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Salmon Consumption during Pregnancy Alters Fatty Acid Composition and Secretory IgA Concentration in Human Breast Milk[1–4]

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Abstract

Fish oil supplementation during pregnancy alters breast milk composition, but there is little information about the impact of oily fish consumption. We determined whether increased salmon consumption during pregnancy alters breast milk fatty acid composition and immune factors. Women ($n = 123$) who rarely ate oily fish were randomly assigned to consume their habitual diet or to consume 2 portions of farmed salmon per week from 20 wk of pregnancy until delivery. The salmon provided 3.45 g long-chain (LC) (n-3) PUFA/wk. Breast milk fatty acid composition and immune factors [soluble CD14, transforming growth factor-β (TGFβ)1, TGFβ2, and secretory IgA] were analyzed at 1, 5, 14, and 28 d postpartum (PP). Breast milk from the salmon group had higher proportions of EPA (80%), docosapentaenoic acid (30%), and DHA (90%) on d 5 PP compared with controls ($P < 0.01$). The LC (n-6) PUFA:LC (n-3) PUFA ratio was lower for the salmon group on all days of PP sampling ($P \leq 0.004$), although individual (n-6) PUFA proportions, including arachidonic acid, did not differ. All breast milk immune factors decreased between d 1 and 28 PP ($P < 0.001$). Breast milk secretory IgA (sIgA) was lower in the salmon group (d 1–28 PP; $P = 0.006$). Salmon consumption during pregnancy, at the current recommended intakes, increases the LC (n-3) PUFA concentration of breast milk in early lactation, thus improving the supply of these important fatty acids to the breast-fed neonate. The consequence of the lower breast milk concentration of sIgA in the salmon group is not clear. J. Nutr. 142: 1603–1610, 2012.

Introduction

The long-chain (LC)[10] (n-3) PUFA DHA is important for early brain, visual, and neural development (1,2). There is also some evidence that early exposure to LC PUFA can influence immune development and function (3). Therefore, it is important that pregnant and lactating mothers can supply sufficient DHA and other LC PUFA to the fetus and neonate to meet its demands for growth and development. In their review of the impact of breast milk DHA status on infant health outcomes, Jensen and Lapillonne (4) stated that there is evidence that higher breast milk DHA is associated with better infant neurodevelopment and visual function and they concluded that increasing the breast milk DHA concentration may confer neurodevelopmental benefits to the recipient breast-fed infant. Seafood, especially oily fish, is a good source of DHA and of another LC (n-3) PUFA, EPA. Consequently, breast milk EPA and DHA were reported to be higher in women with a high habitual consumption of fish (5). Fish oil is rich in EPA and DHA. Providing daily fish oil supplements during pregnancy (6,7), lactation (8), or pregnancy and lactation (9) increases the breast milk DHA concentration. To our knowledge, there are no intervention studies with oily fish in pregnancy reporting on breast milk fatty acids.

In addition to supplying important LC PUFA, human breast milk provides a broad range of immunological factors, which are

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[3] This trial is registered at www.clinicaltrials.gov as NCT00801502.
[4] Supplemental Methods and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[10] Abbreviations used: AA, arachidonic acid 20:4(n-6); ALA, α-linolenic acid 18:3 (n-3); DPA, docosapentaenoic acid 22:5(n-3); % FA, percentage of total fatty acids; LC, long-chain; PP, postpartum; sCD14, soluble CD14; sIgA, secretory IgA; SiPS, Salmon in Pregnancy Study; TGFβ, transforming growth factor-β.
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likely to play a role in early gastrointestinal and immune maturation in neonates and their immune defenses (10,11). These include, among others, transforming growth factor-β (TGF-β) (12), secretory IgA (sIgA) (13), and soluble CD14 (sCD14) (14). TGFβ is one of the most abundant cytokines in human milk (15). It has pleiotropic effects, including promoting oral tolerance (16), inducing cell proliferation and differentiation (17,18), and protecting against allergy-related outcomes in infancy and childhood (12). sCD14 is a key component of the neonate's host defense mechanism, because neonates lack the CD14-independent pathway for dealing with Gram-negative bacterial LPS (19). Newborn infants do not produce their own protective levels of sIgA until 30 d postpartum (PP) (20). Thus, a supply of sIgA from breast milk, providing passive immune protection, is very important in the first month of life (21). A small number of studies have investigated whether supplemental LC (n-3) PUFA during pregnancy and/or lactation affect such immune factors in breast milk (22,23), but to our knowledge, there are no data on the influence of oily fish consumption by pregnant women on breast milk immune factors.

In the UK, pregnant women are recommended to eat 1 or 2 portions of oily fish/wk, with guidance to avoid specific species due to the potential risk of contaminants (24). Similar guidance is offered by the US FDA (25). However, consumption of oily fish is low in these populations, with ~60% nonconsumers among adults in the UK (26). The Salmon in Pregnancy Study (SiPS) is a randomized, controlled intervention investigating the effects of consumption of 2 portions of salmon/wk by women from wk 20 of pregnancy until they give birth (27). Here we tested the hypotheses that increased consumption of salmon by pregnant women would increase breast milk proportions of LC (n-3) PUFA and DHA and would alter breast milk immune factor concentrations.

Participants and Methods

Study design and participant characteristics. The SiPS is a single-blind, randomized, controlled intervention with salmon during pregnancy; the full study design and baseline characteristics of the women were previously reported (27). In brief, pregnant women (n = 123) who rarely ate oily fish and whose unborn infant had a family history of atopy, allergy, or asthma (one or more first-degree relatives of the unborn infant affected by atopy, allergy, or asthma) were randomly assigned to either continue consuming their habitual diet (control group; n = 61) or were provided with farmed salmon and requested to consume two 150 g portions of salmon/wk from 20 wk pregnancy until birth (salmon group; n = 62). Full details of the farmed salmon were previously reported (27) and the 2 portions of salmon typically provided 3.45 g of EPA plus DHA/wk, equivalent to ~0.48 g/d EPA +DHA. Maternal fish and LC (n-3) PUFA intakes and EPA and DHA concentrations in maternal blood were previously reported (27). The study and all procedures were approved by the Southampton and South West Hampshire Research Ethics Committee (07/Q1704/43). The study was conducted according to the principles of the Declaration of Helsinki and all women gave written informed consent.

Breast milk samples were collected during the first month PP to represent colostrum (d 1 PP), transition (d 5 and 14 PP), and mature milk (d 28 PP). The milk was collected after the baby had been fed (i.e., hind milk) and was manually expressed into sterile containers and immediately stored at −20°C until it was transferred to the laboratory, where it was then stored at −80°C until analysis. Not all the women breast fed and of those who did, not all were able to provide breast milk samples at each time point PP. Furthermore, there was insufficient sample in some cases for all analyses to be conducted. **Figure 1** describes the number of breast milk samples available at each time point and for each type of assay. Data were analyzed with linear mixed model to manage missing data points.

Breast milk fatty acid composition. The procedure used for breast milk lipid extraction (28) and breast milk fatty acid composition analysis is described in (**Supplemental Methods**).

Breast milk immune factors. A pilot study identified that whole breast milk was suitable for measurement of immune factor concentrations (**Supplemental Methods**). The concentrations of sCD14, TGFβ1, and TGFβ2 were measured using Quantikine human immunoassays (R & D Systems, Europe), following the manufacturer's instructions. The limits of detection of sCD14, TGFβ1, and TGFβ2 were 125, 7, and 7 ng/L, respectively. Intra- and inter-assay CV were 4 and 11% (sCD14), 5 and 15% (TGFβ1), and 4 and 9% (TGFβ2), respectively. The concentration of sIgA was measured using an ELISA kit reported to have 100% cross-reactivity for human sIgA from breast milk (Demeditec Diagnostics). The manufacturer's instructions were followed. The limit of detection was 1.2 mg/L, and the intra- and inter-assay CV were 7 and 16%, respectively. All plates were read on a GENios Spectra FLUOR plus (Tecan UK).

Breast milk protein concentration. Breast milk protein concentrations were measured using the method of Bradford (29,30) using human serum albumin (Sigma-Aldrich) as standard. The limit of detection was 0.1 g/L and the intra- and inter-assay CV were 7.0 and 9.0%, respectively.>

Statistics. Data were checked for normality with histograms and the Kolmogorov-Smirnov test. Data are expressed as mean ± SEM, median and (25th–75th percentile), or number and percentage as appropriate. Breast milk fatty acids were calculated as a percentage of total fatty acids (% FA) and as a concentration within whole milk (mg/L). They were log- or rank-transformed and the effects of time and treatment group examined by linear mixed model. Breast milk immune factors were log transformed and also compared by linear mixed model. Post hoc pairwise comparisons were carried out with a Student's t test and Bonferroni correction. To adjust for potential confounders, single variables (parity, maternal age, maternal BMI, self-reported maternal atopy, and maternal atopy defined by positive skin prick test) were assessed within the model. Spearman rank correlations or partial correlations on transformed data, controlling for group and time PP, were used to examine the relationships between breast milk immune factors and fatty acids. Models for predicting breast milk DHA and breast milk sIgA for the total population, the control group, and the salmon group were developed with the use of multiple regression analysis. The independent variables considered were breast milk fatty acids and immune factors and maternal characteristics, dietary fatty acid intakes, and fatty acid status. The independent variables selected for inclusion in the model were determined from initial significant bivariate correlations with the dependent variable. Several separate models were fitted because of concerns with multi-colinearity when fitting all variables together in a single model. Breast milk TGFβ1 and TGFβ2 were combined as one variable. All multiple regression analyses were applied in conditions that ensured a suitable fit. These conditions were explored using relevant residual analyses and plots. SPSS was used for all tests and in all cases, $P < 0.05$ was considered significant ($P < 0.05$ adjusted for the number of multiple comparisons).

Results

Subject and pregnancy characteristics. Sixty-one women were enrolled into the control group and 62 into the salmon group and, after attrition during pregnancy, there were 54 births in the control group and 53 in the salmon group (27). A subgroup of the women chose to breast feed and for those who provided breast milk samples, there were no significant differences between the 2 groups with respect to age, height, weight, and BMI at study entry, maternal self-reported atopy, or maternal atopy defined by positive skin prick testing to allergens (31) (**Table 1**). However, the number of women for whom the study pregnancy was their first was higher in the salmon group (Table 1).

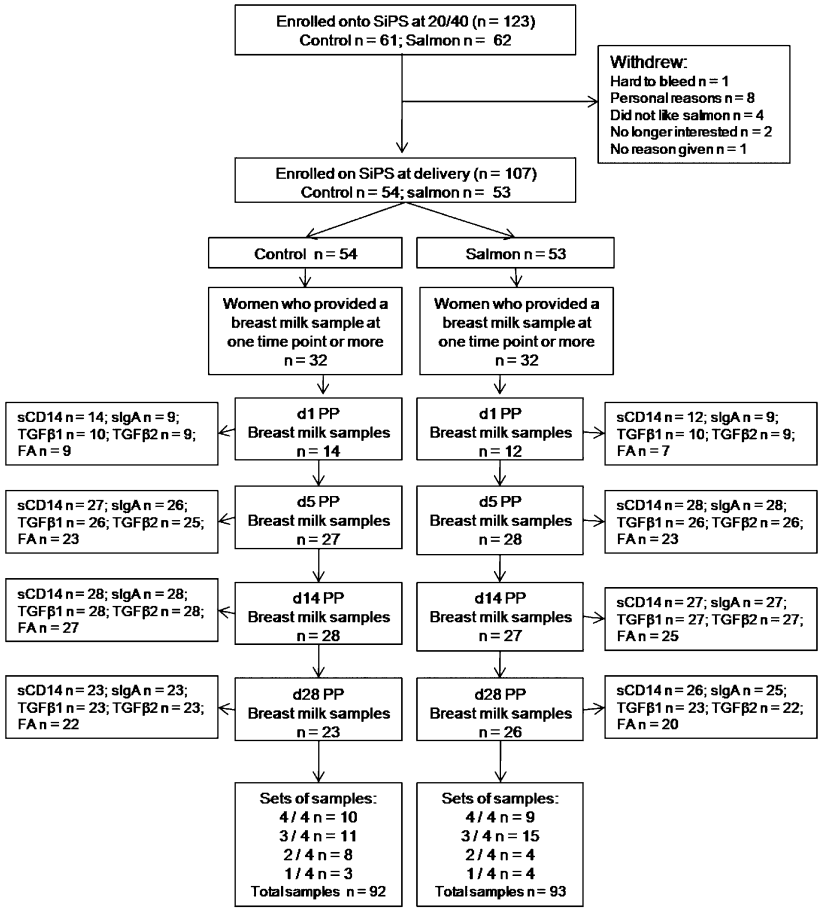

FIGURE 1 CONSORT diagram showing the flow of participants through the SiPS, the collection of breast milk samples, and the analyses completed. FA, fatty acid; PP, postpartum; sCD14, soluble CD14; sIgA, secretory IgA; SiPS, Salmon in Pregnancy Study; TGFβ, transforming growth factor-β.

Breast milk fatty acid composition. There was a significant effect of time on the percentage of a number of fatty acids (**Supplemental Table 1**), including α-linolenic acid [ALA, 18:3 (n-3)], arachidonic acid (AA), docosapentaenoic acid (DPA), and DHA (**Table 2**). There was a significant effect of treatment group on EPA, DPA, and DHA (Table 2). Breast milk from the women who consumed salmon had significantly higher proportions of EPA (80%), DPA (30%), DHA (90%), total (n-3) PUFA (30%), and total LC (n-3) PUFA (60%) on d 5 PP compared with controls (Table 2). The proportion of DHA remained significantly higher in the salmon group at d 14 and 28 PP [i.e., 14 and 28 d after the women had ceased eating the study salmon and had returned to their habitual diet of low oily fish consumption (32)]. The percentages of LC (n-6) PUFA, including AA, did not significantly differ between the 2 groups, but the LC (n-6) PUFA: LC (n-3) PUFA ratio was significantly lower in the milk of the women consuming salmon and remained significantly lower at d 28 PP (Table 2). There was a positive correlation between breast milk AA and DHA, having adjusted for days PP, in both the control group ($r = 0.57$; $P < 0.001$) and the salmon group ($r = 0.41$; $P < 0.001$), although the relationship between milk AA and DHA in the salmon group was best explained by a quadratic function (**Fig. 2**).

There was no significant difference in breast milk total lipid concentration (g/L) between the 2 groups (data not shown). However, the total lipid concentration increased ($P < 0.001$) from 13.2 $\pm$ 2.6 g/L in colostrum to 22.6 $\pm$ 1.9 g/L in mature milk (d 28 PP). Although the absolute concentrations (mg/L) of most fatty acids increased from d 1 to 28 PP, the breast milk of the women who consumed salmon had significantly higher concentrations of EPA (80%), DPA (60%), DHA (100%), and total LC (n-3) PUFA (80%) on d 5 PP compared with controls (overall effects of group $P < 0.03$ for all) (data not shown). Maternal age, BMI, and atopy (self-reported or confirmed by positive skin prick test) did not have a significant effect on either the percentage or absolute concentration of any of the breast milk fatty acids analyzed.

In each of the multiple regression models used, the proportion of breast milk AA was a significant predictor of breast milk DHA (**Table 3**). In the salmon group, in order of greatest effect, plasma phosphatidylcholine DHA in late pregnancy, total dietary (n-3)

TABLE 1 Personal and pregnancy characteristics of the women who provided breast milk samples[1]

	Control group (n = 32)	Salmon group (n = 32)
Age, *y*	30.1 $\pm$ 0.6	30.3 $\pm$ 0.7
Height, *cm*	165.9 $\pm$ 1.2	164.7 $\pm$ 1.0
Weight at 20 wk gestation, *kg*	70.0 (63.6–85.2)	65.9 (61.6–73.5)
BMI at 20 wk gestation, *kg/m^2*	26.1 (23.4–29.8)	23.9 (23.0–27.3)
First pregnancy, *n (%)*	10 (32.3)	19 (59.4)*
Self-reported atopy, *n (%)*	18 (56.3)	19 (59.4)
Positive skin prick test to one or more allergens,[2] *n (%)*	15 (53.6)	20 (64.5)
Duration of pregnancy, *d*	282 $\pm$ 1.5	282 $\pm$ 1.4
Delivery by caesarean section, *n (%)*	6 (18.8)	5 (15.6)

[1] Values are mean $\pm$ SEM, median (25th–75th percentile), or *n* (%). *Different from control, $P = 0.045$.
[2] Not all women agreed to be skin prick tested; in the control group, $n = 28$ were tested and in the salmon group, $n = 31$ were tested.

TABLE 2 The effect of increased salmon consumption by pregnant women on breast milk fatty acid composition at d 1, 5, 14, and 28 PP[1]

	Group	*n*	d 1	*n*	d 5	*n*	d 14	*n*	d 28	Group *P*	Time *P*
			% FA		*% FA*		*% FA*		*% FA*		
Total SFA[2]	Control	9	38.9 ± 0.7	23	38.8 ± 0.9	27	39.4 ± 0.8	22	39.4 ± 1.1	NS	NS
	Salmon	7	41.3 ± 1.4	23	40.7 ± 0.8	25	40.8 ± 0.8	20	39.8 ± 1.0		
18:1(n-9)	Control	9	37.9 ± 0.6	23	38.9 ± 0.6	27	38.2 ± 0.5	22	37.7 ± 0.6	0.027	NS
	Salmon	7	37.7 ± 0.8	23	36.4 ± 0.6*	25	37.0 ± 0.5	20	37.4 ± 0.6		
Total MUFA	Control	9	44.9 ± 0.6	23	45.7 ± 0.7	27	45.0 ± 0.6	22	44.3 ± 0.8	0.032	NS
	Salmon	7	44.2 ± 1.0	23	43.5 ± 0.6	25	43.6 ± 0.6	20	43.4 ± 0.6		
18:2(n-6) (LA)	Control	9	11.6 ± 0.9	23	11.6 ± 0.5	27	11.8 ± 0.5	22	12.6 ± 0.7	NS	NS
	Salmon	7	9.8 ± 0.7	23	11.2 ± 0.6	25	11.5 ± 0.3	20	12.9 ± 0.7		
20:4(n-6) (AA)	Control	9	0.98 ± 0.09^{a}	23	0.62 ± 0.04^{b}	27	0.56 ± 0.03^{bc}	22	0.53 ± 0.04^{c}	NS	<0.001
	Salmon	7	0.88 ± 0.05^{a}	23	0.60 ± 0.02^{b}	25	0.52 ± 0.03^{bc}	20	0.49 ± 0.02^{c}		
Total (n-6) PUFA	Control	9	14.0 ± 0.9	23	13.3 ± 0.5	27	13.4 ± 0.5	22	14.0 ± 0.7	NS	NS
	Salmon	7	12.0 ± 0.7	23	13.2 ± 0.6	25	13.0 ± 0.4	20	14.4 ± 0.7		
Total LC (n-6) PUFA	Control	9	2.37 ± 0.20^{a}	23	1.63 ± 0.06^{b}	27	1.42 ± 0.06^{bc}	22	1.31 ± 0.07^{c}	NS	<0.001
	Salmon	7	2.07 ± 0.10^{a}	23	1.74 ± 0.06^{b}	25	1.36 ± 0.05^{c}	20	1.26 ± 0.06^{c}		
18:3(n-3) (ALA)	Control	9	1.03 ± 0.12	23	1.14 ± 0.07	27	1.31 ± 0.09	22	1.40 ± 0.12	NS	0.007
	Salmon	7	0.92 ± 0.17	23	1.18 ± 0.07	25	1.39 ± 0.12	20	1.37 ± 0.12		
20:5(n-3) (EPA)	Control	9	0.06 (0.00–0.08)	23	0.06 (0.03–0.07)	27	0.08 (0.05–0.08)	22	0.07 (0.06–0.10)	0.004	NS
	Salmon	7	0.09 (0.00–0.11)	23	0.11 (0.06–0.16)*	25	0.10 (0.08–0.21)	20	0.09 (0.07–0.13)		
22:5(n-3) (DPA)	Control	9	0.33 ± 0.06	23	0.23 ± 0.04	27	0.20 ± 0.01	22	0.23 ± 0.01	0.008	<0.001
	Salmon	7	0.45 ± 0.05^{a}	23	0.30 ± 0.02^{ab}*	25	0.22 ± 0.02^{c}	20	0.27 ± 0.04^{bc}		
22:6(n-3) (DHA)	Control	9	0.49 ± 0.04^{a}	23	0.35 ± 0.02^{ab}	27	0.31 ± 0.02^{b}	22	0.32 ± 0.05^{b}	<0.001	<0.001
	Salmon	7	0.66 ± 0.07^{a}	23	0.65 ± 0.04^{a}*	25	0.50 ± 0.05^{b}*	20	0.43 ± 0.05^{b}*		
Total (n-3) PUFA	Control	9	2.01 ± 0.15	23	1.90 ± 0.12	27	2.00 ± 0.10	22	2.13 ± 0.19	0.024	NS
	Salmon	7	2.20 ± 0.20	23	2.40 ± 0.12*	25	2.35 ± 0.18	20	2.25 ± 0.15		
Total LC (n-3) PUFA	Control	9	0.98 ± 0.11	23	0.76 ± 0.07	27	0.68 ± 0.04	22	0.72 ± 0.10	<0.001	<0.001
	Salmon	7	1.28 ± 0.14^{a}	23	1.22 ± 0.07^{a}*	25	0.96 ± 0.09^{b}*	20	0.89 ± 0.09^{b}		
(n-6):(n-3) PUFA	Control	9	7.17 ± 0.54	23	7.49 ± 0.43	27	6.93 ± 0.27	22	7.22 ± 0.45	0.003	NS
	Salmon	7	5.64 ± 0.39	23	5.72 ± 0.34*	25	6.18 ± 0.38	20	6.63 ± 0.39		
LC (n-6):LC (n-3) PUFA	Control	9	2.52 ± 0.19	23	2.35 ± 0.14	27	2.17 ± 0.10	22	2.08 ± 0.13	<0.001	NS
	Salmon	7	1.69 ± 0.16*	23	1.51 ± 0.10*	25	1.60 ± 0.10*	20	1.51 ± 0.07*		

[1] Values are mean ± SEM or median (25th–75th percentile). Values in a row with superscripts without a common letter differ, $P < 0.05$. *Different from control on that day, $P < 0.05$. There were no group × time interactions. AA, arachidonic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid; LA, linoleic acid; LC PUFA, long-chain PUFA; % FA, percentage of total fatty acids; NS, not significant, $P > 0.05$; PP, postpartum.
[2] When the model was adjusted for parity, there was an effect of group for total SFA, $P = 0.011$.

PUFA, and dietary DHA during pregnancy (but not dietary ANA) were individually significant predictors of breast milk DHA in combination with the proportion of breast milk AA (Table 3). This was not the case in the control group where only plasma phosphatidylcholine DHA during late pregnancy was a predictor of breast milk DHA ($P = 0.023$) and dietary DHA ($P = 0.054$) and ALA ($P = 0.09$) tended to be predictors (Table 3).

Breast milk immune factors. There was no significant effect of group, parity, maternal age, BMI, or maternal atopy on breast milk total protein concentration, but there was a reduction in protein concentration with time ($P < 0.001$; data not shown). There was a 100% detection rate for each of the 4 immune factors measured and a significant effect of time on the concentration of all 4 factors (**Table 4**). They all declined significantly (Table 4), even when concentrations were standardized against total protein concentration (**Supplemental Table 2**) and there was a significant effect of group on the concentration of sIgA (Table 4). The sIgA concentration was lower in the salmon group, both per unit volume (36, 80, 75, and 80% of the concentration in the control group at d 1, 5, 14, and 28, respectively) (Table 4) and when standardized against total protein (Supplemental Table 2), even after adjusting for maternal parity, age, and BMI. Maternal BMI had an inverse association with sCD14 ($P = 0.02$; data not shown).

There were positive partial correlations (having controlled for intervention and repeated measures) among all the immune factors: TGFβ1 with TGFβ2 ($r = 0.68$), sIgA ($r = 0.49$), and sCD14 ($r = 0.40$); TGFβ2 with sIgA ($r = 0.50$) and sCD14 ($r = 0.38$); and sIgA with sCD14 ($r = 0.50$) ($P < 0.001$ for all). Milk EPA was negatively associated with all immune factors and there was a positive association of AA with sIgA and TGFβ1 (**Table 5**).

Breast milk TGFβ1 and TGFβ2 expressed as either pg/mg protein (**Fig. 3**) or ng/L were higher in (self-reported) atopic mothers compared with nonatopic mothers (not shown; $P = 0.04$ and $P = 0.02$, respectively). This was not the case for either sCD14 or sIgA.

Discussion

Increased salmon consumption from wk 20 of pregnancy significantly enhanced the LC (n-3) PUFA status of breast milk during early lactation. On d 5 PP, the milk of the women in the salmon group had higher proportions of EPA (80%), DPA (30%), and DHA (90%) compared with those of the control group. The proportion of milk DHA significantly decreased with

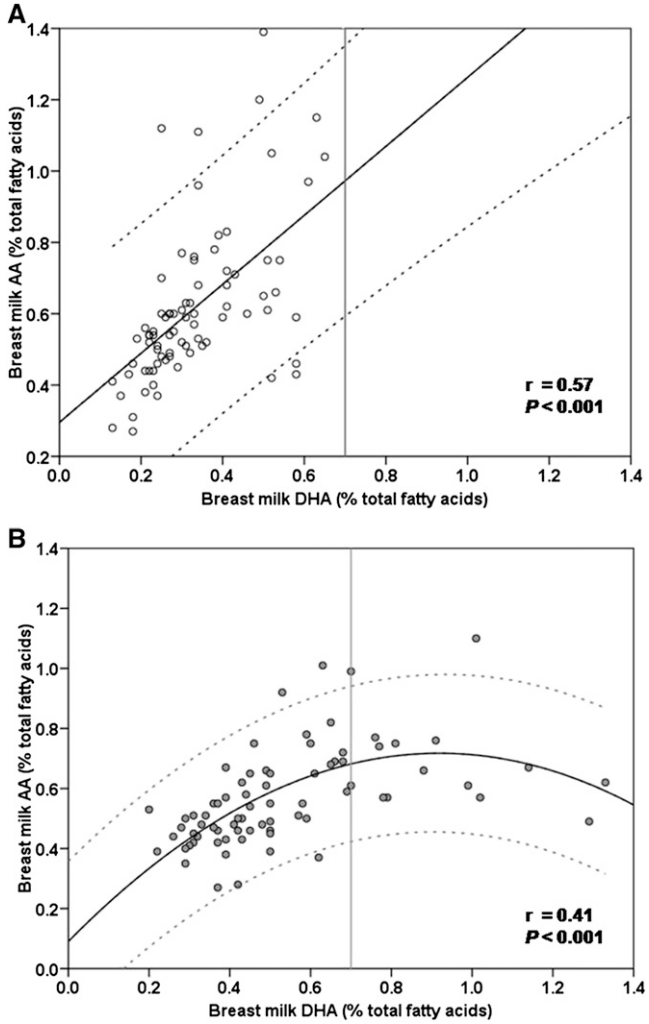

FIGURE 2 Relationship between breast milk AA and DHA for the control group (*A*) and the salmon group (*B*). Solid black line is linear regression (*A*) and quadratic function (*B*). Dotted lines are 95% CI and the vertical solid gray line indicates the arbitrary cutoff point for DHA (0.7% FA). AA, arachidonic acid.

time, as previously reported by others (6,33,34). However, breast milk DHA was still 30% higher in the salmon group compared with the control group (0.43 vs. 0.32% FA, respectively) 4 wk after the consumption of salmon had ceased. Previous studies with fish oil supplements in pregnancy have often used high doses of EPA and DHA, e.g., with daily EPA plus DHA intakes 4.5–6.7 times those in the SiPS (6,9). In one of these studies (6), d 3 PP milk from the fish oil-supplemented group had higher proportions of EPA (170%), DPA (100%), and DHA (130%) compared with the control group and milk DHA remained 70% higher 6 wk after supplementation had ceased (0.42 vs. 0.25% FA, respectively) but was not significantly different at 6 mo PP (6). In contrast, in another study (9), high-dose fish oil during pregnancy did not result in a significant increase in milk DHA at d 4, 16, or 30 PP; however, the supplementation period was confined to the last 10 wk of gestation and the ratio of EPA:DHA in the supplemented oil was 1.4:1 compared with 1:2 in the SiPS and the Dunstan et al. (6) study. The magnitude of the enrichment of breast milk with LC (n-3) PUFA during early lactation in the current study is higher than might be predicted based on the comparatively lower LC (n-3) PUFA intakes relative to studies employing fish oil supplements. The reason for this is unclear. It has been suggested that the bioavailability of LC (n-3) PUFA is greater from fish than that from fish oil (35,36), although not all studies agree on this. For example, in a study of nonpregnant women, consumption of equal amounts of EPA and DHA from either oily fish or fish oil capsules was equally effective at enriching blood lipids with (n-3) PUFA (37). Whatever the reason, the current study suggests that consuming oily fish during pregnancy may be more effective than using fish oil supplements at enhancing breast milk proportions of DHA. A controlled trial comparing oily fish and fish oil in a cohort of pregnant women would be necessary to confirm this. The higher dose of LC (n-3) PUFA provided by fish oil supplements may extend the duration of enrichment of breast milk with these fatty acids, because Dunstan et al. (6) reported elevated proportions of DHA at 6 wk PP. However, samples were not taken beyond 4 wk PP in the current study, so the washout period cannot be determined. Interestingly, the DHA enrichment achieved in the SiPS (0.43% FA at 4 wk PP) was comparable with that reported for relatively high habitual fish consumers (4.6 ± 1.5 fish meals/wk) whose putative baseline percentage milk DHA (i.e., that not affected by dietary intake of oily fish within the last 24 h) at 4 mo PP was 0.41% FA (38). High-dose fish oil during pregnancy resulted in a breast milk DHA percentage of 0.42% FA at 6 wk PP (6). Although the values reported in these 3 diverse studies are very similar, it is important to keep in mind that there were differences between the studies in terms of breast milk processing and the methodology of the FA analysis.

Whether the increased DHA status at birth of both mother and neonate in the salmon group, as previously reported (27), combined with the corresponding increase in milk LC (n-3) PUFA reported here are sufficient to meet the ongoing needs of breast-fed neonates and prevent depletion in mothers during lactation is yet unknown, although previous reports suggest that both may still be at risk (39,40). In this regard, it may be appropriate to reconsider the guidelines recommending only 2 portions of oily fish per week during pregnancy (24,25) and to focus on promoting higher consumption of fish species known to be low in contaminants. It is important to note that the salmon used in the SiPS was tailor made to be very low in contaminants while maintaining a high concentration of LC (n-3) PUFA (27).

Although consumption of salmon increased the percentage of LC (n-3) PUFA in milk, there was no significant decrease in breast milk LC (n-6) PUFA, including AA. This is important, because AA also plays a role in early brain development (1). However, the ratio of LC (n-6) PUFA:LC (n-3) PUFA was significantly lower in the salmon group. This may be of relevance to allergy prevention (41). There was a significant positive correlation between milk DHA and AA, as observed by others (42,43). However, there appears to be a positive relationship between AA and DHA at lower proportions of DHA (arbitrary cutoff of ≤0.7% FA) but an inverse relationship at higher proportions of DHA (arbitrary cutoff >0.7% FA). This is in partial agreement with the findings of Kuipers et al. (5) and a similar relationship between DHA and AA has been reported with varying DHA concentrations in erythrocytes, umbilical arteries, and veins (44). Within the SiPS, only a small number of early lactation samples in the salmon group had DHA >0.7% FA, which may explain why a significant decrease in milk AA was not observed in SiPS, unlike other studies, where milk DHA was >0.7% FA and a significant decrease in milk AA was reported (6,45). Multiple regression analysis aimed at identifying predictors of milk DHA in the SiPS resulted in 22–41% of the variance in milk DHA being positively associated with milk AA. Multiple regression analyses also showed that for the total population, dietary intakes of ALA did not explain the variance

TABLE 3 Predictors of breast milk DHA after increased salmon consumption by pregnant women compared with control and the total population[1]

		Total population		Control group		Salmon group	
	Independent predictors	β[2]	*P*	β	*P*	β	*P*
Model 1	Dietary total (n-3) PUFA in pregnancy[3]	0.48	<0.001	0.15	0.09	0.32	<0.001
	Breast milk 20:4(n-6)	0.53	<0.001	0.67	<0.001	0.58	<0.001
	Adjusted R^2	0.42	<0.001	0.42	<0.001	0.40	<0.001
Model 2	Dietary 18:3(n-3) in pregnancy	0.24	0.001	0.16	0.09	0.07	0.46
	Breast milk 20:4(n-6)	0.44	<0.001	0.63	<0.001	0.55	<0.001
	Adjusted R^2	0.25	<0.001	0.42	<0.001	0.31	<0.001
Model 3	Dietary 22:6(n-3) in pregnancy	0.60	<0.001	0.17	0.054	0.21	0.015
	Breast milk 20:4(n-6)	0.52	<0.001	0.65	<0.001	0.58	<0.001
	Adjusted R^2	0.55	<0.001	0.42	<0.001	0.35	<0.001
Model 4	Plasma PC 22:6(n-3) in late pregnancy[4]	0.60	<0.001	0.22	0.023	0.32	0.001
	Breast milk 20:4(n-6)	0.42	<0.001	0.63	<0.001	0.52	<0.001
	Adjusted R^2	0.55	<0.001	0.48	<0.001	0.40	<0.001

[1] PC, phosphatidylcholine.
[2] β standardized regression coefficient.
[3] Evaluated by FFQ at 34 wk pregnancy covering the previous 3-mo intake (27).
[4] Maternal plasma PC DHA at 38 wk pregnancy (27).

in milk DHA as strongly as did dietary DHA, which is probably reflective of the limited endogenous conversion of ALA in humans (46). However, within the control group only, where intakes of preformed DHA were low (27), dietary ALA showed a trend toward being a predictor of milk DHA ($P = 0.09$).

In the current study, immune factors measured in whole breast milk decreased with time, which is characteristic of the first month of lactation, when human milk is most changeable (47). There was a negative association of all immune factors with milk EPA and a positive association of milk AA with sIgA and TGFβ1. Milk sIgA was significantly lower in the salmon group, which is in contrast to a previous intervention study with high-dose fish oil, where there was no significant difference in milk sIgA between the control and intervention group and there was a positive correlation among milk DHA, DPA, and milk sIgA (22). However, there are significant differences between the 2 studies; the SiPS was a dietary intervention with salmon, not only a source of LC (n-3) PUFA, but also of vitamins A, D, E, and K, and minerals (selenium, zinc), whereas in the Dunstan et al. (22) study, high-dose fish oil supplements were employed, providing LC (n-3) PUFA at a dose >6-fold that used in the SiPS. Some studies have reported that the AA-derived mediator PGE_2 promotes IgA synthesis (48,49); because LC (n-3) PUFA may reduce PGE_2 production (31), this could explain the lower breast milk sIgA in the salmon group in the current study. Published data on the effects of LC PUFA on milk sIgA are limited and therefore further investigation is warranted, particularly in early lactation. This is particularly important, because breast-fed neonates do not produce their own protective levels of sIgA until 30 d PP, but benefit from the supply and passive protection afforded by the sIgA within milk (21). It is not clear whether the lower milk sIgA observed in the salmon group in the current study is of biological significance with respect to defense against pathogens or development of atopy in breast-fed infants.

In the SiPS, infants had a family history of atopy (first-degree relative), but there was no relationship between maternal atopy and breast milk fatty acid composition. The literature is inconsistent on this issue, with reports of lower LC (n-6) PUFA or both lower or higher concentrations of LC (n-3) PUFA being associated with the milk of atopic women compared with nonatopic women (50). In the current study, atopic mothers (self-reported) had higher concentrations of TGFβ1 and TGFβ2 in their milk. This is contradictory to the findings of a review (12), which described lower concentrations of TGFβ1 and TGFβ2 in the milk of atopic compared with nonatopic mothers in some studies and no significant difference in milk TGFβ concentration between atopic and nonatopic mothers in the

TABLE 4 The effect of increased salmon consumption by pregnant women on breast milk immune factors at d 1, 5, 14, and 28 PP[1]Immune factor

	Group	*n*	d 1	*n*	d 5	*n*	d 14	*n*	d 28	Group *P*	Time *P*
sCD14, *mg/L*	Control	14	25.7^{a} (15.7–31.0)	27	12.1^{b} (9.7–14.1)	28	9.2^{bc} (7.5–11.5)	23	8.2^{c} (7.2–9.7)	NS	<0.001
	Salmon	12	17.7^{a} (14.6–28.6)	28	11.4^{b} (10.0–14.4)	27	9.5^{c} (7.5–12.6)	26	8.3^{c} (6.0–10.3)		
sIgA, *g/L*	Control	9	3.13^{a} (1.76–7.04)	26	0.69^{b} (0.51–1.07)	28	0.52^{bc} (0.33–0.63)	23	0.38^{c} (0.31–0.53)	0.006	<0.001
	Salmon	9	1.13^{a} (0.77–3.24)	28	0.55^{b} (0.41–0.68)	27	0.39^{c} (0.27–0.51)	25	0.31^{c} (0.22–0.43)		
TGFβ1, μ*g/L*	Control	10	1.14^{a} (0.60–2.78)	26	0.57^{b} (0.32–0.85)	28	0.30^{b} (0.23–0.47)	23	0.36^{b} (0.24–0.46)	NS	<0.001
	Salmon	10	0.77^{a} (0.29–1.74)	26	0.63^{a} (0.45–0.81)	27	0.36^{b} (0.27–0.46)	23	0.26^{b} (0.20–0.35)		
TGFβ2, μ*g/L*	Control	9	6.61^{a} (3.86–12.52)	25	4.12^{a} (1.98–11.17)	28	2.20^{b} (1.41–5.06)	23	2.81^{b} (1.36–3.53)	NS	<0.001
	Salmon	9	3.36^{ab} (2.92–10.11)	26	5.07^{a} (2.76–8.34)	27	3.03^{bc} (2.33–3.97)	22	2.07^{c} (1.02–3.44)		

[1] Values are median (25th–75th percentile).Values in a row with superscripts without a common letter differ, $P < 0.05$. There were no group × time interactions. NS, not significant, $P > 0.05$; sCD14, soluble CD14; sIgA, secretory IgA; TGFβ, transforming growth factor-β.

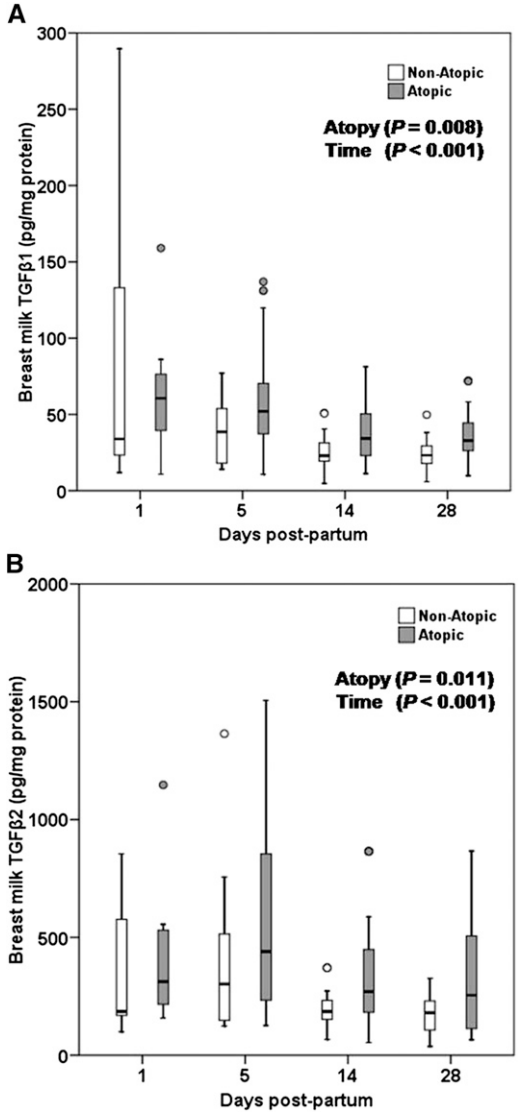

FIGURE 3 Breast milk TGFβ1 (*A*) and TGFβ2 (*B*) concentrations at d 1–28 PP in self-reported atopic and nonatopic mothers in the control and salmon groups. The boxes represent the 25th–75th percentiles, the solid line within the box represents the median, and the whiskers projecting out from the boxes represent the 5th–95th percentiles. d 1 [nonatopic (NA), n = 9; atopic (A), n = 9], d 5 (NA, n = 20; A, n = 31), d 14 (NA, n = 24; A, n = 31), and d 28 (NA, n = 21; A, n = 24). Maternal atopy affected TGFβ1 (P = 0.008) and TGFβ2 (P = 0.011); time affected both ($P < 0.001$). There were no group × time interactions. PP, postpartum; TGFβ, transforming growth factor-β.

largest study. However, in the current study, milk samples were collected during early lactation (1, 5, 14, and 28 d PP), whereas in the studies included in the review, milk was sampled on d 1–4 PP and or 1, 3, 6, and 12 mo PP.

In summary, consumption of salmon during pregnancy, at the current recommended intakes, results in significantly higher proportions of individual and total LC (n-3) PUFA in breast milk during early lactation and in a lower ratio of LC (n-6):LC (n-3) PUFA. The effect of salmon was maintained for at least 1 mo after cessation of salmon consumption. sIgA was significantly lower in the breast milk of women who had consumed salmon during pregnancy, but this effect was apparent only during very early lactation and the biological implications are not clear. The other immune factors measured were not significantly affected by salmon consumption. In conclusion, intake of 2 portions of salmon/wk during pregnancy enhances the percentage LC (n-3) PUFA in breast milk, so improving the supply of these important fatty acids to neonates.

TABLE 5 Relationships between breast milk fatty acids and immune factors for the total population[1]

	Fatty acid				
Immune factor	LA	ALA	AA	EPA	DHA
sCD14	0.01	−0.05	0.08	−0.30†	−0.12
sIgA	−0.21*	−0.18*	0.40†	−0.22**	0.05
TGFβ1	−0.14	−0.08	0.25**	−0.19*	0.04
TGFβ2	−0.06	−0.00	0.18	−0.21*	0.05

[1] Values are partial correlation coefficients. Values were controlled for intervention group and time PP. *$P < 0.05$, **$P < 0.01$, †$P < 0.001$. AA, arachidonic acid; ALA, α-linolenic acid; LA, linoleic acid; PP, postpartum; sCD14, soluble CD14; sIgA, secretory IgA; TGFβ, transforming growth factor-β.

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Literature Cited

1. 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.
2. Innis SM, Friesen RW. Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. Am J Clin Nutr. 2008;87:548–57.
3. Prescott SL, Dunstan JA. Prenatal fatty acid status and immune development: the pathways and the evidence. Lipids. 2007;42:801–10.
4. Jensen CL, Lapillonne A. Docosahexaenoic acid and lactation. Prostaglandins Leukot Essent Fatty Acids. 2009;81:175–8.
5. Kuipers RS, Fokkema MR, Smit EN, van der Meulen J, Boersma ER, Muskiet FA. High contents of both docosahexaenoic and arachidonic acids in milk of women consuming fish from lake Kitangiri (Tanzania): targets for infant formulae close to our ancient diet? Prostaglandins Leukot Essent Fatty Acids. 2005;72:279–88.
6. 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.
7. Imhoff-Kunsch B, Stein AD, Villalpando S, Martorell R, Ramakrishnan U. Docosahexaenoic acid supplementation from mid-pregnancy to parturition influenced breast milk fatty acid concentrations at 1 month postpartum in Mexican women. J Nutr. 2011;141:321–6.
8. Helland IB, Saarem K, Saugstad OD, Drevon CA. Fatty acid composition in maternal milk and plasma during supplementation with cod liver oil. Eur J Clin Nutr. 1998;52:839–45.
9. 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.
10. Chirico G, Marzollo R, Cortinovis S, Fonte C, Gasparoni A. Antiinfective properties of human milk. J Nutr. 2008;138:S1801–6.
11. Walker A. Breast milk as the gold standard for protective nutrients. J Pediatr. 2010;156:S3–7.

12. Oddy WH, Rosales F. A systematic review of the importance of milk TGF-beta on immunological outcomes in the infant and young child. Pediatr Allergy Immunol. 2010;21:47–59.
13. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. J Pediatr. 2010;156:S8–15.
14. Labéta MO, Vidal K, Nores JE, Arias M, Vita N, Morgan BP, Guillemot JC, Loyaux D, Ferrara P, Schmid D, et al. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. J Exp Med. 2000;191:1807–12.
15. Kalliomäki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. J Allergy Clin Immunol. 1999;104:1251–7.
16. Ando T, Hatsushika K, Wako M, Ohba T, Koyama K, Ohnuma Y, Katoh R, Ogawa H, Okumura K, Luo J, et al. Orally administered TGF-beta is biologically active in the intestinal mucosa and enhances oral tolerance. J Allergy Clin Immunol. 2007;120:916–23.
17. Clark DA, Coker R. Transforming growth factor-beta (TGF-beta). Int J Biochem Cell Biol. 1998;30:293–8.
18. Sonoda E, Matsumoto R, Hitoshi Y, Ishii T, Sugimoto M, Araki S, Tominaga A, Yamaguchi N, Takatsu K. Transforming growth factor beta induces IgA production and acts additively with interleukin 5 for IgA production. J Exp Med. 1989;170:1415–20.
19. Cohen L, Haziot A, Shen DR, Lin XY, Sia C, Harper R, Silver J, Goyert SM. CD14-independent responses to LPS require a serum factor that is absent from neonates. J Immunol. 1995;155:5337–42.
20. Brandtzaeg P, Nilssen DE, Rognum TO, Thrane PS. Ontogeny of the mucosal immune system and IgA deficiency. Gastroenterol Clin North Am. 1991;20:397–439.
21. Xanthou M, Bines J, Walker WA. Human milk and intestinal host defense in newborns: an update. Adv Pediatr. 1995;42:171–208.
22. Dunstan JA, Roper J, Mitoulas L, Hartmann PE, Simmer K, Prescott SL. The effect of supplementation with fish oil during pregnancy on breast milk immunoglobulin A, soluble CD14, cytokine levels and fatty acid composition. Clin Exp Allergy. 2004;34:1237–42.
23. 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.
24. UK (Scientific Advisory Committee on Nutrition). Advice on fish consumption: benefits and risks. Joint report of the Scientific Advisory Committee on Nutrition and Committee on Toxicity, UK Food Standards Agency. The Stationery Office, London; 2004.
25. Institute of Medicine. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. Washington, DC: National Academies Press; 2002.
26. Department of Health and Foods Standards Agency. National Diet and Nutrition Survey: adults aged 19–64 years types and quantities of food consumed volume 1. London: The Stationery Office; 2002.
27. Miles EA, Noakes PS, Kremmyda LS, Vlachava M, Diaper ND, Rosenlund G, Urwin H, Yaqoob P, Rossary A, Farges MC, et al. The Salmon in Pregnancy Study: study design, subject characteristics, maternal fish and marine n-3 fatty acid intake, and marine n-3 fatty acid status in maternal and umbilical cord blood. Am J Clin Nutr. 2011;94:S1986–92.
28. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226:497–509.
29. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
30. Bergqvist Y, Karisson L, Fohlin L. Total protein determined in human breast milk by use of coomassie brilliant blue and centrifugal analysis. Clin Chem. 1989;35:2127–9.
31. Noakes PS, Vlachava M, Kremmyda LS, Diaper ND, Miles EA, Erlewyn-Lajeunesse M, Williams AP, Godfrey KM, Calder PC. Increased intake of oily fish in pregnancy: effects on neonatal immune responses and on clinical outcomes in infants at 6 mo. Am J Clin Nutr. 2012;95:395–404.
32. Kremmyda L-S. Salmon in Pregnancy Study (SIPS): the effects of increased oily fish consumption on maternal nutrient intake, fatty acid status and immunity. Southampton: University of Southampton; 2010.
33. 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.
34. Moltó-Puigmarti C, Castellote AI, Carbonell-Estrany X, Lopez-Sabater MC. Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. Clin Nutr. 2011;30:116–23.
35. Visioli F, Rise P, Barassi MC, Marangoni F, Galli C. Dietary intake of fish vs. formulations leads to higher plasma concentrations of n-3 fatty acids. Lipids. 2003;38:415–8.
36. Elvevoll EO, Barstad H, Breimo ES, Brox J, Eilertsen KE, Lund T, Olsen JO, Osterud B. Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. Lipids. 2006;41:1109–14.
37. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. Am J Clin Nutr. 2007;86:1621–5.
38. Lauritzen L, Jorgensen MH, Hansen HS, Michaelsen KF. Fluctuations in human milk long-chain PUFA levels in relation to dietary fish intake. Lipids. 2002;37:237–44.
39. Gibson RA, Neumann MA, Makrides M. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr. 1997;51:578–84.
40. Kuipers RS, Luxwolda MF, Janneke Dijck-Brouwer DA, Muskiet FA. Intrauterine, postpartum and adult relationships between arachidonic acid (AA) and docosahexaenoic acid (DHA). Prostaglandins Leukot Essent Fatty Acids. 2011;85:245–52.
41. Kremmyda LS, Vlachava M, Noakes PS, Diaper ND, Miles EA, Calder PC. Atopy risk in infants and children in relation to early exposure to fish, oily fish, or long-chain omega-3 Fatty acids: a systematic review. Clin Rev Allergy Immunol. 2011;41:36–66.
42. Minda H, Kovacs A, Funke S, Szasz M, Burus I, Molnar S, Marosvolgyi T, Decsi T. Changes of fatty acid composition of human milk during the first month of lactation: a day-to-day approach in the first week. Ann Nutr Metab. 2004;48:202–9.
43. 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.
44. Luxwolda MF, Kuipers RS, Smit EN, Velzing-Aarts FV, Dijck-Brouwer DA, Muskiet FA. The relation between the omega-3 index and arachidonic acid is bell shaped: synergistic at low EPA+DHA status and antagonistic at high EPA+DHA status. Prostaglandins Leukot Essent Fatty Acids. 2011;85:171–8.
45. Helland IB, Saugstad OD, Smith L, Saarem K, Solvoll K, Ganes T, Drevon CA. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. Pediatrics. 2001;108:E82.
46. Burdge GC. Metabolism of alpha-linolenic acid in humans. Prostaglandins Leukot Essent Fatty Acids. 2006;75:161–8.
47. Castellote C, Casillas R, Ramirez-Santana C, Perez-Cano FJ, Castell M, Moretones MG, Lopez-Sabater MC, Franch A. Premature delivery influences the immunological composition of colostrum and transitional and mature human milk. J Nutr. 2011;141:1181–7.
48. Garrone P, Galibert L, Rousset F, Fu SM, Banchereau J. Regulatory effects of prostaglandin E2 on the growth and differentiation of human B lymphocytes activated through their CD40 antigen. J Immunol. 1994;152:4282–90.
49. Pestka JJ. Deoxynivalenol-induced IgA production and IgA nephropathy-aberrant mucosal immune response with systemic repercussions. Toxicol Lett. 2003;140–141:287–95.
50. Sala-Vila A, Miles EA, Calder PC. Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined. Clin Exp Allergy. 2008;38:1432–50.