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ORIGINAL CONTRIBUTION

Probiotics and dietary counselling targeting maternal dietary fat intake modifies breast milk fatty acids and cytokines

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Abstract
Purpose Breast milk fatty acids possess immunomodulatory properties, and new intervention strategies beyond supplementation of maternal diet with single oils are called for. The objective of the present study was to evaluate the effect of dietary intervention during pregnancy and breastfeeding on breast milk fatty acid and cytokine composition.
Methods Pregnant women were randomised into three study groups: dietary intervention with probiotics (diet/probiotic) or with placebo (diet/placebo) and a control group (control/placebo). Dietary intervention included dietary counselling and provision of rapeseed oil-based food products. The probiotics used were *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 in combination. Dietary intake was evaluated by food records at every trimester of pregnancy and 1 month postpartum. Breast milk samples were collected after birth (colostrum) and 1 month after delivery for fatty acid and cytokine analysis ($n = 125$).
Results Dietary intervention improved the quality of fat in the diet. In breast milk, the proportion of α-linolenic acid and total n-3 fatty acids was higher in both dietary intervention groups compared with control group ($p < 0.05$). In the diet/probiotic group, the γ-linolenic acid content was higher compared with the diet/placebo group ($p < 0.05$). The concentrations of TNF-α, IL-10, IL-4 and IL-2 were higher in both dietary intervention groups compared with controls, and furthermore, long-chain n-3 fatty acids were associated with several cytokines in colostrum samples.
Conclusion The present intervention demonstrated the possibility of modifying breast milk immunomodulatory factors by dietary means.

Keywords Breast milk · Fatty acid · Cytokine · Dietary counselling

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Introduction

Maternal diet has a strong influence on breast milk composition and hence for the health of the infant, for example on visual and cognitive development [1, 2]. Polyunsaturated fatty acids (PUFA) in breast milk derived from the diet, liberation from maternal body stores or endogenous synthesis from precursor fatty acids [3] and both long-term and recent maternal dietary PUFA intake are thus crucial for their effector functions, including immunomodulatory properties [4]. Long-chain n-6 and n-3 fatty acids have various effects on anti- and pro-inflammatory eicosanoid mediators, and furthermore, novel findings suggest that n-3 fatty acids may affect cytokine production through transcription factors regulating inflammatory gene expression [5]. Specific PUFA may also support intestinal epithelial barrier integrity [6], and thus breast milk PUFA may promote gastrointestinal maturation.

In addition to fatty acid-derived mediators, breast milk contains various other bioactive compounds such as oligosaccharides, nucleotides and growth factors [7]. Amongst these, molecules mediating the host–microbe interaction are important for the immunologic programming of the infant [8]. Moreover, beneficial microbial products, probiotics, may regulate immunomodulatory factors in breast milk [9]. There are also possible interactions between probiotics and fatty acids, the latter for example possibly affecting surface properties and the adhesion of probiotics [10]. Fatty acids and probiotic bacteria may also have similar signalling pathways, including soluble CD14 [11].

Breast milk is the sole source of nutrition for the infant during the first months of life. Considering various effects of fatty acids on infant health, determinants of PUFA supply via breast milk are important to address. According to previous studies, mothers in allergic families may be a special risk group since they have been observed to have a high dietary intake of saturated fatty acids [12]. Differences in breast milk fatty acid composition between atopic and nonatopic mothers may be explained by the diet [13]. Furthermore, some studies have reported lower amounts of both n-6 and n-3 fatty acids in the serum or breast milk of allergic subjects, but whether these differences are related to diet or altered metabolism of fatty acids amongst allergic subjects remains obscure [4].

Interventions seeking to optimise the balance of immunomodulatory fatty acids have thus far targeted maternal fatty acid intake during pregnancy and lactation by supplementing single oils, mainly fish oil [14, 15]. Recently also new types of oils such as blackcurrant seed oil has been supplemented to pregnant and breastfeeding women [16]. However, these studies do not show consistent results and have ignored the total fatty acid composition of the diet as well as the recently recognised complex interactions of fatty acids with other dietary constituents such as probiotics. The main objective in the present study was to evaluate the effects of dietary intervention (including intensive and repeated dietary counselling and provision of rapeseed oil-based food products) and probiotics on breast milk fatty acid and cytokine composition. Further, the associations between fatty acids and cytokines in breast milk were investigated.

Methods

Subjects and study design

The study protocol for this nutrition intervention study has been described in detail previously [17]. In brief, women from families with a history of allergic disease were recruited at visits to maternal welfare clinics early in pregnancy. The study was approved by the Ethical Committee of the Hospital District of South-West Finland. Written informed consent was obtained from participants. All participants also attended the communal maternal welfare clinics for standard follow-up and health counselling during pregnancy and breastfeeding.

At the first study visit in the first trimester of pregnancy, the participants were randomly assigned to three study groups: dietary intervention groups with probiotics (diet/probiotics) or with placebo (diet/placebo) and a control group (control/placebo). The probiotic capsules used contained *Lactobacillus rhamnosus* GG (ATCC 53103, Valio Ltd, Helsinki, Finland) and *Bifidobacterium lactis* Bb12 (Chr. Hansen, Hoersholm, Denmark) 10^{10} cfu/day each and the placebo capsules microcrystalline cellulose and dextrose anhydrate (Chr. Hansen, Hoersholm, Denmark). Compliance in consumption of study capsules was assessed by interview.

Dietary counselling for the dietary intervention groups emphasised favourable fat intake according to current dietary recommendations; saturated fatty acids (SFA) providing 10% or less of energy intake, monounsaturated (MUFA) 10–15% and polyunsaturated fatty acids (PUFA) 5–10% [18]. Intensive counselling was given by a nutritionist at every study visit, supported with provision of conventional food products with favourable fat composition to be consumed at home (low erucic acid rapeseed oil-based margarines, spreads and salad dressings, Raisio plc, Raisio, Finland). In rapeseed oil, the ratio of n-6 to n-3 fatty acids is approximately 2:1 [19].

The participants visited the study clinic at every trimester of pregnancy and 1 month after delivery, when food records were also collected. Fatty acid and cytokine analyses of breast milk samples (colostrum and 1 month after delivery) were scheduled according to the study plan in the order of entry for half of the original study population. The entry criterion for this study was thus that fatty acid and cytokine analyses were available from colostrum and/or 1 month breast milk samples ($n = 125$).

Collection and analysis of dietary data and breast milk samples

The mothers' dietary intake was evaluated by three-day food records with household measures at every trimester of pregnancy and 1 month after delivery. Intakes of nutrients and key food groups in respect of fat intake were calculated with Micro-Nutrica software (Research Centre of the Social Insurance Institution, Turku, Finland). Maternal background data were collected by interview and infant birth data from birth records.

Breast milk samples were collected by manual expression after birth in the maternity hospital (colostrum) and

1 month after delivery at home. Mothers were given written instructions for standardised collection of samples in the mornings, and the samples were frozen for later analysis. Cytokines in breast milk were analysed as previously described in detail [20].

Total lipids in the breast milk samples were extracted with HPLC grade chloroform:methanol 2:1 v/v [21]. Triheneicosanoin (Larodan AB, Malmö, Sweden) was used as internal standard in the milk samples. Fatty acid methyl esters of the total lipid fractions of breast milk samples were prepared with a base-catalysed transesterification procedure based on the use of 0.5 M sodium methoxide in methanol according to Christie [22]. In this method, the reagents are not heated and it does not contain any aqueous extraction or solvent removal steps. Fatty acid methyl esters were analysed with an Agilent HP 6890 series gas chromatograph (Agilent Technoloqies Inc., USA) equipped with an autosampler, a split/splitless injector, a DB-23 capillary column coated with (50%-Cyanopropyl)-methylpolysiloxane phase (60 m × 0.25 mm i.d. with 0.25 μm film, Agilent J&W Scientific, USA), a flame ionisation detector (FID), and ChemStation software for data processing. The oven temperature programme was the following: from 50 °C (hold 1 min) to 130 °C with 20 °C/min, to 170 °C (hold 5 min) with 6.5 °C/min, to 215 °C (hold 5 min) with 4 °C/min, to 230 °C with 30 °C/min (hold 15 min). An aliquot of the sample (1 μL) was injected in splitless mode keeping the split valve closed for 1 min after injection. The temperature of the injector was 270 °C and that of the detector 280 °C. Helium (purity 99.996) was used as the carrier gas in constant pressure (30 psi) mode. Fatty acid methyl esters were identified based on fatty acid methyl ester reference mixtures and literature data. The quantification of fatty acids in milk samples was based on triheneicosanoin. One determination per sample was performed. The reproducibility of the method was tested before analyses were started and the variation was 2–3% (RSD).

Statistics

Cytokine variables were skewed to the right and were log-transformed before data analyses. The effect of study group (diet/probiotic, diet/placebo and control/placebo) on intakes of nutrients and foods, breast milk fatty acids and cytokines was analysed using mixed model repeated-measures analysis. Study group, time and interaction between study group and time were used as independent variables. If the interaction between time and study group was not significant, the p values presented are based on the main effects of study group. Otherwise the effect of study group was studied separately at all time points. Main effect is the effect of an independent variable on a dependent variable averaging across the levels of any other independent variables.

Associations between cytokines and fatty acids were also studied using the similar mixed model analysis in which fatty acids were used one by one as independent variables instead of study group. As cytokines were log-transformed, these results are presented with ratios of geometric means of cytokines per unit change (per 0.1 unit in EPA) in fatty acids and their 95% confidence intervals. Statistical analysis was made using SAS (version 9.2; SAS Institute, Cary, NC, USA). p values below 0.05 were considered statistically significant.

Results

Clinical characteristics

The mean age of the participating mothers was 30.3 (SD 4.6) years and altogether 78% of them had some allergic disease. The mean duration of gestation was 39.8 (SD 1.6) weeks and the mean birth weight of the infants 3.6 (SD 0.49) kg. There were no differences amongst the three groups in maternal or child birth characteristics.

Dietary intake

The mothers' dietary intake during pregnancy and breastfeeding is presented in Table 1. Means and standard deviations are presented for the first trimester that is the baseline before intervention, and estimated means and confidence intervals are presented for the mean values of the 2nd and 3rd trimesters combined and for the 1 month postpartum. At baseline, nutrient and food intakes were comparable amongst the three groups, and energy and total fat intakes remained comparable throughout the intervention. However, the quality of fat changed in that the intake of SFA was statistically significantly lower and that of MUFA higher in the dietary intervention groups compared with the control group during pregnancy and breastfeeding. PUFA intakes were higher in both dietary intervention groups compared with control group during pregnancy. Mothers in dietary intervention groups consumed more oil whilst there was no difference amongst the groups in the amount of fish eaten during the food recording days (Table 1).

Fatty acids and cytokines in breast milk

The fatty acid and cytokine composition of breast milk (colostrum and 1 month) in the three study groups is presented in Tables 2 and 3. The proportion of α-linolenic acid (ALA, 18:3n-3) and total n-3 fatty acids was higher in both

Table 1 Maternal dietary intake during pregnancy at 1st trimester (baseline), 2nd and 3rd trimester combined and during breastfeeding at 1 month postpartum in the dietary intervention groups (diet/probiotic and diet/placebo) and in the control group (control/placebo)

	Diet/probiotic (n = 41–43) Mean (SD) Mean (95% CI)	Diet/placebo (n = 38–41) Mean (SD) Mean (95% CI)	Control/placebo (n = 39–41) Mean (SD) Mean (95% CI)	p
Nutrients				
Energy (MJ)				
1 trim	8.4 (1.9)	8.4 (1.9)	8.4 (2.0)	0.74
2&3 trim	8.6 (8.1–9.1)	8.7 (8.2–9.3)	8.8 (8.3–9.3)	
1 mo	8.8 (8.1–9.4)	8.7 (8.0–9.3)	9.1 (8.5–9.8)	
Total fat (E%)				
1 trim	31.4 (4.9)	31.6 (5.4)	31.3 (6.4)	0.90
2&3 trim	31.4 (29.8–32.8)	31.9 (30.4–33.5)	31.1 (29.6–32.6)	
1 mo	31.8 (30.1–33.6)	32.1 (30.3–33.9)	32.9 (31.1–34.7)	
SFA (E%)				
1 trim	13.0 (2.5)	13.2 (2.8)	12.8 (3.1)	0.001 A, B
2&3 trim	11.4 (10.6–12.2)	11.4 (10.6–12.2)	13.1 (12.3–13.9)	
1 mo	12.0 (11.1–12.9)	11.7 (10.8–12.6)	13.5 (12.5–14.0)	
MUFA (E%)				
1 trim	10.4 (2.5)	10.3 (2.3)	10.6 (2.7)	0.005 A, B
2&3 trim	11.2 (10.6–11.8)	11.6 (10.9–12.2)	9.9 (9.3–10.5)	
1 mo	11.3 (10.5–12.0)	11.5 (10.7–12.3)	10.8 (10.0–11.6)	
PUFA (E%)				
1 trim	4.9 (1.3)	5.0 (1.7)	5.1 (1.6)	*
2&3 trim	6.0 (5.7–6.4)	6.3 (5.9–6.6)	4.8 (4.5–5.2)	<0.001 A, B
1 mo	5.6 (5.1–6.0)	6.3 (5.8–6.8)	5.6 (5.1–6.1)	0.06 B, C
Protein (E%)				
1 trim	16.6 (2.4)	16.4 (2.7)	16.8 (2.6)	0.24
2&3 trim	16.1 (15.4–16.7)	16.5 (15.9–17.2)	16.9 (16.2–17.5)	
1 mo	16.0 (15.1–16.9)	16.8 (15.9–17.7)	16.7 (15.7–17.6)	
Carboh. (E%)				
1 trim	50.5 (4.6)	50.6 (5.5)	50.4 (6.5)	0.48
2&3 trim	51.1 (49.6–52.7)	50.1 (48.5–51.6)	50.5 (49.0–52.1)	
1 mo	50.7 (48.9–52.5)	49.5 (47.7–51.3)	48.7 (46.7–52.2)	
Fibre (g)				
1 trim	20.5 (6.0)	21.5 (7.0)	20.1 (7.1)	0.09
2&3 trim	21.8 (20.0–23.6)	22.4 (20.5–24.3)	20.1 (18.3–22.0)	
1 mo	19.6 (17.5–21.6)	22.1 (20.0–24.2)	18.5 (16.4–20.7)	
Foods				
Margarine (g)				
1 trim	14.6 (11.3)	14.5 (10.9)	15.5 (9.1)	0.10
2&3 trim	20.2 (17.8–22.7)	21.7 (19.1–24.2)	18.0 (15.5–20.5)	
1 mo	24.0 (20.0–28.0)	25.2 (21.1–29.3)	20.9 (16.8–25.0)	
Oil (g)				
1 trim	9.5 (9.6)	9.0 (8.2)	10.8 (10.5)	0.01 A, B
2&3 trim	14.2 (11.9–16.5)	13.9 (11.6–16.3)	10.1 (7.8–12.5)	
1 mo	11.5 (8.5–14.6)	14.4 (11.3–17.5)	9.8 (6.7–12.9)	
Fish (g)				
1 trim	23.7 (23.4)	25.4 (28.8)	32.9 (37.1)	0.30
2&3 trim	23.8 (16.8–30.7)	30.1 (22.8–37.3)	28.2 (21.2–35.3)	
1 mo	26.1 (16.1–36.1)	31.7 (21.4–41.9)	31.6 (21.3–41.8)	

Pairwise group comparisons $p < 0.05$: A diet/probiotic versus control/placebo, B diet/placebo versus control/placebo, C diet/probiotic versus diet/placebo

trim trimester, *mo* month, *E%* percentage of total energy intake, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

p values are for the main effects of the groups, * Interaction between group × time was significant for PUFA (E%) ($p = 0.02$)

Table 2 Fatty acid composition (% of total fatty acids) in colostrum and breast milk at 1 month in the dietary intervention groups (diet/probiotic and diet/placebo) and in the control group (control/placebo)

n	col/1 mo	Diet/probiotic Mean (SD) 36/40	Diet/placebo Mean (SD) 31/36	Control/placebo Mean (SD) 35/38	*p*
SFA					
10:0	col	0.49 (0.30)	0.53 (0.34)	0.63 (0.51)	0.87
	1 mo	1.40 (0.35)	1.43 (0.37)	1.30 (0.33)	
12:0	col	3.38 (1.29)	3.56 (1.33)	3.89 (2.23)	0.55
	1 mo	5.92 (1.87)	6.46 (2.12)	5.78 (1.75)	
14:0	col	6.39 (1.36)	6.61 (1.40)	6.84 (2.18)	0.16
	1 mo	6.30 (1.84)	7.18 (1.8)	6.93 (1.88)	
16:0	col	25.94 (2.06)	26.08 (2.03)	26.08 (2.09)	0.22
	1 mo	24.01 (2.71)	23.96 (2.56)	25.18 (3.07)	
18:0	col	6.34 (1.29)	6.33 (1.41)	6.80 (1.48)	0.048 A, B
	1 mo	7.41 (2.22)	7.36 (1.58)	8.17 (1.79)	
MUFA					
16:1	col	3.16 (0.62)	3.34 (0.90)	3.18 (0.85)	0.84
	1 mo	2.75 (0.88)	2.59 (0.59)	2.62 (0.55)	
18:1n-9	col	35.55 (3.00)	35.45 (2.73)	35.14 (3.31)	0.33
	1 mo	35.32 (3.87)	34.10 (3.28)	33.93 (3.42)	
18:1n-7	col	2.77 (0.44)	2.70 (0.40)	2.66 (0.47)	0.75
	1 mo	1.96 (0.35)	1.93 (0.27)	1.96 (0.28)	
PUFA					
18:2n-6	col	8.24 (1.24)	8.37 (1.29)	7.89 (1.66)	0.25
	1 mo	9.82 (2.09)	9.46 (1.67)	9.13 (2.04)	
18:3n-6	col	0.052 (0.032)	0.037 (0.015)	0.048 (0.022)	0.038 C
	1 mo	0.083 (0.032)	0.072 (0.028)	0.078 (0.040)	
20:3n-6	col	0.53 (0.14)	0.50 (0.11)	0.57 (0.17)	0.14
	1 mo	0.28 (0.07)	0.29 (0.07)	0.30 (0.10)	
20:4n-6	col	0.61 (0.15)	0.61 (0.11)	0.60 (0.18)	0.80
	1 mo	0.29 (0.09)	0.30 (0.07)	0.28 (0.08)	
18:3n-3	col	1.23 (0.29)	1.32 (0.34)	1.12 (0.27)	0.003 A, B
	1 mo	1.66 (0.51)	1.77 (0.50)	1.46 (0.53)	
20:5n-3	col	0.082 (0.034)	0.080 (0.036)	0.073 (0.036)	0.09
	1 mo	0.084 (0.052)	0.11 (0.097)	0.072 (0.050)	
22:5n-3	col	0.36 (0.16)	0.30 (0.11)	0.29 (0.11)	0.18
	1 mo	0.16 (0.07)	0.19 (0.13)	0.15 (0.08)	
22:6n-3	col	0.63 (0.22)	0.61 (0.19)	0.56 (0.21)	0.25
	1 mo	0.32 (0.19)	0.41 (0.34)	0.31 (0.20)	
Total					
SFA	col	44.02 (4.67)	43.98 (4.01)	45.13 (5.22)	0.17
	1 mo	45.81 (6.01)	47.19 (5.31)	48.16 (5.32)	
MUFA	col	43.32 (3.37)	43.34 (3.09)	42.87 (3.91)	0.39
	1 mo	41.15 (4.57)	39.87 (3.61)	39.74 (3.91)	
PUFA	col	12.66 (1.86)	12.69 (1.83)	12.00 (2.24)	0.11
	1 mo	13.05 (2.51)	12.95 (2.40)	12.10 (2.62)	
n-6	col	9.78 (1.37)	9.84 (1.32)	9.43 (1.84)	0.31
	1 mo	10.56 (2.18)	10.21 (1.73)	9.86 (2.16)	
n-3	col	2.30 (0.52)	2.32 (0.55)	2.05 (0.46)	0.003 A, B
	1 mo	2.22 (0.57)	2.48 (0.80)	1.99 (0.69)	
Ratio n-6/n-3	col	4.37 (0.67)	4.36 (0.65)	4.71 (0.91)	0.004 B
	1 mo	4.96 (1.34)	4.36 (0.91)	5.31 (1.43)	

col colostrum, *mo* month, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

p values are for the main effects of the groups

Group comparisons $p < 0.05$: A diet/probiotic versus control/placebo, B diet/placebo versus control/placebo, C diet/probiotic versus diet/placebo

Table 3 Cytokines (pg/mL) in colostrum and breast milk at 1 month in the dietary intervention groups (diet/probiotic and diet/placebo) and in the control group (control/placebo)

Cytokine pg/mL *n*	col/1 mo	Diet/probiotic Median (IQR) 35/40	Diet/placebo Median (IQR) 31/35	Control/placebo Median (IQR) 34/37	*p*
IFN-γ	col	151.6 (97.2–227.0)	154.6 (97.2–216.1)	131.8 (94.8–164.7)	0.18
	1 mo	225.7 (190.3–244.4)	237.3 (212.3–258.0)	180.5 (141.4–214.7)	
TNF-α	col	11.2 (6.7–15.0)	11.4 (8.8–19.2)	8.0 (5.2–9.4)	0.0004 A, B
	1 mo	13.3 (11.4–14.8)	13.7 (11.3–15.4)	8.2 (6.5–11.3)	
IL-10	col	9.6 (6.2–14.4)	11.0 (7.6–17.8)	7.4 (5.2–11.0)	0.0002 A, B
	1 mo	15.1 (13.0–17.4)	15.5 (12.5–17.9)	9.1 (6.9–11.8)	
IL-6	col	66.7 (34.0–142.6)	60.6 (29.2–106.0)	46.6 (28.4–131.9)	0.22
	1 mo	22.1 (17.9–33.7)	19.4 (16.4–27.7)	15.9 (10.6–22.3)	
IL-4	col	19.0 (13.0–30.1)	20.6 (12.4–39.8)	13.4 (10.5–19.4)	0.0002 A, B
	1 mo	29.2 (20.9–35.5)	29.8 (24.3–33.5)	15.6 (11.3–22.7)	
IL-2	col	40.6 (29.8–51.1)	44.6 (27.8–57.6)	14.2 (12.1–22.9)	<0.0001 A, B
	1 mo	33.1 (26.7–40.1)	31.7 (22.7–41.7)	16.5 (12.6–18.9)	

Group comparisons $p < 0.05$: A diet/probiotic versus control/placebo, B diet/placebo versus control/placebo, C diet/probiotic versus diet/placebo

col colostrum, *mo* month

p values are for the main effects of the groups

Table 4 Association between fatty acids and cytokines in colostrum

	20:5n-3 (EPA) RR (95% CI)	*p*	22:6n-3 (DHA) RR (95% CI)	*p*	Total n-3 RR (95% CI)	*p*
IFN-γ	2.1 (1.3–3.4)	0.002	4.2 (2.0–9.2)	0.0003	1.5 (1.1–2.1)	0.007
TNF-α	1.9 (1.3–2.9)	0.001	4.3 (2.2–8.3)	<0.0001	1.6 (1.2–2.1)	0.002
IL-10	2.0 (1.3–3.0)	0.0008	3.9 (2.1–7.5)	<0.0001	1.5 (1.1–1.9)	0.004
IL-4	2.0 (1.3–3.2)	0.003	4.0 (1.9–8.7)	0.0004	1.6 (1.1–2.1)	0.005
IL-2	1.8 (1.2–2.8)	0.003	3.4 (1.8–6.5)	0.0003	1.6 (1.2–2.1)	0.001

RR describes ratios of geometric means of cytokines per unit change (per 0.1 unit in EPA) in fatty acids

EPA eicosapentaenoic acid, *DHA* docosahexaenoic acid

dietary intervention groups compared with controls. On the other hand, the proportion of stearic (18:0) acid was higher in the control group. In the diet/probiotic group, the content of γ-linolenic acid (18:3n-6) was higher compared with the diet/placebo group. Arachidonic acid (20:4n-6) content was comparable amongst the groups (Table 2). In the case of cytokines, the concentrations of TNF-α, IL-10, IL-4 and IL-2 were higher in both dietary intervention groups compared with control (Table 3).

Association between fatty acids and cytokines in breast milk

In evaluation of the associations between PUFA and cytokines in breast milk, the proportions of eicosapentaenoic (EPA) and docosahexaenoic (DHA) as well as total n-3 fatty acids were found to be positively associated with cytokines IFN-γ, TNF-α, IL-10, IL-4 and IL-2 in colostrum samples (Table 4). Also arachidonic acid was positively associated with IFN-γ, TNF-α, IL-10 and IL-4 in colostrum (all $p < 0.01$). The previous results were studied separately in colostrum and 1-month samples because there was a significant interaction between time and those fatty acids on cytokines. No significant associations between those fatty acids and cytokines were found in 1-month samples. Furthermore, at both time-points, arachidonic acid was positively associated with IL-6 (main effect $p = 0.003$) and total polyunsaturated fatty acids with IL-2 (main effect $p = 0.02$).

Discussion

Breast milk fatty acids and immunomodulatory compounds are vital to the developing immune system and for the later health of the infant. Previous studies have indicated that the

diet of pregnant or breastfeeding women does not always comply with recommendations, for example in respect of the quality of fat in the diet [12, 23]. Attempts to improve the situation have concentrated on supplementing single fatty acids or oils [14–16, 24, 25]. The results of the present study indicate that intensive and repeated dietary counselling and provision of food products with recommended fatty acid composition are efficacious in improving the quality of fat in the maternal diet. Also the proportions of ALA and total n-3 fatty acids were seen to be elevated in the breast milk in the dietary intervention groups.

In the present study, the proportion of γ-linolenic acid (GLA) in breast milk could be promoted by probiotics in conjunction with modulation of dietary fat intake, this suggesting interaction between probiotics and fatty acids. It has previously been observed that probiotics-containing dairy product improved the bioavailability of GLA [26]. Furthermore, GLA has been associated with atopic disease, as atopic infants have been shown to have less GLA in serum phospholipids than healthy infants [27]. Previous studies have also demonstrated that probiotics increase the levels of TGF-β in breast milk, this also resulting in clinical benefits with a lowering of the risk of atopic dermatitis in infants [28].

Wide variations in the concentrations of cytokines in human milk have been observed in different studies making direct comparisons between studies difficult. This variation may be explained by several factors, for example timing of the collection of the samples, different analytical methods and maternal characteristics [29, 30]. Cytokines in breast milk may provide immunological signals during a critical stage of T-cell development [31], but more information is needed on the short and long-term physiological effects of breast milk cytokines on infant health [32–34]. Assessing the complex networks and interactions between cytokines is challenging [34], and it appears that besides cytokines, many other immunomodulatory factors in breast milk are important for the maturation of the immune system and for example, tolerance induction through breastfeeding [35].

The dietary intervention in this study enhanced the levels of various cytokines in breast milk. The effect of fish oil supplementation on breast milk cytokine levels has previously been studied. Dunstan and associates [36] reported that supplementing atopic women fish oil capsules from 20 weeks gestation until delivery increased DHA and EPA levels in breast milk collected 3 days post-partum, and IL-10 and IL-6 levels were found to correlate with n-3 PUFA levels. Correspondingly, in the present study, n-3 fatty acids were found to be positively associated with several cytokines in the colostrum. On the other hand, supplementation of fish oil to lactating women postpartum did not affect cytokine levels in breast milk collected 4 weeks postpartum [37]. Considerable variation has been shown in breast milk n-3 long-chain PUFA depending on fish intake in the previous 24 h, and these fluctuations make it difficult to assess their intake reliably from a single sample [38].

Instead of fish oils, increasing the intake of α-linolenic acid may also increase the levels of longer-chain n-3 fatty acids. The conversion of ALA to EPA and DHA has been found to be higher in women than in men, and it has been suggested that it is further increased during pregnancy [39]. It has been shown that supplemental α-linolenic acid from flaxseed oil may raise EPA and DPA but not DHA in breast milk [25]. Rapeseed oil is a good source of ALA and has a balanced n-6/n-3 fatty acid ratio compared with many other vegetable oils [19], and its dietary intake was also reflected in ALA levels in breast milk in the present study. In Finland, rapeseed oil-based food products are widely available on the food market for everyday use as part of the usual diet.

In addition to the previously known health effects of long-chain PUFA [2], recent findings suggest that fatty acid status may also contribute to the development of obesity in childhood [40]. Therefore, more research on the physiological and health effects of total fatty acid patterns in maternal and infant diets are called for. The strengths of the present study are the individual, intensive and repeated counselling given by a nutritionist, the provision of food products with favourable fat composition and the evaluation of the total macronutrient composition of the diet by repeated food records. The results emphasise the potential of dietary counselling in changing maternal diet and breast milk composition, and the need for evaluating dietary habits and counselling is also stated in consensus recommendations [41]. Provision of single fatty acid supplements is not a feasible public health approach in improving the maternal diet, and general recommendations on healthy diet should therefore be advocated to all pregnant and breastfeeding women. The recommended intake of DHA can be met by consuming two portions of fish per week [41]. Food products with beneficial probiotics might bring further benefits to vulnerable groups at risk of allergic disease [20]. Future studies should undertake an evaluation of interactions amongst nutrients in the maternal diet and breast milk and further, their long-term health effects on the infant.

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Conflict of interest Päivi Laakso was employed by Raisio plc, other authors have no conflict of interest.

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