Total water-soluble choline concentration does not differ in milk from vegan, vegetarian, and non-vegetarian lactating women

By: Maryanne T. Perrin, Roman Pawlak, Lindsay H. Allen, and Daniela Hampel

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Abstract:

Background: Choline is an essential nutrient for brain growth and other processes in the developing neonate. The impact of a maternal plant-based diet on the choline composition of breast milk is unknown. **Objective:** We assessed the water-soluble choline content of milk from lactating women in the United States following 3 dietary patterns: vegan, vegetarian, and nonvegetarian. **Methods:** We conducted a cross-sectional study of 74 healthy lactating women who provided a single breast-milk sample using a standardized collection protocol. Participants completed a food-frequency screener and were classified as follows: nonvegetarians (NONVEG) consumed meat; vegetarians (VEGT) consumed milk, dairy, and/or fish; and vegans (VEGAN) consumed animal products less than monthly. Primary outcomes measured were the concentration (in milligrams per liter) and distribution (percentage) of choline from the following water-soluble forms: free choline, phosphocholine (PCho), and glycerophosphocholine (GPC). Differences between diet groups were evaluated with ANOVA. **Results:** There was a wide range in breast-milk total water-soluble choline (4–301 mg/L), with no significant difference ($P > 0.05$) by maternal diet pattern. There were differences in choline forms, with VEGAN having a greater mean ± SD concentration and distribution of choline derived from GPC (62.7 ± 25.3 mg/L) than VEGT (47.7 ± 21.2 mg/L) and NONVEG (42.4 ± 14.9 mg/L) ($P = 0.0052$). There was a lower mean ± SD percentage of choline from PCho ($P = 0.0106$) in VEGAN (32.5% ± 18.3%) than in VEGT (46.1% ± 18.3%) and NONVEG (44.8% ± 15.7%). Lactation stage and maternal BMI were significantly associated with some choline forms. **Conclusions:** There was a wide range of water-soluble choline concentrations in the milk of healthy lactating women following vegan, vegetarian, and nonvegetarian diets, with no observed difference in total water-soluble choline concentration by maternal diet. This suggests that maternal plant-based diet by itself is not a risk factor for low breast-milk choline.

Keywords: choline | breast milk | vegan | vegetarian | phosphocholine | glycerophosphocholine

Article:

Introduction

Choline is an essential nutrient involved in numerous biological systems including cell membrane structure, nerve signal transmission, lipid transportation, and one-carbon metabolism[1, 2]. Dietary forms of choline include water-soluble free choline (FC), phosphocholine (PCho), and glycerophosphocholine (GPC), and fat-soluble phosphatidylcholine (PtdCho) and sphingomyelin (SM) [3]. The American Academy of Pediatrics has identified choline as a critical nutrient for brain growth in the developing neonate [4]. An imaging study of preterm infant brain development showed that brain choline content increased significantly with age and was lower in infants with cerebellar brain injury [5].

Human milk is considered the gold standard for infant feeding [6, 7]. Total choline concentrations in human milk have been reported to vary significantly between women, increase rapidly in the first week postpartum, and then remain relatively stable in the first 6 mo of lactation [8–11]. The water-soluble forms FC, PCho, and GPC contribute ∼70–80% of the total choline content in mature human milk [8, 9, 12]. Factors that have been shown to influence the choline composition of human milk include maternal choline supplementation, maternal genotype, de novo choline synthesis through the hepatic phosphatidylethanolamine N-methyltransferase (PEMT) pathways, and maternal hormones [12–14]. There is conflicting information regarding the effect of maternal diet on the choline composition of human milk [12, 13, 15].

The Adequate Intake (AI) of choline for lactating women established by the US Institute of Medicine (IOM) is 550 mg/d, and includes all forms of dietary choline (FC, PCho, GPC, PtdCho, and SM). Studies have shown that lactating women often do not meet dietary choline recommendations [12, 15, 16], although intakes below the AI "are likely to be adequate for a significant portion of individuals" according to the IOM, and should thus be interpreted with caution [17]. Eggs are an excellent source of choline, providing 3 times more choline than most meats and grains, and 10 times more than most fruits and vegetables [3]. PtdCho is typically the most abundant form of choline in eggs and meat, whereas FC is more abundant in grains, fruits, vegetables, and dairy products [3]. A simulation of nutrient intake based on a variety of dietary patterns identified vegan diets as at risk of inadequate choline intake [18]. Egg consumption, which is not part of a vegan diet, has been reported as an important dietary factor associated with a significant improvement in meeting choline AI targets [16, 19]. The scientific premise of our study was based on 2 factors: *1*) that vegan diets put individuals at a greater risk of inadequate choline intake [18]; and *2*) that maternal choline intake has an impact on breast-milk choline composition [12, 13]. The purpose of this study was to *1*) assess and compare the water-soluble choline concentrations in breast milk from vegan, vegetarian, and nonvegetarian lactating women; *2*) assess the prevalence of samples that would meet the choline AI for infants age 0–6 mo (125 mg/d); and *3*) evaluate the relation of other maternal factors with breast-milk choline

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Abbreviations used: AI, Adequate Intake; FC, free choline; GPC, glycerophosphocholine; IOM, Institute of Medicine; NONVEG, nonvegetarian classification (consumed meat); PCho, phosphocholine; PEMT, phosphatidylethanolamine N-methyltransferase; PtdCho, phosphatidylcholine; SM, sphingomyelin; VEGAN, vegan classification (consumed animal products less than monthly); VEGT, vegetarian classification (consumed milk, dairy, and/or fish).

content. We hypothesized that women following a vegan diet would have lower milk choline concentrations.

Methods

The study was approved by the Institutional Review Boards at the University of North Carolina at Greensboro and East Carolina University. Details of the recruitment of subjects, collection of samples, and dietary information have been described in detail elsewhere [20, 21]. Briefly, healthy lactating women who were >2 wk postpartum and living in the United States were recruited between November 2016 and April 2017 to provide 1 breast-milk sample by complete expression of 1 breast, and to complete a diet survey. Samples were collected in the morning during the first or second feeding of the day. Information regarding the participant's last meal and whether they were in the fasted state was not collected. Subjects were classified based on their reported dietary intake as vegan, vegetarian, or nonvegetarian. Vegans (VEGAN) were defined as participants who did not ingest any meat but may have ingested other animal products rarely (<1/mo). Vegetarians (VEGT) consisted of participants who did not eat meat but sometimes (at least monthly and less than weekly) or often (at least once per week) ingested other animal products, such as eggs, fish, or dairy. Nonvegetarians (NONVEG) consumed meat and other animal products. This report is a secondary analysis of samples from a study that was powered to detect differences in breast-milk vitamin B-12 concentrations [20].

Water soluble-choline compounds were assessed at the USDA/Agricultural Research Service Western Human Nutrition Research Center (Davis, CA) based on Wang et al. [22] with modifications. Briefly, 5-µL whole milk samples were diluted 1:500 in water:methanol (1:4, vol:vol) and analyzed after the addition of stable isotope internal standards—choline chloride-d9 (Cambridge Isotope Laboratories); L-α-glycerophosphoryl(choline-d9) (Avanti Polar Lipids); and phosphocholine-(trimethyl-d9) (Millipore-Sigma)—using a Waters ACQUITY I-class ultra-performance liquid chromatograph coupled to a SCIEX 4500QT mass spectrometer (UPLC-MS/MS) in positive ion mode and multiple reaction monitoring. Injection volume was 10 µL onto a Phenomenex Luna Silica [2] column protected by a Phenomenex silica SecurityGuard using a gradient of aqueous 0.1% propionic acid and acetonitrile (Hampel D, Shahab-Ferdows S, Allen LH, unpublished data). Choline forms were quantified by area response ratios using the internal standards and an 8-point calibration curve was prepared in water:methanol (1:4, vol:vol). Relative recovery for FC, PCho, and GPC was 108.3%, 110.3%, and 112.8%, respectively. Pooled human milk was used as quality control (n = 8) with CVs of 4.2% (FC), 2.6% (PCho), and 3.2% (GPC).

SAS Software Enterprise Edition 9.4 (SAS Institute Inc.) was used to analyze the data. Primary outcome variables included the concentration of choline from each of the water-soluble forms, expressed as milligrams of choline per liter (e.g., 1 mg PCho contains 0.566 mg choline and would be reported as 0.566 mg choline/L from PCho); and the distribution of choline from each form expressed as a percentage of the total water-soluble choline concentration. Descriptive statistics were computed by maternal dietary pattern. Kurtosis and skewness values were evaluated for the data distribution of all outcome variables to determine whether data analysis was appropriate using an ANOVA test. Kurtosis values were between −0.24 and 2.89 and skewness values were between 0.53 and 1.17, which supports the use of an ANOVA as a robust test to assess differences in nonnormally distributed data [23]. Tukey's honestly significant difference test was used for multiple comparisons. Fisher's exact test was used to assess differences in categorical variables. Total choline intake from breast milk was estimated using the following assumptions: an infant consumes 0.78 L breast milk daily [24] and the fat-soluble choline fraction represents 16% of total choline [8, 9]. Regression analysis was performed to probe for significant factors associated with the outcome variables including lactation stage, maternal BMI, maternal age, and parity.

Results

A total of 74 women participated in this study: VEGAN (n = 26); VEGT (n = 22); and NONVEG (n = 26). Demographic information on study participants and their reported dietary and supplement patterns are summarized in Table 1. There was a significant difference between diet groups ($P < 0.05$) in maternal BMI, lactation stage, and diet duration, with lower BMI and diet duration in VEGAN than in NONVEG, and longer lactation stage in VEGT than in NONVEG. There was also a significant difference between diet groups in reported meat, dairy, fish, and egg intakes ($P < 0.001$).

Table 1. Summary of study participants and dietary habits[1]

	VEGAN	**VEGT**	**NONVEG**	***P***
n	26	22	26	
Maternal age, y	32.7 ± 5.2	32.2 ± 4.6	31.0 ± 4.7	0.438
Maternal parity, *n*	1.8 ± 0.8	1.6 ± 0.8	2.3 ± 1.8	0.158
Maternal BMI, kg/m^2	22.8 ± 3.1^{b}	23.9 ± 3.8^{a,b}	25.8 ± 4.5^{a}	0.021
Lactation stage, wk	36.6 ± 27.7^{a,b}	54.6 ± 46.0^{a}	27.5 ± 19.8^{b}	0.017
Diet duration, y	6.2 ± 5.5^{b}	7.5 ± 5.6^{b}	25.8 ± 11.5^{a}	<0.001
Ethnicity				0.900
Black	0 (0.0)	0 (0.0)	0 (0.0)	
Asian	1 (3.9)	0 (0.0)	0 (0.0)	
Hispanic	1 (3.9)	1 (4.6)	1 (3.9)	
White	22 (84.6)	20 (90.9)	21 (80.8)	
Mixed/other	2 (7.7)	1 (4.6)	4 (15.4)	
Education				0.279
HS/GED/other	1 (3.9)	0 (0.0)	1 (3.9)	
Some college/technical	2 (7.7)	3 (13.6)	7 (26.9)	
4-y college degree	8 (30.8)	11 (50.0)	9 (34.6)	
Graduate degree	15 (57.7)	8 (36.4)	9 (34.6)	
Food consumption patterns				
Meat products				<0.001
Never[2]	26 (100.0)	18 (81.8)	0 (0.0)	
Rarely	0 (0.0)	4 (18.2)	0 (0.0)	
Sometimes	0 (0.0)	0 (0.0)	3 (11.5)	
Often	0 (0.0)	0 (0.0)	23 (88.5)	
Dairy products				<0.001
Never	23 (88.5)	1 (4.6)	0 (0.0)	
Rarely	3 (11.5)	1 (4.6)	1 (3.9)	
Sometimes	0 (0.0)	5 (22.7)	0 (0.0)	
Often	0 (0.0)	15 (68.2)	25 (95.2)	
Eggs				<0.001
Never	25 (96.2)	4 (18.2)	0 (0.0)	
Rarely	1 (3.9)	3 (13.6)	1 (3.9)	
Sometimes	0 (0.0)	6 (27.3)	8 (30.8)	

	VEGAN	VEGT	NONVEG	*P*
Often	0 (0.0)	9 (40.9)	17 (65.4)	
Fish				<0.001
Never	26 (100.0)	18 (81.8)	1 (3.9)	
Rarely	0 (0.0)	1 (4.6)	10 (38.5)	
Sometimes	0 (0.0)	3 (13.6)	12 (46.2)	
Often	0 (0.0)	0 (0.0)	3 (11.5)	
Vitamin use				
Prenatal vitamin	14 (53.9)	13 (59.1)	14 (53.9)	0.915
Multivitamin	4 (15.4)	0 (0.0)	5 (19.2)	0.083
B-complex vitamin	2 (7.7)	0 (0.0)	1 (3.9)	0.771

1. Numerical values are means ± SDs and are evaluated with 1-factor ANOVA and Tukey's honestly significant difference test for multiple comparisons. Categorical values are *n* (%) and are evaluated using Fisher's exact test. Labeled means in a row without a common superscript letter are significantly different, $P < 0.05$. GED, general education diploma; HS, high school; NONVEG, nonvegetarian; VEGAN, vegan; VEGT, vegetarian.

2. Food frequency definitions: rarely (less than monthly); sometimes (at least monthly and less than weekly); often (≥1 times/wk). Summary information on study participants has previously been reported [20, 21].

Table 2. Water-soluble choline composition of breast milk by maternal dietary pattern[1]

	VEGAN	VEGT	NONVEG	*P*
n	26	22	26	
Total water-soluble choline, mg/L	134 ± 49.5 (58.5, 301)	132 ± 60.0 (4.0, 261)	128 ± 28.0 (91.7, 195)	0.6273
FC, mg/L	24.5 ± 17.6 (4.1, 69.4)	24.4 ± 16.1 (0.0, 75.0)	26.0 ± 10.8 (8.1, 49.6)	0.4055
Choline in PCho, mg/L	47.2 ± 35.2 (2.4, 126)	60.4 ± 36.8 (3.9, 150)	59.4 ± 28.2 (11.8, 104)	0.5117
Choline in GPC, mg/L	62.7 ± 25.3[a] (21.7, 112)	47.7 ± 21.2[b] (0.0, 85.2)	42.4 ± 14.9[b] (22.0, 86.4)	0.0052
Distribution of choline, %				
FC, %	20.0 ± 14.4 (2.3, 58.8)	18.1 ± 9.5 (0.0, 32.5)	21.4 ± 10.4 (6.0, 42.5)	0.3265
Choline in PCho, %	32.5 ± 18.3[b] (4.1, 69.3)	46.1 ± 18.3[a] (13.3, 97.6)	44.8 ± 15.7[a] (10.1, 69.7)	0.0106
Choline in GPC, %	47.5 ± 14.0[a] (28.5, 78.6)	35.7 ± 13.3[b] (1.0, 56.7)	33.8 ± 10.7[b] (14.3, 57.2)	0.0026

1. Values are mean ± SD (range) for concentration of choline (expressed as milligrams of choline from each choline form per liter of milk) and distribution of choline (expressed as percentage of total choline contribution from each choline form). Comparisons between dietary groups were evaluated by ANOVA, controlling for differences in maternal BMI and lactation stage, with Tukey's honestly significant difference test for multiple comparisons. Labeled means in a row without a common superscript letter are significantly different, $P < 0.05$. FC, free choline; GPC, glycerophosphocholine; NONVEG, nonvegetarian; PCho, phosphocholine; VEGAN, vegan; VEGT, vegetarian.

Milk choline composition by vegan, vegetarian, and nonvegetarian diet patterns

The total water-soluble choline concentration (mean ± SD) of breast milk was 132 ± 46.3 mg/L and ranged from 4.0 mg/L to 301 mg/L (quartile 1: 104 mg/L; quartile 3: 148 mg/L). When controlling for differences in maternal BMI and lactation stage, the FC, PCho, and total breast-milk choline concentrations did not differ by maternal diet pattern ($P > 0.05$). The concentration of choline from GPC in breast milk in the VEGAN group was significantly higher ($P < 0.05$) than in VEGT and NONVEG. VEGAN also differed significantly from VEGT and NONVEG in the percentage of total choline from GPC and in the percentage of total choline from PCho. Total choline concentrations and distribution of choline across different choline forms are summarized by maternal diet pattern in Table 2.

AI of choline

Fifty-nine percent (44 of 74) of the samples did not meet the AI for choline assuming intake of 0.78 L milk/d. There was no observed difference ($P > 0.05$) in the prevalence of samples meeting the AI for choline based on maternal diet pattern (VEGAN, VEGT, or NONVEG). Limiting this analysis to samples that were from a stage of lactation of 6 mo or earlier, when breast milk is recommended as the sole diet for an infant, 63% of the samples (24 of 38) would not meet the AI of 125 mg/d at intake volumes of 0.78 L/d, with no observed difference by maternal diet ($P > 0.05$).

Maternal factors associated with milk choline composition

Lactation stage was positively associated with the concentration of choline (milligrams per liter) for FC, GPC, and total water-soluble choline. Lactation stage was negatively associated with the percentage of choline found in PCho. Maternal BMI, but not maternal age or parity, was significantly associated with some choline forms (Table 3).

Table 3. Maternal factors associated with choline concentrations and distributions[1]

Maternal factor	**β (95% CI)**	**Standardized β**	R^2	P
Lactation stage				
FC	0.15 (0.05, 0.25)	0.33	0.11	0.0036
Choline in PCho	NS	NS	NS	NS
Choline in GPC	0.58 (0.21, 0.94)	0.35	0.12	0.0023
Total water-soluble choline	0.51 (0.21, 0.81)	0.37	0.14	0.0013
Choline				
% from FC	NS	NS	NS	NS
% from PCho	−0.001 (−0.003, −0.001)	−0.24	0.06	0.0381
% from GPC	NS	NS	NS	NS
Maternal BMI				
GPC	−2.10 (−3.33, −0.88)	−0.37	0.14	0.0010
% from PCho	0.01 (0.00, 0.02)	0.28	0.08	0.0179
% from GPC	−0.01 (−0.02, −0.01)	−0.40	0.16	0.0004
Other choline fractions	NS	NS	NS	NS
Maternal age				
All choline fractions	NS	NS	NS	NS
Maternal parity				
All choline fractions	NS	NS	NS	NS

1. Values are β, standardized β, and R^2 values from linear regression. FC, free choline; GPC, glycerophosphocholine; NS, nonsignificant ($P > 0.05$); PCho, phosphocholine.

Discussion

We observed wide variability in the total water-soluble choline composition of human milk, which has also been reported by others [11, 25]. Contrary to our hypothesis, we did not observe significant differences in the total water-soluble choline concentration of breast milk from women who reported limited animal-product intake. Zeisel et al. [26] reported a high prevalence of low FC concentrations from lactating women in Ecuador who consumed low-animal-product diets compared with lactating women in Boston. Only FC was measured in that study, which has been reported to contribute 7–15% of the total choline concentration in breast milk [8, 9, 12]; therefore, differences in overall choline concentrations could not be established. Wiedeman et al.[15] compared the water-soluble choline content of breast milk from lactating women in Canada (*n* = 301) and Cambodia (*n* = 67) and found no differences, despite differences in food availability in these countries. In a subset of the Canadian cohort where dietary intake data were available, there were no significant relations between maternal choline intake during pregnancy and the choline composition of breast milk at 2 mo postpartum. The lag between the assessment of maternal intake and the assessment of breast-milk composition is one possible reason that no relation was detected. Fischer et al. [12] measured maternal choline intake using a 3-d food record immediately before collecting a breast-milk sample and the only breast-milk choline form that was significantly associated with maternal choline intake in unsupplemented lactating women was PtdCho, which represented only 9% of the total choline in study samples. Fischer et al.'s finding of a lack of relation between maternal dietary choline intake and breast-milk water-soluble choline compounds supports our observation that maternal plant-based diet was not associated with breast-milk total water-soluble choline composition.

Although we did not observe differences in the total water-soluble choline composition of breast milk by maternal diet pattern, we did observe significant differences in the form of choline in breast milk by maternal diet pattern, with VEGAN having higher concentrations and distribution of GPC, and lower concentrations of PCho. This is a novel finding, and the potential biological significance of the different choline forms warrants further investigation.

Understanding how choline is derived in human milk may provide insights into our findings. In a study of lactating and nonlactating women consuming 2 different amounts of choline (480 mg/d and 930 mg/d), the lactating women had almost 30% higher serum FC concentrations than nonlactating women, suggesting that lactation may preserve maternal serum choline, theoretically enabling a greater supply of choline available for milk synthesis [13]. Within lactating women, those at the higher choline intake amounts (930 mg/d compared with 480 mg/d) had significantly higher concentrations of serum FC, as well as higher concentrations of breast-milk PCho, GPC, and total choline after a 10-wk intervention, which supports the link between higher choline intake amounts and increased maternal serum choline being available for milk production. The 94% increase in maternal choline intake between the 930 mg/d and the 480 mg/d groups resulted in a 20% increase in mean breast-milk choline concentrations (1200 ± 60 µmol/L compared with 1000 ± 50 µmol/L; $P = 0.041$), suggesting that the effect of maternal intake above certain amounts may be limited. In a study that used an isotopically labeled choline supplement, the labeled choline supplement was not found intact in the breast milk, but had instead been used to synthesize new choline forms via hepatic PEMT pathways, which were present in the milk [13]. Fischer et al. [12] probed for genetic variations in lactating women with high choline concentrations in their milk and found polymorphisms in the methylenetetrahydrofolate dehydrogenase gene (*MTHFD1*). Others have reported that adult choline requirements are significantly shaped by genetic variations in the ability to synthesize choline, with 37% of adults showing no signs of choline deficiency after consuming a low-choline diet for 42 d [27]. Collectively, these studies suggest that the choline composition in human milk is affected by a variety of factors including choline intake, differences in maternal choline metabolism in the lactating compared with the nonlactating state, genetic differences in maternal choline metabolism, and de novo synthesis of choline forms via hepatic PEMT pathways. Of relevance to our study population is that plant-based diets tend to be high in folate, and there was a high use of B-12 supplements among our study participants with no difference in milk B-12 concentration [20], suggesting the availability of methyl donor nutrients to support elevated choline synthesis via the PEMT pathway.

Our findings regarding a high theoretical prevalence of samples that would not meet the current AI of choline for infants age 0–6 mo are consistent with those reported by Wiedeman et al. [15] who evaluated water-soluble choline compounds in breast milk from 368 lactating women residing in Canada and Cambodia. In their study, 81% of samples did not meet the AI for choline, compared with 63% of the samples from our study. Wiedeman et al. [15] noted that the current AI for choline for infants age 0–6 mo is 20% higher than the observed choline content in the breast milk of apparently healthy women, and questioned whether the AI might be too high after finding that <20% of healthy lactating women in Canada (*n* = 301) had choline concentrations that would meet the current AI. The mean total water-soluble choline content for all samples in our study was 132 mg/L, which is comparable with the mean water-soluble choline concentration of human milk in unsupplemented lactating women reported by others[8, 9, 12, 15]. Notably, the mean water-soluble choline content we observed is 25% more than the 105 mg/L of total water-soluble choline reported in a tightly controlled 10-wk intake study where lactating women received 930 mg choline/d through food and supplements [13], which is almost double the current AI for lactating women. Total breast-milk choline concentrations of 130 mg/L would translate to an infant intake of 101 mg total choline/d, assuming volumes of 0.78 L breast milk/d, providing further support that the current AI of 125 mg/d for infant choline intake may be too high. Even at a lower AI of 105 mg/d, one-third of the samples in our study would not meet this target, which warrants further investigation.

Limitations

The results are based on a convenience sample of lactating women, which might not be generalizable to other populations, including those with low food security. We did not measure the fat-soluble choline fraction in the milk; however, studies have shown that the water-soluble choline fraction represents the majority of the choline in breast milk [8, 9, 12, 13]. We did not measure maternal choline intake or maternal serum choline concentrations; however, others have reported significantly lower serum choline concentrations after consuming vegan meals than after omnivore meals [28]. Over 50% of participants reported using prenatal and multivitamin supplements, which may have contributed to the sparing of choline from use in one-carbon metabolism. We did not ask about exclusivity of breastfeeding; therefore, comparisons to AI were hypothetical, assuming that human milk was the only feeding source.

Conclusions and future research

We observed a wide range of water-soluble choline concentrations (4–301 mg/L) in the mature breast milk of women following vegan, vegetarian, and nonvegetarian diets, with no observed difference in the total choline content by maternal plant-based diet pattern. This large variation warrants further investigation, given the important role of choline in infant development. It also suggests that maternal plant-based diet by itself is not a risk factor for low breast-milk water-soluble choline concentrations. Maternal diet pattern was significantly associated with the forms of water-soluble choline in breast milk, with vegans having higher concentrations and proportions of choline coming from GPC. The biological significance of differences in dietary choline forms is an important area for future research. This research also supports the conclusions of others regarding a need to revisit current choline AI recommendations for infants aged 0–6 mo.

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Notes

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