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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

C.L. Sherry *, J.S. Oliver, B.J. Marriage

Abbott Nutrition, Scientific and Medical Affairs, 3300 Stelzer Road, Columbus OH 43219, United States

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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

* Corresponding author. Tel.: +1 614 624 3341; fax: +1 614 727 3341.
E-mail address: christina.sherry@abbott.com (C.L. Sherry).

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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