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ORIGINAL CONTRIBUTION

A cross-sectional study of fatty acids and brain-derived neurotrophic factor (BDNF) in human milk from lactating women following vegan, vegetarian, and omnivore diets

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Abstract

Purpose Essential fatty acids are critical for brain growth and neurodevelopment in infancy. Maternal diet and supplement use have a significant impact on the fat composition of human milk. The objective of this study is to assess supplement utilization patterns and fatty acid and brain-derived neurotrophic factor (BDNF) concentrations in the breast milk of women following vegan, vegetarian, and omnivore diet patterns.

Methods This is a cross-sectional, observational study of 74 lactating women in the United States following a vegan ($n=26$), vegetarian ($n=22$), or omnivore ($n=26$) diet pattern. A single breast milk sample was collected from each participant and assessed for fatty acids and BDNF.

Results Median unsaturated fatty acids in the breast milk of vegan, vegetarian, and omnivores, as a percentage of total fatty acids, was 66.0, 57.8, and 56.2%, respectively ($p<0.001$). Total omega-3 percentages were 2.29% for vegans, 1.55% for vegetarians, and 1.46% for omnivores ($p<0.001$). Docosahexaenoic acid percentages were not different by diet pattern, but over 80% of participants had milk concentrations below 0.30% of total fatty acids. Reports of omega-3 supplements use (10/74) and weekly seafood consumption (3/74) were limited. BDNF was not detectable in any samples.

Conclusions Breast milk from vegans had significantly higher unsaturated fat and total omega-3 fats, and lower saturated fats, *trans* fats, and omega-6 to omega-3 ratios than their vegetarian and omnivore counterparts. Docosahexaenoic acid concentrations in breast milk were low regardless of maternal diet pattern, and were reflective of low seafood intake and supplement use.

Keywords Human milk · Breast milk · Brain derived neurotrophic factor · Docosahexaenoic acid · Vegetarian · Vegan

Abbreviations

ALA	Alpha-linolenic acid
BDNF	Brain derived neurotrophic factor
BSQ	Basic screening questionnaire
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
LA	Linoleic acid (LA)
LCPUFA	Long chain polyunsaturated fatty acids

Introduction

The lipid fraction of human milk provides a major energy source for the rapidly growing infant as well as essential omega-3 and omega-6 polyunsaturated fatty acids which may be important for visual and neural development. On average, over 50% of calories in human milk comes from fat [1], although significant variation exists among lactating women, with total fat consistently being the most variable macronutrient [2–6]. Likewise, significant variability in omega-3 and omega-6 fatty acid composition in human milk has been reported [7–9].

Alpha-linolenic acid (ALA), an essential omega-3 fatty acid, and linoleic acid (LA), an essential omega-6 fatty acid,

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are predominantly found in plant-based foods. The Recommended Dietary Allowance (RDA) for lactating women in the United States is 1.3 and 13.0 g/day for ALA and LA, respectively, though actual infant requirements are impacted by a complex set of factors including the kinetics of maternal dietary transfer into breast milk, maternal and infant stores, and endogenous synthesis of some nutrients. The long chain polyunsaturated fatty acids (LCPUFA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can be obtained primarily through fish-based foods or synthesized from ALA, though conversion rates are low, while arachidonic acid (AA) can be obtained from animal-based foods or derived from LA [10].

Reviews regarding the long-term effects of breastfeeding suggest a modest benefit to intelligence [11, 12]. There is considerable interest in the role of DHA as a potential mechanism for improved neurodevelopment due to evidence of higher DHA in the brain and plasma of breastfed infants compared to infants fed formula that was void of DHA [13–15]. Observational studies have shown a correlation between breast milk DHA levels and visual and neurodevelopment outcomes, though other components in human milk may be contributing to these observations [16]. In animal studies, dietary DHA was positively associated with brain-derived neurotrophic factor (BDNF), a growth factor that plays an important role in neurodevelopment [17–19]. In human studies, results on the relationship between dietary DHA and serum BDNF are conflicting [20, 21]. Nassar et al. reported higher levels of serum BDNF in breastfed infants, compared to formula fed infants, though breast milk was not measured as a potential source of the BDNF [22]. Limited studies have assessed BDNF in human milk, with a 100-fold difference in concentrations reported between studies [23–25]. No studies have investigated a potential relationship between BDNF concentrations in human milk and maternal diet.

Maternal diet has a strong influence on the lipid profile of human milk [26–28]. Vegetarian and vegan diets provide differing content of total, saturated, unsaturated, and essential fats compared to omnivore diet patterns [29, 30], which likely influences breast milk composition. Conflicting findings regarding the LCPUFA content in human milk of women who followed a plant-based diet have been reported [31–33]. DHA supplements, but not ALA supplements, have been shown to increase breast milk DHA concentrations [34–38], which may be an important strategy for lactating women who consume little dietary DHA.

The purpose of this study is to assess DHA supplement utilization patterns, and fatty acid and BDNF concentrations in the breast milk of healthy, lactating women in the United States who follow three different diet patterns: vegan, vegetarian, and omnivore. We hypothesize that there will be significant differences in BDNF and fatty acid profiles between diet groups, and that DHA supplement use and fish intake will predict breast milk DHA concentrations.

Methods

Subject recruitment

This was a cross-sectional, observational study of lactating women in the United States who were recruited between November 2016 and April 2017 through online communities focused on breastfeeding or vegetarianism, and through local religious communities with a vegetarian population. Inclusion criteria were: maternal age of 18 or older; stage of lactation 2 weeks or more postpartum; healthy, term birth; willingness to provide one breast milk sample for the study and complete a short diet survey. Exclusion criteria included any of the following conditions: pregnant; taking metformin; Crohn's disease; Celiac disease; MTHFR gene mutation; liver disease; myeloproliferative disorders; hyperthyroid or hypothyroid. Interested individuals were asked to complete an online basic screening questionnaire (BSQ) to determine eligibility and self-identify their diet pattern (vegan, vegetarian, omnivore). The BSQ was completed by 371 individuals, 229 were excluded because they did not qualify or because target sample size had been reached, 142 were invited to participate, and 74 completed the study. This study was part of a project to assess vitamin B12 composition in human milk, with sample size calculations, and B12 results reported elsewhere [39]. Briefly, over 90% of vegans reported using supplements containing B12 and there was no observed difference in the breast milk B12 concentrations by maternal diet pattern. Subjects were provided with a digital informed consent and received a $25 gift card for participation. This study was approved by the Institutional Review Boards at East Carolina University and the University of North Carolina at Greensboro.

Diet survey and sample collection

Participants were asked to complete a digital questionnaire which included demographic, diet, and supplement information. Consumption of meat (beef, lamb, pork, and poultry), dairy (milk, cheeses, and yogurts), egg, fish, and omega-3 supplemented margarine were categorized as follows: Never; Rarely (less than 1 times per month); Sometimes (1–4 times per month), Often (more than 1 time per week). A diet category was assigned to each participant based on detailed responses to the diet survey as follows: vegans never consumed meat and never or rarely consumed other animal products; vegetarians never or rarely consumed meat and sometimes or often consumed other animal products; omnivores consumed meat sometimes or often. To ensure

a representative milk sample was collected, participants received written collection instructions. Briefly, samples were collected in the morning during the first or second feeding of the day, and at least 2 h since the previous feeding, by complete expression of one breast in a dimly lit room, to help protect light-sensitive nutrients [40]. The entire sample was transferred to a storage container appropriate for breast milk storage, dated, wrapped in aluminum foil, and placed in the freezer until the sample was collected by a researcher, or shipped on dry ice to the University of North Carolina Greensboro laboratory. Upon receipt, samples were thawed, gently mixed by hand, and aliquoted in a dimly lit lab, and stored at − 20 °C until analysis. Total storage time of samples (at subject's home and in the lab) averaged 197 days (minimum 93 days; maximum 257 days) prior to completion of fatty acid and BDNF analysis. Average total storage time prior to creamatocrit analysis was 340 days (minimum 236 days; maximum 400 days).

Milk analysis

Total fat was measured by creamatocrit, using previously described methods for human milk that were validated against reference methods [41]. Briefly, samples were thawed, vortexed for 10 s, and drawn into a 75 µL capillary tube which was centrifuged at 11,200 RPM for 12 min on a Zip-IQ PCV flatbed centrifuge (LW Scientific, Lawrenceville, GA). Capillary tubes were immediately read on a Creamatocrit Plus (EKF Diagnostics, Boerne, TX) by a trained researcher. Samples were measured in duplicate and the average coefficient of variation (CV) was 2.3%.

The fatty acids present in the samples were measured as fatty acid methyl esters according to the method of Bannon et al. [42]. Briefly, 0.2 g of milk was hydrolyzed using 1.0 mL of 0.5 N methanolic sodium hydroxide (Thermo Fisher Scientific, Fair Lawn, NJ) for 5 min. The resulting fatty acids were converted to their methyl esters by the addition of 1.0 mL of boron trifluoride (Sigma Chemical Corp., St. Louis, MO) as a catalyst and refluxing for an additional 10 min. The methyl esters were extracted into 1.0 mL of hexane. The resulting fatty acid methyl esters were analyzed using a Perkin Elmer Autosampler XL GC (Perkin Elmer Instruments, Norwalk, CN) and a BPX-70 column (SGE Analytical Science, Austin, TX) with a flame ionization detector (FID) and a capillary column containing 70% cyanopropyl polysilphenylene-siloxane as the stationary phase (30 m length, 0.25 mm i.d., 0.25 mm film thickness). Helium was used as the carrier gas at 1.85 mL/min. A temperature program was used with an initial temperature of 60 °C held for 2 min. The temperature was increased to 180 °C at 10°C/min, then to a final temperature of 235 °C at 4°C/min. The injector was heated to 265 °C and the split flow was 76.9 mL/min. The detector temperature was 265 °C. Fatty acids were identified by comparison with fatty acid methyl ester standards purchased from Matreya (Matreya, Inc., Pleasant Gap, PA). The amount of each fatty acid present was calculated by normalizing the peak area for each fatty acid identified against the total peak area according to AOCS Method Ce 1b-89 [43]. Fatty acid composition is reported as g/100 g total fatty acids and is detectable to 0.01 g per 100 g of total fatty acids. Values that fell below the limit of detection were set to zero. Duplicate readings were performed on 33 of the samples and the average CV was 7.6%.

Total BDNF was measured in duplicate using a Quantikine Total BDNF ELISA kit (part number DBNT00, R&D Systems, Minneapolis, MN) which has a standard curve range of 15.6–1000 pg/mL. This method was selected because it was used in two of the three published studies on BDNF in human milk, and was the only published methods available at the time that had been validated by the manufacturer for use with human milk [23, 25]. Samples were prepared according to the manufacturer's instructions to centrifuge for 15 min at 1000 RPM, remove the aqueous fraction, and repeat two more times. Alternative sample preparation methods were also used based on those reported by others including centrifuging at increased speeds of 10,000 RPM and acidification to potentially improve BDNF binding capacity [23, 25, 44]. The average CV for duplicate samples read on a BioTek Synergy Plate Reader (BioTek Instruments, Winooski, VT) at 450 nm was 1.8% and the average R^2 value for the standard curves was 0.999.

Statistical analysis

Descriptive statistics were computed for all outcome variables. Distribution of data for normalcy was evaluated using a Shapiro–Wilk test. Normally distributed outcome variables were evaluated using a one-way ANOVA. Distributions of non-parametric variables were evaluated using a Kruskal–Wallis test. Due to the large number of statistical tests performed on numerical outcome variables, a Bonferroni correction to the p value was computed as 0.05/41 which resulted in a significance threshold of 0.001. Differences in categorical variables were evaluated using a Fisher's exact test with a significance threshold of 0.05. Linear regression was used to identify other significant predictor variables including maternal age, parity, BMI, and stage of lactation. Analysis was conducted using SAS Enterprise Software 9.4 (SAS Corporation; Cary, NC).

Results

A total of 74 participants completed this study, with 26 classified as vegan, 22 classified as vegetarian, and 26 classified as omnivores based on responses to the diet survey. There

was no significant difference in age, parity, ethnicity, or education ($p > 0.05$), but maternal BMI and lactation stage differed ($p < 0.05$) by diet type. Ten participants (13.5%) reported using DHA/EPA supplements, with differences in supplement use by diet pattern approaching significance (vegan 26.9%; vegetarian 9.1%; omnivore 3.9%; $p = 0.052$). There was a significant difference in the frequency of consumption of meat, dairy, eggs, and fish by diet group ($p < 0.001$). Consumption patterns of omega-3 containing margarines did not differ by group. Descriptive statistics of study participants and their diet patterns are summarized in Table 1.

Fat content in human milk by diet pattern

Thirty-one of the 41 outcome variables measured followed a non-parametric distribution; therefore, median and interquartile descriptive statistics are provided in Table 2 by maternal diet pattern. There was a significant difference ($p \leq 0.001$) in the breast milk composition of saturated fat, unsaturated fat, and *trans* fats by diet. Long chain fatty acids containing 15 or more carbons were the predominant fatty acids in all samples (mean = 84.3%; min = 61.3%; max = 91.3%), and no differences in the distribution of total long chain ($p = 0.906$) or total medium chain ($p = 0.897$) fatty acids between diet groups were observed. Nine fatty acids made up 93% or more of the fatty acids in all milk samples, regardless of maternal diet: C10:0; C12:0; C14:0; C16:0; C16:1,*cis*; C18:0; C18:1,*cis*; C18:2,*cis* n-6; and C18:3,*cis* n-3. Of these most abundant fatty acids, five of them were significantly different by diet group: C16:0; C16:1,*cis*; C18:0; C18:1,*cis*; and C18:3,*cis* n-3 ($p \leq 0.001$). Fatty acids that were present in smaller quantities were also significantly different by diet group.

Omega-3, omega-6, and BDNF

The omega-3 fatty acids measured included ALA, EPA, and DHA. DHA was undetectable in 11/74 (14.9%) samples and EPA was undetectable in 39/74 (52.7%) samples, with no observed difference in prevalence of undetectable samples by diet groups ($p > 0.05$). A significant difference in total omega-3 fatty acids was observed by diet group, driven by higher percentages of ALA ($p \leq 0.001$), but not EPA ($p = 0.057$) or DHA ($p = 0.543$). Eighty-two percent (61/74) of study participants had DHA milk concentrations below 0.3%, and there was no difference in the prevalence of low milk DHA (< 0.3%) by diet group ($p = 0.555$).

The omega-6 fatty acids measured included LA, gamma linolenic acid (C18:3,cis,gamma), dihomo-gamma linolenic acid (C20:3,cis), and AA. No difference in total omega-6 fatty acids was observed by diet pattern, but there were significant differences in gamma linolenic acid ($p \leq 0.001$). The ratio of LA to ALA (median, IQR) was significantly lower ($p \leq 0.001$) in the breast milk of vegans (9.3, 2.1) compared to vegetarians (12.2, 4.9) and omnivores (12.7, 6.2). When excluding the 10 participants who reported taking DHA/EPA supplements from the analysis, there were no changes to the significant findings related to omega-3 fatty acids. Use of a DHA/EPA supplement was a significant positive predictor of milk ALA, DHA, and total omega-3 composition, and a significant negative predictor of omega-6:omega-3 ratios (Table 3). There was no measurable BDNF in any samples.

Other factors predicting human milk fat profiles

Stage of lactation, maternal BMI, and maternal Age were modeled against individual fatty acids, and fat subcategories (total, saturated, unsaturated, trans, medium chain, long chain, omega-3, and omega-6) to evaluate other potential relationships in our dataset. A summary of β coefficients, and R square values are presented in Table 3 for variables with a significant relationship.

Discussion

Polyunsaturated fatty acids

We observed a significant difference in the saturated, unsaturated, and omega-3 fatty acid profiles of breast milk from lactating women by maternal diet pattern. This supports our hypothesis that maternal plant-based diets, which are rich in unsaturated fats, have a significant impact on breast milk lipid profiles; however, within the family of omega-3 fatty acids we did not observe a difference in DHA, which was unexpected. There are conflicting findings in the literature regarding the impact of maternal plant-based diets on DHA composition of breast milk from women living in the United States. Two small studies reported no difference in breast milk DHA between vegetarians and omnivores, but both studies were small, and occasional fish consumption in one vegetarian group may have influenced findings [31, 32]. Sanders et al. studied the breast milk of 19 vegans, 5 vegetarians, and 21 omnivores and reported DHA composition of 0.14, 0.30, and 0.37%, respectively, which was significantly different by diet [33]. While we did not observe differences in DHA by diet type, it is important to note that the median DHA composition across all diet groups in our study was much lower than those reported by Sanders et al. and compared to worldwide averages [8, 9, 33]. We observed median DHA of 0.14, 0.17, and 0.18% for vegans, vegetarians, and omnivores, respectively. Two independent reviews of worldwide DHA breast milk concentrations reported averages of 0.32–0.37%, with concentrations in US populations often reported at 0.20% or lower, which is similar to our findings

Table 1 Summary of study participants and dietary habits

	Vegan ($n=26$)	Vegetarian ($n=22$)	Omnivores ($n=26$)	p value
Maternal age (years)	32.7 (5.2)	32.2 (4.6)	31.0 (4.7)	0.438
Maternal parity (births)	1.8 (0.8)	1.6 (0.8)	2.3 (1.8)	0.158
Maternal BMI (kg/m^2)	22.8^{a} (3.1)	23.9^{ab} (3.8)	25.8^{b} (4.5)	0.021
Lactation stage (weeks)	36.6^{ab} (27.7)	54.6^{a} (46.0)	27.5^{b} (19.8)	0.017
Diet duration (years)	6.2^{a} (5.5)	7.5^{a} (5.55)	25.8^{b} (11.5)	< 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-year 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	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)	
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)	
Omega-3 margarine				1.00
Never	23 (88.5)	20 (90.9)	22 (84.6)	
Rarely	2 (7.7)	1 (4.6)	2 (7.7)	
Sometimes	1 (3.9)	1 (4.6)	1 (3.9)	
Often	0 (0.0)	0 (0.0)	1 (3.9)	
Use DHA/EPA supplement: # (%)	7 (26.9)	2 (9.1)	1 (3.9)	0.052

Numerical data are presented as means (standard deviations) and are evaluated with one-way ANOVA and Tukey HSD for multiple comparisons. Values in the same row with a common letter in the superscript are not significantly different. Categorical data are presented as # (%) and are evaluated using Fisher's exact test. Food frequency definitions: never; rarely (less than 1 times per month); sometimes (1–4 times per month), often (more than 1 time per week)

Table 2 Fat composition of human milk by maternal diet pattern

	Vegan ($n=26$)	Vegetarian ($n=22$)	Omnivores ($n=26$)	*p value
Total fat (g/dL)	3.0 (1.7)	4.0 (2.9)	4.0 (2.9)	0.041
% Saturated*	33.1 (6.2)	40.0 (8.6)	42.3 (8.4)	< 0.001
C8:0	0.21 (0.13)	0.18 (0.15)	0.17 (0.18)	0.052
C10:0	1.46 (0.71)	1.50 (0.74)	1.65 (0.43)	0.332
C12:0	7.12 (3.63)	5.88 (2.01)	6.22 (3.07)	0.186
C14:0	5.55 (3.89)	6.81 (3.06)	6.56 (3.36)	0.571
C15:0*	0.06 (0.08)	0.24 (0.20)	0.26 (0.16)	< 0.001
C16:0*	13.32 (2.49)	18.57 (5.22)	20.02 (4.43)	< 0.001
C17:0*	0.12 (0.02)	0.22 (0.11)	0.26 (0.07)	< 0.001
C18:0*	3.98 (1.27)	5.85 (2.52)	6.12 (1.72)	< 0.001
C20:0	0.16 (0.14)	0.20 (0.16)	0.09 (0.15)	0.002
C22:0	0.06 (0.09)	0.05 (0.08)	0.00 (0.04)	0.015
C24:0	0.00 (0.07)	0.06 (0.08)	0.00 (0.08)	0.765
% Unsaturated*	66.0 (6.5)	57.8 (9.8)	56.2 (8.5)	< 0.001
Total omega-3*	2.29 (0.77)	1.55 (0.56)	1.46 (0.94)	< 0.001
ALA (C18:3 *cis*)*	2.09 (0.75)	1.40 (0.70)	1.19 (0.80)	< 0.001
EPA (C20:5 *cis*)	0.0 (0.05)	0.0 (0.05)	0.04 (0.18)	0.057
DHA (C22:6 *cis*)	0.14 (0.09)	0.17 (0.14)	0.18 (0.18)	0.543
Total omega-6	19.65 (2.88)	18.69 (6.76)	17.11 (6.39)	0.492
LA (C18:2 *cis*)	18.86 (2.29)	17.98 (6.91)	16.28 (6.40)	0.366
C18:3 *cis*, gamma*	0.0 (0.0)	0.0 (0.15)	0.08 (0.19)	< 0.001
C20:3 *cis*	0.27 (0.09)	0.34 (0.11)	0.31 (0.13)	0.106
AA (C20:4)	0.38 (0.21)	0.38 (0.13)	0.45 (0.16)	0.014
LA : ALA*	9.3 (2.1)	12.2 (4.9)	12.7 (6.2)	< 0.001
Omega-6: omega-3*	8.8 (2.4)	11.4 (3.7)	11.2 (3.8)	< 0.001
C14:1 *cis**	0.00 (0.07)	0.20 (0.13)	0.23 (0.15)	< 0.001
C15:1 *cis*	0.00 (0.00)	0.00 (0.05)	0.00 (0.04)	0.002
C16:1 *cis 9**	1.01 (0.89)	1.44 (0.86)	2.04 (0.63)	< 0.001
C17:1 *cis**	0.03 (0.08)	0.12 (0.09)	0.16 (0.06)	< 0.001
C18:1 *cis**	39.86 (7.56)	35.09 (7.96)	33.09 (8.50)	< 0.001
C20:1 *cis*	0.38 (0.16)	0.33 (0.10)	0.29 (0.12)	0.003
C20:2 *cis*	0.26 (0.15)	0.26 (0.08)	0.27 (0.11)	0.873
C22:1 *cis*	0.00 (0.09)	0.05 (0.07)	0.00 (0.05)	0.278
C24:1 *cis*	0.00 (0.01)	0.00 (0.03)	0.00 (0.0)	0.430
% Total Trans*	0.44 (0.19)	0.66 (0.71)	1.09 (0.55)	< 0.001
C14:1 *trans*	0.00 (0.00)	0.00 (0.03)	0.00 (0.00)	0.108
C16:1 *trans*	0.36 (0.17)	0.34 (0.10)	0.36 (0.10)	0.863
C18:1 *trans**	0.00 (0.07)	0.14 (0.61)	0.62 (0.45)	< 0.001
C18:2 *trans*	0.00 (0.07)	0.14 (0.34)	0.15 (0.29)	0.021
Medium chain (%)	14.9 (6.3)	14.6 (5.6)	14.9 (6.7)	0.897
Long chain (%)	85.1 (6.9)	85.4 (5.9)	85.6 (6.7)	0.906

Data represent median (interquartile range, computed as quartile 3 subtract quartile 1). Bold font indicates outcome variables that followed a normal distribution and mean differences were evaluated using a one-way ANOVA; regular font indicates outcome variables that followed a non-parametric distribution and distributions were evaluated using a Kruskal–Wallis test

*The p value for significance was determined using a Bonferroni's correction of 0.05/41 ($p \leq 0.001$)

[8, 9]. While use of a DHA supplement is well supported in the literature as a way to increase breast milk DHA [34–38], less than one-third of vegans in our study reported using DHA supplements. Similarly, fish consumption was low, with only 3/26 (11.5%) of omnivores and 0/22 (0.0%) vegetarians reporting more than weekly consumption. This suggests low adherence to recommendations in the 2015 Dietary Guidelines for Americans (DGAs) for seafood consumption

Table 3 Bivariate regression analysis of significant maternal factors predicting human milk fat composition

	β	Standard error	Standardized β	Adjusted R^2	p value
Use of DHA/EPA supplement					
DHA	0.10	0.040	0.277	0.064	0.017
Total omega-3	0.71	0.196	0.392	0.142	< 0.001
Omega-6:omega-3 ratio	− 2.84	0.986	− 0.321	0.091	0.005
C18:3,*cis* (ALA)	0.62	0.194	0.352	0.112	0.002
Stage of lactation					
C14:0	0.03	0.009	0.398	0.147	< 0.001
C16:1,*cis*	− 0.01	0.002	− 0.353	0.112	0.002
Medium chain FFA	0.04	0.020	0.228	0.039	0.050
Long chain FFA	− 0.04	0.020	− 0.239	0.044	0.041
Maternal BMI					
C16:0	0.31	0.119	0.291	0.072	0.012
C16:1,*cis*	0.05	0.018	0.286	0.069	0.014
C18:1,*cis*	− 0.37	0.177	− 0.241	0.045	0.038
Maternal age					
Total fat	0.10	0.047	0.245	0.047	0.036
C8:0	0.01	0.00	0.365	0.121	0.001
C20:5 (EPA)	− 0.01	0.00	− 0.305	0.080	0.008

of eight ounces per week [45]. A recent study on seafood consumption patterns in the United States reported over 80% of Americans were not meeting DGA seafood recommendations, which is consistent with our observation of limited seafood intake, even among omnivores [46].

There is limited research on infant outcomes related to breast milk lipid composition. Observational studies have shown a relationship between breast milk DHA and infant visual and neurodevelopment outcomes [14, 16]. A recent review by Innis suggested that infant DHA biomarkers are likely influenced by many factors including maternal DHA status during pregnancy, breast milk DHA concentration, and genetic differences in DHA synthesis pathways [16]. Intervention studies of DHA supplementation among lactating women have reported inconclusive findings, which may be attributed to a wide range of supplement doses and varying baseline DHA levels [16, 47, 48]. While there is currently no RDA for DHA, several researchers have recommended establishing an RDA of 200 mg/day of DHA during pregnancy and lactation which would yield breast milk DHA of 0.30% [47, 49, 50]. Eighty-two percent of our study participants had breast milk DHA concentrations below 0.30%, suggesting potential widespread inadequacy of breast milk DHA levels in lactating women in the United States, independent of maternal diet pattern.

Trans fats

In 2015, the Food and Drug Administration (FDA) removed the "generally regarded as safe" designation from *trans* fats due to growing evidence of health risks [51]. Studies conducted prior to this ruling suggest that trans fats were present in breast milk, with reported averages of 7% [52, 53]. Friesen et al. documented a decline in breast milk *trans* fats between 2004 (mean 6.2%) and 2006 (mean 4.6%) in Canadian women after changes in *trans* fat food labeling requirements went into effect [54]. In our study, median *trans* fat breast milk concentrations were below 1.1%, with vegans having lower levels than vegetarians and omnivores. Our findings of overall low breast milk *trans* fat composition is supported by evidence that *trans* fat intake declined steadily in the United States between 1999 and 2010 [55].

Brain derived neurotrophic factor

Using an assay validated for human milk, we found that BDNF was not detectable in any of our samples, which is inconsistent with the findings of others who used the same commercial ELISA [23, 25]. Li et al. measured BDNF in the breast milk of 42 healthy Chinese women at 3, 10, and 30 days postpartum and reported average BDNF levels of 10–13 pg/mL, which is below the standard curve range of 15.6–1000 pg/mL [23]. Protein levels of human milk drop significantly in the first weeks postpartum, which may explain differences in findings, since our study excluded participants from the early postpartum period. Alternatively, our samples generated low levels of BDNF similar to Li et al., prior to deducting absorption values of a blank on the 96-well plate; therefore, calculation methods may also explain differences in reported results. Ismail et al. studied BDNF in the breast milk of 30 women with epileptic infants and 15 healthy controls and reported significantly

higher breast milk BDNF concentrations (min–max) in the mothers of epileptic infants (1285–2077 pg/mL) compared to controls (585–995 pg/mL) [25]. While breast milk DHA was not reported in either of these studies, it is important to note that these studies were conducted in Egypt and China, where DHA intake may have differed from our study population. Dangat et al. used a different ELISA kit not specifically identified for use with human milk and reported breast milk BDNF concentrations in the range of 300 pg/mL, with significant differences between women with preeclampsia and healthy controls [24, 56]. Breast milk DHA concentrations were also reported and were significantly higher in women with preeclampsia, though mean concentrations in both groups were well below the worldwide average of 0.30% [56]. Both Li and Ismail reported centrifuging samples at speeds higher than those provided in the manufacturer's instruction while Dangat did not describe sample preparation protocols. Our lab tried a variety of published sample preparation techniques including using increased centrifuge speed and sample acidification and did not find measurable BDNF in any samples when adjusting for the absorption readings of a blank. The ELISA manufacturer reported average recovery of 94% in human milk samples spiked with BDNF, and that BDNF was undetectable in the breast milk of 10 healthy volunteers, which is in alignment with our findings. The 100-fold difference in breast milk BDNF composition reported in the literature suggests that more research is needed in understanding the potential presence and role of BDNF in human milk and its association with maternal DHA intake. Detailed descriptions of sample preparation protocols are important for comparing results across multiple studies given the inconsistencies in the literature.

Limitations

Study recruitment was conducted primarily online and represents a convenience sample with limited ethnic or educational diversity; therefore, study findings may not reflect nutritional status of other populations consuming low animal-product diets (e.g., food insecure). This was a cross sectional analysis, with differences in maternal BMI, stage of lactation, and supplement use between diet groups; however, neither maternal BMI nor stage of lactation were significant predictors of total unsaturated fat, *trans* fat, or omega-3 fatty acid composition. Because this was a cross-sectional study with only one sample collected per subject, we were not able to assess how fatty acid profiles of human milk change within a subject who follows a defined diet pattern. The sample size for this study was based on detecting differences in B12 concentration of human milk and may not have been adequate to detect differences in all fatty acids assessed, though several significant differences were observed.

Conclusions

In this cross-sectional study of human milk from women following three distinct dietary patterns, we observed significant differences in breast milk from vegan women compared to vegetarian and omnivores, including higher unsaturated fat, total omega-3 fats, and ALA, and lower saturated fats, *trans* fats, and ALA to LA ratios. There was no difference in milk DHA composition by diet group, but over 80% of study participants had milk concentrations below the 0.30% target suggested by expert committees. Supplement use was associated with higher breast milk DHA composition, which may be an important strategy for lactating women who consume low dietary DHA to ensure an adequate DHA supply for the breastfeeding infant. BDNF was not detectable in the breast milk of any participants, which conflicts with the findings of others. More research is needed to understand the relationship between maternal DHA status during pregnancy, breast milk DHA and BDNF concentrations, and the role of DHA supplementation in lactating women as it relates to infant outcomes.

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Author contributions MTP and RP designed the study. MTP, RP, LLD, AC and LF conducted research. MTP analyzed data and wrote paper. MTP has primary responsibility for final content. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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