for choline during pregnancy (15) alters the PC:PE ratio in erythrocytes or plasma.

This study tested whether choline intake influenced erythrocyte and plasma PC:PE ratios or PC fatty acid composition in pregnant women in the third trimester and in nonpregnant women. To achieve the latter aim, relative proportions of characteristic products from the PEMT pathway (ie, PC-DHA and PC-ARA) and CDP-choline pathway (ie, PC–linoleic acid and PC–oleic acid) were analyzed. Because estrogen is a modiﬁer of PEMT expression (16) and increases during gestation (17), and pregnancy is associated with alterations in lipid metabolism (18), the effects of the third trimester of pregnancy on erythrocyte and plasma PC fatty acid constituents and PC:PE ratios were also examined.

SUBJECTS AND METHODS

Study participants

Nonpregnant women and pregnant women in the third trimester (27 wk of gestation) aged ≥21 y were recruited from the Ithaca, NY, area from January 2009 to October 2010 as described by Yan et al (19). During the screening phase, interested individuals provided a blood sample for blood chemistry proﬁling and complete blood count analyses and completed a health history and demographics questionnaire. Important inclusion criteria were as follows: 1) general healthiness as determined by the questionnaire, blood chemistry proﬁle, and complete blood count; 2) no drug or alcohol use; 3) normal kidney and liver function; and 4) willingness to comply with the study protocol, including agreement to eat ≤5 meals/wk at the on-site location and to not consume food or beverages outside what was provided by the study. Exclusion criteria for screened women included the following: 1) use of prescription medications known to affect liver function, 2) multiple pregnancies (where applicable), or 3) pregnancy-associated complications, eg, pre-eclampsia, gestational diabetes (where applicable). Eligible pregnant women were admitted to the study on a rolling basis at 26–29 wk of gestation, and eligible nonpregnant women were added as scheduling and space constraints allowed until the desired number of participants completed the study; a diagram detailing the ﬂow of participants through the study phases can be found in the article by Yan et al (19).

The study protocol was reviewed and approved by the Institutional Review Board for Human Study Participant Use at Cornell University and at the Cayuga Medical Center (the hospital where pregnant participants delivered their infants; Ithaca, NY). Study participants were compensated for participation, and informed consent was obtained from all participants before their entry into the study.

Study design, diet, and supplements

Design

This study was part of a controlled feeding study conducted in nonpregnant and pregnant women randomly assigned to consume 480 or 930 mg choline/d for 12 wk (19) through a combination of dietary choline (∼380 mg/d) and supplemental choline chloride (100 or 550 mg choline/d). Throughout the controlled feeding period, all participants also consumed a daily 200-mg DHA supplement to achieve the recommended intake of this nutrient for pregnant women (20). Blood was collected and processed as previously described (19) at baseline and study weeks 6, 10, and 12.

Diet

All study participants consumed the 7-d cyclic diet described in detail by Yan et al (19) throughout the duration of the study. The study diet provided ∼380 mg choline/d (19). Lipid-soluble forms of choline including PC, sphingomyelin, and lysophosphatidylcholine contributed 236 mg choline/d, and water-soluble forms including free choline, phosphocholine, and glycerophosphocholine contributed 142 mg choline/d (19). In addition, the diet provided 100 mg betaine/d (19) and ∼400 μg dietary folate equivalent natural food folate/d (21) as measured by our laboratory. The diet also provided 5.1 μg vitamin B-12 and 1.3 mg vitamin B-6 as calculated by Nutritionist Pro software (version 4.2.0; Axxya Systems). The study diet supplied ∼2000 kcal/d, which could be modiﬁed to meet caloric requirements by the addition or subtraction of nonnutritive food items (19). Food was prepared in the Francis A. Johnston and Charlotte M. Young Human Metabolic Research Unit (HMRU) at Cornell University. Study participants consumed ≥5 meals/wk under the supervision of study personnel at the HMRU; all other food and beverages were provided as take-aways.

Supplements

Choline supplements were prepared by study personnel as described by Yan et al (19). Briefly, pharmaceutical-grade choline chloride (Balchem) was dissolved in autoclaved drinking water, and amounts containing 100 and 550 mg choline were dispensed into sterile 50-mL conical tubes containing cranberry-grape juice and stored at −20°C in HMRU freezers; 1–2 d before consumption, supplements were thawed at 4°C.

In addition to the study diet and choline supplements, study participants consumed dietary supplements to achieve recommended nutrient intakes not met with the study diet (20, 22). Supplements included a daily DHA supplement containing 200 mg DHA (Neuramins; Nature’s Way Products); a daily over-the-counter prenatal multivitamin supplement (Pregnancy Plus; Fairhaven Health LLC) containing 750 μg folic acid (21), 2.6 μg vitamin B-12, and 1.9 mg vitamin B-6; and a thrice-weekly potassium/magnesium supplement (General Nutrition Corp). The DHA, vitamin B-12, and vitamin B-6 supplement levels were not veriﬁed by our laboratory. When eating on site, participants consumed supplements under the supervision of study personnel. Otherwise, supplements were provided in plastic bags along with take-away meals, and the participants were instructed to consume the supplements with a meal of their choice.

Compliance

The study protocol was well tolerated, and 92% of the enrolled participants completed the study (21 of 22 nonpregnant and 26 of 29 pregnant women). Reasons for stopping the study included nausea, early delivery, personal challenges, and food dislikes (19). Study participants completed daily checklists indicating that they received and consumed all menu items and supplements. For meals consumed off-site, participants were asked to return all empty conical tubes, plastic bags, and take-away food containers to study...
personnel during their next visit to the HMRU. In addition, study personnel had daily contact with participants throughout the study to maintain a positive rapport and to enhance compliance.

Sample collection and processing
Fasting venous blood was drawn at baseline and at study weeks 6, 10, and 12 in the HMRU ward by a trained phlebotomist. Blood samples were collected into EDTA and serum separator tubes, processed within 2 h, and stored in cryostat tubes at −80°C until analyzed as previously described (19).

Analytic measurements

Erythrocyte and plasma PC fatty acid constituents
Erythrocyte and plasma PC fatty acid constituents, including PC-DHA, PC-ARA, PC-linoleic acid, and PC–oleic acid, were quantified via gas chromatography (23). Fatty acid constituent data are presented as % by weight total PC fatty acids.

Erythrocyte and plasma phospholipids
PC and PE were quantified from washed, packed red blood cells and from plasma with the use of an HPLC-evaporative light-scattering detection method (24).

Genotyping
Select genetic variants that may affect choline metabolism, including PEMT G5465A (rs7946) (25), MTHFR C677T (rs1801133) (26), MTHFD1 G1958A (rs2236225) (27), and BHMT G742A (rs3733890) (28) were ascertained. Genotyping for the PEMT G5465A was performed by using a fluorescent TaqMan probe commercially available kit (Applied Biosystems). All other genotypes were determined by sequencing the double-stranded DNA templates with an Applied Biosystems Automated 3730 DNA analyzer.

Statistical methods
The effect of choline intake and reproductive state on dependent variables—including plasma and erythrocyte PC-DHA and PC-ARA and the sum of PC-linoleic plus PC–oleic—and the plasma and erythrocyte PC:PE ratios were examined. Mann-Whitney U tests were used to test baseline differences in participant characteristics and dependent variable values between reproductive groups and baseline differences by choline intake level within reproductive groups.

Two sets of linear mixed models (LMMs) were used to achieve the study aims. The first set of models stratified data by reproductive state (ie, pregnancy) and used LMMs to determine the effect of choline intake on dependent variables within reproductive groups. Because baseline status could influence the response to the choline intake treatment, in this set of models the baseline dependent variable value was entered as a covariate. Time (study week), choline intake (480 or 930 mg/d), and their interactions were entered as fixed factors; subject identifier was entered as a random factor. No main effect of choline intake level, 3-factor interaction (ie, reproductive state × choline × time), or interaction between reproductive state and choline intake was detected. One influential outlier each was excluded from erythrocyte PC-DHA and PC-linoleic+oleic analyses.

For both sets of LMMs, additional covariates/factors considered in initial models included age, ethnicity, BMI, serum folate concentration, and the PEMT G5465A, MTHFR C677T, MTHFD1 G1958A, and BHMT G742A genotypes. Nonsignificant interactions, covariates, and factors were progressively removed until the final models were derived. Bonferroni corrections were made for multiple comparisons where applicable. All statistical analyses were performed with IBM SPSS software (version 19; SPSS Inc).

RESULTS

Participant characteristics and baseline values
Forty-seven women were included in the final analyses. Twenty-one nonpregnant and 23 pregnant women completed 12 wk of the study, and 3 pregnant women completed 10 wk of the study. The study end time point was used in all statistical analyses and reflects the last sample collection for each participant. The ethnicity/race of the participants and the MTHFR C677T, MTHFD1 G1958A, and BHMT G742A genotypes were balanced by reproductive state and choline intake group (Table 1), whereas the distribution of PEMT G5465A genotype varied (P = 0.032) by reproductive state, but not (P = 0.809–1.0) by choline intake group (Table 1).

In erythrocytes at baseline, pregnant women had a greater (P = 0.001) proportion of PC-DHA and a lower (P = 0.001) proportion of PC-ARA than did nonpregnant women, whereas the proportion of erythrocyte PC-linoleic+oleic did not differ (P = 0.487) by reproductive state (Table 1). In plasma, pregnant women had a greater proportion of PC-DHA (P = 0.010) and PC-linoleic+oleic (P = 0.047) and a lower proportion of PC-ARA (P < 0.001) than did nonpregnant women (Table 1). Pregnant women had lower erythrocyte (P < 0.001) and plasma (P = 0.014) PC:PE ratios than did nonpregnant women at baseline (Table 1). No dependent variables varied by choline intake at baseline (Table 1).

Effect of choline intake on PC fatty acids by reproductive state

Pregnant women in the third trimester

Erythrocytes. After baseline values and covariates were controlled for, choline intake did not affect (P = 0.617) the proportion of erythrocyte PC-DHA. However, pregnant women consuming 930 mg choline/d had a lower (P = 0.02) proportion of erythrocyte PC-ARA when compared with pregnant women consuming

...
480 mg/d (Figure 1). No statistically significant differences were found in the concentrations of any other fatty acid between women consuming 930 mg choline/d and those consuming 480 mg choline/d. Choline intake at any level did not affect (P = 0.698) the proportion of plasma PC-DHA in pregnant women. Analyses of the data as mg/100 g PC fatty acids yielded an identical result.

**Plasma.** After baseline values and covariates were controlled for, choline intake did not affect the proportion of plasma PC-DHA (P = 0.737), PC-ARA (P = 0.596), or PC-linoleic+oleic (P = 1.0) in pregnant women.

**Nonpregnant women**

**Erythrocytes.** After baseline values and covariates were controlled for, the proportion of PC-DHA was greater (P < 0.001) in nonpregnant women consuming 930 mg choline/d than in nonpregnant women consuming 480 mg/d (Figure 2A); however, choline intake did not affect (P = 0.154) the proportion of erythrocyte PC-ARA. The proportion of PC-linoleic+oleic was lower (P = 0.021) in nonpregnant women consuming 930 mg choline/d than in women consuming 480 mg/d (Figure 2B).

**Plasma.** After baseline values and covariates were controlled for, choline intake interacted with time (P = 0.002) to influence the proportion of plasma PC-DHA (Figure 3). Nonpregnant women consuming 930 mg choline/d had a greater (P < 0.001) proportion of PC-DHA than did women consuming 480 mg/d at week 6. However, no difference (P = 0.434) in PC-DHA by choline intake level was found at study end because of a decrease (P = 0.019) in the proportion of plasma PC-DHA in nonpregnant women consuming 930 mg choline/d and an increase (P = 0.017) in women consuming 480 mg choline/d from

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**TABLE 1**

Participant characteristics and baseline lipid values for pregnant women at ~27 wk of gestation and for nonpregnant women of reproductive age randomly assigned to consume 480 or 930 mg choline/d.

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<thead>
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<th>Ethnicity (n)</th>
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<th>930 mg/d (n = 13)</th>
<th>All (n = 26)</th>
<th>480 mg/d (n = 10)</th>
<th>930 mg/d (n = 11)</th>
<th>All (n = 21)</th>
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**Erythrocyte PC fatty acids (% of total)**

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<th>DHA</th>
<th>ARA</th>
<th>Linoleic+oleic</th>
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<td>930 mg/d</td>
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<td>7.6</td>
<td>35.5 ± 2.2</td>
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<td>All</td>
<td>3.1</td>
<td>7.4</td>
<td>35.6 ± 2.1</td>
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**Plasma PC fatty acids (% of total)**

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<th></th>
<th>DHA</th>
<th>ARA</th>
<th>Linoleic+oleic</th>
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<tr>
<td>480 mg/d</td>
<td>4.4</td>
<td>12.4</td>
<td>33.2 ± 2.3</td>
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<tr>
<td>930 mg/d</td>
<td>4.1</td>
<td>12.7</td>
<td>36.6 ± 2.7</td>
</tr>
<tr>
<td>All</td>
<td>4.1</td>
<td>12.2</td>
<td>33.4 ± 2.5</td>
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</table>

**Note:** The effect of pregnancy (ie, all pregnant compared with all nonpregnant) was analyzed by using the Mann-Whitney U test. All pregnant compared with all nonpregnant: *P ≤ 0.001, *P ≤ 0.01, *P ≤ 0.05. Differences by choline intake level within physiologic groups (ie, pregnant in the 930-mg/d group compared with pregnant in the 930-mg/d group) were analyzed with Mann-Whitney U tests. No dependent variables varied by choline intake at study baseline. ARA, arachidonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine.  

1 Mean ± SD (all such values).  
2 Median; 95% CI in parentheses (all such values).
Choline intake did not affect the proportion of plasma PC-ARA ($P = 0.670$) or PC-linoleic+oleic ($P = 0.361$) in nonpregnant women.

**Effect of choline intake on the PC:PE ratio**

After baseline values and covariates were controlled for, choline intake did not affect the erythrocyte ($P = 0.545–0.590$) or plasma ($P = 0.259–0.340$) PC:PE ratio in pregnant or nonpregnant women.

**Effect of pregnancy on erythrocyte and plasma PC fatty acids**

**Erythrocytes**

After the covariates were controlled for, reproductive state interacted with time ($P < 0.001$) to influence the proportion of erythrocyte PC-DHA (Figure 4A). At baseline, the proportion of erythrocyte PC-DHA in pregnant women was greater ($P = 0.002$) than that in nonpregnant women; however, no difference ($P = 0.453–0.865$) was found by week 6 or at study end by reproductive state. The proportion of erythrocyte PC-DHA increased ($P \leq 0.002$) from baseline to study end in both pregnant and nonpregnant groups (Figure 5A). The proportion of plasma PC-ARA was lower ($P < 0.001$) in pregnant women than in nonpregnant women throughout the study; in all women, the proportion of plasma PC-ARA decreased ($P = 0.001$) from baseline to study end by reproductive state ($P \times$ choline intake level interaction was significant ($P = 0.193–0.860$).

**Plasma**

After the covariates were controlled for, reproductive state interacted with time ($P < 0.001$) to influence the proportion of plasma PC-DHA (Figure 5A). At baseline, the proportion of plasma PC-DHA was greater ($P = 0.002$) in pregnant women than in nonpregnant women; however, no difference ($P = 0.453–0.865$) was found by week 6 or at study end by reproductive state. The proportion of plasma PC-DHA increased ($P \leq 0.002$) from baseline to study end in both pregnant and nonpregnant groups (Figure 5A). The proportion of plasma PC-ARA was lower ($P < 0.001$) in pregnant women than in nonpregnant women throughout the study; in all women, the proportion of plasma PC-ARA decreased ($P = 0.001$) from baseline to study end by reproductive state ($P \times$ choline intake level interaction was significant ($P = 0.193–0.860$).

**FIGURE 1.** Erythrocyte PC-ARA in pregnant women in the third trimester who consumed 480 ($n = 13$) or 930 ($n = 13$) mg choline/d and supplementary DHA (200 mg/d) under controlled dietary conditions for 12 wk. Statistical analyses were performed with a linear mixed model that included baseline PC-ARA as a covariate; plotted data are predicted means with 95% CIs derived from the statistical model. Different lowercase letters indicate a significant main effect ($P < 0.05$) of choline intake. Choline intake groups were sampled at the same time points; $x$-axis data points were staggered slightly so that the error bars would not overlap. ARA, arachidonic acid; PC, phosphatidylcholine; wt, weight.

**FIGURE 2.** Erythrocyte PC-DHA (A) and erythrocyte PC-linoleic+oleic (B) in nonpregnant women who consumed 480 ($n = 10$) or 930 ($n = 11$) mg choline/d and supplementary DHA (200 mg/d) under controlled dietary conditions for 12 wk. Statistical analyses were performed with linear mixed models that included baseline PC-DHA or PC-linoleic+oleic as a covariate; plotted data are predicted means with 95% CIs derived from the statistical models. Different lowercase letters indicate a significant main effect ($P < 0.05$) of choline intake. Choline intake groups were sampled at the same time points; $x$-axis data points were staggered slightly so that the error bars would not overlap. linoleic+oleic, linoleic acid plus oleic acid; PC, phosphatidylcholine; wt, weight.
end in pregnant women, but decreased (P < 0.001, respectively) in pregnant women (Figure 5C). The proportion of plasma PC-linoleic+oleic did not change (P = 0.796) in pregnant women compared with nonpregnant women (P = 0.705) changed from baseline to study end. Neither the reproductive state x choline x time interaction nor the re-productive state x choline intake level interaction was significant (P = 0.432–0.981) for erythrocyte or plasma PC:PE.

**Effect of pregnancy on the PC:PE ratio**

**Erythrocytes**

After the covariates were controlled for, pregnant women had lower erythrocyte (P < 0.001) PC:PE than did nonpregnant women throughout the study. Erythrocyte PC:PE did not change (P = 0.511; Figure 6A) during the study.

**Plasma**

After the covariates were controlled for, reproductive state tended to interact with time (P = 0.062; Figure 6B) to influence the PC:PE ratio response. At baseline, the plasma PC:PE ratio did not differ (P = 0.112) by reproductive group; however, at week 6 and study end, plasma PC:PE was lower (P = 0.031 and P = 0.006, respectively) in pregnant than in nonpregnant women. In pregnant women, plasma PC:PE decreased (P < 0.001) from baseline to study end; however, in nonpregnant women this ratio did not change significantly (P = 1.0). In pregnant women, both plasma PC and PE increased (P = 0.002 and P < 0.001, respectively) from baseline to study end; however, PC increased by ~11%, whereas PE increased by ~40%; thus, the PC:PE ratio decreased in pregnant women. In nonpregnant women, neither plasma PC (P = 0.796) nor plasma PE (P = 0.705) changed from baseline to study end. Neither the reproductive state x choline x time interaction nor the re-productive state x choline intake level interaction was significant (P = 0.432–0.981) for erythrocyte or plasma PC:PE.

**DISCUSSION**

This study offered a unique opportunity to examine the inter-relations of choline and lipid metabolism in pregnant women in the third trimester and nonpregnant women consuming 480 or 930 mg choline/d plus supplemental DHA. The analyses found that 1) a higher choline intake (930 compared with 480 mg/d) increased the rise in PC-DHA in nonpregnant women and 2) choline-related lipid metabolism is altered during the third trimester of pregnancy.

**Choline intake influenced PC-DHA in nonpregnant women**

Not unexpectedly (29, 30), the DHA supplement included in the study protocol increased erythrocyte and plasma PC-DHA in all nonpregnant women; however, the results of the current study indicate that a higher choline intake along with supplementary DHA acted synergistically to produce the greatest enrichment of erythrocyte PC-DHA. Specifically, a higher choline intake yielded a greater proportion of erythrocyte PC-DHA throughout the study and a more rapid increase in plasma PC-DHA. The simultaneous increase in erythrocyte PC-DHA (Figure 2A) and decrease in erythrocyte PC-linoleic+oleic (Figure 2B) suggest that additional choline was directed toward methylation (ie, PEMT pathway) rather than the CDP-choline pathway in the 930-mg/d group than in the 480-mg/d group.

A higher choline intake may facilitate flux through the PEMT pathway by increasing the availability of methyl donors via its metabolite betaine. Indeed, studies using isotopically labeled choline (d9-choline) have confirmed that methyl groups derived from the choline molecule are used in converting PE to PC via PEMT (31, 32). Importantly, a recent study suggesting that plasma PC-DHA is a proxy for hepatic PEMT activity (33) also found that PC-DHA decreased during choline deficiency in premenopausal women—a result that underlines the relation between PEMT activity and the availability of methyl donors.

5-Methyltetrahydrofolate is the other carrier of labile methyl groups used to generate SAM. In the current study, folate status was "supranutritional" in nonpregnant women, as evidenced by high serum folate concentrations and urinary excretion of ~40% of daily folate intake (21). Importantly, serum 5-methyltetrahydrofolate did not differ (P = 0.328) by choline intake group. Thus, in nonpregnant women, consumption of 930 compared with 480 mg/d choline yielded greater erythrocyte PC-DHA and faster enrichment of plasma PC-DHA under conditions of equal and abundant 5-methyltetrahydrofolate, which suggests that flux through PEMT may rely to some extent on the availability of choline-derived methyl groups.
Choline altered PC-ARA but not PC-DHA in pregnant women in the third trimester

In our pregnant participants, the proportion of PC-DHA did not vary by choline intake level in erythrocytes or plasma; however, the proportion of erythrocyte PC-ARA was lower in pregnant women consuming 930 mg choline/d than in those consuming 480 mg/d. The small but significant difference in the relative amount of erythrocyte PC-ARA between choline intake groups suggests that additional choline may affect PC biosynthetic pathways and influence PC fatty acid composition during the third trimester. However, this unexpected result is difficult to interpret because there was no accompanying change in plasma PC-ARA or other PC fatty acids examined, and the primary source of PC-ARA (i.e., PEMT compared with CDP-choline pathway) during human pregnancy is ambiguous (34, 35). Thus, until replication, the finding that choline intake affects erythrocyte PC-ARA during the third trimester of pregnancy should be considered with caution.

Although a higher choline intake did not increase PC-DHA enrichment in our pregnant women in the third trimester, PC-DHA production may be enhanced with greater choline intakes earlier in gestation. Additional studies are needed to examine whether increased choline intake can enhance the proportion of PC-DHA during the first and second trimesters, when estrogen concentrations and hepatic lipid metabolism more closely resemble those of nonpregnant women, in whom an effect of choline intake was found.

Third trimester of pregnancy alters phospholipid biosynthesis

Several indicators of choline-related lipid metabolism, including erythrocyte and plasma PC fatty acid composition and PC:PE ratios, were affected during the third trimester of pregnancy. The proportions of erythrocyte and plasma PC-DHA were greater in pregnant women at study baseline (Figure 4A and Figure 5A), which confirms prior research reporting increased PC-DHA enrichment in pregnant women in mid- to late gestation (36, 37). Assuming that PC-DHA is primarily produced via the PEMT pathway during human pregnancy, the greater proportion of PC-DHA in both erythrocytes and plasma at baseline in pregnant women is consistent with increased PEMT activity, which may be related to elevated estrogen (38), increased DHA substrate mobilization/availability associated with pregnancy (36, 39), or both.

Pregnancy-induced upregulation of PEMT may explain why choline intake did not affect the proportion of erythrocyte or plasma PC-DHA in pregnant women and why the rise in erythrocyte and plasma PC-DHA was less (erythrocyte: 17% compared with 53%; plasma: 15% compared with 48%) in...
pregnant than in nonpregnant women consuming the same DHA supplement. That is, if PEMT was already upregulated at study baseline (ie, week 27 of gestation), it is possible that the capacity of additional choline-derived methyl donors to further stimulate PC biosynthesis via PEMT was attenuated. Nevertheless, elevated activity of the PEMT pathway, a significant consumer of SAM (40), underscores the increased demand for methyl groups during pregnancy.

Notably, despite evidence of increased PEMT activity, PC-ARA was lower in pregnant women than in nonpregnant women at baseline and remained so throughout the study (Figure 4B and Figure 5B). Pynn et al (1) showed that PC-ARA is preferentially produced via the PEMT pathway in nonpregnant women, but also reported that PC-ARA synthesis exhibits less biosynthetic pathway specificity than PC-DHA (1). Thus, the results of the current study and those of Pynn et al suggest that erythrocyte and plasma PC-ARA enrichment are less sensitive indicators of PEMT activity than is PC-DHA enrichment during pregnancy.

Data from the current study indicate that PC synthesis is increased through both the CDP-choline and PEMT pathways during the third trimester of pregnancy. Evidence of increased PEMT activity during the third trimester was discussed above, and, unlike in nonpregnant women, pregnant women maintained the same proportion of plasma PC-linoleic+oleic throughout the study (Figure 5C), which indicated greater PC synthesis via the CDP-choline pathway. Whereas these data are evidence of increased production from both PC biosynthetic pathways, the decline in erythrocyte PC-DHA relative to total erythrocyte PC fatty acids from week 6 to study end in pregnant women (Figure 4A) suggests that CDP-choline pathway PC production is augmented to a greater degree during the third trimester than is PEMT PC production. Indeed, fatty acids and diacylglycerol are activators of the CDP-choline pathway (41); thus, the increased lipolysis and fatty acid synthesis that occurs late in gestation would be expected to significantly boost the production of PC via the CDP-choline pathway. Thus, the percentage of PC produced via the CDP-choline pathway (compared with PEMT) during the third trimester may exceed 70%—the ratio most often cited for hepatic PC synthesis (42, 43). It is important to note that the decline in erythrocyte PC-DHA in the third trimester in pregnant women during the last half of the study also corresponds to the period of peak fetal accretion (39).

Both the erythrocyte and plasma PC:PE ratios were lower in pregnant women in the third trimester than in nonpregnant women. Importantly, neither these differences nor the decrease in the plasma PC:PE ratio observed in pregnant women was modified by a higher choline intake. This indicates that the altered PC:PE ratio during pregnancy is not governed by choline intake/availability and may be due to conditions associated with this

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**FIGURE 5.** Plasma PC-DHA (A), PC-ARA (B), and PC-linoleic+oleic (C) in pregnant women in the third trimester (n = 26) and in nonpregnant women (n = 21) who consumed a constant choline intake (480 or 930 mg/d) and supplementary DHA (200 mg/d) for 12 wk. Statistical analyses were performed with linear mixed models; plotted data are predicted means with 95% CIs derived from the statistical models. A: Reproductive state × time interaction (P = 0.001); different lowercase letters indicate a significant difference (P = 0.002) between reproductive groups at denoted time points. *Significant change in enrichment (P < 0.01) from baseline to the end of the study within reproductive group. B: Different lowercase letters indicate a main effect of reproductive group (P < 0.001). *Significant main effect of time (P < 0.001). C: Reproductive state × time interaction (P = 0.019); different lowercase letters indicate significant differences (P < 0.05) between reproductive groups at the denoted time points. *Significant change in enrichment (P = 0.01) from baseline to the end of the study within reproductive group. Neither the reproductive state × choline × time nor reproductive state × choline intake level interaction was significant (P = 0.231–0.856) for PC-DHA, PC-ARA, or PC-linoleic+oleic. Reproductive groups were sampled at the same time points; x-axis data points were staggered slightly so that the error bars would not overlap. ARA, arachidonic acid; linoleic-oleic, linoleic acid plus oleic acid; PC, phosphatidylcholine; wt, weight.
FIGURE 6. Erythrocyte PC:PE (A) and plasma PC:PE (B) ratios in pregnant women in the third trimester (n = 26) and in nonpregnant women (n = 21) who consumed a constant choline intake (480 or 930 mg/d) and supplementary DHA (200 mg/d) for 12 wk. Statistical analyses were performed with linear mixed models; plotted data for erythrocytes and plasma are predicted means with 95% CIs and geometric predicted means with 95% CIs, respectively, derived from the statistical models. A: Different lowercase letters indicate a main effect of reproductive state (P < 0.001). B: Reproductive state × time interaction (P = 0.062); different lowercase letters indicate significant differences (P < 0.05) between reproductive groups at the denoted time points. *Significant change in PC:PE ratio (P < 0.001) from baseline to the end of the study within reproductive group. Neither the reproductive state × choline × time nor reproductive state × choline intake level interaction was significant (P = 0.432–0.981) for erythrocyte or plasma PC:PE. Reproductive groups were sampled at the same time points; x axis data points were staggered slightly so that the error bars would not overlap. PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Conclusions

Increasing the concentration of circulating PC-DHA is often desirable in pregnant and nonpregnant women. The strong specificity for PC-DHA generation via the SAM-dependent PEMT pathway increases the use of and demand for methyl groups, with perhaps an explicit requirement for methyl groups derived from choline. Thus, efforts to better understand and improve DHA nutriture should fully consider factors influencing the PEMT pathway, including methyl donors such as choline.

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