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Review

Docosahexaenoic acid supplementation in lactating women increases breast milk and plasma docosahexaenoic acid concentrations and alters infant omega 6:3 fatty acid ratio

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ABSTRACT

This study investigated the effects of docosahexaenoic acid (DHA) supplementation on the fatty acid composition of breast milk and plasma concentrations in lactating women and their infants. Eighty-nine lactating women 4–6 weeks post-partum received placebo, 200 mg or 400 mg DHA for 6 weeks with usual diets. Breast milk fatty acids and maternal plasma fatty acids were measured at the beginning and end of the study and infant plasma at the end of the study. Breast milk and maternal plasma DHA were significantly greater with 200 mg and 400 mg DHA compared with placebo (50% and 123% breast milk $p < 0.05$; 71% and 101% plasma, $p < 0.0001$), respectively. Infant plasma omega 6:3 and arachidonic acid (AA):DHA were significantly greater in the placebo group compared to both supplement groups (67% and 106%; 71% and 116%, respectively, $p < 0.05$). DHA supplementation impacts infant fatty acids important for brain development and breast milk fatty acid composition.

1. Introduction

The composition of human breast milk reflects the nutritional status and dietary intake of the lactating mother. Some vitamins and minerals can be impacted when the mother is under and/or malnourished [1]. Other nutrients in breast milk, including fatty acids, are influenced by maternal nutrition [2]. Linoleic (LA) and α-linolenic acid (ALA) are omega 6 (n-6) and omega 3 (n-3) fatty acids, respectively, that are essential in the diet [3,4], and it does not appear that the secretion of these fatty acids in breast milk is regulated [5]; therefore, dietary intake by lactating women can greatly influence the concentration in breast milk. Docosahexaenoic acid (DHA) is an n-3 fatty acid that has gained increased attention over the last 20 years in pregnancy and lactation for its role in brain development, as it accounts for over 10% of brain fatty acids [6] and is essential for infant development [7]. Over the first 6 months of life, which is the time exclusive breast feeding is recommended [8], the infant brain doubles in weight, and the large brain to body weight ratio for infants (0.1) compared with adults (0.02) [9] may put the infant at greater risk to deficits in nutrients and energy. Much of the increase in brain weight is attributed to increased gray matter, corresponding to the formation of neural synapses [10] which are rich in DHA [11,12]. Neurite outgrowth, dendritic complexity and neurotransmitter metabolism are also highly reliant on DHA [11]. Although DHA can be synthesized from its n-3 precursor (ALA) [13], studies have shown that DHA from the maternal diet is a more efficient source of neural tissue DHA than an equivalent amount of ALA [14,15], given that less than 10% ALA is converted to DHA [16].

The amount of DHA in breast milk is influenced by maternal diet and parity [17] and the reported level of DHA in breast milk (by weight) of total fatty acids is $0.32 \pm 0.22\%$ (mean ± standard error) with a range of 0.06–1.4% [18]. Marine mammals have high amounts of DHA as it can be synthesized in aquatic phytoplankton and subsequently transferred up through the aquatic food chain; therefore, fatty fish are a rich dietary source of DHA [19]. Dietary intake of DHA in many parts of the world is low and has been related to dietary fish intake. In a review of worldwide DHA breast milk levels, 4 of the 5 top locations reporting the highest breast milk DHA concentrations are all coastal or island populations with a diet high in marine foods. In contrast, those with the lowest reported levels are either inland or developed countries with low amounts of marine foods in their diets [18]. Many U.S. women, including those who are pregnant, do not consume the recommended 8–12 oz (225–350 g) of

Abbreviations: ALA, α-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAMEs, fatty acid methyl esters; FFQ, Food Frequency Questionnaire; GC, gas chromatography; GLA, γ-linolenic acid; LA, linoleic acid; n-6, omega 6; n-3, omega-3; PUFA, polyunsaturated fatty acids

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seafood per week [20], resulting in a very low consumption of DHA during pregnancy and lactation of about 30–70 mg/day [21]. Additionally, there has been a trend in increased consumption of refined vegetable oils, such as soybean and canola, as the source of fat in the diet and subsequent decreased animal fat consumption. This has resulted in an overall increase in n-6 in the diet as well as decreased plant sources of n-3, such as flax and walnuts [22], suggesting an even greater importance for intake of preformed DHA. These dietary changes, taken together with the overall shift in available dietary fatty acids over the past several decades has resulted in a significant increase in the ratio of n-6:3 fatty acids to 15–20:1, whereas ratios closer to 2–4:1 have been recommended for health [23,24].

Although it is well established that DHA supplementation to lactating women results in increased DHA concentrations, few studies have examined the impact of this increase on infant fatty acid parameters. Therefore, as part of a larger multi-site, prospective, randomized, blinded, placebo-controlled dose response study to examine DHA, lutein and vitamin E supplementation in lactating women, the aims of this portion of the study were to determine the impact of DHA supplementation on fatty acid status of lactating women and their infants. The objectives in this dose response model were to (1) examine the impact of DHA supplementation on infant fatty acids (2) and confirm the responsivity of DHA supplementation to lactating women.

2. Subjects and methods

2.1. Subjects and study design

Eighty-nine (89) U.S. mothers $\geq$ 18 years who had delivered full-term singleton infants that were 4–6 weeks post-partum, had been continuously successfully lactating, and planned to continue breast-feeding for at least 6 weeks were enrolled as part of an umbrella study of lutein, DHA and vitamin E supplementation previously described [25]. Seven were excluded for reasons documented in Fig. 1. Only fatty acid data are currently presented. Subjects (n=9 placebo, n=6 low dose and n=6 high dose) consuming a supplement with DHA/fish oil participated in a 10-day washout prior to starting the study and all subjects were asked to discontinue these supplements during the study. Subjects were randomly assigned to 1 of 3 groups and were asked to consume 2 study capsules daily in the morning with food for 6 weeks; no groups were given other dietary advice. The 3 groups were (1) 2 placebo capsules (placebo), (2) 1 placebo+1 experimental capsule containing 200 mg DHA (low dose) or (3) 2 capsules containing 400 mg DHA (high dose). The placebo capsules were identical in appearance to the experimental capsules and did not contain DHA (Table 1).

Subjects provided a baseline breast milk and blood sample prior to starting the supplement, weekly breast milk samples and an optional blood sample and infant blood sample at the end of study for determination of fatty acids as described below in Section 2.3. Infant birth weight and length were collected at the end of the study. For those infants providing a blood sample, an infant food record was also collected. All subjects completed a food frequency questionnaire (FFQ) modified from a previously published version [26] and completed a weekly 3 day food record as previously described [25]. This study was approved by the Copernicus Group Institutional Review Board. Written informed consent was obtained from all subjects before enrollment.

2.2. Breast milk and blood sample collections

Subjects were instructed on breast milk collection as previously described [25]. Briefly, approximately 20 ml mid-milk sample was collected and it was recommended to collect milk at the same time of the day for each visit in the afternoon. Samples were either frozen immediately or kept under refrigerated temperatures for $\leq$ 12 h prior to freezing. Venous blood samples ($\sim$6 ml mother and $\sim$4 ml infant) were collected in Na heparin vacuum-tubes. The plasma layer was transferred into Eppendorf tubes and stored at $-20\,^{\circ}C$ for not more than 6 weeks until the analysis was performed as described below.

2.3. Fatty acid analysis

All samples were stored at $-20\,^{\circ}C$ for no more than 4–6 weeks, and shipped to the analytical laboratory (Craft Technologies, Inc., Wilson, NC, USA). Fatty acids were analyzed as previously described [27]. Lipids and fatty acids were extracted from breast milk by organic solvents and lipids extracts were hydrolyzed and methylated to fatty acid methyl esters (FAMEs) using BF3 in methanol. Heparinized plasma samples had an internal standard (C17:0 or C23:0 in chloroform) added and lipids were extracted twice in 2 ml of chloroform: methanol (1:1 v/v) by a vortex-mixing for 1 min. After centrifugation, the chloroform extract was combined, taken to dryness in a centrifugal evaporator and hydrolyzed and methylated to FAMEs using BF3 in methanol. Breast milk and plasma sample FAMEs were extracted twice with n-hexane and quantitatively measured by a capillary gas chromatography (GC). Saturated and monounsaturated fats were calculated as the sum of respective fatty acids. Prepared FAMEs were analyzed by GC instrumentation equipped with a fused silica column coated with bonded polyglycol liquid phase, oxygen scrubber in carrier gas line and flame ionization detector.

2.4. Sample size and statistical analysis

The sample size was obtained from the software package nQuery Advisor 5.0 (Statistical Solutions Ltd., Cork, Ireland). The primary outcome of the umbrella study was breast milk lutein; therefore, the sample size was calculated on this parameter [25] and the sample size achieved in this study allowed for an observed power of 92% for DHA, confirming that there was adequate power to detect a difference in breast milk DHA. Analysis of variance was used in baseline comparisons of continuous variables, while the Cochran–Mantel–Haenszel test statistics were used in baseline comparisons of categorical variables. Fatty acids were compared among treatment groups using analysis of variance techniques. Relationships among treatment groups were explored using linear regression. Comparisons between paired data were analyzed using t-tests and correlations, as appropriate. If the residuals were not from a normal distribution, a transformation of the data (e.g. natural log) may have been used in order to improve the model fit. SAS® (Cary, NC) Version 9.2 was used to perform the statistical analyses.

3. Results

3.1. Subject characteristics

Of the 89 subjects enrolled in the study, 82 provided data for the analysis. Five subjects were lost to follow-up and did not continue with study visits (Fig. 1). One subject was found not to be eligible after study completion and one subject never consumed the study supplement. All were similar in age and body mass index (BMI) and the significant majority of the subjects were white with an average age of 29 $\pm$ 0.5 and pre-pregnancy BMI of 24 $\pm$ 0.33. The average self-reported intake of study supplements was 95% (93–97%) and was not different among study groups. There was no reported difference in dietary intake of fish or DHA fortified foods in the month prior to start of the study as assessed by FFQ. No significant differences in dietary intake of DHA were reported throughout the study as

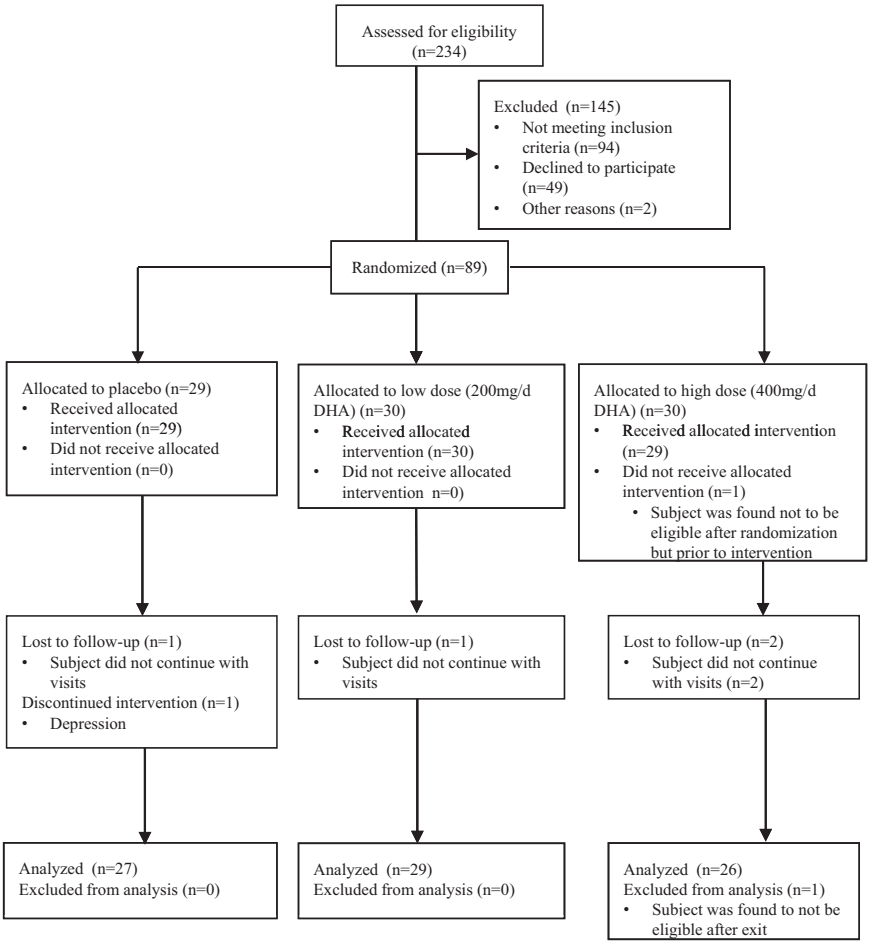

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Table 1
Composition of study supplements.

Nutrient	Placebo	Experimental
Calories	5	5
Palmitic acid (16:0), mg	59.9	27.7
Stearic acid (18:0), mg	24.9	9.11
Oleic acid (18:1n9), mg	123.6	13.3
LA (18:2n-6), mg	305.3	25.8
EPA (20:5n-3), mg	0	39.2
DHA (22:6n-3), mg	0	207
Lutein, mg	0	6
D-α-tocopherol, IU	0	30
DL-α-tocopheryl acetate, IU	50.6	0

LA, linoleic acid; EPA, eicosapentaenoic acid; DHA docosahexaenoic acid.
Placebo group consumed 2 placebo capsules, low dose consumed 1 placebo capsule and 1 experimental capsule and high dose consumed 2 experimental capsules.

determined by self-reported 3 day food records (60.02 ± 11.25 placebo, 78.39 ± 24.05 low dose and 51.71 ± 9.14 high dose mg/day, $p=0.64$). The characteristics of the infants are summarized elsewhere [25]; there was no difference among the groups in feeding the infants received. There was no difference in adverse events or serious adverse events among groups in the mothers or infants and none were related to the study intervention.

3.2. Characteristics of fatty acids in breast milk and plasma

At the beginning of the study there were no differences in absolute (Table 2) or percent total (data not shown) fatty acids in breast milk among treatment groups; however, there were differences in several plasma fatty acids among the groups (Table 3). Baseline values were used as covariates in the model accounting for these differences when determining the impact of supplementation. For all subjects, polyunsaturated fatty acids (PUFAs) was the most abundant in maternal plasma while saturated fatty acids were the most abundant in breast milk at baseline (Fig. 2A). The individual fatty acid with the highest concentration was LA in maternal and infant plasma; oleic acid had the highest concentration in breast milk with LA third after palmitic acid (Fig. 2B). Infant plasma (placebo group only) more closely matched the fatty acid distribution of maternal plasma compared with breast milk with the highest concentration being PUFA and LA (Fig. 2A and B). Also the concentrations of infant plasma n-3 and n-6 fatty acids were more similar to maternal plasma compared with breast milk, and plasma concentrations were greater than breast milk (Fig. 2A). There was a significant correlation between breast milk and maternal plasma DHA, ALA and eicosapentaenoic acid (EPA) at baseline ($r=0.41$, $p=0.0002$ DHA; $r=0.22$, $p=0.05$ ALA; $r=0.24$, $p=0.03$ EPA, respectively) and the strength of these correlations increased with supplementation (data not shown). In addition, γ-linolenic acid (GLA) demonstrated a significant correlation after supplementation ($r=0.23$ and $p=0.04$).

3.3. Breast milk fatty acids

After 6 weeks of supplementation with the low or high dose of DHA, absolute breast milk DHA was significantly greater compared with placebo (Table 2), which was also significant when represented

Table 2
Fatty acid composition of breast milk.

Fatty acid (mg/dl)	Placebo ($n=27$)		Low dose ($n=29$)		High dose ($n=26$)	
	Baseline	6 weeks	Baseline	6 weeks	Baseline	6 weeks
Palmitic acid (16:0)	621.50 ± 47.14	675.41 ± 51.95	665.27 ± 57.55	547.93 ± 42.53	574.19 ± 45.63	616.67 ± 68.53
Oleic acid (18:1n-9)	981.80 ± 67.76	1032.83 ± 82.26	936.37 ± 89.13	785.19 ± 66.63	895.96 ± 73.45	882.25 ± 95.26
LA (18:2n-6)	557.85 ± 48.05	609.68 ± 49.35^{a}	522.87 ± 48.23	423.15 ± 37.34^{b}	485.49 ± 43.65	467.30 ± 48.33^{a,b}
ALA (18:3n-3)	42.26 ± 4.09	45.09 ± 4.12	41.83 ± 4.67	34.91 ± 3.55	34.27 ± 2.83	35.09 ± 3.96
GLA (18:3n-6)	3.90 ± 0.37	4.38 ± 0.50	4.48 ± 0.39	3.35 ± 0.32*	3.73 ± 0.36	3.05 ± 0.40
AA (20:4n-6)	14.51 ± 1.03	14.83 ± 1.23	16.10 ± 1.49	11.58 ± 0.91*	13.62 ± 1.06	12.12 ± 1.23
EPA (20:5n-3)	1.55 ± 0.10	1.61 ± 0.20	1.84 ± 0.21	1.90 ± 0.23	1.53 ± 0.14	2.06 ± 0.21*
DPA (22:5n-3)	3.70 ± 0.25	3.99 ± 0.38	3.99 ± 0.42	3.43 ± 0.34	3.30 ± 0.30	3.58 ± 0.41
DHA (22:6n-3)	5.78 ± 0.50	5.87 ± 0.73^{a}	6.98 ± 0.94	8.83 ± 1.03^{b}	5.14 ± 0.51	13.08 ± 1.69^{b,*}
Monounsaturated	1141.99 ± 75.86	1192.56 ± 92.79	1100.17 ± 103.00	906.55 ± 76.15	1041.39 ± 85.26	1015.96 ± 108.00
Polyunsaturated	668.83 ± 55.51	721.97 ± 58.52^{a}	638.86 ± 58.70	513.67 ± 44.53^{b,*}	583.55 ± 49.51	566.53 ± 58.24^{a,b}
Saturated	1207.80 ± 84.08	1329.34 ± 114.29	1300.74 ± 114.08	1042.91 ± 78.83*	1102.16 ± 88.49	1259.82 ± 141.56
Total n-3	61.45 ± 4.96	64.21 ± 5.64	63.04 ± 6.45	55.16 ± 5.14	51.50 ± 4.05	60.63 ± 6.66
Total n-6	605.53 ± 50.76	655.80 ± 53.22^{a}	573.66 ± 52.28	456.81 ± 39.54^{b,*}	530.12 ± 45.70	503.78 ± 51.76^{a,b}
Total fatty acids	3018.61 ± 196.52	3243.87 ± 256.89	3039.76 ± 262.84	2463.12 ± 188.75*	2727.10 ± 215.13	2842.32 ± 300.49
Total n-6:3	9.95 ± 0.24	10.53 ± 0.33^{a}	9.37 ± 0.28	8.49 ± 0.26^{b,*}	10.33 ± 0.40	8.67 ± 0.34^{b,*}
AA:DHA	2.64 ± 0.11	2.81 ± 0.12^{a}	4.02 ± 1.51	1.53 ± 0.13^{b}	2.81 ± 0.31	1.09 ± 0.10^{c,*}

LA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. All values are means ± SEMs mg/dl. Values with different letters indicate significant difference by ANCOVA after log transformation among treatment groups at a visit ($p<0.05$) and symbols (*) indicate significant difference by t test between visits within a treatment group ($p<0.05$).

Table 3
Fatty acid composition of maternal plasma.

Fatty acid (mg/dl)	Placebo ($n=27$)		Low dose ($n=29$)		High dose ($n=26$)	
	Baseline	6 weeks	Baseline	6 weeks	Baseline	6 weeks
Palmitic acid (16:0)	70.95 ± 5.68	62.42 ± 4.25^{x,*}	59.72 ± 2.31	61.56 ± 3.31^{y}	64.78 ± 4.08	56.45 ± 2.95^{x,*}
Oleic acid (18:1n-9)	59.18 ± 5.76^{a}	53.11 ± 5.52^{x}	44.44 ± 2.36^{b}	48.67 ± 3.11^{y}	52.01 ± 4.82^{a,b}	43.55 ± 3.29^{x,*}
LA (18:2n-6)	92.46 ± 5.05^{a}	88.95 ± 3.99	75.43 ± 2.98^{b}	79.83 ± 3.03	84.40 ± 3.47^{a}	78.24 ± 3.38*
ALA (18:3n-3)	2.74 ± 0.29	2.35 ± 0.22*	1.98 ± 0.15	2.10 ± 0.18	2.22 ± 0.19	1.95 ± 0.18
GLA (18:3n-6)	1.33 ± 0.17	1.21 ± 0.10	1.40 ± 0.15	1.25 ± 0.09	1.15 ± 0.12	1.06 ± 0.10
AA (20:4n-6)	20.42 ± 1.18	19.41 ± 0.81^{x}	20.47 ± 0.84	20.04 ± 0.76^{x}	20.84 ± 0.72	17.29 ± 0.70^{y,*}
EPA (20:5n-3)	1.54 ± 0.11	1.26 ± 0.10^{x,*}	1.86 ± 0.10	1.86 ± 0.15^{y}	1.64 ± 0.17	1.79 ± 0.12^{y}
DPA (22:5n-3)	1.32 ± 0.09	1.26 ± 0.07^{x}	1.27 ± 0.07	1.19 ± 0.06^{x}	1.29 ± 0.07	1.02 ± 0.06^{y,*}
DHA (22:6n-3)	3.82 ± 0.26	2.98 ± 0.17^{x,*}	4.13 ± 0.19	5.09 ± 0.27^{y,*}	3.50 ± 0.16	5.99 ± 0.32^{z,*}
Monounsaturated	72.41 ± 7.15^{a}	64.40 ± 6.37	55.20 ± 3.00^{b}	59.34 ± 3.67	63.78 ± 5.82^{a,b}	52.91 ± 4.11*
Polyunsaturated	130.80 ± 6.93^{a}	123.79 ± 4.90	113.34 ± 4.08^{b}	117.48 ± 3.93	121.81 ± 4.28^{a,b}	112.83 ± 4.02*
Saturated	101.27 ± 7.53	90.70 ± 5.64*	86.82 ± 3.25	90.48 ± 4.57	93.66 ± 5.78	84.05 ± 4.39*
Total n-3	9.86 ± 0.65	8.30 ± 0.42^{x,*}	9.67 ± 0.34	10.69 ± 0.53^{y,*}	9.05 ± 0.45	11.13 ± 0.54^{y,*}
Total n-6	120.71 ± 6.36^{a}	115.24 ± 4.64	103.45 ± 3.82^{b}	106.53 ± 3.52	112.52 ± 4.05^{a}	101.44 ± 3.75*
Total fatty acids	304.48 ± 21.23^{a}	278.89 ± 16.34	255.37 ± 9.49^{b}	267.30 ± 11.34	279.25 ± 15.24^{a,b}	249.79 ± 11.46*
Total n-6:3	12.70 ± 0.48^{a}	14.41 ± 0.63^{x,*}	10.82 ± 0.32^{b}	10.35 ± 0.36^{y}	12.93 ± 0.62^{a}	9.38 ± 0.43^{z,*}
AA:DHA	5.58 ± 0.26^{a,b}	6.82 ± 0.31^{x,*}	5.14 ± 0.23^{a}	4.23 ± 0.26^{y,*}	6.16 ± 0.26^{b}	3.03 ± 0.19^{z,*}

LA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. All values are means ± ± SEMs mg/dl. Values with different letters indicate significant difference by ANCOVA after log transformation among treatment groups at baseline (a–c) and 6 weeks (x–z) ($p<0.05$) and symbols (*) indicate significant difference by t test between baseline and 6 weeks within a treatment group ($p<0.05$).

as percent total (0.35 ± 0.02 and 0.45 ± 0.02 vs. 0.18 ± 0.02, $p<0.0001$). There was also a 29% increase in the high dose as compared to low dose groups as a percent total ($p<0.0005$) and a trend towards an increase as an absolute concentration ($p=0.06$), however this did not reach significance (Table 2). The high dose group demonstrated a 154% increase over time (baseline to 6 weeks) in breast milk DHA (Table 2) and there was a significant, positive linear relationship among the three groups ($R^2=0.20$). The placebo group had a ~40% increase in each of the following: LA, PUFA and n-6 fatty acids compared with the low dose group after 6 weeks of supplementation. After 6 weeks of supplementation, the ratio of total n-6:3 was ~20% greater in the placebo group compared with both treatment groups; there was no difference between treatment groups. In the low and high dose groups, the total n-6:3 was reduced by 10% and 19%, respectively, after 6 weeks of supplementation. Given there was no difference in the ratio of LA:ALA (data not shown) among the groups, and the ratio of AA:DHA was 84% and 158% greater in the placebo group compared with both treatment groups, respectively, the change in the total n-6:3 appears to be driven specifically by the changes in DHA. Additionally, the low dose group AA:DHA ratio was 40% greater than the high dose group at the end of the study. Eicosapentaenoic acid increased 35% over time in the high dose group, but was not different among treatment groups. Several fatty acids decreased over time in the low dose group (GLA, AA, PUFA, saturated fatty acids, n-6, total identified fatty acids and n-6:3), but no changes were seen in the other groups (Table 2).

3.4. Plasma fatty acids

After 6 weeks of supplementation with low or high dose of DHA, maternal plasma DHA increased by 71% and 101%, respectively (Table 3), compared with placebo which was also significantly represented as a percent total (1.92 ± 0.09 and 2.45 ± 0.11 vs. 1.13 ± 0.09, $p<0.0001$). The high dose supplement was 18% greater than low dose

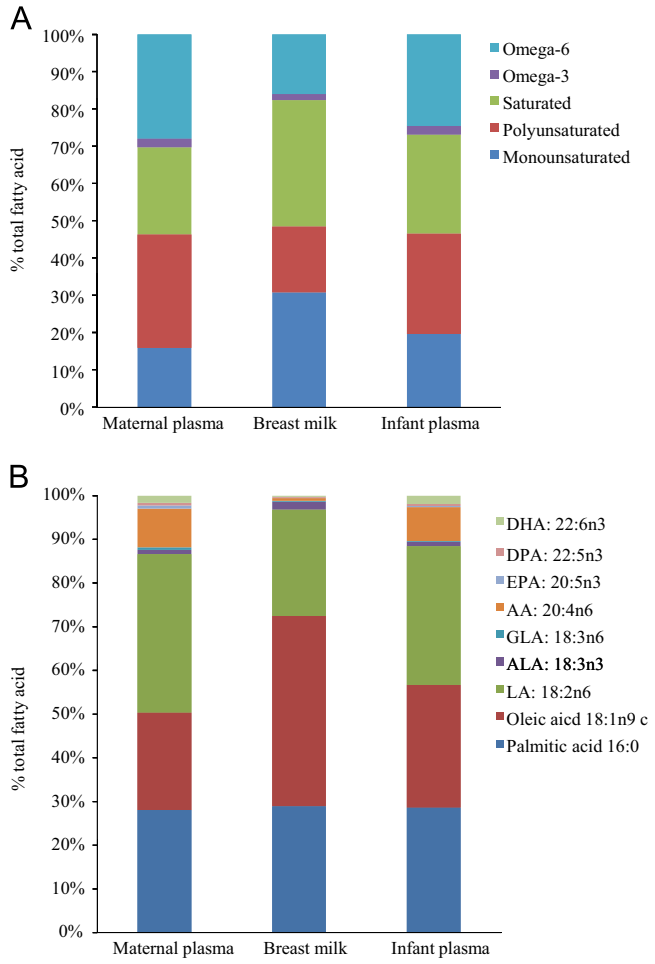

Fig. 2. Comparison of fatty acids in maternal plasma and breast milk and infant plasma. Maternal data is all subjects reported at baseline and infant plasma placebo group reported at study completion. Values are means and represented as percentage of fatty acids in the tissue. DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; GLA, γ-linolenic acid; ALA, α-linolenic acid; LA, linoleic acid.

(Table 3), and significant when expressed as percent total. Both supplement groups demonstrated a significant increase in plasma DHA over time, whereas the placebo group had a significant decrease in plasma DHA over time (Table 3). There was a significant, positive linear relationship among the three groups ($R^2=0.56$). The placebo group had the highest n-6:3 and AA:DHA ratios, and increasing the amount of DHA in the supplement resulted in lower ratios (Table 3). There was no difference in LA:ALA among the groups (data not shown), suggesting that the changes in n-6:3 are driven by the changes in DHA. Over time, the ratio of AA:DHA decreased in both the low and high dose groups, the ratio of n-6:3 decreased only in the high dose group and both ratios increased in the placebo group (Table 3). Several other fatty acids changed after 6 weeks of supplementation in maternal plasma. Of note, EPA and n-3 fatty acids were significantly increased in the low and high dose groups compared with placebo and docosapentaenoic acid (DPA) was significantly greater in the placebo and low dose groups compared with the high dose group. Infant plasma n-6:3 and AA:DHA ratios were higher for those whose mothers received the placebo ($n=11$) compared with the low ($n=11$) and high ($n=5$) dose supplement groups (Supplemental Table 1); there were no differences in LA:ALA (data not shown). Infants whose mothers received the placebo had higher plasma palmitic acid, oleic acid, LA, ALA, DPA and n-6 compared with the high dose group, of which oleic acid, ALA and DPA were also greater as compared to the low dose group.

4. Discussion and conclusions

4.1. Discussion

One of the primary objectives of this supplementation study was to determine the impact of maternal DHA supplementation on infant fatty acids, and due to limitations of sample size there was no conclusive evidence that DHA in infants was directly impacted. Our data demonstrated a significant increase in infant DHA as percent total fatty acids for high dose compared with placebo. There was a trend towards a decrease in total fatty acids in the infants whose mothers received the low dose ($p=0.088$) or high dose ($p=0.051$) of DHA compared with placebo. Therefore, given the high dose group had almost half the total fatty acids as the placebo group, it is the opinion of these investigators that the increase as percent total was not a true representation of the data. Nonetheless, these data are the first to demonstrate that the infants from both the low and high dose supplemented mothers reported a 40% and 51%, respectively, lower n-6:3 fatty acid ratio, as compared to infants from the placebo mothers. A lower n-6:3 ratio diet fed to rodents during brain development has been shown to result in the greatest relative percentage DHA accumulation in three critical regions of the brain [28], and it has been suggested that an imbalance in n-6:3 early in life may lead to irreversible changes in the hypothalamus [29]. Additionally, an increased amount of n-3 has been shown to benefit learning and memory [30], in addition to overcoming deficits induced by neurotoxins in a rodent model [31]. A ratio of 4:1 has been shown to be the most effective in improving neuro-cognitive measures [30] and is recommended as optimal for healthy adults [32]. Infants from mothers on the high dose achieved a ratio closest to this recommendation (5.44:1) among all the groups. A recent study has demonstrated that maternal DHA supplementation can confer a long-term benefit on neurodevelopment 5 years later [33]. Only one other study has looked at infant blood concentrations with maternal DHA supplementation and demonstrated that 0.35–1.13 g/day DHA given to lactating women for 12 weeks increased infant plasma phospholipid DHA as percent total [34].

Several studies over a decade ago examined the impact of DHA supplementation in lactating women [13,35–39] from numerous different populations. Baseline breast milk DHA (0.21 ± 0.01% total fatty acids) in this cohort was less than the worldwide mean of 0.32 ± 0.22% [18]. Our data demonstrate that in lactating women with relatively low fish intake, which is the primary dietary source of DHA, supplementation significantly increased breast milk and circulating DHA in mothers as well as decreased the ratio of n-6:3 in infants. Our results in breast milk are in agreement with several others [13,35–37,39] that used similar doses of DHA during several different time periods of supplementation. Additionally, a recent analysis [40] of a previous dose response study [34] provides an equation relating maternal intake and breast milk DHA output ($r^2=0.998$), which is in agreement with the current data ($r^2=0.995$). Both doses of DHA increased breast milk DHA compared with placebo; however, this was not the case in a previous dose response study, which only showed increases at 400 mg or more of DHA [38]. Women in the previous study started supplementation 5 days post-partum for 12 weeks and it is known that breast milk DHA decreases over the first 6–16 weeks post-partum [41]; therefore, it is possible in the current study that breast milk DHA had stabilized at 6 weeks enough for a 200 mg dose to have an impact.

Baseline plasma DHA in the current study is similar to previous studies with a collection about the same period post-partum, ~4–8 weeks [39] and greater than others [42] with very early post-partum collection (~3 days post-partum) and the early changes in fatty acids profiles after delivery may account for these differences. There was no difference among groups in baseline fish intake as

assessed by FFQ or DHA intake over the course of the study determined by 3 day food records, supporting that the increase was due to the supplement. Both doses of supplement increased plasma DHA which is consistent with previous research [35,36] and demonstrated a significant difference between the two doses, which is similar to Hawkes et al. [35] that supplemented with 300 and 600 mg of DHA for 4 weeks. Another study with increasing doses only showed a significant difference in plasma with a much higher dose (1021 mg/day DHA as cod liver oil) but demonstrated increase in breast milk DHA at all doses [39]. It is likely that the shorter supplementation (14 days) time or DHA delivery as cod liver oil which were not able to impact plasma phospholipids levels.

Several other PUFA responded to DHA supplementation. Eicosapentaenoic acid was increased over time in breast milk with the high dose of DHA. Additionally in maternal plasma both doses had significantly higher EPA after 6 weeks of supplementation compared with placebo. Others have reported increases in breast milk [35,39] and plasma [35] EPA with a DHA supplement. There is inherent EPA that is present in many supplements and may be the reason for increased levels. In both the low and high dose groups, n-3 fatty acids increased in plasma and n-6 decreased slightly in breast milk resulting in a decrease in the ratio of n-6:3 in both tissues, which is similar to some [37] but not others [39]. A lower n-6:3 ratio is considered to provide possible health benefits [23]. Docosapentaenoic acid, an elongated metabolite of EPA and an intermediary product between EPA and DHA, decreased in maternal plasma with DHA supplementation which has been seen in similar studies [38] with longer periods of supplementation, but not in others with short supplementation periods [37,39].

4.2. Conclusions

Numerous consensus statements recommend at least 200 mg per day of DHA for pregnant and lactating women [3,4] and data from our study and others [13,39] demonstrate that many lactating women are only receiving around 25% of this recommended amount. The breast milk DHA concentration was 4–6 times lower in the current baseline sample compared with those with the highest fish intake [18,43] and 1.5 times lower than the worldwide mean [18]. Limitations of this study include the plasma phospholipids were not analyzed due to the secondary analysis from a multi-nutrient supplementation trial and the enrollment time post-partum resulted in a shorter washout. Nonetheless, supplementation was able to achieve a concentration similar (low dose) or slightly higher (high dose) than the worldwide mean, suggesting that supplementation may be necessary for those with low dietary intake. Additionally, the current study demonstrated that maternal supplementation impacts fatty acids in infants that have been shown important for brain development. Results are inconclusive on the long-term cognitive impact of DHA supplementation during pregnancy on the offspring [44,45]; however, the importance of adequate PUFA in the infant diet for normal growth and development is well established [46]. Taken together, this study demonstrates that in a population with low dietary intake of DHA, supplementation results in an increase in breast milk and maternal DHA at levels that would reflect adequate dietary intake and beneficially impact fatty acid ratios in infants important for brain development.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plefa.2015.01.005.

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