



Fatty acid composition of human milk in atopic Danish mothers¹⁻³

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ABSTRACT

Background: Atopic dermatitis has been related to a disturbed metabolism of polyunsaturated fatty acids (PUFAs).

Objective: We tested whether the PUFA composition of breast milk differs significantly between mothers with atopic dermatitis, mothers with other types of atopy, and nonatopic mothers. We also investigated whether differences in diet can explain possible observed differences.

Design: Mothers with current or previous asthma ($n = 396$) were divided into 3 groups according to history of atopic dermatitis and allergic rhinitis. Breast-milk samples were collected from 314 women ≈ 3 wk after delivery. The habitual diet of the women was assessed with food-frequency questionnaires in the 25th week of gestation ($n = 207$). Breast-milk samples and simultaneous dietary data from 14 nonatopic mothers were used for comparison.

Results: Compared with the milk of nonatopic mothers, that of atopic mothers had significantly higher concentrations of 22:5n-6 and lower concentrations of 20:5n-3; moreover, 20:4n-6/20:5n-3, 22:5n-6/22:6n-3, and long-chain n-3 PUFA/18:3n-3 were shifted toward n-6 PUFA and 18:3n-3 in nonatopic and atopic mothers, respectively. No differences in breast-milk PUFA composition were evident between the subject groups. The diets of the groups differed only slightly with respect to protein intake. However, the PUFA composition of the breast milk was associated with diet and time of milk sampling, and the above difference in milk PUFAs disappeared when those factors were taken into account.

Conclusion: Our data do not support the possibility that the fatty acid composition of breast milk is affected by atopic dermatitis or atopy in general, because most differences in breast-milk PUFA composition appear to be explained by the diet. *Am J Clin Nutr* 2006;84:190-6.

KEY WORDS Human milk, long-chain n-3 polyunsaturated fatty acids, n-3 LC-PUFA, atopy, diet, lactation

INTRODUCTION

The relation between polyunsaturated fatty acid (PUFA) composition and atopic disease has been an issue for many decades. Manku et al (1) suggested impaired function of Δ^6 -desaturase to explain the observed higher concentrations of linoleic acid (18:2n-6) and lower concentrations of the derived PUFAs (18:3n-6, 20:3n-6, and 20:4n-6) in the plasma of adults and children with atopic dermatitis than were seen in healthy control subjects. Several studies also found low concentrations of long-chain n-6 PUFAs (n-6 LC-PUFAs) in the breast milk of mothers with various types of atopy (2-5). However, other studies

observed no differences in the PUFA composition of breast milk (6, 7), and some have found indications for other types of disturbances in the breast-milk PUFA composition (2, 8). As an alternative, Yu et al (2) hypothesized that atopy may be associated with a disturbance in the transition from low 18:2n-6 and 18:3n-3 and high LC-PUFAs in colostrum to high 18:2n-6 and 18:3n-3 and low LC-PUFAs in mature milk. An increased utilization of LC-PUFAs for eicosanoid production in atopic subjects has also been put forward as a possible explanation (5). Two of the studies investigated whether the fatty acid (FA) composition of breast milk varied with the types and numbers of atopic diseases, but no effects were found (4, 6). The studies have generally been rather small (17-43 atopic mothers), and only one study has included dietary assessment (5).

The FA composition of milk is highly influenced by diet, especially the ratio of n-3 PUFAs to n-6 PUFAs and the content of LC-PUFAs (9). Atopic mothers may have other dietary habits due to additional food intolerance (10). Dietary intake in general has also been suggested as a contributing factor to atopy; among the proposed factors is the intake of PUFAs (11-17). It is therefore possible that a specific dietary pattern is associated with atopy.

The Copenhagen Prospective Study on Asthma in Childhood (COPSAC) investigated the relation among genetic, environmental, and lifestyle factors, including diet, in the development of atopic disease in 411 infants born to women with a history of asthma as previously described in detail (18). COPSAC provides a unique possibility to study whether atopic diseases affect the FA composition of breast milk and to explore whether differences can be ascribed to dietary habits or to the atopic disease per se. In the current substudy, we tested the hypothesis that the relative content of LC-PUFAs and the ratio of n-3 PUFAs to

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n-6 PUFAs would be significantly different between the breast milk of mothers with atopic dermatitis (AD) and that of mothers with other types of atopy and a small random selection of nonatopic mothers.

SUBJECTS AND METHODS

Subjects and data collection

Participants were mainly recruited from the Danish National Birth Cohort (DNBC; 19) and from among pregnant women attending maternity clinics. Women with a history of asthma were invited during pregnancy, and their infants were enrolled during the first month of life. Children from the Greater Copenhagen area were eligible for inclusion in COPSAC if their mothers had a confirmed clinical diagnosis of asthma (present or past) and had received daily asthma treatment for ≥ 2 wk at least twice since the age of 7 y or for ≥ 1 y since the age of 7 y. Atopic history was ascertained by interviewing the mother. Children who were born before 36 wk of gestation, who were born with known severe congenital diseases, or who had systemic illness or a history of lower respiratory symptoms were not included. The children were born between August 1998 and December 2001. Initially, 1479 pregnant women indicated an interest in study participation, 815 fulfilled enrollment criteria, and 453 accepted participation, but only 411 attended the enrollment visit after delivery. Seventeen mothers were included with 2 infants—9 sets of twins and 8 sibling couples—for a total of 394 atopic mothers (18).

The participants were seen for the first time in the COPSAC clinical research unit when their infants were ≈ 1 mo old. The mothers were asked to collect a sample of their breast milk during or soon after the visit. The atopic mothers were divided into 3 groups according to types of atopy: those with bronchial asthma (BA) only, those with asthma plus allergic rhinitis (BA+AR), and those with asthma and atopic dermatitis (BA+AD). Most of the mothers in the latter group (76%) also had a history of AR.

The nonatopic comparison group was recruited by the home health visitors in the municipality of Frederiksberg, who at their first visit (≈ 1 wk after delivery; February to April of 2004) provided all breastfeeding mothers with information about the study (given to ≈ 250 mothers). Sixteen mothers showed an interest in the study, and 14 of these 16 complied with the inclusion criteria: no history of atopy (defined in the same way as in COPSAC), infant age at milk sampling 13–48 d, the infant born at term (>34 wk of gestation), and the infant exclusively breastfed. The mothers were asked to visit the Department of Human Nutrition; during that visit, they were interviewed and asked to fill out the food-frequency questionnaire (FFQ). The mothers were asked to collect a milk sample immediately after nursing their baby at some time between the afternoon of the day before the visit and their visit to the department.

The participants gave written informed consent to participate after the study had been explained to them orally and in writing. The protocols for COPSAC and the current substudy were approved by the local scientific ethics committee (KF 01-289/96, KF 11-035/02, and KF 11-152/03) and the Danish Data Protection Agency (1996-1200-360 and 2002-54-0938).

Fatty acid analysis of breast milk

Milk samples (2–5 mL) were delivered immediately or stored in a freezer at home for up to 2 wk until they were delivered to the

research unit. Two-mL aliquots of the milk samples were then added to 0.01% 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene; Sigma Chemical Co, St Louis, MO) and frozen at -80°C . All milk samples were analyzed within 1 y after collection.

Lipids from 1-mL aliquots of the milk samples were extracted according to a method of Bligh and Dyer (20) and methylated with potassium hydroxide in methanol (21). The resulting fatty acid methyl esters were extracted with heptane and separated by gas-liquid chromatography as described previously (22). All peaks from lauric acid (12:0) to docosaheptaenoic acid (22:6n-3) were identified from the retention times of commercial standards (Nu-Chek-Prep Inc, Elysian, MN) as described previously (23). A mean ($\pm$ SEM) of $97.06 \pm 0.06\%$ ($n = 329$) of the chromatogram areas were identified. The FA composition of all breast-milk samples was determined in duplicate, and all analyses were successful.

Eighty of the 394 COPSAC mothers did not deliver a milk sample. The actual sampling time for the 314 breast-milk samples from the atopic mothers varied from 5 to 151 days after delivery ($\bar{x} \pm \text{SEM}$: 25.2 ± 0.9 d). Twenty-eight mothers delivered milk samples that were taken <13 d after delivery (15, 10, and 3 samples from the BA+AD, BA+AR, and BA groups, respectively), and milk samples of 22 mothers were taken >48 d after delivery (12, 7, and 3 samples from the BA+AD, BA+AR, and BA groups, respectively). These 50 samples were excluded because previous studies showed that the time of milk sampling has a pronounced influence on PUFA composition (2). Of the remaining 264 COPSAC subjects, 144 also had dietary data and were included in the current analysis along with the 14 control subjects. The milk of the included subjects was sampled at an infant age of 22.0 ± 0.6 d ($n = 158$), which according to Yu et al (2) is the time of the greatest difference in PUFA composition of breast milk between atopic and nonatopic mothers.

Dietary data

Dietary data were collected by a comprehensive self-administered, semiquantitative 300-item FFQ. The questionnaire was a modified version of that used by Tjønneland et al (24) in the DNBC. In the current version, the women were questioned about their diet during the 4 wk before their visit to the Department of Human Nutrition. The FFQ contained questions about the total diet and detailed questions about fish intake for lunch (in Denmark, as topping on bread), hot meals, and fish-oil and cod liver supplements (n-3 LC-PUFA supplements were used by only 5 of the mothers, 4 from the BA+AD group and 1 from the BA+AR group). Using assumptions of portion sizes, standard recipes, and the nutrient content of foods [calculated from the Danish Food Tables of The Danish Food Agency, which contained comprehensive information on LC-PUFAs (25)], we estimated the average daily intake of macronutrients and PUFAs (in grams) by using the FOODCALC program (version 1.3; the Danish Cancer Society, Copenhagen, Denmark; www.foodcalc.dk). The current version of the FFQ was validated in pregnant women with respect to, among other nutrients, n-3 PUFA, which was found to correlate with intake estimated from 7-d weighed food diaries and n-3 LC-PUFA in erythrocytes (26). The FFQ was given to the control subjects at the time of breast-milk collection. Dietary information for the COPSAC participants was acquired via the DNBC, in which the FFQs were mailed to the participants in the 25th week of gestation. DNBC

TABLE 1Characteristics of participants in each of the subject groups¹

	Group			
	BA + AD (<i>n</i> = 63)	BA + AR (<i>n</i> = 58)	BA (<i>n</i> = 23)	Nonatopic (<i>n</i> = 14)
Age (y)	29.2 ± 0.5 ^b	29.4 ± 0.6 ^{a,b}	29.0 ± 1.1 ^{a,b}	32.8 ± 1.2 ^a
Previous pregnancies (<i>n</i>)	1.8 ± 0.1	2.1 ± 0.2	2.0 ± 0.3	1.6 ± 0.2
Gestational age (wk)	39.8 ± 0.2	39.7 ± 0.2	40.0 ± 0.3	39.6 ± 0.4
Birth weight (kg)	3.50 ± 0.07	3.52 ± 0.08	3.54 ± 0.10	3.44 ± 0.14
Infant age at milk sampling (d)	21.3 ± 0.9	22.7 ± 1.0	22.1 ± 1.6	21.9 ± 1.2

¹ All values are $\bar{x} \pm \text{SEM}$. BA, bronchial asthma; AD, atopic dermatitis; AR, allergic rhinitis. Participants are grouped by their medical history of disease. Data are compared with ANOVA and post hoc Bonferroni tests. Values in a row with different superscript letters are significantly different, $P < 0.05$.

dietary data were available for 207 of the COPSAC subjects (53%) and for 144 of those with milk samples. Only these subjects are included in the data presented here. There were no differences in the FA composition of milk between those with and without dietary data (data not shown) except with respect to the content of 18:3n-6 (0.15 ± 0.01 and $0.18 \pm 0.01\%$ of FA, respectively; $P = 0.028$). There also were no differences in the diet between those with and without milk samples (data not shown), and the included subjects did not differ from the rest of the COPSAC subjects with respect to the number of atopic diseases, maternal age, parity, gestational age, or infant birth weight (data not shown). Characteristics of participants in each of the atopy groups are shown in **Table 1**. The subjects in the 3 atopy groups did not differ from each other, but the subjects in the nonatopic group were on average older than those in the COPSAC study (32.8 ± 1.2 and 29.2 ± 0.3 y, respectively; $P = 0.004$).

Statistical analysis

All data were analyzed with SPSS software (version 12.0; SPSS Inc, Chicago, IL), and a significance level of $P < 0.05$ was used. Normality was checked with histograms and the Shapiro-Wilk test of normality. Results are given as mean $\pm$ SEM for Gaussian-distributed data and as median (25th–75th percentile) for non-Gaussian-distributed data. All group comparisons, eg, those of included and excluded mothers, were performed by using Student's *t* test. Multiple comparisons of diet and breast-milk FA composition in the atopy groups were performed with an analysis of variance (ANOVA) and Bonferroni post hoc test, except for non-Gaussian-distributed variables, which were compared by using an ANOVA of the log-transformed values. Analyses of bivariate correlations between diet and breast-milk FA composition were performed with Pearson's product-moment correlation for Gaussian-distributed variables and with Kendall's τ correlation analysis for non-Gaussian-distributed variables. The analysis of the combined effects of atopy and diet on the PUFA composition of breast milk was performed with a 2-way ANOVA with the inclusion of infant age at the time of the breast-milk sample and an interaction term for atopy $\times$ sampling time. Non-Gaussian-distributed variables that were included in these analyses were made to comply with a normal distribution by log transformation. All analyses were performed in the selected group and in all available COPSAC participants with similar results.

RESULTS

The FA composition of the breast milk of the women in each of the atopy groups is shown in **Table 2**. No significant differences were observed in the overall FA composition [saturated FAs (SFAs), monounsaturated FAs (MUFAs), PUFAs, or the ratio of n-6 to n-3 PUFAs]. However, some marked differences were observed in the minor PUFA components of breast milk. The content of 22:5n-6 was higher and that of 20:5n-3 was lower in all of the atopic groups ($P = 0.016$, 0.003 , and < 0.001 and $P = 0.001$, 0.007 , and 0.007 , respectively, for the comparison of the BA, BA + AR, and BA + AD groups with the nonatopic group). No significant differences were found in the major LC-PUFAs of the 2 families, 20:4n-6 and 22:6n-3, but the ratios of 20:4n-6 to 20:5n-3 and of 22:5n-6 to 22:6n-3 were found to be higher in the milk from atopic mothers than in that from nonatopic mothers ($P = 0.001$, 0.045 , and 0.008 for the comparison of BA, BA + AR, and BA + AD with NA; $P = 0.003$ for the comparison of BA with NA; and $P < 0.001$ for the comparisons of BA + AR and BA + AD with NA). The ratio of n-3 LC-PUFA to 18:3n-3 was significantly lower in all 3 groups of atopic subjects ($P = 0.002$, 0.014 , and 0.039 , respectively) than in atopic mothers, but no significant differences were observed in the overall "metabolic index" (LC-PUFA/18:2n-6 + 18:3n-3). Similar results were observed after exclusion of those atopic mothers who used n-3 LC-PUFA supplements (data not shown).

We found only one significant difference in the diet between the 4 atopy groups—the energy intake from protein differed significantly between groups ($P = 0.023$, ANOVA). However, no significant differences were detected between the individual groups in the post hoc test (**Table 3**). The largest difference was detected between the nonatopic group and the BA + AR group (1.6 ± 0.6 ; $P = 0.063$). Similar results were observed after exclusion of those atopic mothers who used n-3 LC-PUFA supplements (data not shown).

Correlations between the FA composition of milk and diet were examined in a correlation matrix between all variables that focused on those variables showing significant group differences in the group comparisons. The milk content of 22:5n-6 was not in itself significantly associated with any of the dietary variables, but the best trend was found with respect to energy intake ($\tau = 0.086$, $P = 0.120$). The milk content of 20:5n-3 was significantly associated with all measures of n-3 PUFA intake; the highest association was with the total intake of n-3 PUFAs ($\tau = 0.182$, $P = 0.001$). The milk content of n-6 and n-3 PUFAs was

TABLE 2

Breast-milk fatty acid composition in each of the subject groups¹

	Group			
	BA + AD (n = 63)	BA + AR (n = 58)	BA (n = 23)	Nonatopic (n = 14)
MC-SFA, 12:0 + 14:0 (%)	11.6 ± 0.4 ²	12.0 ± 0.6	10.7 ± 0.7	10.4 ± 0.7
SFA (%)	41.2 ± 0.5	41.8 ± 0.6	41.5 ± 0.9	39.7 ± 1.4
MUFA (%)	41.2 ± 0.4	41.1 ± 0.5	41.1 ± 0.7	42.6 ± 1.1
18:1 ^{trans} (%)	0.74 ± 0.05	0.68 ± 0.06	0.56 ± 0.05	0.10 ± 0.04
PUFA (%)	14.0 ± 0.3	13.6 ± 0.3	13.8 ± 0.4	14.9 ± 0.8
18:2n-6 (%)	10.1 ± 0.3	9.8 ± 0.3	10.0 ± 0.4	10.8 ± 0.6
18:3n-6 (%)	0.13 (0.09–0.21) ⁴	0.13 (0.09–0.21)	0.12 (0.09–0.19)	0.12 (0.07–0.18)
20:2n-6 (%)	0.30 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.02
20:3n-6 (%)	0.42 ± 0.01	0.41 ± 0.01	0.38 ± 0.02	0.46 ± 0.04
20:4n-6 (%)	0.59 ± 0.01	0.57 ± 0.01	0.58 ± 0.02	0.60 ± 0.03
22:4n-6 (%)	0.11(0.09–0.15)	0.12(0.10–0.14)	0.11(0.10–0.16)	0.12(0.10–0.15)
22:5n-6 (%)	0.13 (0.06–0.16) ^b	0.08 (ND–0.14) ^b	0.10 (ND–0.16) ^b	0.06 (0.02–0.07) ^a
n-6 PUFA (%)	11.8 ± 0.3	11.4 ± 0.3	11.7 ± 0.4	12.5 ± 0.6
n-6 LC-PUFA (%)	1.55 ± 0.04	1.47 ± 0.03	1.47 ± 0.05	1.59 ± 0.11
18:3n-3 (%)	1.28 ± 0.05	1.33 ± 0.05	1.32 ± 0.07	1.10 ± 0.11
20:5n-3 (%)	0.11 (0.08–0.15) ^b	0.11 (0.08–0.14) ^b	0.10 (0.07–0.13) ^b	0.17 (0.14–0.20) ^a
22:5n-3 (%)	0.20 (0.18–0.24)	0.21 (0.16–0.23)	0.21 (0.19–0.23)	0.21 (0.19–0.25)
22:6n-3 (%)	0.48 (0.40–0.63)	0.47 (0.35–0.57)	0.50 (0.35–0.56)	0.51 (0.43–0.65)
n-3 PUFA (%)	2.09 (1.71–2.53)	2.11 (1.73–2.42)	2.11 (1.84–2.36)	1.84 (1.72–2.47)
n-3 LC-PUFA (%)	0.79 (0.67–1.02)	0.77 (0.63–0.95)	0.82 (0.60–0.92)	0.87 (0.78–1.09)
n-6/n-3 PUFA	5.8 ± 0.2	5.5 ± 0.2	5.6 ± 0.2	6.1 ± 0.5
n-6/n-3 LC-PUFA	2.02 ± 0.09	2.04 ± 0.09	1.90 ± 0.08	1.63 ± 0.14
20:4n-6/20:5n-3	5.80 ± 0.36 ^b	5.40 ± 0.27 ^b	6.52 ± 0.60 ^b	3.45 ± 0.31 ^a
22:5n-6/22:6n-3	0.23 (0.13–0.34) ^b	0.16 (ND–0.27) ^b	0.17 (ND–0.31) ^b	0.11 (0.03–0.14) ^a
LC-PUFA/18:2n-6 + 18:3n-3	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.24 ± 0.14
n-6LC-PUFA/18:2n-6	0.158 ± 0.004	0.155 ± 0.004	0.149 ± 0.006	0.151 ± 0.010
n-3LC-PUFA/18:3n-3	0.75 ± 0.06 ^b	0.69 ± 0.07 ^b	0.64 ± 0.04 ^b	1.25 ± 0.39 ^a

¹ BA, bronchial asthma; AD, atopic dermatitis; AR, allergic rhinitis; MC, medium-chain; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected; LC, long-chain. Participants are grouped by their medical history of disease. Individual fatty acids are named by the number of carbon atoms—the number of double bonds followed by the position of the last double bond—given as a percentage of total fatty acid content. The content of individual fatty acids or fatty acid classes is given as a percentage of total fatty acid content. Normally distributed data are compared with ANOVA and post hoc Bonferroni tests. Values in a row with different superscript letters are significantly different. $P < 0.05$.

² $\bar{x} \pm \text{SEM}$ (all such values).

³ Not included in the comparison because the *trans* fatty acid content in Danish products was changed during the course of the study.

⁴ Median; 25th–75th percentile in parentheses (all such values). Log-transformed data compared by ANOVA.

not inversely correlated to the intake of the opposite PUFA family, and only a weak association was found between n-6:n-3 PUFAs in milk and diet. There was, however, a significant association between n-6:n-3 LC-PUFAs in the diet and the milk ($r = 0.338$, $P < 0.001$). The ratio of 20:4n-6 to 20:5n-3 in milk was associated with intake of n-3 PUFAs, but most significantly with 20:4n-6/n-3 LC-PUFAs in the diet ($r = 0.231$, $P = 0.005$). The 22:5n-6/22:6n-3 in the milk was not significantly associated with any of the dietary variables, but the best trend was found with the intake of 20:5n-3 ($r = -0.081$, $P = 0.135$). All 3 metabolic indexes and the relative content of LC-PUFAs in milk were significantly associated with the total energy intake ($P = 0.011$ for n-6 LC-PUFA/18:2n-6) and the best association for n-3 LC-PUFA/18:3n-3 was observed with (log)percentage of energy from fat ($r = -0.222$, $P = 0.005$).

When differences in the milk content of (log)22:5n-6 and (log)20:5n-3 and in the ratios of 20:4n-6 to 20:5n-3, (log)22:5n-6 to 22:6n-3, and n-3 LC-PUFAs to 18:3n-3 were analyzed in multiple ANOVAs by using general linear modeling that included atopy group, the above-described most significant dietary factor, infant age at milk sampling, and the atopy group ×

infant age interaction, no significant effects of atopy were observed. No significant interactions between atopy and infant age were observed in these multiple analyses. The effects of the included dietary factors remained significant ($P = 0.034$ for the effect of energy intake on milk 22:5n-6, $P < 0.001$ for that of n-3 PUFA intake on milk 20:5n-3, $P = 0.001$ for that of dietary 20:4n-6/n-3 LC-PUFAs on milk 20:4n-6/20:5n-3, $P = 0.001$ for that of 20:5n-3 intake on 20:5n-6/22:6n-3 in milk, and $P = 0.016$ for that of energy from fat on n-3 LCPUFA/18:3n-3 in the milk).

DISCUSSION

The current study is the largest study of the FA composition of breast milk from atopic mothers. Compared with breast milk from nonatopic mothers, breast milk from atopic mothers was found to have higher concentrations of 22:5n-6, lower concentrations of 20:5n-3 and 20:4n-6/20:5n-3, 22:5n-6/22:6n-3 and n-3 LC-PUFA/18:3n-3 that were shifted toward n-6 PUFAs and 18:3n-3, respectively. No differences in the PUFA composition of breast milk were evident between mothers with

TABLE 3

Dietary intake in each of subject groups¹

	Groups			
	BA + AD (n = 63)	BA + AR (n = 58)	BA (n = 23)	Nonatopic (n = 14)
Energy (MJ/d) ²	10.4 (8.5–12.2) ³	10.2 (8.8–12.3)	10.6 (8.6–13.1)	11.1 (8.7–14.0)
Protein (% of energy) ⁴	14.7 ± 0.3 ⁵	15.2 ± 0.3	14.1 ± 0.4	13.6 ± 0.5
Carbohydrate (% of energy)	53.8 ± 0.8	54.1 ± 0.7	53.7 ± 1.4	56.6 ± 1.6
Alcohol (% of energy) ²	0.23 (ND–0.59)	0.30 (ND–0.78)	0.18 (ND–1.02)	0.30 (0.06–0.68)
Fat (% of energy) ²	30.4 (26.6–36.0)	29.6 (25.0–32.2)	30.4 (25.3–36.5)	28.0 (25.1–33.6)
SFA (% of energy)	12.6 ± 0.4	12.2 ± 0.4	12.7 ± 0.7	11.3 ± 0.6
MUFA (% of energy)	9.4 ± 0.3	9.1 ± 0.3	9.7 ± 0.5	9.8 ± 0.8
PUFA (% of energy)	4.4 ± 0.1	4.2 ± 0.1	4.6 ± 0.2	4.4 ± 0.5
18:2n–6 (% of FAs)	12.5 ± 0.3	12.5 ± 0.4	12.7 ± 0.6	12.8 ± 0.9
20:4n–6 (% of FAs)	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.03	0.11 ± 0.02
n–6 PUFA (% of FAs)	12.6 ± 0.3	12.6 ± 0.4	12.8 ± 0.6	12.9 ± 0.9
18:3n–3 (% of FAs)	2.12 ± 0.05	2.15 ± 0.05	2.21 ± 0.10	2.38 ± 0.10
20:5n–3 (% of FAs) ²	0.17 (0.07–0.24)	0.16 (0.09–0.28)	0.15 (0.10–0.26)	0.15 (0.08–0.20)
22:5n–3 (% of FAs) ²	0.08 (0.06–0.10)	0.09 (0.05–0.11)	0.08 (0.06–0.11)	0.07 (0.05–0.10)
22:6n–3 (% of FAs) ²	0.42 (0.22–0.61)	0.42 (0.25–0.58)	0.40 (0.32–0.70)	0.34 (0.20–0.48)
n–3 LC-PUFA (% of FAs) ²	0.65 (0.37–0.95)	0.67 (0.38–0.93)	0.64 (0.48–1.16)	0.54 (0.35–0.78)
n–3 PUFA (% of FAs) ²	2.91 (2.43–3.32)	2.92 (2.50–3.31)	3.02 (2.48–3.57)	3.06 (2.77–3.28)
n–6/n–3 PUFA	4.5 ± 0.2	4.4 ± 0.1	4.2 ± 0.3	4.3 ± 0.3
20:4n–6/n–3 LC-PUFA	0.22 ± 0.02	0.23 ± 0.02	0.19 ± 0.03	0.24 ± 0.05
LC-PUFA (% of PUFA)	5.6 ± 0.4	5.7 ± 0.5	6.6 ± 1.0	4.5 ± 0.6

¹ BA, bronchial asthma; AD, atopic dermatitis; AR, allergic rhinitis; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids; LC, long-chain. Subjects are grouped by medical history of disease. Individual fatty acids are named by the number of carbon atoms—the number of double bonds followed by the position of the last double bond. Normally distributed data are compared with ANOVA and post hoc Bonferroni tests.

² Comparisons performed by using ANOVA of log-transformed data.

³ Median; 25th–75th percentile in parentheses (all such values).

⁴ Protein intake differed significantly between groups, $P = 0.023$ (ANOVA). None of the post hoc individual group comparisons found significant differences between the individual groups.

⁵ $\bar{x} \pm \text{SEM}$ (all such values).

different types of atopy. The results indicate that most differences between the groups were due to differences in diet—not only PUFA intakes but also energy intake and macronutrient composition. Our results did not support the hypothesis that Δ^6 -desaturase activity is impaired in women with AD, because products of 18:2n–6 metabolism, if anything, were higher in the AD group as well as in the other atopic groups, both in absolute amounts and relative to 18:2n–6. Moreover, in multiple analyses including infant age at milk sampling, we did not find any effect of atopy on the PUFA composition of milk that could support the hypothesis that atopic mothers have a disturbed metabolic regulation (2). Previous studies found lower concentrations of 18:2n–6 metabolites (2, 3, 5, 8), but others have not detected any differences (6, 7, 16). Duchén et al (27) found lower concentrations of all PUFAs and higher n–6:n–3 PUFAs in milk from atopic mothers. Higher n–6:n–3 PUFAs have also been reported by Yu et al (2) and are indicated by higher 20:4n–6/20:5n–3 and 22:5n–6/22:6n–3 in the current study, whereas Kankaanpää et al (5) found lower n–6:n–3 PUFAs. In agreement with Wijga et al (4), our data showed a lower ratio of n–3 LC-PUFAs to the precursor in milk from atopic mothers. The ratio of LC-PUFA to precursor has been used as a metabolic index. However, n–6 LC-PUFA/18:2n–6 and n–3 LC-PUFA/18:3n–3 do not reflect metabolism in the same way, because the relative contributions of diet and of endogenous LC-PUFA synthesis differ between the 2 PUFA families. The n–3 LC-PUFA

content of breast milk is strongly influenced by diet (23). PUFA metabolism may also be influenced by diet, as indicated by the observed association between energy intake and energy from fat and the metabolic indexes. The dietary habits of atopic and nonatopic women may differ between cultural settings, and this may explain the inconsistencies in the previous studies.

Only one previous study has included diet, and that study included only 20 atopic and nonatopic mothers (5). Our study also has some limitations. Data from the nonatopic group were not collected simultaneously with data from the atopic groups, which resulted in some dietary differences due to changes in the society (eg, lower content of *trans* FAs in margarine). It was difficult to attract nonatopic mothers, which resulted in a small, highly selected group that differed from the atopic mothers in age and possibly also in factors such as lifestyle and socioeconomic characteristics. However, the FA composition of the breast milk from the nonatopic mothers matched previous observations in healthy Danish mothers (22). The atopic groups are limited by the fact that most of the mothers had more than one atopy diagnosis. More well-defined atopic groups potentially could have provided better information as to whether the type of atopy has any effect on the PUFA composition of breast milk. Furthermore, milk samples and dietary information were not collected for all of the COPSAC mothers. However, the results were verified in the entire group because excluded subjects did not differ from included subjects with respect to diet or the FA composition of



milk. In addition, similar differences were observed when all subjects were included in the group comparisons. A priori, it seems odd that the observed significant differences in the PUFA composition of milk were explained by diet, when no significant differences were observed in the diet. This seeming paradox may be explained by large variances in diet within the groups. Dietary intake varies considerably, and the applied retrospective FFQ method has limited sensitivity (26); both of these factors may in part explain why differences in such variables as fat intake ($\approx 10\%$ of energy) did not result in any significant difference between groups. There was a delay of ≈ 18 wk between the collection of dietary data and of milk samples in the COPSAC study. A closer coupling in time would probably result in better correlations. Some associations between diet and the FA composition of breast milk (especially for SFA, MUFA, and total PUFA content) were stronger when examined in the nonatopic group alone, in which collections were close together in time. The overall pattern of associations between diet and those milk PUFA components that differed between atopy groups was generally similar, although somewhat weaker in the nonatopic group, but there was no significant association between breast-milk $n-3$ LC-PUFAs and the intake of LC-PUFAs in the nonatopic group alone. This lack of association could be due to the large reduction in sample size, but it also may be explained by a narrower range in intakes combined with large day-to-day variations in the intake of these FAs. As expected, the associations between intake and milk content of $n-3$ LC-PUFAs are strong, even with the delay. Thus, the obtained dietary information seems to reflect the habitual diet in that sense. The observed differences in alcohol intake between the atopic and nonatopic mothers (although they were not significant) probably reflect the fact that the dietary information from atopic mothers is first collected during pregnancy. Comparison of atopic and nonatopic mothers gives a possibility of confounding-by-indication in this case by the use of fish-oil supplements. However, only a few of the atopic mothers used fish-oil supplements, and exclusion of these mothers from the statistical analysis did not change the overall results.

Atopic mothers appeared to eat more protein, less carbohydrate, and more fat (although the last 2 were not significant) than did nonatopic mothers. A high intake of fat and a low intake of carbohydrate have also been observed in atopic Finnish and Norwegian women (10, 28). Solvoll et al (10) found a higher intake of SFAs, lower intake of LC-PUFAs, and higher dietary $n-6:n-3$ PUFAs in women with AD than in nonatopic women. No significant differences were observed in the PUFA composition of the diets in the current study, but the reported differences in the PUFA composition of breast milk are in agreement with the dietary differences in the study by Solvoll et al. Intake of fat was found to be a contributing factor that, together with the intakes of energy and LC-PUFAs, influenced the PUFA composition of breast milk in the current study. All observed atopy-related differences in the PUFA composition of breast milk disappeared when the comparisons were controlled for diet.

Several studies have examined the FA composition of breast milk in relation to atopic sensitization in the children (8, 16, 27–34). Some of these studies find that a high $n-3$ LC-PUFA content (16, 27, 29), a low $n-6$ PUFA content (16), or low $n-6:n-3$ PUFAs (27) in breast milk is associated with lower atopic risk, although one study found that breast milk $n-3$ LC-PUFAs were associated with a greater risk of atopy (30). Other

studies have shown that other FA variables, eg, SFAs (29) and the content of $18:2n-6$ relative to the sum of its metabolites (31, 32), could play a role. Several studies have investigated the effect of PUFA supplementation in AD and have concluded that there is no relevant clinical effect (33). Most of these studies were performed in older children and adults, and all of these studies were observational in nature. To our knowledge, there have been only 3 randomized controlled trials—Mihirshahi et al (34), Dunstan et al (35), and Lauritzen et al (36)—in which infant immune function was investigated after fish-oil supplementation of pregnant women or breastfeeding mothers. Mihirshahi et al found that wheezing at 18 mo of age was significantly reduced in children after higher exposure to $n-3$ LC-PUFAs. The other 2 trials were small, and the examined immune functions were not directly related to atopic sensitization (35, 36s). Diet, and thus the FA composition of breast milk, reflects lifestyle and culture, which are passed on to the children. Therefore, associations in observational studies may be due to confounding factors. It is planned that COPSAC will investigate the extent to which the FA composition of breast milk influences the risk of atopy in the offspring, and possible influences from diet and lifestyle will also be included to contribute to an elucidation of the importance of all of these factors.

In conclusion, our data do not support a hypothesis that the FA composition of breast milk is affected by atopy or that it differs between mothers with various types of atopic diseases. Although differences were observed in the PUFA composition of breast milk from atopic and nonatopic mothers, these differences were small and appear to be explained by the diet. It is therefore important to include dietary assessment in future studies. Because of the small size of the nonatopic reference group and the delay between sampling of milk and diet in the current study, further studies are needed to ascertain whether breast milk is affected by atopy. Furthermore, large randomized controlled trials are necessary to elucidate whether differences in the PUFA composition of breast milk play a role in atopic sensitization in the offspring.

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HB designed and planned the COPSAC study and, together with LL and KFM, was responsible for the original idea and design of the current sub-study. LL was responsible for analysis of fatty composition in breast milk, statistical analysis, and writing of the manuscript. TBM and SFO were responsible for analysis of maternal FFQ. LBH and LLo were responsible for COPSAC data management. All authors were involved to some extent in data analysis and interpretation of the results, and all approved the final manuscript. None of the authors had any personal or financial conflict of interest.

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