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Docosahexaenoic Acid Supplementation from Mid-Pregnancy to Parturition Influenced Breast Milk Fatty Acid Concentrations at 1 Month Postpartum in Mexican Women[1–4]

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Abstract

(n-3) PUFA, including DHA, are essential for neural development and accumulate extensively in the fetal and infant brain. (n-3) PUFA concentrations in breast milk, which are largely dependent on maternal diet and tissue stores, are correlated with infant PUFA status. We investigated the effect of prenatal DHA supplementation on PUFA concentrations in breast milk at 1 mo postpartum. In a double-blind, randomized, controlled trial conducted in Mexico, pregnant women were supplemented daily with 400 mg DHA or placebo from 18–22 wk gestation to parturition. Fatty acid concentrations in breast milk obtained from 174 women at 1 mo postpartum were determined using GLC and were expressed as % by weight of total detected fatty acids. Breast milk DHA concentrations in the DHA and placebo groups were (mean ± SD) 0.20 ± 0.06 and 0.17 ± 0.07 ($P < 0.01$), respectively, and those of α-linolenic acid (ALA) were 1.38 ± 0.47 and 1.24 ± 0.46 ($P = 0.01$), respectively. Concentrations of EPA and arachidonic acid did not differ between groups ($P > 0.05$). Maternal plasma DHA concentrations at 1 mo postpartum correlated positively with breast milk DHA at 1 mo postpartum in both the placebo and DHA groups ($r = 0.4$; $P < 0.01$ for both treatment groups). Prenatal DHA supplementation from 18–22 wk gestation to parturition increased concentrations of DHA and ALA in breast milk at 1 mo postpartum, providing a mechanism through which breast-fed infants could benefit. J. Nutr. 141: 321–326, 2011.

Introduction

Long-chain PUFA (LCPUFA)[8] such as DHA [22:6(n-3)] and arachidonic acid [AA; 20:4(n-6)] are major components of brain and retinal cell membrane phospholipids and accumulate extensively in the fetal and infant brain during the last trimester of pregnancy and infancy (1–4). LCPUFA, especially DHA, are preferentially transported across the placenta to the developing fetus (5,6). Dietary (n-3) PUFA and parity influence availability of DHA to the fetus (7,8). LCPUFA are present in breast milk; their concentration depends upon maternal stores, dietary intake, and synthesis in the mammary glands; however, synthesis of DHA in the mammary gland is likely minimal (9,10). The DHA concentration in breast milk decreases as lactation progresses and supplementation of lactating women improves DHA breast milk concentrations (11–14). Because developing fetuses and breast-fed infants rely on maternal supply of DHA, adequacy of DHA in the maternal diet during pregnancy and lactation can be expected to be critical for optimal support of neurological development.

Dietary intake of (n-3) PUFA in many parts of the world, including the US, is low, although intake of (n-6) PUFA has increased (15). In the US, intake of (n-3) PUFA and the combination of DHA and EPA [20:5(n-3)] are estimated to be 1.6 and 0.1–0.2 g/d, respectively, and the ratio of dietary (n-6):(n-3) PUFA is ~9.8:1 (16). A study of pregnant Canadian women showed that daily (n-3) PUFA and DHA intake was 1.45 and 0.082 g/d, respectively (17). Populations living in coastal countries such as Japan and Norway, where fish is widely consumed, have higher dietary intakes of (n-3) LCPUFA and correspondingly high concentrations of DHA in their breast milk (15,18). Although there is no official dietary recommendation for EPA and DHA intake in the US, several expert groups suggest DHA intakes of at

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[3] This trial is registered at clinicaltrials.gov as NCT00646360INSP and at INSP in Mexico as CI-011.
[4] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
[8] Abbreviations used: AA, arachidonic acid, 20:4(n-6); ALA, α-linolenic acid, 18:3 (n-3); IMSS, Instituto Mexicano del Seguro Social; INSP, Instituto Nacional de Salud Pública; LA, linoleic acid, 18:2(n-6); LCPUFA, long-chain PUFA.
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least 200 mg/d (up to 1 g/d) for pregnant and lactating women and 1.4–2.7 g/d of (n-3) PUFA, and the suggested (n-6):(n-3) fatty acid ratio is ~2–5:1 (16,19–21). Ecological data show that high DHA intake correlates with high breast milk DHA concentration, and fish oil supplementation during lactation influences breast milk composition (9). Additionally, variations in genes that encode the enzymes responsible for desaturation of LA and α-linolenic acid (ALA) to AA and DHA, respectively, influence DHA content in breast milk (22). Because DHA content in breast milk is dependent on maternal diet and stores, low DHA intake, as seen in many populations, results in low concentration of DHA in breast milk, which might adversely influence growth and development of breast-fed infants.

In this article, we assess the influence of algae-derived DHA supplementation from gestation wk 18–22 to parturition on breast milk fatty acid composition 1 mo postpartum.

Methods

Study population and setting. We recruited study participants at the Mexican Institute of Social Security [Instituto Mexicano del Seguro Social (IMSS)] General Hospital I, a large hospital located in Cuernavaca, Mexico, and 3 small health clinics within the IMSS system in Cuernavaca during routine prenatal care visits between February 2005 and February 2007 in the Prenatal DHA (n-3) fatty acid Supplements on Infant Growth and Development (POSGRAO) Study. The IMSS health care system provides employed persons access to medical care. Women were considered for inclusion in the study if they were in gestation wk 18–22, planned to predominantly breast-feed for at least 3 mo, were 18–35 y old, planned to deliver at the IMSS General Hospital in Cuernavaca, and planned to live in the area for 2 y after delivery. Women were excluded if any of the following criteria were present: high risk pregnancy, lipid metabolism or absorption disorders, regular intake of fish oil or DHA supplements, or chronic use of certain medications, such as those for epilepsy. Study team members who were employed by Instituto Nacional de Salud Pública (INSP) and IMSS conducted the field work. This study is an ongoing collaborative project between the INSP in Cuernavaca, Mexico and Emory University, and is a registered clinical trial.

Ethics. The study protocol and all informed consent documents were approved by Emory University's Institutional Review Board and by the INSP Biosafety and Ethics committees. Written informed consent was obtained from each participant after a thorough explanation of the study details, and participants were free to withdraw from the study at any time without consequence for their care or treatment. The welfare of the participants was monitored by an external Data Safety Monitoring Committee.

*Study design.*Women were assigned to receive 2 DHA or placebo capsules daily from gestation wk 18–22 to parturition. The DHA capsules contained 200 mg DHA, each derived from an algal source (Martek Biosciences), and the placebo capsules, which were similar in appearance and taste to the DHA capsules, contained olive oil. We deemed olive oil an appropriate placebo, because it is considered safe in pregnancy, the very small dose of 400 mg/d was not expected to influence study outcomes, and it has been used as a placebo in several similar trials of fish oil supplementation in pregnancy and/or lactation (14,23). Fieldworkers visited the women's homes and/or workplaces weekly to deliver a new bottle of 14 capsules, and compliance was monitored by counting any remaining pills and through interviews with participants.

All eligible women were randomized to either the treatment or the control group using a computer-generated list created by the study biostatistician at Emory University. We used a block randomization method to randomly create balanced replication of 4 treatments (2 colors for DHA and 2 for control) using a block size of 8. Success of randomization was assessed by comparison of a variety of baseline maternal characteristics between the 2 treatment groups. All participants and members of the study team were unaware of the treatment scheme throughout the intervention period of the study. Data were disclosed to the analytical study team after the last baby in the study was born and was 6 mo of age. The study is ongoing for follow-up of child health and development and therefore participants and field workers in Mexico remain unaware of treatment allocation.

Ascertainment of prenatal dietary intake. We assessed maternal dietary intake at 18–22 wk gestation using a 110-item FFQ that included specific questions about consumption of sources of DHA such as freshwater fish, seafood, canned tuna and sardines, salmon, trout, and cod liver oil in the previous 3 mo (24,25). Estimates of (n-3) PUFA intake ascertained using this FFQ correlated reasonably well with erythrocyte membrane (n-3) PUFA phospholipid concentration (26).

Blood and milk samples. Breast milk samples were collected during routine study visits at 1 mo postpartum. The milk samples were taken during a morning feed between 0800 and 1200. Mothers were asked to feed their infant before coming to the hospital so that the milk sample was not from the first feed of the day. Initially, 5 mL of foremilk was hand-expressed before infants were allowed to suckle the nipple for 10 min; afterwards, an expression of 5 mL of hindmilk was collected into the same 14-mL plastic test tube and was thoroughly mixed with the initial 5-mL foremilk sample. The samples were then aliquoted into multiple cryovials and frozen in a nitrogen environment at −70°C.

Maternal blood (7 mL) was obtained by venipuncture by trained technicians at the IMSS hospital at 18–22 wk gestation, delivery, and 1 mo postpartum. All samples were collected into tubes containing EDTA. Plasma and erythrocytes were separated by centrifugation at 800 × *g* for 10 min at room temperature. Plasma and erythrocytes were then aliquoted into multiple cryovials and immediately frozen in a nitrogen environment and stored at −70°C.

We analyzed a random subsample of 174 breast milk samples. The sample size was based on power calculations that estimated that a sample of 85 participants/group would have at least 90% power to detect a difference of 0.5 SD units in mean breast milk DHA levels between the DHA and placebo groups using a 2-tailed test and confidence level < 0.05 (27). We also analyzed a random subsample of 198 maternal plasma samples. The total fat from milk and plasma was extracted with a chloroform-methanol mixture (2:1). Lipids were saponified and the resulting fatty acid methylated using boron fluoride, as described by Morrison and Smith (28). The extracts were injected into a gas chromatograph, Hewlett Packard Mod. 5890 Series II, using a 100-m long × 0.25-mm i.d. Supelco SP 2560 column for milk and a 30-m long × 0.25-mm i.d. DB-FAP column, using nitrogen as carrier. The injector temperature was set at 250°C and the detector temperature at 275°C. The temperature ramps were programmed at 120, 175, and 235°C for milk and at 150, 200, and 245°C for plasma. The response factor for DHA was 1.1. The program for temperature ramps was: ramp 0: T° initial, 145°C, kept for 5 min, increase 0.3°C/min until reaching 155°C, 0 min. Ramp 1: 1°C/min until 170°C, 0 min. Ramp 2: 2°C/min until 220°C for 20 min. The chromatographic peaks were identified using true reference standards for 37 fatty acids (Supelco). The fatty acid content is expressed as % by weight of total detected fatty acids.

Statistical methods. Continuous characteristics at randomization and at birth were tested for normality, and between-group differences were assessed using *t* tests or Wilcoxon's rank sum tests, as appropriate. Categorical characteristics were tested using chi-square tests. Socioeconomic status was estimated using principal components analysis to construct a wealth index based on the materials used to construct the house, availability of running water, presence of an indoor lavatory, and ownership of household appliances (e.g., videocassette recorder, DVD, refrigerator, microwave oven, washer, dryer, car, motorcycle, phone, stereo, computer) (29). Extreme outliers in breast milk PUFA concentrations, defined as exceeding the sample 75th percentile by >3 times the IQR, were deleted and outcomes were log-transformed to improve normality, if necessary (16 of the 2958 PUFA determinations were considered extreme outliers and were deleted). Between-group differences between breast milk fatty acid concentrations were evaluated using either Wilcoxon's rank sum

tests or *t* tests, as appropriate. We examined the effect of treatment and time on maternal plasma PUFA concentrations using PROC MIXED models with repeated measures. Within-group differences in maternal plasma PUFA concentrations were assessed using paired *t* tests when the effect of treatment and time was significant. We computed Spearman correlation coefficients to describe the relationships among breast milk fatty acids and dietary intake and plasma DHA, EPA, and AA levels. We conducted statistical analyses using TTEST, NPAR1WAY, and CORR procedures in the Statistical System Software version 9.2 (SAS Institute). Significance was defined as $P \leq 0.05$. Values in the text are means ± SD.

Results

CONSORT statement. Among the 1836 women screened, 1094 were randomized to treatment (25). A total of 485 women in the DHA group and 488 women in the placebo group completed treatment by remaining in the study through parturition. Compliance, measured as the proportion of capsules consumed of the capsules distributed, was high (>94%) and was similar between the 2 groups ($P = 0.6$). Primary study outcomes, including birth outcomes, are described elsewhere (25). A total of 851 women attended their 1-mo postpartum study visit and 842 provided breast milk samples (**Supplemental Fig. 1**).

Baseline characteristics. Baseline maternal characteristics for the subsample of 174 mothers whose breast milk samples were analyzed were similar between treatment groups (**Table 1**). Additionally, baseline characteristics of the subsample of 174 women were similar to those of the main study population of 1094 women, indicating that the subsample was representative of the study population (25). At baseline, women were on average 26 y old and 20.6 wk pregnant and 34.5% were primigravid. The women had completed an average of 11.6 y of school and SES levels were similar across groups. Characteristics at delivery of the 174 infants whose mothers' breast milk samples were analyzed were similar between groups. Daily dietary intakes of energy, fat, and PUFA at 18–22 wk gestation did not differ between treatment groups (**Table 2**).

Fatty acid composition of maternal plasma and breast milk. Mean maternal plasma DHA and total (n-3) PUFA concentrations increased significantly between baseline and delivery in both treatment groups, with a greater increase in the DHA group [group × time interaction, $P < 0.0001$ for DHA and $P = 0.02$ for total (n-3) PUFA] (**Table 3**). Concentrations of both DHA and ALA in breast milk, expressed as % by weight of total detected fatty acids, were higher in the DHA group compared with the placebo group ($P < 0.01$ for DHA and $P = 0.01$ for ALA) (**Table 4**). Breast milk DHA and ALA concentrations in the DHA and placebo groups were 0.20 ± 0.06 and 0.17 ± 0.07, and 1.38 ± 0.47 and 1.24 ± 0.46, respectively. Breast milk concentrations of other PUFA, including 18:1 (n-9) (oleic acid), did not differ between treatment groups. Correlations among DHA, EPA, and AA in breast milk and PUFA concentrations at baseline and delivery and dietary PUFA intake, by group, are shown in **Table 5**. Maternal plasma total lipid DHA concentrations at 1 mo postpartum correlated positively with breast milk DHA at 1 mo postpartum in both the placebo and DHA groups ($r = 0.4$; $P < 0.01$ for both treatment groups).

TABLE 1 Selected characteristics at randomization and delivery of 174 women whose 1 mo postpartum breast milk samples were analyzed, in DHA and placebo groups[1]

	DHA	Placebo	*P*-value
At randomization			
Age, *y*	26.4 ± 5.1	25.8 ± 4.8	0.42
Gestational age, *wk*	20.7 ± 2.0	20.6 ± 1.9	0.74
Primigravid, *%*	32.2	36.8	0.52
Parity	2.1 ± 1.0	2.0 ± 1.1	0.57
Weight, *kg*	61.1 ± 9.9	62.0 ± 10.0	0.56
Height, *cm*	154.4 ± 5.9	155.6 ± 5.4	0.16
BMI, *kg/m²*	25.6 ± 3.6	25.6 ± 3.7	0.95
Schooling, *y*	11.4 ± 3.6	11.7 ± 3.4	0.66
SES[2]	−0.13 ± 1.0	−0.08 ± 1.0	0.76
At delivery			
Gestational age, *wk*	39.1 ± 2.0	39.4 ± 1.8	0.34
Premature, <37 wk, *%*	8.1	7.0	0.79
Birth weight, *g*	3245.6 ± 445.5	3254.1 ± 455.4	0.90

[1] Values are means ± SD or percentages, $n = 87$ for each group.
[2] Using principal components analysis.

TABLE 2 Daily dietary intakes in women at randomization in DHA and placebo groups[1,2]

	DHA	Placebo	*P*-value
Energy,[3] *kcal/d*	3200 ± 1130	3444 ± 1070	0.41
Total fat, *g/d*	89.3 ± 35.8	97.4 ± 30.5	0.51
Saturated fat, *g/d*	28.1 ± 11.8	29.3 ± 10.0	0.36
MUFA, *g/d*	32.3 ± 16.2	34.8 ± 13.2	0.77
PUFA, *g/d*	19.2 ± 9.1	20.6 ± 8.4	0.48
∑(n-6) fatty acids, *g/d*	18.3 ± 8.6	18.7 ± 7.8	0.57
18:2(n-6) (LA), *g/d*	18.2 ± 8.5	18.5 ± 7.8	0.44
20:4(n-6) (AA), *g/d*	0.14 ± 0.1	0.13 ± 0.1	0.79
∑(n-3) fatty acids, *g/d*	1.51 ± 0.8	1.60 ± 1.0	0.12
18:3(n-3) (ALA), *g/d*	1.35 ± 0.7	1.54 ± 0.9	0.30
20:5(n-3) (EPA), *g/d*	0.02 ± 0.03	0.02 ± 0.06	0.21
22:6(n-3) (DHA), *g/d*	0.06 ± 0.06	0.05 ± 0.1	0.43
(n-6):(n-3) fatty acids	12.6 ± 4.6	11.9 ± 4.6	0.40

[1] Values are medians ±SD, $n = 87$ for each group.
[2] Evaluated by FFQ (24).
[3] 1 kcal = 4.18 kJ.

Discussion

We showed that supplementing women with 400 mg/d of algae-derived DHA from 18–22 wk gestation through delivery resulted in significantly higher concentrations of both DHA and ALA in breast milk at 1 mo postpartum. Breast milk concentrations of other PUFA such as EPA and AA acid did not differ between groups. The finding of higher levels of ALA in the DHA-supplemented group was unexpected and might be explained through conservation due to less conversion to DHA. We also found a positive correlation between DHA concentrations in maternal plasma at 1 mo postpartum and breast milk at 1 mo postpartum.

Previous studies have examined the influence of maternal supplementation with (n-3) PUFA on breast milk PUFA content but have focused on supplementation during lactation, supplementation during lactation and pregnancy, and supplementation with fish oil (DHA+EPA) rather than DHA alone. Demonstrating the efficacy of algae-derived DHA in improving breast milk DHA concentrations has important implications; specifically, this source of DHA provides an option other than fish oil and fish for persons who do not consume seafood. DHA from algal oil is bioequivalent to DHA from salmon (30). Our study allowed us to examine the influence of prenatal DHA on breast milk DHA

TABLE 3 Fatty acid concentrations in plasma at baseline and delivery in women in the DHA and placebo groups[1]

Fatty acids	Group	Baseline	Delivery	Group × time *P*-value
		% by wt of total fatty acids in maternal plasma		
∑(n-6)	DHA	31.0 ± 3.6	28.1 ± 3.4	0.29
	Placebo	30.8 ± 3.9	28.7 ± 3.0	
18:2(n-6) (LA)	DHA	26.6 ± 3.4	24.7 ± 3.1	0.36
	Placebo	26.4 ± 3.7	25.2 ± 3.0	
20:4(n-6) (AA)	DHA	4.4 ± 1.0	3.3 ± 0.9	0.28
	Placebo	4.4 ± 1.0	3.5 ± 0.8	
∑(n-3)	DHA	3.0 ± 0.7	3.5 ± 0.8*	0.02
	Placebo	2.8 ± 0.6	3.1 ± 0.7*	
18:3(n-3) (ALA)	DHA	0.8 ± 0.3	0.7 ± 0.2	0.15
	Placebo	0.8 ± 0.2	0.7 ± 0.2	
22:5(n-3) (EPA)	DHA	0.8 ± 0.7	1.0 ± 0.7	0.67
	Placebo	0.8 ± 0.6	1.0 ± 0.6	
22:6(n-3) (DHA)	DHA	1.3 ± 0.4	1.7 ± 0.5*	<0.0001
	Placebo	1.2 ± 0.3	1.4 ± 0.4*	
(n-6):(n-3) fatty acids	DHA	11.1 ± 3.1	8.5 ± 2.4	0.13
	Placebo	11.5 ± 3.2	9.7 ± 2.2	

[1] Values are means ± SD, $n = 99$ for each group. *Different from baseline, $P < 0.05$ (paired t tests when group × time $P < 0.05$).

concentrations without having to consider EPA, the other (n-3) PUFA contained in fish oil. This provides insight into the influence of an individual LCPUFA, DHA, on outcomes of interest. Additionally, unlike many fish oil studies, the dose of DHA used in our study is attainable through dietary intake of foods rich in DHA, such as coldwater fish.

Although DHA supplementation resulted in a 12% higher level of DHA in breast milk, the concentration of DHA in breast milk in the study population remained low. Differences in breast milk DHA levels were likely much more pronounced at delivery and would have declined within the first 1–2 mo postpartum, as shown by 2 previous studies of fish oil supplementation in pregnancy (14,23). We expect that more notable differences in breast milk PUFA levels would have been observed had the women received the DHA supplement through early lactation. Breast milk PUFA composition varies across populations; DHA concentrations range from 0.17 to 0.99% of total fatty acids, with a mean concentration worldwide of 0.32% (18,31). Mean breast milk DHA concentrations in our study population are similar to those seen in women in the US and other countries where dietary DHA consumption is lower than recommended (16,18). A study of Otomi women living in rural Mexico found a lower concentration of ALA, a similar concentration of AA, and a higher concentration of LA in breast milk (32).

TABLE 4 Fatty acid concentrations in breast milk measured at 1 mo postpartum, by treatment group[1]

Fatty acid	DHA	Placebo	*P*-value
	% by wt of total fatty acids		
Saturated	40.4 ± 4.6	41.5 ± 5.1	0.15
Unsaturated	39.6 ± 3.1	39.5 ± 3.1	0.82
18:1(n-9)	35.5 ± 2.7	35.5 ± 3.0	0.95
∑(n-6)	18.1 ± 3.2	17.2 ± 3.5	0.11
18:2(n-6) (LA)	16.5 ± 3.0	15.8 ± 3.4	0.11
18:3(n-6)	0.13 ± 0.07	0.13 ± 0.07	0.63
20:2(n-6)	0.45 ± 0.10	0.47 ± 0.12	0.88
20:3(n-6)	0.43 ± 0.09	0.45 ± 0.11	0.28
20:4(n-6) (AA)	0.41 ± 0.12	0.43 ± 0.12	0.27
22:4(n-6)	0.11 ± 0.04	0.12 ± 0.05	0.15
∑(n-3)	1.92 ± 0.57	1.79 ± 0.58	0.10
18:3(n-3) (ALA)	1.38 ± 0.47	1.24 ± 0.46	0.01
20:3(n-3)	0.07 ± 0.04	0.07 ± 0.05	0.74
20:5(n-3)	0.14 ± 0.13	0.16 ± 0.15	0.25
22:5(n-3)	0.10 ± 0.04	0.12 ± 0.05	0.07
22:6(n-3) (DHA)	0.20 ± 0.06	0.17 ± 0.07	<0.01
(n-6):(n-3) fatty acids	9.9 ± 2.0	10.1 ± 2.1	0.43

[1] Values are means ± SD, $n = 87$ for each group.

Although our findings show that DHA supplementation in pregnancy improved breast milk DHA concentrations, we do not know if this difference is biologically significant for the breast-fed infant. However, because breast milk DHA levels have been shown to decline as lactation progresses, any significant increase in breast milk DHA concentration should be deemed desirable (14,23,33). A higher concentration of DHA in breast milk indicates better maternal DHA status, which could benefit both the infant and the mother. DHA in breast milk correlates positively with DHA status of the infant, and infant DHA status may influence cognitive development (2). Additionally, improved DHA status in the mother might provide benefits such as a lower risk of preeclampsia and postpartum depression, although for the latter condition, recent results do not support a benefit of DHA supplementation in pregnancy alone (34–39).

This study was conducted in a developing country in a population in which dietary DHA intake is lower than recommended (24). Our study setting and population were unique, because most of the (n-3) PUFA supplementation trials have been conducted in Europe, Australia, and the United States. The conduct of such trials in developing countries is especially important, because DHA intake in many developing countries is particularly low due to the high cost of marine foods.

Several studies have shown that maternal intake of DHA, most often in the form of fish oil, in pregnancy and/or lactation results in improved maternal, breast milk, and infant (n-3) PUFA status (14,23,40–46). Overall, fish oil supplementation from mid-pregnancy through early lactation results in greater in-

TABLE 5 Spearman rank-order correlation between breast milk DHA, EPA, and AA concentrations at 1 mo postpartum and maternal intake of fatty acids in pregnancy and plasma phospholipid fatty acid concentrations at baseline, delivery, and 1 mo postpartum, by treatment group

	Breast milk	Maternal DHA plasma			Dietary DHA intake	Maternal AA plasma			Dietary AA intake	Maternal EPA plasma			Dietary EPA intake
		Baseline	Delivery	1 mo PP	Baseline	Baseline	Delivery	1 mo PP	Baseline	Baseline	Delivery	1 mo PP	Baseline
Placebo group	DHA	0.06	0.06	0.41*	0.22†	0.31†	0.22	0.22†	0.24	0.08	0.15	−0.06	0.22†
	AA	−0.03	0.04	0.40*	0.18	0.34*	0.42*	0.54*	0.24†	0.23	0.18	−0.06	0.12
	EPA	−0.13	0.00	−0.05	−0.12	0.12	0.04	0.08	−0.19	0.04	0.15	−0.03	−0.12
DHA group	DHA	−0.08	−0.00	0.41*	−0.05	−0.12	0.02	0.27†	−0.10	0.11	0.07	0.10	−0.07
	AA	0.01	−0.10	0.30*	−0.09	0.10	0.26†	0.47*	0.00	0.32†	0.04	0.05	−0.12
	EPA	−0.34	−0.19	0.11	−0.10	−0.20	0.11	0.08	0.00	0.32†	0.04	−0.04	−0.12

*$P < 0.01$; †$P < 0.05$.

creases in DHA concentration in breast milk than fish oil only in pregnancy and higher doses of fish oil result in higher breast milk DHA concentrations. Boris et al. (23) supplemented pregnant Danish women with fish oil from wk 30 of pregnancy through 30 d postpartum or through delivery. This study demonstrated that women who were supplemented with fish oil in pregnancy and lactation had higher concentrations of breast milk DHA at 30 d postpartum (1.4% total fatty acids) compared with women supplemented during pregnancy alone (0.6% total fatty acids) (23). A study by Dunstan et al. (14) showed that women who were supplemented with the high dose of 2.1 g DHA from 20 wk gestation through delivery had a 40% higher concentration of breast milk DHA at 6 wk postpartum than controls, with an effect size of 1.1, showing that higher concentrations of dietary DHA result in a large effect size. The above 2 studies also demonstrated that fish oil supplementation influenced 20:3(n-6) concentrations in breast milk. This finding was not observed in our study, likely because women in our study were supplemented with a lower dose of DHA and no EPA.

In conclusion, adequate DHA concentration in breast milk is essential for the exclusively breast-fed infant, because breast milk provides the sole source of his or her nutrition. We showed that supplementing women from 18–22 wk gestation to delivery with 400 mg algal-derived DHA/d resulted in higher concentrations of breast milk DHA and ALA compared with placebo. Improvements in breast milk DHA status can be achieved by a dose obtainable through diet, equivalent to ~2 fish meals/wk. Intake of DHA is inadequate in many populations, including pregnant women in the US, and dietary guidance and/or supplementation might be indicated to ensure an optimal supply of DHA for both the mother and her infant.

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Literature Cited

1. Innis SM. Polyunsaturated fatty acids in human milk: an essential role in infant development. Adv Exp Med Biol. 2004;554:27–43.
2. Innis SM. Dietary (n-3) fatty acids and brain development. J Nutr. 2007;137:855–9.
3. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: Implications for fatty acid requirements. Early Hum Dev. 1980;4:121–9.
4. Innis SM. Dietary omega 3 fatty acids and the developing brain. Brain Res. 2008;1237:35–43.
5. Hanebutt FL, Demmelmair H, Schiessl B, Larque E, Koletzko B. Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. Clin Nutr. 2008;27:685–93.
6. Koletzko B, Larque E, Demmelmair H. Placental transfer of long-chain polyunsaturated fatty acids (LC-PUFA). J Perinat Med. 2007;35 Suppl 1:S5–11.
7. Ozias MK, Carlson SE, Levant B. Maternal parity and diet (n-3) polyunsaturated fatty acid concentration influence accretion of brain phospholipid docosahexaenoic acid in developing rats. J Nutr. 2007;137:125–9.
8. Al MD, van Houwelingen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. Am J Clin Nutr. 2000;71:S285–91.
9. Innis SM. Human milk: maternal dietary lipids and infant development. Proc Nutr Soc. 2007;66:397–404.
10. Fidler N, Sauerwald T, Pohl A, Demmelmair H, Koletzko B. Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. J Lipid Res. 2000 Sep;41:1376–83.
11. Makrides M, Simmer K, Neumann M, Gibson R. Changes in the polyunsaturated fatty acids of breast milk from mothers of full-term infants over 30 wk of lactation. Am J Clin Nutr. 1995;61:1231–3.
12. Bonham MP, Duffy EM, Wallace JM, Robson PJ, Myers GJ, Davidson PW, Clarkson TW, Shamlaye CF, Strain JJ. Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study). Prostaglandins Leukot Essent Fatty Acids. 2008;78:343–50.
13. Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. Am J Clin Nutr. 2000;71:S307–11.
14. Dunstan JA, Mitoulas LR, Dixon G, Doherty DA, Hartmann PE, Simmer K, Prescott SL. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: a randomized controlled trial. Pediatr Res. 2007;62:689–94.
15. Hibbeln JR, Nieminen LRG, Blasbalg TL, Riggs JA, Lands WEM. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. Am J Clin Nutr. 2006;83:S1483–93.
16. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr. 2000;71:S179–88.
17. Denomme J, Stark KD, Holub BJ. Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations. J Nutr. 2005;135:206–11.
18. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007;85:1457–64.
19. Koletzko B, Cetin I, Brenna JT. Dietary fat intakes for pregnant and lactating women. Br J Nutr. 2007;98:873–7.
20. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current

knowledge and consensus recommendations. J Perinat Med. 2008;36: 5–14.
21. Institute of Medicine Food and Nutrition Board. Dietary Reference Intakes for energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: The National Academies Press; 2002/2005.
22. Xie L, Innis SM. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J Nutr. 2008;138:2222–8.
23. Boris J, Jensen B, Salvig JD, Secher NJ, Olsen SF. A randomized controlled trial of the effect of fish oil supplementation in late pregnancy and early lactation on the n-3 fatty acid content in human breast milk. Lipids. 2004;39:1191–6.
24. Parra-Cabrera S, Stein AD, Wang M, Martorell R, Rivera J, Ramakrishnan U. Dietary intakes of polyunsaturated fatty acids among pregnant Mexican women. Matern Child Nutr. Epub 2010 Jun 21.
25. Ramakrishnan U, Stein AD, Parra-Cabrera S, Wang M, Imhoff-Kunsch B, Juarez-Marquez S, Rivera J, Martorell R. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food Nutr Bull. 2010;31:S108–16.
26. Parra MS, Schnaas L, Meydani M, Perroni E, Martinez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. Public Health Nutr. 2002;5:931–7.
27. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale (NJ): Lawrence Erlbaum Associates, Publishers; 1988.
28. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. J Lipid Res. 1964;5:600–8.
29. Maluccio JA, Melgar P, Mendez H, Murphy A, Yount KM. Social and economic development and change in four Guatemalan villages: demographics, schooling, occupation, and assets. Food Nutr Bull. 2005;26: S25–45.
30. Arterburn LM, Oken HA, Bailey Hall E, Hamersley J, Kuratko CN, Hoffman JP. Algal-oil capsules and cooked salmon: nutritionally equivalent sources of docosahexaenoic acid. J Am Diet Assoc. 2008;108:1204–9.
31. Yuhas R, Pramuk K, Lien EL. Human milk fatty acid composition from nine countries varies most in DHA. Lipids. 2006;41:851–8.
32. Villalpando S, Butte NF, Flores-Huerta S, Thotathuchery M. Qualitative analysis of human milk produced by women consuming a maize-predominant diet typical of rural Mexico. Ann Nutr Metab. 1998;42:23–32.
33. van Goor SA, Dijck-Brouwer DA, Hadders-Algra M, Doornbos B, Erwich JJ, Schaafsma A, Muskiet FA. Human milk arachidonic acid and docosahexaenoic acid contents increase following supplementation during pregnancy and lactation. Prostaglandins Leukot Essent Fatty Acids. 2009;80:65–9.
34. Hibbeln JR. Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. J Affect Disord. 2002;69:15–29.
35. Su KP, Huang SY, Chiu TH, Huang KC, Huang CL, Chang HC, Pariante CM. Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. J Clin Psychiatry. 2008;69:644–51.
36. Freeman MP. Omega-3 fatty acids and perinatal depression: a review of the literature and recommendations for future research. Prostaglandins Leukot Essent Fatty Acids. 2006;75:291–7.
37. Olsen SF, Secher NJ. A possible preventive effect of low-dose fish oil on early delivery and pre-eclampsia: indications from a 50-year-old controlled trial. Br J Nutr. 1990;64:599–609.
38. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA. 2010;304:1675–83.
39. Golding J, Steer C, Emmett P, Davis JM, Hibbeln JR. High levels of depressive symptoms in pregnancy with low omega-3 fatty acid intake from fish. Epidemiology. 2009;20:598–603.
40. Lauritzen L, Jorgensen MH, Olsen SF, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. Reprod Nutr Dev. 2005;45:535–47.
41. Lauritzen L, Jorgensen MH, Mikkelsen TB, Skovgaard M, Straarup EM, Olsen SF, Hoy CE, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes. Lipids. 2004;39:195–206.
42. Hawkes JS, Bryan DL, Makrides M, Neumann MA, Gibson RA. A randomized trial of supplementation with docosahexaenoic acid-rich tuna oil and its effects on the human milk cytokines interleukin 1 beta, interleukin 6, and tumor necrosis factor alpha. Am J Clin Nutr. 2002;75:754–60.
43. Hawkes JS, Bryan DL, Neumann MA, Makrides M, Gibson RA. Transforming growth factor beta in human milk does not change in response to modest intakes of docosahexaenoic acid. Lipids. 2001;36:1179–81.
44. Jensen CL, Maude M, Anderson RE, Heird WC. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr. 2000;71:S292–9.
45. Helland IB, Saugstad OD, Saarem K, Van Houwelingen AC, Nylander G, Drevon CA. Supplementation of n-3 fatty acids during pregnancy and lactation reduces maternal plasma lipid levels and provides DHA to the infants. J Matern Fetal Neonatal Med. 2006;19:397–406.
46. Bergmann RL, Haschke-Becher E, Klassen-Wigger P, Bergmann KE, Richter R, Dudenhausen JW, Grathwohl D, Haschke F. Supplementation with 200 mg/day docosahexaenoic acid from mid-pregnancy through lactation improves the docosahexaenoic acid status of mothers with a habitually low fish intake and of their infants. Ann Nutr Metab. 2008;52:157–66.