A randomized trial of supplementation with docosahexaenoic acid–rich tuna oil and its effects on the human milk cytokines interleukin 1β, interleukin 6, and tumor necrosis factor α[1–3]

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ABSTRACT

Background: Increased consumption of n−3 long-chain polyunsaturated fatty acids (PUFAs) has been recommended during pregnancy and lactation. The production of proinflammatory cytokines by human peripheral blood mononuclear cells (PBMCs) can be modified by dietary n−3 PUFAs.

Objective: We sought to determine whether dietary supplementation of lactating women with docosahexaenoic acid (DHA) can modulate the concentration of cytokines in the aqueous phase of human milk and the production of cytokines by human milk cells (HMCs) and PBMCs.

Design: In this double-blind, prospective, randomized trial, mothers of healthy full-term infants were asked to consume daily a nutritional supplement of 2000 mg oil containing either placebo ($n = 40$), 300 mg DHA + 70 mg eicosapentaenoic acid (EPA; $n = 40$), or 600 mg DHA + 140 mg EPA ($n = 40$). The fatty acid composition of plasma, PBMCs, milk, and HMCs from lactating mothers at 4 wk postpartum was measured by gas chromatography. The concentration of interleukin 6 and tumor necrosis factor α in milk and the production of interleukin 1β, tumor necrosis factor α, and interleukin 6 by PBMCs and HMCs after stimulation with lipopolysaccharide was measured by enzyme-linked immunosorbent assay.

Results: At 4 wk postpartum, 82 mothers were still breast-feeding their infants. DHA increases in maternal plasma, PBMCs, milk, and HMCs were proportional to dietary DHA. There was no relation between tissue DHA status and cytokine concentrations.

Conclusions: Consumption of ≤600 mg DHA and 140 mg EPA/d for 4 wk increased n−3 PUFA concentrations in relevant tissues but did not cause perturbations in cytokine concentrations in human milk.   *Am J Clin Nutr* 2002;75:754–60.

KEY WORDS Docosahexaenoic acid, human milk, cytokines, n−3 fatty acids, human milk cells, interleukin 1β, interleukin 6, tumor necrosis factor α, randomized controlled trial, breast-feeding, women

INTRODUCTION

There is considerable evidence that breast-feeding provides infants with protection against several types of infection, including respiratory tract infection, gastrointestinal illness, otitis media, urinary tract infections, and necrotising enterocolitis (1, 2). In addition to factors with direct antimicrobial effects, human milk contains components with immunomodulating potential, such as cytokines, growth factors, and leukocytes. We documented the concentrations of many cytokines in the aqueous phase of human milk of healthy Australian mothers during the first 3 mo of lactation (3, 4). Cytokine concentrations were variable, ranging from undetectable to >50 μg/L. The dominant cell types in human milk are neutrophils and macrophages, which together make up ≈90% of the milk cells (5, 6). The remaining cells are lymphocytes (both T and B cells) and a relatively small number of epithelial cells (5–7). We have also shown the capacity of mature milk cells to produce increased amounts of interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and IL-6 after in vitro stimulation with lipopolysaccharide (8). The precise functions of these and other cytokines identified in human milk have not been elucidated; however, it is possible that they provide immunologic help to the infant in the absence of a fully developed immune system during the neonatal period.

Many human studies have reported that supplementing the diet with dietary fish-oil containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) suppresses peripheral blood mononuclear cell (PBMC), IL-1β, TNF-α, and IL-6 production ex vivo (9–12). Both EPA and DHA influence many immune cell functions, although some studies indicate that these fatty acids differ in their effects and act through different mechanisms.

The polyunsaturated fatty acid (PUFA) composition of human milk is partly determined by the PUFA content of the maternal

TABLE 1
Fatty acid composition of the dietary supplements[1]

	Tuna oil	Placebo oil[2]
	% of total fatty acids	
Total saturates	31.0	9.0
Total monounsaturates	23.4	78.2
LA, 18:2n−6	1.7	12.0
AA, 20:4n−6	2.0	—
Total n−6	6.9	12.0
ALA, 18:3n−3	0.5	0.7
EPA, 20:5n−3	6.7	—
DHA, 22:6n−3	28.8	—
Total n−3	38.2	0.7

[1] LA, linoleic acid; AA, arachidonic acid; ALA, α-linoleic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[2] The placebo oil was a highly monounsaturated sunflower seed oil.

diet (13). Because the DHA status of infants has been associated with improved visual and neural outcomes (14, 15), some dietary recommendations stress the need for increased consumption of n−3 PUFAs during pregnancy and lactation (16, 17).

We conducted a randomized, dietary intervention trial to examine the long-term effects of supplementation with tuna oil on aspects of fatty acid status of lactating and nonlactating women. We evaluated the effect of supplementing the diet of lactating women with DHA on the concentration of cytokines in the aqueous phase of human milk and the production of cytokines by human milk cells (HMCs) at 4 wk postpartum.

SUBJECTS AND METHODS

Subjects

This was a double-blind, randomized, prospective study conducted in Adelaide, South Australia. One hundred twenty healthy women aged ≥18 y who had delivered full-term singleton infants and intended to breast-feed for ≥12 wk were enrolled in the study from postnatal wards at Flinders Medical Centre. Mothers with a known history of inflammatory disorders or who were currently taking antiinflammatory medication or fish-oil supplements were excluded from participation in the study. All participating women were asked to limit their fish and seafood intake to a maximum of one meal per week. Women who had ceased lactating by the time of sample collection (4 wk postpartum) were excluded because the primary outcome variables related to human milk cytokines. Subject characteristics were collected by interview at the time of enrolment into the study (eg, date of birth, smoking status, and number of alcoholic drinks consumed per week) or at the 4 wk clinic appointment (eg, number of fish meals per week and side effects). The present study was approved by the Flinders Clinical Research Ethics Committee and written, informed consent was obtained from all participants.

Study design and dietary intervention

Mothers were randomly allocated to 1 of 3 groups and were asked to consume 4 × 500 mg capsules of oil from day 3 postpartum until the end of their 12th postpartum week. The 3 groups were assigned to consume *1*) placebo (n = 40; 4 × 500 mg placebo oil capsules), *2*) 300 mg DHA/d and 70 mg EPA/d from tuna oil [n = 40; 2 × 500 mg tuna oil capsules + 2 × 500 mg

placebo oil capsules (LoDHA)], or *3*) 600 mg DHA/d and 140 mg EPA/d from tuna oil [n = 40; 4 × 500 mg tuna oil capsules (HiDHA)]. The placebo capsules were identical in appearance to the tuna oil capsules and contained no DHA and no EPA. Four capsules were packaged into 84 individual bags for daily consumption. These 84 bags were then grouped into a single package designed to meet the requirements of the entire study period. Each treatment package was labeled with a consecutive number according to a computer-generated randomization schedule with a block size of 6. This was performed before commencement of the trial and by an investigator not associated with the day-to-day management of the trial to ensure that neither the participating mothers nor the research personnel conducting the clinic and laboratory assessments were aware of the dietary allocation. The fatty acid composition of the capsules is shown in **Table 1**. All capsules were provided by Clover Corporation Pty Ltd, Altona North, Australia.

Clinic visit at 4 wk

Participating mothers were taught to hand express their milk while in the hospital and were phoned weekly to encourage successful breast-feeding and compliance with the dietary intervention. During the 4th week of the study, mothers were asked to express a small sample (2 mL) of milk daily for 5 d. Samples were frozen until transport to the clinic at 4 wk postpartum. At that visit, a milk sample of 50 mL expressed on the morning of the appointment was collected in a sterile polypropylene container, and peripheral blood (20 mL) was drawn by venipuncture. The mothers participating in this study also attended clinics at 3, 6, and 9 mo postpartum to assess aspects of fatty acid status in postpartum women; these data will be reported elsewhere.

Preparation of human milk samples

The 5 daily milk samples were thawed, pooled, and retained for total fatty acid analysis. The fresh 50-mL samples were centrifuged at 890 × g for 30 min at 4°C. The fat layer from each sample was discarded and the aqueous fraction was frozen at −80°C for analysis of aqueous phase cytokines by enzyme-linked immunosorbent assay (ELISA). The cellular component was washed and adjusted to a concentration of 5 × 10^9 cells/L in RPMI containing 5% fetal calf serum. Aliquots of cells were retained for immunophenotyping by flow cytometry and fatty acid determinations when the cell yield was sufficient. HMCs were cultured (1 × 10^6/well) in 96-well flat-bottomed plates (Nalge Nunc International, Naperville, IL) at 37°C in the presence or absence of lipopolysaccharide (500 μg/L; Sigma Chemical Co BioSciences, St Louis). After 24 h, cell culture supernatant fluid was harvested and stored at –80°C before analysis for secreted cytokines. The cells were resuspended in fresh media, lysed by one freeze-thaw cycle, and the supernatant fluid was harvested and stored at –80°C before analysis for intracellular cytokines.

Preparation of peripheral blood samples

Venous blood samples were divided as described below. One milliliter was placed into lithium heparin for the separation of plasma and erythrocytes, and each component was retained for fatty acid analysis. The remaining sample was added to EDTA (4.5%, 4 mL) and dextran T500 (6%, 4 mL) (Pharmacia Biotech AB, Uppsala, Sweden) for 30 min at 37°C to enable sedimentation of erythrocytes. PBMCs were isolated from the leukocyte-enriched
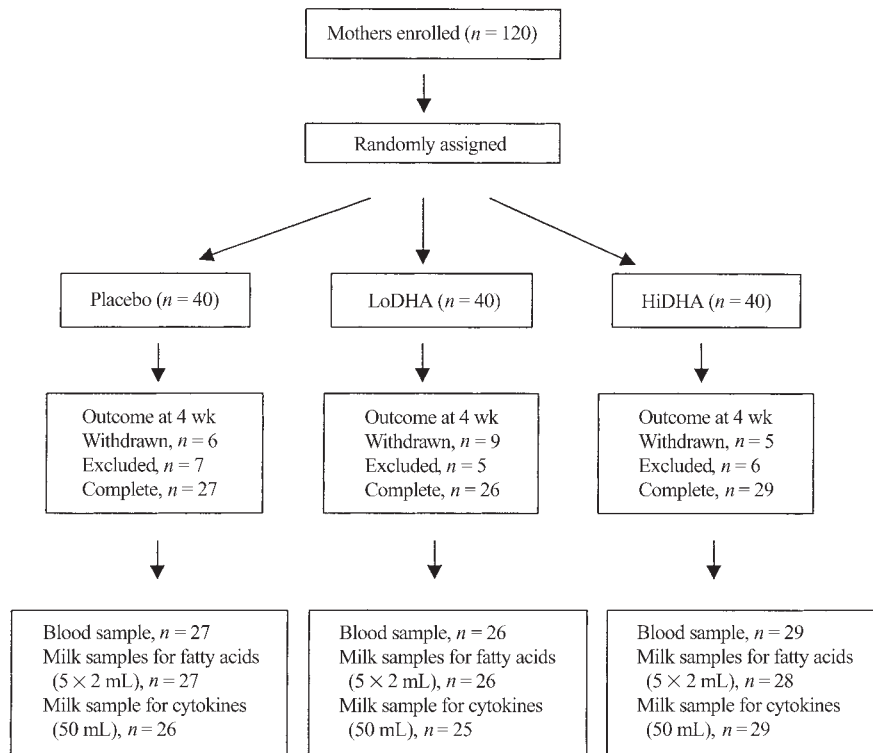
FIGURE 1. Flow chart showing the distribution of the 120 subjects enrolled in the present study. LoDHA, low–docosahexaenoic acid group; HiDHA, high–docosahexaenoic acid group.

plasma by density gradient centrifugation with use of Lympho-prep (Nycomed Pharma, Oslo). Aliquots of cells were retained for fatty acid analysis. PBMCs (2×10^5/well) were cultured in the presence or absence of lipopolysaccharide (200 μg/L), as described for the HMCs.

Cytokine assays

Human milk aqueous phase samples were analyzed for IL-6 and TNF-α and cell culture supernatant fluid (intracellular and secreted cytokines) was analyzed for total IL-1β, IL-6, and TNF-α by using double-antibody sandwich ELISA, as described previously (3). IL-1β was omitted from the analysis of human milk aqueous phase samples because our previous study showed that it is present in only a small proportion of samples at 4 wk post-partum and is generally found in low concentrations. The limit of detection for each ELISA was as follows: IL-1β, 8 ng/L; IL-6, 2 ng/L; TNF-α, 6 ng/L. Validation studies for each assay are described in our previous study (3). Results are expressed as total (intracellular + secreted) cytokine produced. All cytokine analyses were completed within 8 mo of sample collection.

Immunophenotyping

HMCs (1×10^6 cells) were stained with fluorochrome-labeled monoclonal antibodies against CD45 (leukocyte common anti-gen) and CD14 (monocytes and macrophages). Washed cells were analyzed with the use of 2-color direct immunofluores-cence on a FACScan instrument by using Lysis II software (Bec-ton Dickinson, San Jose, CA). The percentages of monocytes, lymphocytes, and granulocytes were determined on the basis of CD45/CD14 fluorescence and forward and side scatter parame-ters. Results are given as a percentage of total leukocytes.

Fatty acid analysis

Fatty acid methyl esters were prepared, separated, and ana-lyzed as described previously (13). Briefly, total lipids were extracted from the 5 pooled human milk samples and methylated in 1% H_2SO_4 in methanol for 2 h at 70 °C. The phospholipid frac-tions of plasma, PBMC, and HMC lipid extracts were obtained by thin-layer chromatography before methylation. The resulting methyl esters were extracted into *n*-heptane and transferred into vials containing anhydrous Na_2SO_4 as the dehydrating agent. Fatty acid methyl esters were separated and quantified on a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) and were identified based on the retention time of authentic lipid standards (NuChek Prep Inc, Elysian, MN).

Sample size and statistical analysis

The target sample size for each group was based on the num-ber required to complete the entire study to 9 mo postpartum. With regard to the fatty acid of interest, DHA, a total of 30 sub-jects in each group would enable the detection of a 0.5% differ-ence between the means of the 3 populations with >90% power and an α of 0.05 (10). On the basis of data reported in previous studies in which dietary supplementation with n−3 fatty acids inhibited cytokine production by PBMCs (9, 10), calculations indicated that a total of ≈30 subjects in each group would be adequate to enable the detection of significant differences in cel-lular cytokine production between the 3 groups.

One-way analysis of variance (ANOVA) and post hoc analysis by multiple comparisons test with a Bonferroni's correction were used to determine the differences in proportions of cell types and plasma, milk, PBMC, and HMC fatty acids between diet groups.

TABLE 2
Characteristics of the mothers still breast-feeding at the time of sample collection according to dietary supplementation group[1]

Characteristic	Placebo (n = 27)	LoDHA (n = 26)	HiDHA (n = 29)
Maternal age (y)	30.8 ± 5.5[2]	30.1 ± 5.0	29.7 ± 4.3
No. of fish meals/wk	1.1 (0.6, 1.6)[3]	0.7 (0.4, 0.9)	0.7 (0.4, 0.9)
Side effects [n (%)]	4/27 (15)	4/26 (15)	2/29 (7)
Smoker [n (%)]	2/27 (7)	5/25 (20)[4]	2/28 (7)[4]
No. of alcoholic drinks/wk	1.4 (0.1, 2.7)	1.3 (0.6, 2.2)	0.9 (0.3, 1.4)

[1] LoDHA, low–docosahexaenoic acid group; HiDHA, high–docosahexaenoic acid group. LoDHA group, 300 mg DHA/d and 70 mg EPA/d from tuna oil; HiDHA group, 600 mg DHA/d and 140 mg EPA/d from tuna oil. There were no significant differences between dietary groups for any of the variables described.
[2] $\bar{x}$ ± SD.
[3] $\bar{x}$; 95% CI in parentheses.
[4] Two subjects, one from the LoDHA group and one from the HiDHA group, did not answer the question of whether they smoked. Thus, n = 25 and 28 for the LoDHA and HiDHA groups, respectively.

Data are expressed as means ± SDs. Because the distributions of the cytokine values were asymmetric, data were log transformed and significant differences between dietary groups were calculated by Kruskal-Wallis ANOVA and Pearson's chi-square tests. Samples with cytokine concentrations below the limit of detection for IL-1β, IL-6, and TNF-α were assigned a value of one-half the limit of detection (4, 1, and 3 ng/L, respectively) for statistical calculations. Associations between fatty acid and cytokine concentrations were tested by using Spearman's rank-order correlation coefficient. All analyses were completed by using SPSS for WINDOWS 10.0 (SPSS Inc, Chicago).

RESULTS

Subject characteristics

Of those who were enrolled in the present study, 27 subjects in the placebo group, 26 in the LoDHA group, and 29 in the HiDHA group were still breast-feeding their infants at the time of the 4 wk appointment (**Figure 1**). Ninety-three percent of the mothers were providing >80% of their infants' diet as breast milk. Mothers who completed the study were between 20 and 42 y of age and, on average, consumed less than one fish meal per week throughout the study (**Table 2**). Seven to 15% of the women reported some form of side effect that they attributed to the supplementation, which included gas (n = 4), fishy burps (n = 4), unsettled babies (n = 1), and rash (n = 1). There was no significant difference between dietary groups in the number of alcoholic drinks consumed per week and in the percentage of mothers who smoked cigarettes.

Sample collection

Peripheral blood (82/82 samples) and milk (81/82 samples for fatty acid analysis and 80/82 fresh 50-mL samples) were collected from lactating mothers on day 35 (95% CI: 33.6, 36.1; Figure 1). All 50-mL expressions were completed in the morning and most of the mothers expressed before a feed (67%) from a single breast only (62%).

Blood and human milk fatty acids

Fatty acid analyses were completed on all samples provided of plasma, PBMCs, and milk and on a subset (40/80) of HMC samples (**Table 3**). Maternal dietary compliance was confirmed by observing n−3 PUFA concentrations. DHA concentrations in plasma, PBMCs, milk, and HMCs increased in a linear manner in response to dietary DHA. The effect of tuna oil consumption on EPA concentration was more modest, with higher concentrations of PBMC, milk, and plasma EPA at the highest tuna oil intake only (P = 0.08). Tuna oil consumption caused a reduction in plasma AA concentrations, but the changes in other tissues were not significant.

Types of human milk cells

The relative proportions of the 3 main leukocyte populations in human milk were determined in 65 of the 80 samples. There was considerable variability ($\bar{x}$ ± SD) between individual samples with regard to the proportions of monocytes and macrophages [placebo (n = 21), 33 ± 19%; LoDHA (n = 22), 34 ± 13%; and

TABLE 3
Maternal plasma, peripheral blood mononuclear cell (PBMC), and human milk cell (HMC) phospholipid fatty acids and milk total fatty acids after 4 wk of dietary supplementation[1]

Fatty acid	Placebo (n = 27)	LoDHA (n = 26)	HiDHA (n = 29)
	% of total fatty acids		
Plasma			
DHA	3.4 ± 1.0[a]	4.1 ± 0.5[b]	5.7 ± 0.9[c]
EPA	1.07 ± 0.32[a,b]	1.03 ± 0.31[a]	1.25 ± 0.25[b]
AA	10.6 ± 1.3[a]	9.4 ± 1.2[b]	9.5 ± 1.4[b]
PBMC			
DHA	2.1 ± 0.5[a]	2.8 ± 0.5[b]	3.3 ± 0.7[c]
EPA	0.43 ± 0.12[a]	0.47 ± 0.13[a]	0.56 ± 0.10[b]
AA	23.1 ± 1.6	22.4 ± 1.3	22.0 ± 2.4
Milk[2]			
DHA	0.26 ± 0.08[a]	0.39 ± 0.09[b]	0.66 ± 0.18[c]
EPA	0.11 ± 0.02[a]	0.11 ± 0.02[a]	0.14 ± 0.03[b]
AA	0.46 ± 0.07	0.44 ± 0.06	0.47 ± 0.08
HMC[3]			
DHA	1.27 ± 0.27[a]	1.89 ± 0.45[b]	2.53 ± 0.73[c]
EPA	0.37 ± 0.08[4]	0.38 ± 0.11[4]	0.43 ± 0.13[5]
AA	7.0 ± 2.3	6.3 ± 1.7	6.2 ± 2.5

[1] $\bar{x}$ ± SD. LoDHA, low–docosahexaenoic acid group; HiDHA, high–docosahexaenoic acid group; EPA, eicosapentaenoic acid; AA, arachidonic acid. LoDHA group, 300 mg DHA/d and 70 mg EPA/d from tuna oil; HiDHA group, 600 mg DHA/d and 140 mg EPA/d from tuna oil. Values in the same row with different superscript letters are significantly different, P < 0.05.
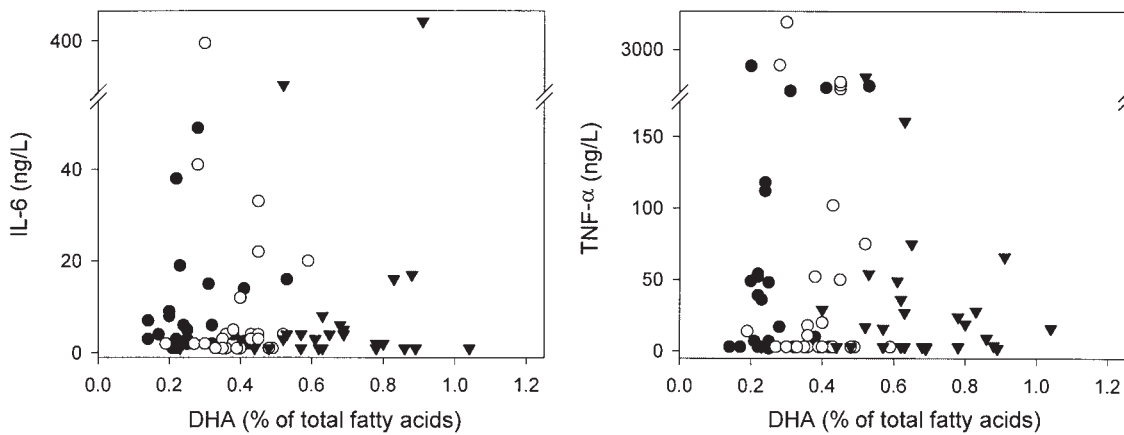[2] n = 28 for the HiDHA group.
[3] n = 12, 13, and 15 for the placebo, LoDHA, and HiDHA groups, respectively.
[4] n = 10.
[5] n = 14.

FIGURE 2. Human milk aqueous phase cytokines interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) plotted as a function of milk docosa-hexaenoic acid (DHA) after 4 wk of dietary supplementation [●, placebo group, n = 26; ○, LoDHA group (300 mg DHA/d and 70 mg EPA/d from tuna oil), n = 25); ▼, HiDHA group (600 mg DHA/d and 140 mg EPA/d from tuna oil), n = 28]. There was no correlation between either cytokine and breast milk DHA.

HiDHA (n = 22), 38 ± 18%], granulocytes (placebo, 45 ± 24%; LoDHA, 36 ± 16%; and HiDHA, 39 ± 24%) and lymphocytes (placebo, 22 ± 21%; LoDHA, 30 ± 15%; and HiDHA, 23 ± 18%). There was no significant difference between the dietary groups in the relative proportions of these human milk leukocytes.

Human milk aqueous phase cytokines (IL-6 and TNF-α) at 4 wk

There was no significant difference between the dietary groups in mean rank concentrations of IL-6 or TNF-α in the aqueous phase of milk. In addition, there were no correlations between milk DHA (**Figure 2**), EPA, or total n−3 PUFAs and cytokine concentrations. IL-6 and TNF-α were detected in ≈50–60% of all samples collected, and with each of these cytokines there was a wide range of concentrations in each dietary group. There was an even distribution of samples below the limit of detection for TNF-α across treatment groups. However, a greater proportion of

values for IL-6 were <2 ng/L with DHA treatment compared with placebo (chi-square = 8.17, P < 0.05).

Cellular cytokine production (IL-1β, IL-6, and TNF-α) at 4 wk

There was no significant difference in mean rank concentrations between the dietary groups for any of the cytokines produced by cells isolated from human milk (**Table 4**) or peripheral blood (**Table 5**) after in vitro stimulation with lipopolysaccharide or in the absence of stimulation (data not shown). In addition, there were no correlations between cellular n−3 fatty acids (DHA, EPA, and total n−3 PUFA) and the cellular production of cytokines (data for DHA shown in **Figures 3** and **4**).

DISCUSSION

This is the first study to report the effect of dietary n−3 PUFAs on human milk aqueous phase cytokine concentrations

TABLE 4
Cytokine production by expressed human milk cells after 4 wk of dietary supplementation[1]

	Placebo (n = 25)	LoDHA (n = 24)	HiDHA (n = 29)
		ng/L	
Interleukin 1β			
Median	816	507[2]	521
5th–95th percentile	36–3961	21–4560	24–8301
Interleukin 6			
Median	882	845	981
5th–95th percentile	50–2028	53–2598	40–8603
Tumor necrosis factor α			
Median	473[3]	669	350[4]
5th–95th percentile	27–2350	25–4149	11–5607

[1] LoDHA, low–docosahexaenoic acid group; HiDHA, high–docosahexaenoic acid group. LoDHA group, 300 mg DHA/d and 70 mg EPA/d from tuna oil; HiDHA group, 600 mg DHA/d and 140 mg EPA/d from tuna oil. There were no significant differences between dietary groups for any of the variables described.
[2] n = 25.
[3] n = 24.
[4] n = 28.

TABLE 5
Cytokine production by peripheral blood mononuclear cells after 4 wk of dietary supplementation[1]

	Placebo (n = 26)	LoDHA (n = 26)	HiDHA (n = 27)
		ng/L	
Interleukin 1β			
Median	5532	7105	6572
5th–95th percentile	3599–11751	3936–12058	3098–11485
Interleukin 6			
Median	1886	1929	1824
5th–95th percentile	1223–2850	1286–2593	974–2517
Tumor necrosis factor α			
Median	1733	1760	1477
5th–95th percentile	282–4221	543–3770	199–5961

[1] LoDHA, low–docosahexaenoic acid group; HiDHA, high–docosahexaenoic acid group. LoDHA group, 300 mg DHA/d and 70 mg EPA/d from tuna oil; HiDHA group, 600 mg DHA/d and 140 mg EPA/d from tuna oil. There were no significant differences between dietary groups for any of the variables described.
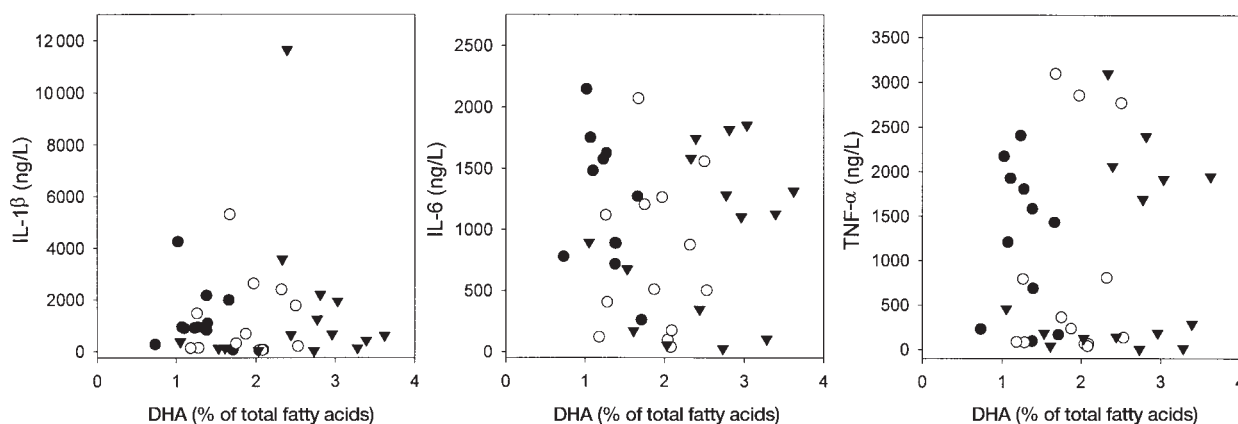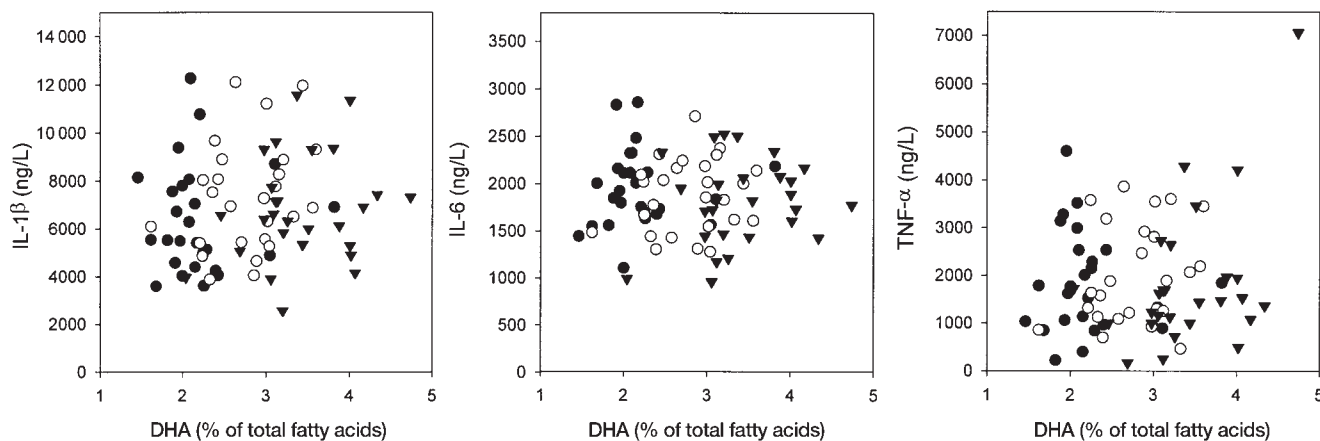
FIGURE 3. Interleukin 1β (IL-1β), IL-6, and tumor necrosis factor α (TNF-α) production by expressed human milk cells (HMCs) plotted as a function of HMC docosahexaenoic acid (DHA) after 4 wk of dietary supplementation [●, placebo group, *n* = 11; ○, LoDHA group (300 mg DHA/d and 70 mg EPA/d from tuna oil), *n* = 13; ▼, HiDHA group (600 mg DHA/d and 140 mg EPA/d from tuna oil), *n* = 15]. There was no correlation between any of the cytokines measured and HMC DHA.

and HMC cytokine production. Supplementation with tuna oil rich in DHA resulted in a dose-dependent increase in plasma and milk DHA content, and the membrane DHA phospholipid content of both HMCs and PBMCs clearly indicated the success of this dietary intervention. However, within the limits of the present study, we could not detect an effect of the dietary intervention on the concentration of cytokines in the aqueous phase of human milk or on the production of cytokines by HMCs or PBMCs. There was extensive variability in the concentration of cytokines measured in the aqueous phase of milk samples and in the supernatant fluid from stimulated HMCs, which is comparable with results from previous studies by us (3, 8) and by others (18, 19). The number of human milk aqueous phase samples with concentrations of IL-6 below the level of detection were greater in the groups receiving dietary fish oil, but we are unsure of the relevance of this observation.

Numerous human studies have examined the effect of n−3 PUFAs on cytokine production, with many describing sup-

pressed production of the proinflammatory cytokines IL-1β, IL-6, and TNF-α by PBMCs (9–12, 20, 21). A possible explanation for the lack of cytokine inhibition in the present study could lie in the different PUFA compositions of tuna and other fish oils used in dietary interventions. Tuna oil is rich in DHA (≈30%) but contains little EPA (≈6%). This specific composition, together with the relatively low dose of supplementation, resulted in minimal changes in the EPA content of tissues compared with 5–6-fold increases in cellular EPA concentrations described in other studies where both doses and ratios of EPA to DHA were higher (9, 12). These results suggest that it is the 20-carbon n−3 fatty acid (EPA) that is the major contributor in the regulation of cytokine production.

However, Kelley et al (21) described the inhibition of PBMC-derived cytokines after the consumption of DHA alone (6 g/d for 3 mo), which resulted in a 3-fold increase in cellular DHA concentrations in the absence of any change in EPA concentrations, suggesting that DHA in high doses also has the capacity to alter

FIGURE 4. Interleukin 1β (IL-1β), IL-6, and tumor necrosis factor α (TNF-α) production by peripheral blood mononuclear cells (PBMCs) plotted as a function of PBMC docosahexaenoic acid (DHA) after 4 wk of dietary supplementation [●, placebo group, *n* = 26; ○, LoDHA group (300 mg DHA/d and 70 mg EPA/d from tuna oil), *n* = 26; ▼, HiDHA group (600 mg DHA/d and 140 mg EPA/d from tuna oil), *n* = 27]. There was no correlation between any of the cytokines measured and PBMC DHA.

immune function. The changes in n−3 PUFA concentrations in both PBMCs and HMCs collected from subjects in the present study are significant but not of the same order of magnitude as those described by other investigators. These and other data suggest that the inhibition of cytokines by n−3 PUFAs is dose dependent, requiring higher doses than that used in the present study. Given the large biological variability in cytokine production, particularly by HMCs, the present trial was not adequately powered to detect modest differences should they exist.

Human studies addressing the effects of n−3 PUFAs on immune response usually include healthy young male volunteers, although there is no clear data to suggest that the immunomodulatory effects of n−3 PUFAs are likely to be different in women. One study comparing immune responses of young and older women after oral n−3 PUFA supplementation showed that the suppressive effects of n−3 PUFAs are more dramatic in older women, accompanied by larger increases in plasma n−3 PUFAs (10). The possibility of changes in the hormone balance of lactating women during the first 4 wk postpartum influencing the response to dietary n−3 PUFAs cannot be discounted.

There is increasing community awareness of the benefits of a high n−3 PUFA intake, particularly with regard to cardiovascular health and amelioration of symptoms of some autoimmune and inflammatory diseases. The doses of dietary DHA in this study provided the range of milk DHA to be expected from healthy mothers consuming 400–500 g red salmon/wk (22). The lower concentration of DHA supplementation in the present study is also in line with additional n−3 PUFA consumption that is recommended during lactation by nutrition committees (23) and commercial nutritional supplement ventures.

The immunologic effects of human milk cytokines and leukocytes are currently being defined, although these components are clearly present in quantities that have the potential to contribute to the immunoprotection of the recipient infant. The results of the present study suggest that consumption of ≤600 mg DHA/d and 140 mg EPA/d for 4 wk (equivalent to consuming one or more fish meals per day), although increasing PUFA concentrations in relevant cell types does not cause perturbations in cytokine concentrations. Thus, it is possible for lactating women to follow dietary guidelines beneficial for their own health without potential risk to their infant.

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