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Effects of phosphatidylcholine and betaine supplements on women's serum choline

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Abstract

Background: Maternal phosphatidylcholine supplements have shown benefit in the development of the human fetal brain, as assessed both by newborn physiological measurements and by a related decrease in later childhood behavioral abnormalities. However, the relatively low choline component of phosphatidylcholine mandates high doses that are difficult for pregnant women to consume.

Objective: Betaine can substitute for some choline effects. The hypothesis was that betaine supplementation would significantly increase women's serum choline.

Design: A three-arm crossover clinical trial was used to assess serum concentrations of choline after betaine supplements at two doses, in comparison with phosphatidylcholine supplementation. The effects of both a single dose and of one-week twice-daily doses were assessed in normal non-pregnant women.

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Conflicts of interest

None for all authors.

CRediT authorship contribution statement

M. Camille Hoffman: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing - original draft. **Ann Olincy:** Data curation, Investigation, Methodology, Project administration, Supervision. **Angelo D'Alessandro:** Data curation, Methodology, Formal analysis. **Julie A. Reisz:** Methodology, Formal analysis. **Kirk C. Hansen:** Methodology. **Sharon K. Hunter:** Conceptualization, Formal analysis, Methodology. **Robert Freedman:** Conceptualization, Formal analysis, Writing - review & editing. **Randal G. Ross:** Conceptualization, Investigation, Methodology, Funding acquisition.

Results: Betaine supplements at two doses failed to increase serum choline concentrations after single administration or one-week twice-daily dosing. Phosphatidylcholine supplements raised choline concentrations after both single doses (mean change from baseline 8.34 ± 7.29 ng/ml, paired t = 3.24, df 7, p = 0.014, range 1–21 ng/ml, d' = 1.15) and one-week twice-daily doses (mean change from baseline 4.58 ± 3.68 ng/ml standard deviation; paired t = 3.51, df 7, p < 0.001, range 2–13 ng/ml, d' = 2.65). Betaine concentrations rose after both betaine and phosphatidylcholine supplementation.

Conclusions: Betaine supplements did not substitute for phosphatidylcholine supplements, which raise serum choline concentrations both after a single dose and after repeated administration. However, serum betaine concentrations did rise after both betaine and phosphatidylcholine consumption and, therefore, betaine may be a stable indicator of choline intake.

Keywords

Phosphatidylcholine; Choline; Betaine; Dimethylglycine; Pregnancy

1. Introduction

Increasing clinical evidence and findings from animal model experiments point to the value of raising maternal serum choline concentrations during gestation. Choline reaching the fetus and amniotic fluid has multiple roles in fetal membrane synthesis, one-carbon metabolism including DNA methylation, and activation of fetal cholinergic receptors prior to their receiving innervation from acetylcholine-containing synapses [1,2]. Laboratory dams who received choline supplementation have enhanced development of inhibitory synaptic function and behavioral competencies [3,4]. In humans, increased maternal dietary choline intake during pregnancy is associated with enhanced cognitive performance in the offspring for up to 7 years [5].

Phosphatidylcholine has been used in the clinical trials of choline supplementation because boluses of choline can reach the large intestine, where the gut flora produce trimethylurea, which can be "foulsmelling," and the potentially atherogenic trimethylamine N-oxide [1,6]. Phosphatidylcholine is resistant to this degradation by gut flora, well absorbed, and increases maternal serum choline concentrations [7]. A dose of 6300 mg/day of phosphatidylcholine after 17 weeks of pregnancy resulted in children who at 40 months had fewer symptoms of attention deficits and social isolation, both developmental signs of future mental illness [8,9]. A major problem with phosphatidylcholine voiced by the women in the trial was the requirement to take 7 large capsules to achieve their 6300 mg dose. Choline itself accounts for only 900 mg, with the majority of the mass being the phosphatidyl moiety.

Most choline is used in one of two pathways: it can be metabolized to betaine by oxidation to an aldehyde and a subsequent dehydrogenase-mediated step or it can be used as a substrate for the synthesis of phosphatidylcholine that is used to form cell membranes (Fig. 1). In human pregnancy, there is increasing diversion of choline consumption to fuel phosphatidylcholine synthesis [10]. In a chicken model of choline deficiency, betaine supplements replaced up to 50% of the choline requirement, presumably by sparing the

consumption of choline to synthesize betaine [11]. In mice with deficient methionine synthesis, the opposite effect has been observed with the consumption of choline to form betaine at the expense of its availability in the brain to produce acetylcholine [12]. Betaine concentrations are decreased in obese pregnant baboons and their fetuses via a similar mechanism, but in this model choline concentrations are maintained [13]. We hypothesized that betaine supplements, which unlike choline do not require the volume consumed of the phosphatidyl supplement, might substitute for a portion of the maternal choline requirement. One clinical trial of choline in pregnancy found that increased betaine concentrations in the placebo control group mothers were associated with later infant cognition, although it is not clear if the concentrations reflected increased consumption of choline-containing or betaine-containing foods [14]. Betaine supplements are given safely to humans in the treatment of homocystinuria [15]. As a first step, we compared the effects of phosphatidylcholine and betaine supplements on serum choline concentrations in 8 non-pregnant women of childbearing age.

2. Subjects and methods

2.1. Procedure

Healthy non-pregnant women, ages 18–45, were recruited to participate in this prospective cross-sectional pilot study using standard clinical recruitment processes at our center. Participants were excluded if they were pregnant or attempting to become pregnant, had co-morbid medical conditions requiring daily medications, or if they used tobacco or other illicit drugs. All participants were of childbearing age and had an assured method of contraception.

The clinical trial compared three treatments: phosphatidylcholine (PhosChol 900 mg, Nutrasal, Portland ME) 3600 mg daily, anhydrous betaine (Life Extension, Ft Lauderdale, FL) 588 mg daily, and betaine (Life Extension, Ft Lauderdale, FL)1000 mg daily. Each treatment was given for 7 days per month. Thus, the trial lasted for 3 consecutive months for each participant. The 7 days of dosing occurred during the luteal phase of the menstrual cycle (7–14 days before the woman's expected menstrual period). Each participant received treatments in the following order: 7 days of phosphatidylcholine (3600 mg is equivalent to approximately 514 mg choline) during month one; 7 days of anhydrous betaine (588 mg dissolved in juice is approximately equimolar to the 514 mg choline dose) during month two; and 7 days of betaine 1000 mg (which is 170% of the molarity of the two previous doses), during month three.

For each monthly initial dose (Day 1 of 7), the women were admitted after an overnight fast as day inpatients to the Clinical Translational Science Institute service at University of Colorado Hospital. An intravenous catheter was placed to sample their baseline blood concentrations. Then the test compound was administered, and further samples were drawn at 2-h intervals for the next 12 h. Vital signs and adverse effects were monitored. The participants received a low choline and betaine diet while hospitalized.

The women were discharged at 12 h with phosphatidylcholine capsules or betaine powder and instructed to take phosphatidylcholine 3600 mg each morning and 2700 mg each

evening for one week in the first arm, betaine 588 mg each morning and 412 mg each evening for the second arm, and betaine 1000 mg both morning and evening for the third arm. No constraints were placed on diet. They returned one week later for monitoring of side effects, vital signs, and weight. A blood sample was taken fasting, 12 h after their last dose. They then received no treatment before returning for the next arm of the trial the following month.

2.2. Analysis of choline and its metabolites

All blood samples were obtained in serum separator tubes and immediately centrifuged and refrigerated to protect the serum from contamination by choline from the red blood cell membranes. Three samples were lost to clotting: one baseline, one two-hours post dosing, and one four-hours post dosing; all three samples were from the same subject on the same day at the time of testing the higher dose of betaine.

Choline, betaine, dimethylglycine, and trimethylamine N-oxide were analyzed via ultra-high performance liquid chromatography (UHPLC) coupled online with high resolution mass spectrometry (MS), as reported with minor modifications described as follows [16]. Briefly, 10 μl of serum were re-suspended in 240 μl of ice cold lysis buffer (methanol:acetonitrile:water 5:3:2) before vortexing at 4 °C for 30 min and then centrifugation at 10,000 g at 4 °C for 10 min. The lysis buffer was supplemented with deuterated isomers of the compounds of interest, namely D6-dimethylglycine, D9-betaine and 1,1,2,2-D-choline (D-2464, D3509, D-3352 – Cambridge Isotope Laboratories, Tewksbury, MA, USA) at 1 μM concentration. Supernatants were dried down and reconstituted in equal volume of double distilled water supplemented with 0.1% formic acid. Ten microliters of reconstituted samples were injected in a Vanquish UHPLC system (Thermo Fisher) and separated on a Kinetex C18 column (1.7u 2.1 × 150) through a 6 min gradient at 250 μl/min (Phase A: ddH_2O + 0.1% formic acid; phase B: acetonitrile + 0.1% formic acid). The gradient was designed as follows: 2 min at 2% B, 0.5 min from 2 to 25% B, 1.5 min isocratic at 25% B, 2 min equilibration at 2% B. The method was linear within the 50 pM to 50 μM range, baseline separated isobaric isomers of compounds of interest (e.g. RT for betaine and valine isomers were 1.23 and 1.47 min, respectively). UHPLC was coupled online with a high-resolution quadrupole orbitrap instrument (Q Exactive, Thermo Fisher) operated in positive ion mode at 70,000 resolution. Quantitation was performed by determining the ratio of light endogenous/heavy internal standards x dilution factor x heavy standard concentration for each compound of interest. Technical mixes were run every fifteen samples to ensure CV < 10% for all tested compounds.

2.3. Ethics

All procedures including the informed consent were approved and monitored by the Colorado Multi-Institutional Review Board.

2.4. Statistics

Student's t-test was used to assess the pre-specified primary outcome of the study, the level of serum choline obtained 4 h after initial treatment. The entire data set for the 12 h of serum concentrations during all three arms was then analyzed by an analysis of

variance with repeated measures with the Greenhouse-Geyser correction for non-sphericity. Analyses were performed for choline, betaine, dimethylglycine, and trimethylamine N-oxide concentrations. Post-hoc paired t-tests were used at each time point to illustrate the time course of change in serum concentration compared to each subject's baseline. The one-week concentrations were similarly analyzed.

2.5. Results

All 8 female subjects tolerated the treatments and were able to complete all three phases of the study. Their mean age was 26.3 ± 3.6 years. The mean participant body mass index (BMI) was 23.7 ± 4.5. Seven subjects had normal BMI and one was obese (BMI 33.8). All 8 (100%) were white; one was of Hispanic ethnicity. One subject complained of mild nausea and stomach discomfort before administration of the higher betaine dose and mild nausea 12 h later. There were no other complaints of side effects, including depression, anxiety, vomiting, unusual odor, or incontinence. There were no changes in BMI or vital signs.

Serum choline concentrations rose significantly after single-dose administration of phosphatidylcholine (Fig. 2). Choline concentrations did not increase after the administration of either the lower or higher dose of betaine. The analysis of variance found a significant effect of treatment type, $F = 19.24$, df 1.803, $p < 0.001$, and a significant treatment type*time effect, $F = 7.121$, df 3.989, $p = 0.001$. For the primary outcome, serum choline concentrations 4 h after treatment, administration of phosphatidylcholine produced elevated choline concentrations in all 8 subjects. The mean change from baseline was 8.34 ± 7.29 ng/ml, paired $t = 3.24$, df 7, $p = 0.014$, range 1–21 ng/ml, d' = 1.15. This increase after phosphatidylcholine was significantly different from both the lower dose and higher doses of betaine. The choline concentrations fell slightly for both betaine doses, −0.08 ± 2.49 ng/ml change from baseline for the lower dose and −0.62 ± 2.98 ng/ml for the higher dose. Additional post-hoc t-tests found significant increase in choline concentrations compared to baseline from 2 to 10 h after phosphatidylcholine administration.

After 1-week of twice daily supplement administration, fasting serum choline concentrations 12–14 h after the last dose were increased only after phosphatidylcholine administration. The analysis of variance found a significant effect of the treatment type, $F = 7.203$, df 1.614, $p = 0.015$, and a significant treatment type*time effect, $F = 29.700$, df 1.527, $p < 0.001$. The fasting serum choline concentration after phosphatidylcholine administration was significantly different from baseline, with all 8 subjects' concentrations elevated. The mean change was 4.58 ± 3.68 ng/ml standard deviation; paired $t = 3.51$, df 7, $p < 0.001$, range 2–13 ng/ml, d' = 2.65. There was no increase in fasting serum choline concentration after 1 week of either low or high dose betaine treatment.

Serum betaine concentrations peaked two hours after single-dose administration of both low and high betaine doses and they also rose significantly after phosphatidylcholine treatment (Fig. 3). The analysis of variance found a significant effect of the treatment type, $F = 15.46$ df 1.246 $p = 0.003$, and a significant treatment type*time effect, $F = 5.378$ df 1.925 $p = 0.023$. Post-hoc t-tests found significant increases in betaine concentrations compared to baseline from 2 to 12 h after both phosphatidylcholine and betaine administration. After 1-week treatment with both betaine doses, betaine concentrations increased significantly

compared to baseline. They also increased with phosphatidylcholine treatment. The effect for the equimolar doses of phosphatidylcholine and betaine (lower-dose betaine) were identical, while the effect was greater for the higher betaine dose. The analysis of variance showed non-significant effects of both treatment type and treatment type*time.

There were no significant effects of any treatment on serum dimethylglycine. At 4 h after administration, the change in mean level was −0.39 ± 2.06 ng/nl for phosphatidylcholine treatment, 7.98 ± 20.94 ng/ml for the lower dose of betaine, and 12.75 ± 38.07 ng/ml for the higher dose. There was no change after 1-week administration of any of the treatments.

The only obese woman in the study (BMI = 33) had the largest rise in choline concentrations, an increase of 21.1 ng/ml at 4 h after single-dose and an increase of 13.0 ng/ml after 1-week. However, her baseline fasting concentration, 8.2 ng/ml, was close to the group mean. For the other 7 women, there was no significant effect of BMI on the fasting level or increase after supplementation.

2.6. Discussion

The study demonstrated that betaine supplementation does not achieve an increase in serum choline concentrations in women. Because choline is the active compound for fetal membrane synthesis and activation of cholinergic receptors prior to their synaptic innervation, the failure of betaine to increase choline concentrations eliminates the possibility of using it as a substitute for phosphatidylcholine. The study, nonetheless, provides some worthwhile information. First, it replicates Zeisel's 1980 report that phosphatidylcholine supplementation raises choline concentrations [7]. Second, it extends that report to show that the supplementation effect is maintained after administration for a week and that choline is not sequestered or metabolized to maintain the previous baseline concentrations. Third, betaine concentrations from lower dose betaine are closely reflected by betaine concentrations resulting from phosphatidylcholine supplementation. Equimolar phosphatidylcholine and betaine produced essentially equal betaine concentrations after 1-week administration. Cheatham et al. suggested this possible interpretation for their clinical trial of maternal choline supplementation, in which they found higher concentrations of betaine in the control group [14]. For ethical reasons, controlled trials of choline supplementation have provided mothers with extensive dietary recommendations for adequate choline intake regardless of whether they were assigned to phosphatidylcholine supplements or placebo. Increased betaine concentrations indicate that mothers have heeded this advice. Furthermore, the betaine concentrations do not peak as markedly or fall as quickly as choline concentrations after phosphatidylcholine supplementation even though the range of serum concentrations of betaine is much wider in our cohort. Thus, betaine concentrations might be a more long-term marker of choline consumption than serum choline concentration in clinical practice, where fasting status cannot be assured.

Limitations of the study include the study of non-pregnant subjects, who have somewhat different use of choline and betaine than pregnant women [10] and the lack of genotypic information. Genotypic variants in both folate metabolizing enzymes and phosphatidylethanolamine methyltranferase have been associated with differences in the metabolism of choline and betaine [14,17].

In this study, betaine supplementation did not achieve serum choline concentrations comparable to phosphatidylcholine supplementation, eliminating betaine as a candidate to supplement adequate choline during pregnancy in a smaller volume of daily capsules. The experience with folic acid supplementation during pregnancy indicates that pharmacological supplementation is markedly more effective than good diet alone [18]. In the case of phosphatidylcholine supplementation, incorporation into baked goods has been used experimentally, but no commercial product exists [14]. Such a product would need to be appealing to pregnant women but not be confused with cookies or other foods that might be diverted to other family members and thus deprive the pregnant woman of the supplement.

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HIGHLIGHTS

- Phosphatidylcholine supplementation during pregnancy increases maternal choline levels.
- Phosphatidylcholine supplementation during pregnancy positively influences fetal and child development and behavior.
- Consumption of a large quantity of phosphatidylcholine is required during pregnancy to improve outcomes for the child.
- Unfortunately, betaine did not increase choline levels in women of childbearing age.

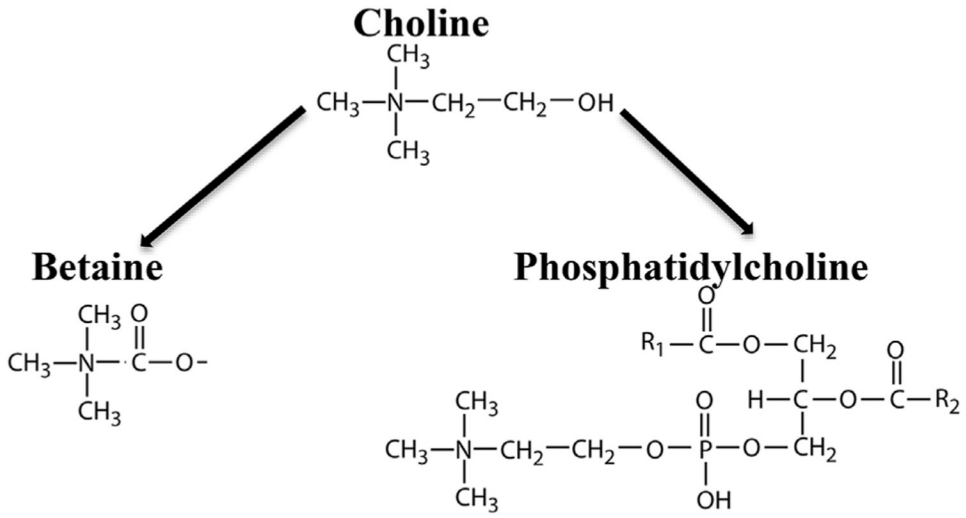

Fig. 1.
Biochemical structures of choline, betaine and phosphatidylcholine.

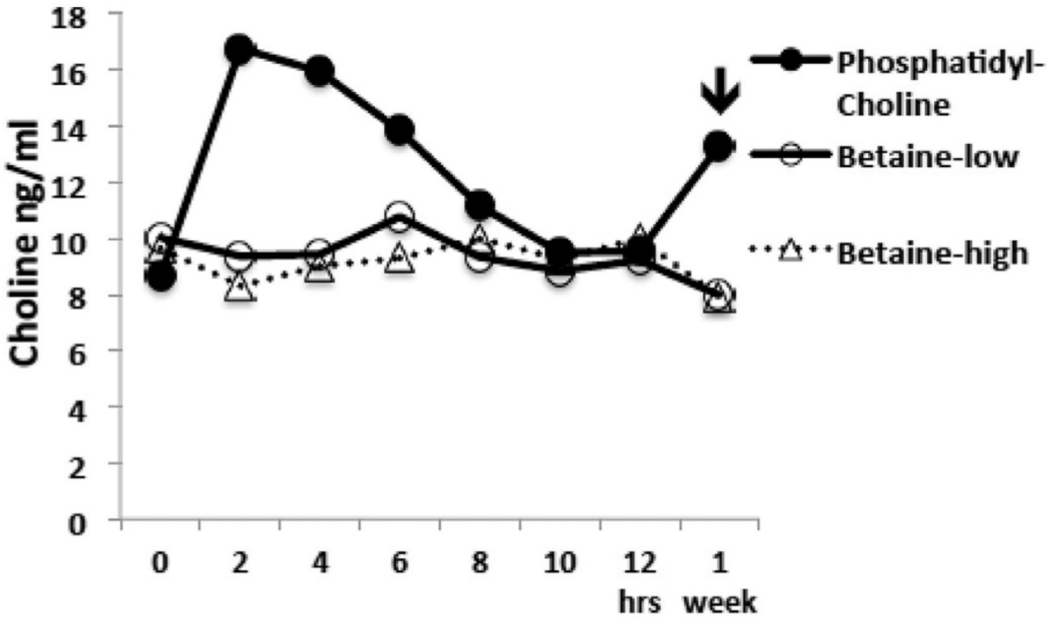

Fig. 2.

Serum choline estimated mean levels following acute and 1-week treatment with phosphatidylcholine and two doses of betaine. *All choline levels after acute phosphatidylcholine choline treatment are significantly elevated over the 0-h baseline, until 12 h post treatment ($p < 0.05$).* The arrow points to fasting levels obtained 12–14 h after the last dose in a 1-week twice daily dosing regimen. Choline levels were not elevated after either betaine treatment.

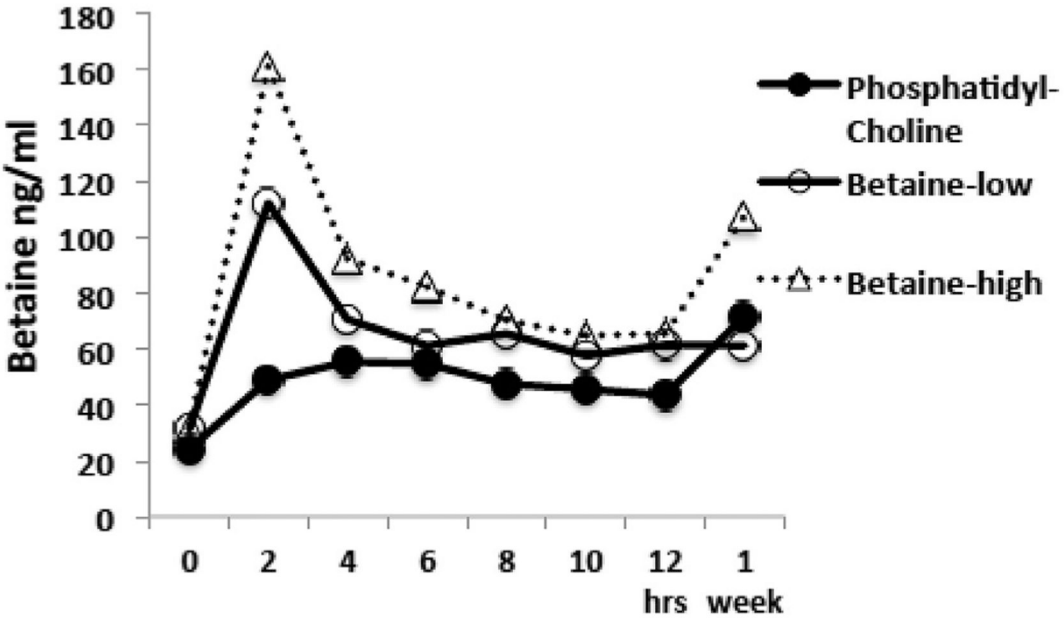

Fig. 3.

Serum betaine estimated mean levels following acute and 1-week treatment with phosphatidylcholine and two doses of betaine. *All choline and betaine levels after both acute phosphatidylcholine and betaine treatment were significantly elevated over the 0-h baseline ($p < 0.05$).*