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## Maternal nutritional determinants of colostrum fatty acids in the EDEN mother-child cohort

Martine Armand <sup>a,\*</sup>, Jonathan Y Bernard <sup>b,c</sup>, Anne Forhan <sup>b,c</sup>, Barbara Heude <sup>b,c,1</sup>, Marie-Aline Charles <sup>b,c,1</sup>, and the EDEN mother-child cohort study group

<sup>a</sup>*Aix Marseille Univ, CNRS, CRMBM, Marseille, France*

<sup>b</sup>*INSERM UMR1153 Centre of Research in Epidemiology and Statistics Sorbonne Paris Cité (CRESS), Developmental Origins of Health and Disease (ORCHAD) Team, F-94807 Villejuif, France*

<sup>c</sup>*Paris Descartes University, F-75006, Paris, France*

\* Corresponding author. CRMBM, UMR CNRS 7339, Faculté de Médecine Timone, 27 Bd Jean Moulin, 13385 Marseille cedex 05, France. E-mail address: [martine.armand@univ-amu.fr](mailto:martine.armand@univ-amu.fr)

<sup>1</sup> Equally contributed as the last authors.

Abbreviations used: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DGLA, dihomo  $\gamma$ -linolenic acid; DHA, docosahexaenoic acid; DNL, *de novo* lipogenesis; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; E, energy; EPA, eicosapentaenoic acid; GLA,  $\gamma$ -linolenic acid; LA, linoleic acid; LC-PUFA, long chain polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD1, Stearoyl-CoA-Desaturase 1; SFA, saturated fatty acids.

### SUMMARY

**Background & aims:** Programming of infant development and later health may depend on early-milk polyunsaturated fatty acids (PUFA) contents, that are very variable between women for reasons not well elucidated. Indeed, a high n-6/n-3 PUFA in milk was associated with higher adiposity, arterial pressure and lower psychomotor scores in childhood. We aimed to explore the respective contribution of several maternal and perinatal factors to the variability of linoleic (LA),  $\alpha$ -linolenic (ALA), arachidonic (AA), and docosahexaenoic (DHA) acid levels in early milk.

**Methods:** Fatty acids of 934 colostrum samples from the EDEN mother-child cohort were analysed by gas chromatography. The dietary intakes during the last trimester of pregnancy were estimated using a quantitative food frequency questionnaire. Relationship between milk PUFA and dietary fatty acids, and other maternal or pregnancy variables were analysed by multiple linear regression.

**Results:** The means ( $\pm$  SD) of colostrum LA, ALA, AA and DHA levels were, respectively,  $9.85 \pm 1.85$ ,  $0.65 \pm 0.22$ ,  $0.86 \pm 0.16$ , and  $0.64 \pm 0.19$  % of total fatty acids. Obese mothers colostrum contained the highest level of LA and AA and the lowest level of ALA and DHA. Colostrum LA, AA and DHA levels were higher in primiparous women. Mother's age was positively associated with colostrum AA and DHA. Dietary n-6 PUFA were associated with higher LA and lower DHA levels in colostrum, while dietary n-3 PUFA were related to higher LA and lower AA levels. Contrary to what was observed for DHA, AA level in colostrum was not related to its dietary intake. High dietary AA/DHA and total n-6/n-3 ratios were critical for the content of DHA in colostrum lipids.

**Conclusions:** Our study brings new insights in the understanding of the main maternal factors involved in PUFA levels variability in early milk. These data are important to consider for dietary counseling for women prior to and during pregnancy.

**Keywords:** maternal determinants, colostrum PUFA, pregnant women diet, BMI class, cohort study

## 1. Introduction

Environmental exposures during the first 1,000 days of life (from conception until 2 years of age), a critical window recently targeted by the World Health Organization (WHO), influences children growth, development, and later health. Early nutrition, and especially through mothers dietary intakes, is one of the essential exposure factors in the “Developmental Origin of Health and Diseases” (DOHaD) framework, first via nurturing the preimplantation embryo, then via the placental transfer of nutrients to the fetus, and lastly via human milk to breastfed infants [1-3].

Human milk is considered the most appropriate food for optimal development of the infant partly because of its n-6 and n-3 polyunsaturated fatty acid (PUFA) content and specifically, linoleic (LA) and arachidonic (AA) acids, and  $\alpha$ -linolenic (ALA) and docosahexaenoic (DHA) acids [4]. Indeed, PUFA are constituents of tissue cells membrane and display many regulatory functions as in the inflammatory process through metabolites or for the set-up of gene expression levels [4-6]. Therefore they are essential for general growth and the development of several key organs in the newborn, such as brain, cardiovascular system and intestine [4,7-10]. PUFA content in human milk is quite variable. It originates from several maternal sources (storage in white adipose tissue, long chain (LC) PUFA biosynthesis from the food precursors LA and ALA in liver), and is sensitive to mother’s diet [11,12], lactation period [13] and maternal overweight [14,15]. Although knowledge about the individual importance of these factors would be helpful to optimize PUFA human milk content, few studies have considered them, and none concomitantly, in a large number of mothers (available studies comprise from 5 to 180 women).

The “early milk”, i.e. colostrum, may be of considerable biological importance since it provides the initial supply of PUFA to the breastfed newborns. In the EDEN cohort, we previously reported a lower cognitive development in 2-3 year-old children when formerly breastfed by mothers whose colostrum had the higher LA levels (9.7-15.9% of total fatty acids) as compared to the lower LA levels (5.4-9.7%) [16]. In addition, the intelligence quotient at 5-6 years was lower for children previously fed colostrum with highest LA levels combined with lowest DHA (< 0.64%) and AA (< 0.86%) levels [17]. In this context, we aimed at better understanding the sources of variability of colostrum PUFA levels, especially the modifiable maternal and pregnancy factors, using data from the EDEN mother-child cohort [18].

## 2. Methods

### 2.1. Study population

The participants are part of the EDEN mother-child cohort study, a French prospective cohort designed to investigate the role of pre- and postnatal environmental factors involved in child development and health. Recruitment started in 2003 in the university hospitals of Poitiers (mid-West part of France) and Nancy (North-East of France) and ended up in 2006 with the inclusion of 2,002 mothers. A detailed protocol of the EDEN cohort study has been previously published [18]. Briefly, the cohort consisted of pregnant women enrolled before 24 wk of gestation and of their newborn (95% full-term) whom development has been followed through numerous parameters until the age of 10 y. For the present study, we focused on 934 women (flowchart on **Fig. 1**) with available data on fatty acids in colostrum. The local ethics research committee (CCPP for biomedical research, Bicêtre Hospital) and the Data Protection Authority (CNIL) approved the study. Informed written consents were obtained from the mother for herself at inclusion.

### 2.2. Colostrum sampling and analysis of the fatty acid composition

Colostrum samples of about 5 mL were collected at the hospitals between 2 and 5 days after delivery (Mean  $\pm$  SD: 3.9  $\pm$  1.1 d) during one feed by manual expression and were stored

at  $-80^{\circ}\text{C}$  until analysis. A direct methylation procedure was performed on  $100\ \mu\text{L}$  of colostrum at  $100^{\circ}\text{C}$  for 1 h with methanol/hexane (4:1, v/v) and acetyl chloride by adapting a published method [19]. This quicker procedure was compared to a more time consuming method routinely used in the laboratory (milk lipid extraction using chloroform/methanol 2:1 followed by methylation using boron trifluoride [10]) and showed comparable results (data not shown). Fatty acid methyl-esters (FAME) were analyzed by gas chromatography (Perkin Elmer Clarus 600, flame ionization detector, Totalchrom software, hydrogen as gas carrier) using a fused silica capillary fast column (BPX 70, 10 m x 0.1 mm i.d., 0.2  $\mu\text{m}$  film thickness, Sigma-Supelco). The initial oven temperature was set at  $60^{\circ}\text{C}$ , raised to  $200^{\circ}\text{C}$  at  $20^{\circ}\text{C}/\text{min}$ , then to  $225^{\circ}\text{C}$  at  $7^{\circ}\text{C}/\text{min}$  with one min hold, and to  $250^{\circ}\text{C}$  at  $160^{\circ}\text{C}/\text{min}$  with one min hold. The control of the proportionality between FAME peaks area (in  $\mu\text{V}\cdot\text{sec}$ ), and their identification based on their retention time, were performed using an external calibrator FAME standard (GLC 674, Nu-Chek Prep, Waterville, NM, USA). Each Fatty acid level was expressed as the proportion of total fatty acids present in the chromatogram (weight percent). Intra-assay reproducibility of the direct method was obtained by repeated analysis of three different human milk samples (5 or 10 aliquots each). Coefficients of variability for fatty acids contributing for more than 1% or for 0.1-1% of total fatty acids were comprised from 0.12 to 1.48 or from 0.84 to 2.28, respectively. Internal standard (tridecanoic acid, Sigma-Fluka) was added to quantify total lipid content. We further calculated the sum or ratios of specific fatty acids as surrogate indexes of *de novo* lipogenesis (DNL) in the liver (sum of 16:0 + 16:1n-7 + 18:1 n-9 + 20:1 n-9), of DNL in the breast (sum of 10:0 + 12:0 + 14:0 + 14:1), of Stearoyl-CoA-Desaturase 1 (SCD1) i.e.  $\Delta 9$ -desaturase activity (SCD1-16 product/substrate ratio: 16:1 n-7/16:0; SCD1-18: 18:1 n-9/18:0), of  $\Delta 5$ -desaturase activity (20:4 n-6/20:3 n-6 i.e. AA/DGLA) and of  $\Delta 6$ -desaturase activity (20:3 n-6/18:2 n-6 i.e. DGLA/LA), as previously published for milk [20,21]. The DHA Sufficiency Index was also calculated (22:6 n-3/22:5 n-6 i.e. DHA/n-6 DPA) [22].

### 2.3. Dietary assessment

A food-frequency questionnaire (FFQ), adapted from a validated one [23], based on 137 different foods or food groups combined with pictures from 12 food types for portion size evaluation, was completed by the women to assess their usual diet during the third trimester of pregnancy, as previously described [24]. Total energy, protein, carbohydrate, lipid and alcohol intakes were calculated by using the SU.VI.MAX nutrient database, whereby the majority of the nutrient information comes from French nutrient composition database [24]. The 137 items were therefore clustered into 33 food groups according to their nature (e.g. Meat, Eggs, Poultry, Fish & Seafood, Cheese, Fruits, Vegetables, etc).

Except for some cooked dishes (ready meals or dishes with well-known recipes), the nutrient composition of food items was assessed without potential added fat. A specific food group named "Added-Fat after cooking" inquired about the frequency of added fat (butter, margarine, cream), and another one called "Sauces" about added sauces (dressing, ketchup, mayonnaise), in the plate. Intake of different fatty acids including LA, ALA, AA, DHA, eicosapentanoic acid (EPA) and n-3 docosapentaenoic acid (n-3 DPA) was calculated from the nutrient composition table and by summation within a same food group. We therefore calculated the respective contribution (percentage) of each food group to the total maternal intake of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), total PUFA, total n-6 PUFA, total n-3 PUFA, LA, ALA, AA, EPA, n-3 DPA and DHA.

In addition, mothers were asked about their habits in fat use for cooking, frying or seasoning (butter, margarine, coconut butter, and different types of vegetable oil). Oils were combined into three groups according to their richness in n-3 PUFA (colza, mixtures), in n-6 PUFA

(sunflower, corn) or in MUFA (peanut, olive). Women were therefore categorized into users vs. non-users for each type of added fat and each group of vegetable oil.

#### 2.4. Other collected data

Women declared their weight just before pregnancy, and midwives measured mothers height (wall Seca 206 stadiometer, Hamburg Germany) during the first visit scheduled between 24 and 28 weeks of amenorrhea. Pre-pregnancy BMI was calculated (weight kg/height m<sup>2</sup>), and categorized according to the WHO four classes: underweight (BMI < 18.5), normal weight (18.5-24.9), overweight (25-29.9) and obesity (BMI ≥ 30). Gestational age at delivery was determined from the date of the last menstrual period and early standard ultrasound fetal measurement. Maternal age at delivery, parity and newborn sex were extracted from clinical records. Smoking habits during pregnancy were collected from self-administered questionnaire during pregnancy and after delivery, and women were qualified as smokers or not smokers during pregnancy. Maternal education level was estimated based on the highest diploma obtained and expressed in years of attended school.

#### 2.5. Statistical analyses

Data are means ± SD unless otherwise indications. The impact of the number of days between birth and colostrum sampling (referred as “sampling day” throughout the paper) on fatty acid colostrum level was investigated by Spearman’s correlation coefficients. For each fatty acid, the colostrum level was compared between BMI categories using multivariable linear regression analysis adjusted for center and sampling day.

Correlations between fatty acids intake during pregnancy (expressed as % of total lipid intake) and fatty acids in colostrum (expressed as % of total fatty acids) were investigated using partial Spearman’s correlation coefficients after adjustment for center and sampling day. Because of the existence of strong correlations between the different fatty acids, we did not perform any correction for multiple testing.

We then focused on the levels of LA, ALA, DHA and AA in colostrum to explore their main independent determinants in multivariable linear regression models. Their values were standardized in order to compare the effect size of the different contributors between models. All models initially included center, sampling day, mother’s age at delivery, gestational age at delivery, pre-pregnancy BMI categories, primiparous status, smoking, maternal education level, main food groups contributors, potential competitors intake (n-3 PUFA intake for LA and AA, n-6 PUFA intake for ALA and DHA), and Added-Fat intake (for cooking and after cooking). The main food contributors were those accounting for about 33% of the total intake for ALA and LA and for about 55% of total intake for DHA and AA (“Cheese”, “Added-fats”, and “Fresh/dried fruits” for ALA, “Fish and seafood” for DHA, “Sauces” and “Bread” for LA, “Eggs and meats” regrouping intake from “Eggs”, “Poultry” and “Meats” groups for AA). Factors with P-values over 15% were excluded from the models using backward selections.

All analyses were performed with SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA) and significance was assumed at  $P < 0.05$ .

### 3. Results

#### 3.1. Descriptive results

Main characteristics of the population are reported in **Table 1**.

**Table 2** displays the colostrum fatty acid levels in the whole study population, and shows that sampling day was positively correlated with LA and ALA levels, and negatively with most of their derivatives LC-PUFA, and with LC-MUFA levels including nervonic acid (24:1 n-9).

**Table 3** shows the women’s dietary intake during the last trimester of pregnancy and information on fatty acid food sources. The item “Sauces” was the main food group

contributing to LA intake, and “Eggs, Meats & Poultry” were the main sources of AA intake. The two main sources of ALA were “Cheese” and “Added-Fat after cooking”, and the main dietary contributors to DHA maternal intake were in decreasing order “Fish and seafood”, “Fast foods” (i.e. hamburger, pizza, sandwich, savory pie, toasties and cheese sandwich, puff pastry roll), and “Eggs”.

### 3.2. Relationship between colostrum fatty acid levels and maternal pre-pregnancy BMI

Total SFA in colostrum, and especially the pentadecanoic acid (15:0), palmitic acid and stearic acid (18:0), displayed the strongest negative associations with pre-pregnancy BMI (**Table 4**). On the opposite, total MUFA (mainly palmitoleic acid i.e. 16:1 n-7, 16:1 n-9, and oleic acid) and total n-6 PUFA (mainly LA, dihomo  $\gamma$ -linolenic (DGLA), AA and docosatetraenoic acid (DTA)) levels were positively associated with BMI categories. Among n-3 PUFA, only the DPA showed a positive association with BMI, whereas total n-3 PUFA, ALA and DHA displayed inverse U-shaped associations, the highest values being observed in women with normal BMI. LA/ALA ratio was positively associated with increasing BMI categories. AA/DHA and total n-6/total n-3 PUFA ratios followed an U-shaped association with BMI, i.e. women with normal pre-pregnancy BMI had the lowest ratios and obese mothers the highest. The colostrum DHA Sufficiency Index followed an inverse U-shaped association with BMI. The colostrum indexes for DNL-liver, and for SCD1-16, SCD1-18 and  $\Delta$ 6-desaturase enzymatic activities were all positively associated with BMI, while indexes for DNL-breast and for  $\Delta$ 5-desaturase (P-trend 0.053) enzymatic activity were negatively associated with it.

### 3.3. Correlations between fatty acids dietary intake during the last trimester of pregnancy and colostrum fatty acid levels

**Table 5** shows that, after adjusting for center and sampling day, the correlations between maternal intake in a given fatty acid and the corresponding fatty acid in colostrum were in general positive, the strongest correlations being for DHA and EPA. Noticeable exception was observed for AA with no correlation. Regarding ratios, the correlation was strong for AA/DHA, and moderate for total n-6/n-3 PUFA. Correlations between different fatty acid families were noticeable: maternal dietary SFA were negatively associated with colostrum PUFA (especially LA and ALA), and similarly total dietary PUFA (total n-6 and n-3 PUFA) were negatively associated with milk SFA; dietary MUFA were positively associated with colostrum n-6 PUFA, and especially LA, and negatively with colostrum DHA, leading to positive correlations with AA/DHA and total n-6/n-3 PUFA ratios. ALA intake by mothers correlated moderately with EPA and DHA in milk. Dietary AA/DHA and total n-6/n-3 PUFA were negatively correlated with the content in EPA, DPA n-3 and DHA in colostrum lipids.

### 3.4. Respective contribution of maternal factors to ALA, DHA, LA and AA levels in colostrum

Results of the multivariable linear regression models explaining ALA and DHA, and LA and AA colostrum levels variability are shown in **Tables 6** and **7**, respectively.

The strongest contributors of ALA levels in colostrum, showing a positive association, were sampling day and n-6 PUFA intake, then use of MUFA-rich oils (i.e. olive and peanut oils) and mother's age at delivery. Main ALA contributors with a negative association were the use of coconut butter (rich in SFA) and pre-pregnancy overweight status. The strongest predictor of DHA was fish and seafood intake with an increase of 0.039 SD of the DHA level for one additional serving per month. The relationship between dietary intake of DHA and DHA level in colostrum was, however, not strictly proportional (**Fig. 2**). Maternal age at delivery and primiparous status also significantly contributed to the level of DHA in colostrum through a positive association, while sampling day was negatively associated. Abnormal pre-pregnancy

BMI and smoking tended to be predictors of lower DHA level in colostrum. All together, these factors explained 16.3% of colostrum DHA content variability.

The consumption of n-6 PUFA-rich vegetable oils (i.e., sunflower and corn oils) was the main contributor of LA levels in colostrum, followed by pre-pregnancy obesity and primiparous status, all showing a positive association. Predictors with a negative association were an underweight status before pregnancy and energy intake. The most important contributors to AA colostrum level were sampling day (negative association) and maternal pre-pregnancy BMI (negative association with underweight status and positive association with overweight/obese status, when normal BMI class was taken as a reference). Colostrum AA level was also positively associated with maternal age and primiparous status, and was lower in mothers who declared using MUFA-rich oil. The variables included in this model explained 13.5% of the colostrum AA content variability. Alcohol consumption was not significantly associated with the colostrum PUFA levels (data not shown). Nevertheless, taken all together the factors we explored explained only a part of the PUFA variability (i.e. 5 to 16% of variance explained).

#### 4. Discussion

In the EDEN mother-child cohort, we found several maternal (BMI, age, diet, smoking status) or perinatal (parity, lactation period) factors independently associated with LA, ALA, AA and DHA levels in early milk. Albeit the factors we explored showed a modest overall contribution to the PUFA variation in colostrum, the knowledge of their respective impact is valuable due to recent associations reported between early/mature milk PUFA levels and offspring outcomes later in life. Indeed, high LA level and high LA/ALA and total n-6/n-3 PUFA ratios in milk were found correlated to lower psychomotor scores at 14-18 months of age [25,26] or at 2-3 or 5-6 years old [16,17], a negative correlation was reported between milk DHA level (from 0.34 to 0.59% of total fatty acids) and fat mass in girls of 6-9 years old [27], and diastolic and systolic pressure was lower in 12 years old infants previously breastfed with a milk containing at least 0.51% of n-3 PUFA [9].

The rapid changes in colostrum PUFA profile over time after birth are consistent with other observations reported between day 3 and day 5 [28]. This factor turned out to be the strongest predictor of the variability of the colostrum AA level while it explained only about 10% of the total variance for DHA level. This is probably linked to the mobilization of fatty acids stored in the maternal white adipose tissue, which was reported to be richer in AA than DHA [29].

Pre-pregnancy BMI represented another important predictor of PUFA levels in early milk. Colostrum of obese mothers had the highest level of n-6 PUFA and lower levels of ALA and DHA, indicating a worse balanced PUFA profile (higher LA/ALA, AA/DHA and total n-6/n-3 PUFA ratios) than women with lower pre-pregnancy BMI. This observation is consistent with what was reported in the colostrum of Swedish obese mothers [14], although the n-3 PUFA difference between obese and normal women was lower in our study. A higher level in LA was also reported in mature milk from Argentine obese mothers [15]. All these PUFA variations due to high pre-pregnancy BMI may worsen during lactation by being more pronounced in mature milk [14,15]. A novelty raised herein is that being underweight before pregnancy was associated with lower DHA level to the same extent than obesity, leading to higher total n-6/n-3 PUFA and AA/DHA ratios. Interestingly the average intake in DHA during the last trimester of pregnancy in each BMI class subgroup was similar (0.17-0.18 g/d) and so did not seem to intervene in these differences. In contrast, colostrum fatty acids indexes orientated towards specific maternal metabolic anomalies. Indeed, increase in DNL liver index together with a decrease in DNL breast index, an increase in SCD1-16, SCD1-18 and  $\Delta 6$ -desaturase indexes,

and a decrease in  $\Delta 5$ -desaturase index, were evocative of the known decrease in insulin sensitivity associated with increasing BMI [20,30]. Furthermore, a recent meta-analysis highlighted a high plasma lipids DGLA level in overweight/obese subjects as a very relevant biomarker in the pathogenesis of obesity, that reflects disturbances in  $\Delta 6$  (elevated through DGLA/LA ratio) and  $\Delta 5$  (decreased through AA/DGLA ratio) desaturases activities [31]. We found that this altered LC-PUFA status also characterized the fatty acid profile of early milk from overweight/obese mothers. Since foods DGLA content is very low, the increased level of DGLA in the colostrum of obese women may reflect an increased conversion rate of LA and a decreased conversion rate of DGLA in AA, as reported by others in plasma lipids [31]. The lower  $\Delta 5$ -desaturase activity consecutive to adiposity [32] can explain the lower DHA level in their colostrum, while the higher AA level (as well as the higher LA level) could be due to a higher supply from the elevated amount of white adipose tissue, since a higher storage of AA was reported to correlate with obesity and metabolic dysregulations [31]. This explains the consistent linear relationship between colostrum AA levels and pre-pregnancy BMI. The lower colostrum DHA level in underweight women could be linked to a lower DHA status of these women as suggested by their lower milk DHA sufficiency index.

Maternal diet is known to influence milk fatty acids composition, however, the share attributable to diet is less documented [11,12,33]. The inverse relationship we report between mother dietary SFA and PUFA in milk is consistent with others [12,33]. In addition, a specific source of SFA (coconut butter) led to low ALA level in our colostrums samples. We confirm a positive association between dietary MUFA and colostrum PUFA level [12], especially with LA, and to a lesser extent with ALA. MUFA oil was one contributor influencing n-6 and n-3 PUFA in colostrum probably because of the existing competition between the n-9 (via oleic acid), n-6 (via LA as precursor) and n-3 (via ALA as precursor) pathways for the same desaturase and elongase enzymes in the body. The colostrum of mothers declaring using MUFA oil was thus higher in ALA and lower in AA possibly because of a lower conversion rate of ALA into DHA and of LA into AA. Due to competition, dietary n-6 fatty acids were predictive of a higher ALA and a lower DHA levels in colostrum, while dietary n-3 fatty acids would be associated with higher LA and lower AA levels. Moreover, while the daily dietary intake of LA was high (mainly from sauces and the use of n-6 PUFA oils), it correlated moderately to LA level in colostrum. The daily dietary supply in AA (mainly from meat, chicken and eggs) was close to the DHA supply (mainly from fish and seafood), but the AA level in early milk was not related to the dietary intake [33], whereas the DHA intake showed the highest association with colostrum level [33]. The moderate relationship we found between the dietary LA and its level in colostrum is consistent with a study estimating that about 30% of dietary LA is transferred into milk lipids [34]. In addition, the AA converted from dietary LA represents about 1% of total milk AA secreted [34]. It means that a major proportion of LA and AA of milk lipids derives from storage in the adipose tissues [29]. A strong positive association between fish and other seafood consumption and DHA in milk has been known for long [35], but herein the association was moderately high ( $Rho = 0.29$ ,  $P < 0.0001$ ) and not proportional to the amount of DHA intake (from 19 to 735 mg/d) in accordance with the literature [11,12]. This can be explained by the fact that DHA in early milk is likely to originate from many sources i.e. diet, mobilization from adipose tissue storage and biosynthesis from dietary ALA ( $Rho = 0.13$ ,  $P < 0.0001$ , herein). In addition, maternal variants of genes involved in LC-PUFA biosynthesis such as the FADS1/FADS2 cluster [36-38], also capable of modifying the association between fish intake and DHA level in milk [37], and the ELOVLs [21], are powerful factors controlling the levels of AA and DHA in milk.

Other maternal factors were found independently associated with ALA, DHA, LA or AA levels in colostrum. Primiparous status was one of them possibly because optimal essential fatty acids and LC-PUFA maternal status is not attained after several pregnancies [22]. Maternal age could explain AA and DHA levels through an increased in  $\Delta 5$ -desaturase activity with age [32]. The negative association of smoking with colostrum DHA, but not AA level, could be due to a decreased conversion rate of ALA via a specific inhibition of the  $\Delta 5$  desaturation by toxic xenobiotics [39].

Some limitations and strengths of our study can be pointed out. A first limitation is the collection of a fraction of colostrum (5 mL) taken during breast-feeding instead of a sample from a total production (i.e. from 15-60 mL). However, the colostrum fatty acids profile is quite homogeneous throughout a single production, at the contrary of mature milk (fore versus hind milk), and the analysis of sample collected any time during suckling is representative of the entire produced colostrum [28]. Another limitation is the collection of colostrum at the maternity unit where mothers received a standard hospital diet instead of following their usual diet. However, it is more likely that the high inter-individual variability obtained resulted from the impregnation of the personal diet and maternal status rather than of the homogeneous food received during the few days of hospitalization [12]. The main strength of this study is to rely on a large sample of women through the use of the Eden cohort's database, which renders these results very robust.

In conclusion, our study brings new insights in the understanding of the main maternal factors involved in PUFA levels variability in early milk. These data are important to consider for dietary counseling for women prior to and during pregnancy. For instance, a normal pre-pregnancy BMI and a better choice in dietary fat sources for improving PUFA's storage quality and biosynthesis, represent two main targets to achieve for improving the PUFA status in early milk.

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### **Statement of authorship**

MA: colostrum samples analysis, data folders; JYB, BH, and AF: dietary records analysis, statistical analyses; MAC and BH: principal investigators of the EDEN mother-child cohort

study; MA, JYB, AF, BH, MAC: interpretation of the data and critical revision of the manuscript for important intellectual content; MA and BH: writing of the manuscript. All authors read and approved the final manuscript.

### Conflict of interest

None of the authors declared a conflict of interest.

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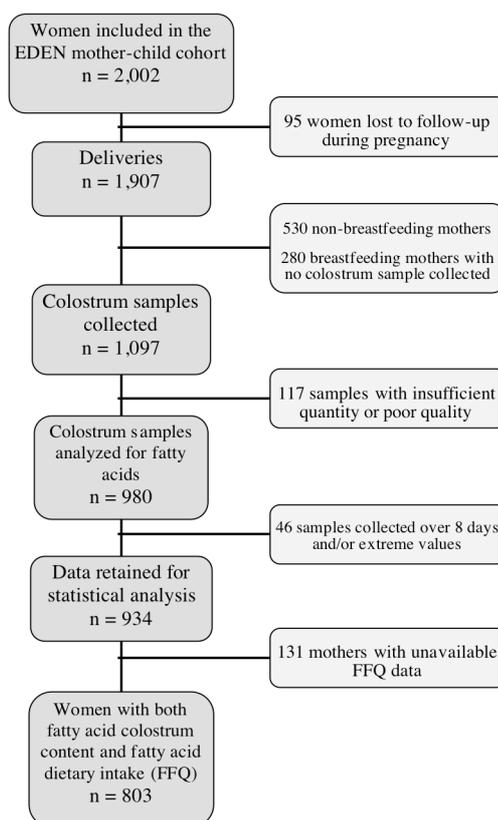
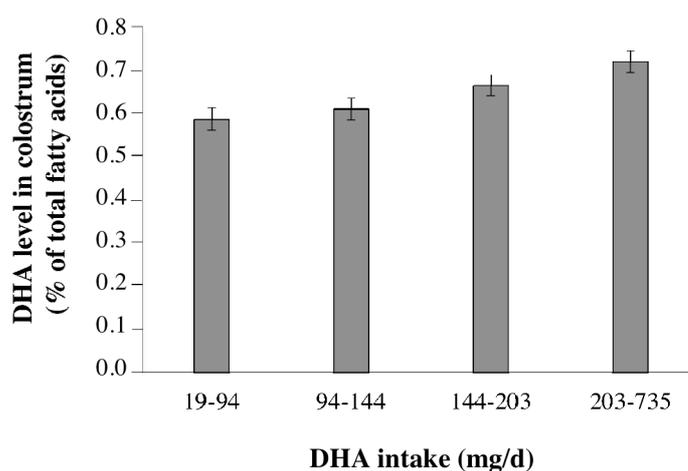


Fig. 1.

**Fig. 1.** Flowchart of selected women for whom colostrum fatty acid content was analyzed and who answered the Food Frequency Questionnaires within the EDEN mother-child cohort study.



**Fig. 2.** Colostrum DHA level (mean  $\pm$  SEM; expressed as % of total fatty acids) according to quartiles of DHA maternal intake during pregnancy in the EDEN cohort ( $n = 802$ ). The y-axis represents least-square means after adjustment for BMI (4 classes), center of recruitment, sampling day, primiparous status, mothers' age at delivery, gestational age at delivery, smoking during pregnancy, total n-6 PUFA intake, fish and seafood intake. P-trend was  $< 0.001$ .

## TABLES

**Table 1:** Characteristics of the population from the EDEN mother-child cohort study (n = 934).

Variables	% (n)	or	Mean $\pm$ SD
Center (Poitiers)	33.6 (314)		
Mother's age at delivery, y			29.6 $\pm$ 4.7
Gestational age at delivery, wks			39.3 $\pm$ 1.6
Primiparous (yes)	45.6 (426)		
BMI, kg/m <sup>2</sup>			22.8 $\pm$ 4.3
Underweight	10.0 (93)		
Normal	68.5 (640)		
Overweight	14.5 (135)		
Obese	7.1 (66)		
Smoker	23.1 (216)		
Maternal education, y			14.1 $\pm$ 2.6
Sampling day, d			3.9 $\pm$ 1.1
Type of fat used for cooking/seasoning <sup>1</sup>			
Butter (yes)	19.1 (154)		
Margarine (yes)	25.9 (208)		
Coconut butter (yes)	27.2 (218)		
n-3 PUFA oil (yes)	27.9 (224)		
n-6 PUFA oil (yes)	40.6 (326)		
MUFA oil (yes)	58.3 (468)		
Sauces and bread intake <sup>1</sup> , time per mo			85.7 $\pm$ 37.4
Cheeses, added-fat, fresh/dried fruits <sup>1</sup> , time per mo			114.0 $\pm$ 71.3
Fish & seafood intake <sup>a</sup> , time per mo			8.6 $\pm$ 6.9
Meat/Poultry/Eggs intake <sup>a</sup> , time per mo			13.5 $\pm$ 11.5

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

<sup>a</sup>n = 803 for dietary intakes variables.

**Table 2:** Colostrum total lipid content and fatty acid levels, and correlation with sampling day in the EDEN cohort<sup>a</sup>.

Lipids	All samples (n = 934)	Range	Correlation with sampling day <sup>b</sup>
<b>Total lipid g/L</b>	23.31 ± 12.15	3.1 - 91.6	0.09**
<b>SFA</b>	44.85 ± 3.59	34.8 - 57.6	-0.03
10:0	1.87 ± 1.63	0 - 19.2	0.01
12:0	3.07 ± 1.21	0.7 - 8.3	0.40***
14:0	6.38 ± 1.45	2.8 - 16	0.03
15:0	0.42 ± 0.07	0 - 0.7	-0.01
16:0	26.12 ± 1.82	20.4 - 33.5	-0.19***
17:0	0.28 ± 0.05	0.1 - 0.4	0.12***
18:0	6.72 ± 1.06	3.6 - 10.6	-0.19***
<b>MUFA</b>	40.90 ± 2.89	28.6 - 51.3	0.05
14:1 n-5	0.28 ± 0.07	0.1 - 0.5	0.16***
15:1	0.05 ± 0.03	0 - 0.2	-0.18***
16:1 n-9	0.59 ± 0.13	0 - 0.9	-0.11**
16:1 n-7	2.39 ± 0.66	0.8 - 5.1	0.20***
18:1 n-9	36.32 ± 2.63	24.4 - 47.5	0.04
20:1 n-9	0.96 ± 0.42	0 - 12.3	-0.34***
24:1 n-9	0.31 ± 0.09	0 - 0.8	-0.41***
<b>n-6 PUFA</b>	12.20 ± 1.92	7.8 - 19.0	-0.02
18:2 n-6 (LA)	9.85 ± 1.85	5.4 - 16.8	0.07*
18:3 n-6 (GLA)	0.12 ± 0.05	0 - 0.4	0.11**
20:3 n-6 (DGLA)	0.61 ± 0.16	0.3 - 1.2	-0.14***
20:4 n-6 (AA)	0.86 ± 0.16	0.4 - 1.5	-0.24***
22:4 n-6 (DTA)	0.55 ± 0.18	0 - 1.3	-0.46***
22:5 n-6 (DPA)	0.11 ± 0.04	0 - 0.3	-0.27***
<b>n-3 PUFA</b>	2.05 ± 0.41	0.8 - 3.5	-0.07*
18:3 n-3 (ALA)	0.65 ± 0.22	0.3 - 1.6	0.17***
18:4 n-3	0.28 ± 0.07	0 - 0.5	-0.15***
20:4 n-3	0.24 ± 0.07	0.1 - 0.6	-0.24***
20:5 n-3 (EPA)	0.06 ± 0.03	0 - 0.2	0.11**
22:5 n-3 (DPA)	0.28 ± 0.10	0.1 - 0.6	-0.26***
22:6 n-3 (DHA)	0.64 ± 0.19	0.2 - 1.5	-0.12***
<b>Ratio</b>			
LA/ALA	16.58 ± 5.47	6.4 - 36.7	0.04
AA/DHA	1.43 ± 0.38	0.6 - 3.4	-0.13***
Total n-6/n-3 PUFA	6.18 ± 1.52	3.3 - 13.1	-0.05

Abbreviations: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DGLA, dihomo  $\gamma$ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; GLA,  $\gamma$ -linolenic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. <sup>a</sup> Data are means  $\pm$  SD and range (minimal and maximal values), and fatty acid levels are expressed as % total fatty acids. <sup>b</sup> Spearman's correlation coefficients between fatty acid and sampling day; \* <0.05, \*\* <0.01, \*\*\* <0.001.

**Table 3:** Maternal macronutrient and fatty acid intakes during the last trimester of pregnancy and description of lipid main food group sources in the EDEN cohort (n = 803)<sup>a</sup>.

Nutritional factors	Daily intakes (m ± SD [min-max]) or food group contribution (%)	
Total energy intake, kcal/d <sup>b</sup>	2231 ± 726	[1003 - 4929]
Total energy intake, kJ/d	9327 ± 3035	[4192 - 20604]
Proteins, E%	16.6 ± 3.1	[7.4 - 31.2]
Carbohydrates, E%	43.6 ± 7.5	[19.9 - 70.3]
Lipids, E%	39.3 ± 6.6	[21.8 - 62.4]
SFA, E%	18.0 ± 3.7	[7.9 - 33.1]
SFA, g/d	45.2 ± 19.7	[13.6 - 150.4]
Cheese, SFA%		21.0
Added-Fat <sup>c</sup> , SFA%		16.6
Fermented milk, SFA%		8.1
Fast Foods, SFA%		6.2
Cakes, SFA%		6.2
MUFA, E%	14.2 ± 2.6	[7.3 - 24.9]
MUFA, g/d	35.4 ± 14.4	[11.0 - 124.6]
Cheese, MUFA%		13.4
Added-Fat, MUFA %		12.1
Sauces <sup>d</sup> , MUFA %		10.1
Meats, MUFA %		8.5
PUFA, E%	4.7 ± 1.2	[2.1 - 10.6]
PUFA, g/d	11.6 ± 5.0	[3.1 - 38.9]
Sauces, PUFA%		21.8
Bread, PUFA%		8.0
Snacks, PUFA%		6.2
Added-Fat, PUFA%		5.9
Cakes, PUFA%		5.7
Fast Foods, PUFA%		5.6
PUFA n-6, E%	4.0 ± 1.2	[1.6 - 9.6]
PUFA n-6, g/d	9.9 ± 4.5	[2.3 - 34.4]
LA, E%	3.9 ± 1.2	[1.6 - 9.5]
LA, g/d	9.7 ± 4.5	[2.3 - 33.8]
Sauces, LA%		25.1
Bread, LA%		8.4
Snacks, LA%		6.9
Cakes, LA%		5.8
Added-Fat, LA%		5.6
Fast Foods, LA%		5.4
AA, E%	0.06 ± 0.03	[0.02 - 0.21]
AA, g/d	0.15 ± 0.08	[0.02 - 0.66]
Eggs, AA%		19.3
Meats, AA%		16.8
Poultry, AA%		14.6

Fishes & seafoods, AA%	11.2	
PUFA n-3, E%	0.46 ± 0.10	[0.17 - 0.85]
PUFA n-3, g/d	1.15 ± 0.45	[0.32 - 2.89]
ALA, E%	0.36 ± 0.06	[0.15 - 0.67]
ALA, g/d	0.89 ± 0.35	[0.27 - 2.30]
Cheese, ALA%	13.5	
Added-Fat, ALA%	11.9	
Fresh/dried fruits, ALA%	7.2	
EPA, E%	0.03 ± 0.02	[0.00 - 0.16]
EPA, g/d	0.08 ± 0.06	[0.01 - 0.39]
Fishes & seafoods, EPA%	66.5	
Fast Foods, EPA%	15.3	
Meats, EPA%	9.5	
DPA, E%	0.02 ± 0.01	[0.003 - 0.07]
DPA, g/d	0.04 ± 0.02	[0.007 - 0.23]
Fishes & seafoods, DPA%	30.7	
Meats, DPA%	19.6	
DHA, E%	0.07 ± 0.05	[0.01 - 0.33]
DHA, g/d	0.17 ± 0.12	[0.02 - 0.73]
Fishes & seafoods, DHA%	56.1	
Fast Foods, DHA%	13.5	
Eggs, DHA%	11.9	
LA/ALA	10.99 ± 2.71	[3.78 - 20.51]
AA/DHA	1.06 ± 0.53	[0.2 - 4.2]
Total n-6/n-3 PUFA	8.74 ± 2.43	[3.19 - 17.56]

Abbreviations: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid; n-3 DPA, n-3 Docosapentaenoic acid; E, energy; EPA, eicosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

<sup>a</sup>Data are means ± SD [min-max] or percentages.

<sup>b</sup>Alcohol consumption represents 0.53% ± 1.6% of total energy intake.

<sup>c</sup>“Added-Fat” corresponds to any fat added after cooking by the mother in the plate (butter, margarine, cream).

<sup>d</sup>“Sauces” corresponds to any fat added by the mother in the plate (dressing, ketchup, mayonnaise).

**Table 4:** Total lipid content, fatty acid levels and fatty acid indexes for enzymatic activities of colostrum according to maternal pre-pregnancy BMI categories in the EDEN cohort (n = 934)<sup>a,b</sup>.

Lipids	Underweight (n = 93)	Normal (n = 640)	Overweight (n = 135)	Obese (n = 66)	P
<b>Total lipid g/L</b>	22.81 ± 1.26	23.35 ± 0.48	23.07 ± 1.05	24.10 ± 1.51	0.9
<b>SFA</b>	46.27 ± 0.37	45.01 ± 0.14	43.97 ± 0.3	43.09 ± 0.44	<.0001
10:0	2.09 ± 0.16	1.88 ± 0.06	1.75 ± 0.14	1.69 ± 0.20	0.32
12:0	3.32 ± 0.11	3.06 ± 0.04	2.94 ± 0.09	3.06 ± 0.13	0.07
14:0	6.77 ± 0.15	6.37 ± 0.06	6.14 ± 0.12	6.31 ± 0.18	0.01
15:0	0.43 ± 0.01	0.43 ± 0.00	0.40 ± 0.01	0.38 ± 0.01	<.0001
16:0	26.40 ± 0.18	26.21 ± 0.07	25.95 ± 0.15	25.30 ± 0.22	0.0003
17:0	0.27 ± 0.01	0.28 ± 0.00	0.28 ± 0.00	0.27 ± 0.01	0.7
18:0	6.99 ± 0.11	6.80 ± 0.04	6.51 ± 0.09	6.07 ± 0.13	<.0001
<b>MUFA</b>	39.94 ± 0.29	40.76 ± 0.11	41.74 ± 0.24	41.94 ± 0.35	<.0001
14:1 n-5	0.29 ± 0.01	0.28 ± 0.00	0.27 ± 0.01	0.26 ± 0.01	0.02
15:1	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.8
16:1 n-9	0.56 ± 0.01	0.58 ± 0.00	0.61 ± 0.01	0.62 ± 0.01	0.001
16:1 n-7	2.25 ± 0.07	2.37 ± 0.03	2.55 ± 0.05	2.55 ± 0.08	0.001
18:1 n-9	35.53 ± 0.27	36.20 ± 0.10	37.00 ± 0.22	37.20 ± 0.32	<.0001
20:1 n-9	0.95 ± 0.04	0.97 ± 0.02	0.95 ± 0.04	0.94 ± 0.05	0.9
24:1 n-9	0.31 ± 0.01	0.31 ± 0.00	0.31 ± 0.01	0.32 ± 0.01	0.8
<b>n-6 PUFA</b>	11.81 ± 0.20	12.15 ± 0.08	12.29 ± 0.16	12.98 ± 0.24	0.001
18:2 n-6 (LA)	9.56 ± 0.19	9.83 ± 0.07	9.84 ± 0.16	10.39 ± 0.23	0.05
18:3 n-6 (GLA)	0.13 ± 0.01	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.01	0.5
20:3 n-6 (DGLA)	0.57 ± 0.02	0.60 ± 0.01	0.64 ± 0.01	0.69 ± 0.02	<.0001
20:4 n-6 (AA)	0.82 ± 0.02	0.85 ± 0.01	0.90 ± 0.01	0.93 ± 0.02	<.0001
22:4 n-6 (DTA)	0.51 ± 0.02	0.54 ± 0.01	0.58 ± 0.01	0.63 ± 0.02	<.0001
22:5 n-6 (DPA)	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.6
<b>n-3 PUFA</b>	1.98 ± 0.04	2.08 ± 0.02	2.00 ± 0.04	1.99 ± 0.05	0.03
18:3 n-3 (ALA)	0.65 ± 0.02	0.66 ± 0.01	0.60 ± 0.02	0.63 ± 0.03	0.02
18:4 n-3	0.28 ± 0.01	0.28 ± 0.00	0.27 ± 0.01	0.25 ± 0.01	0.001
20:4 n-3	0.24 ± 0.01	0.24 ± 0.00	0.24 ± 0.01	0.25 ± 0.01	0.7
20:5 n-3 (EPA)	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.3
22:5 n-3 (DPA)	0.26 ± 0.01	0.28 ± 0.00	0.30 ± 0.01	0.31 ± 0.01	0.001
22:6 n-3 (DHA)	0.59 ± 0.02	0.65 ± 0.01	0.63 ± 0.02	0.61 ± 0.02	0.03
<b>Ratio</b>					
LA/ALA	15.83 ± 0.53	16.27 ± 0.20	17.84 ± 0.44	18.09 ± 0.63	0.0004
AA/DHA	1.50 ± 0.04	1.39 ± 0.01	1.50 ± 0.03	1.60 ± 0.04	<.0001
Total n-6/n-3 PUFA	6.25 ± 0.16	6.07 ± 0.06	6.39 ± 0.13	6.65 ± 0.19	0.006
<b>Fatty acid indexes</b>					
DNL liver <sup>c</sup>	65.13 ± 0.29	65.74 ± 0.11	66.44 ± 0.24	65.98 ± 0.35	0.0057
DNL breast <sup>d</sup>	12.47 ± 0.29	11.59 ± 0.11	11.10 ± 0.24	11.33 ± 0.35	0.0036

SCD1-16 <sup>c</sup>	0.086 ± 0.003	0.091 ± 0.001	0.098 ± 0.002	0.102 ± 0.003	<.0001
SCD1-18 <sup>f</sup>	5.21 ± 0.1	5.46 ± 0.04	5.85 ± 0.08	6.25 ± 0.12	<.0001
Δ5 desaturase <sup>g</sup>	1.51 ± 0.04	1.49 ± 0.01	1.46 ± 0.03	1.40 ± 0.04	0.2385
Δ6 desaturase <sup>h</sup>	0.061 ± 0.002	0.063 ± 0.001	0.067 ± 0.002	0.069 ± 0.002	0.0061
DHA Sufficiency <sup>i</sup>	6.18 ± 0.28	6.75 ± 0.11	6.37 ± 0.23	6.24 ± 0.34	0.0953

Abbreviations: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DGLA, dihomo  $\gamma$ -linolenic acid; DHA, docosahexaenoic acid; DNL, *de novo* lipogenesis; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; GLA,  $\gamma$ -linolenic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SCD, Stearoyl-CoA-Desaturase; SFA, saturated fatty acids.

<sup>a</sup>Data are means ± SEM, and fatty acid levels are expressed as % total fatty acids.

<sup>b</sup>Means are adjusted for center and sampling day.

<sup>c</sup>Sum of 16:0 + 16:1n-7 + 18:1n-9 + 20:1n-9, for DNL index in the liver.

<sup>d</sup>Sum of 10:0 + 12:0 + 14:0 + 14:1, for DNL index in the breast.

<sup>e</sup>Product/substrate ratio for SCD 1 (or  $\Delta$ 9 desaturase) activity index: 16:1n-7/16:0.

<sup>f</sup>Product/substrate ratio for SCD 1 (or  $\Delta$ 9 desaturase) activity index: 18:1n-9/18:0.

<sup>g</sup>Product/substrate ratio for  $\Delta$ 5 desaturase activity index: 20:4 n-6/20:3 n-6 (AA/DGLA).

<sup>h</sup>Product/substrate ratio for  $\Delta$ 6 desaturase activity index: 20:3 n-6/18:2 n-6 (DGLA/LA).

<sup>i</sup>DHA Sufficiency Index: 22:6 n-3/22:5 n-6 (DHA/n-6 DPA).

**Table 5:** Correlations between fatty acid intake during pregnancy and colostrum fatty acid levels in the EDEN cohort (n = 803)<sup>a,b</sup>.

Fatty acids in diet (% lipids)	Fatty acids in colostrum (% total fatty acids)													
	SFA	MUFA	PUFA	PUFA n-6	LA	AA	PUFA n-3	ALA	EPA	DPA	DHA	LA/ALA	AA/DHA	n-6/n-3
SFA	<b>0.10</b>	-0.02	<b>-0.13</b>	<b>-0.14</b>	<b>-0.15</b>	0.00	-0.04	<b>-0.10</b>	-0.01	0.02	0.01	0.00	-0.01	-0.06
MUFA	-0.02	-0.04	<b>0.09</b>	<b>0.11</b>	<b>0.11</b>	-0.01	-0.04	0.06	-0.05	<b>-0.08</b>	<b>-0.09</b>	0.01	<b>0.10</b>	<b>0.11</b>
PUFA	<b>-0.12</b>	0.04	<b>0.15</b>	<b>0.14</b>	<b>0.16</b>	-0.01	<b>0.11</b>	<b>0.12</b>	0.06	0.02	0.05	-0.02	-0.06	0.01
n-6 PUFA	<b>-0.11</b>	0.03	<b>0.14</b>	<b>0.14</b>	<b>0.16</b>	-0.01	<b>0.09</b>	<b>0.12</b>	0.04	0.00	0.02	-0.02	-0.04	0.02
LA	<b>-0.11</b>	0.03	<b>0.14</b>	<b>0.14</b>	<b>0.16</b>	-0.01	<b>0.09</b>	<b>0.12</b>	0.04	0.00	0.02	-0.02	-0.04	0.02
AA	0.00	-0.02	0.03	0.02	0.01	-0.01	0.04	0.05	0.02	0.02	0.02	-0.04	-0.03	-0.02
n-3 PUFA	<b>-0.07</b>	0.03	<b>0.09</b>	0.04	0.05	-0.02	<b>0.23</b>	0.07	<b>0.24</b>	<b>0.20</b>	<b>0.27</b>	-0.03	<b>-0.34</b>	<b>-0.17</b>
ALA	-0.07	0.03	<b>0.08</b>	0.05	0.07	-0.01	<b>0.17</b>	<b>0.10</b>	<b>0.13</b>	<b>0.10</b>	<b>0.13</b>	-0.06	<b>-0.17</b>	<b>-0.11</b>
EPA	-0.04	0.02	0.05	0.00	0.00	0.01	<b>0.20</b>	0.02	<b>0.25</b>	<b>0.21</b>	<b>0.30</b>	-0.01	<b>-0.35</b>	<b>-0.17</b>
DPA	-0.02	-0.02	0.06	0.03	0.02	0.01	<b>0.11</b>	0.06	<b>0.14</b>	<b>0.11</b>	<b>0.15</b>	-0.03	<b>-0.17</b>	<b>-0.08</b>
DHA	-0.02	0.00	0.04	-0.01	-0.01	-0.01	<b>0.20</b>	0.03	<b>0.24</b>	<b>0.21</b>	<b>0.29</b>	-0.02	<b>-0.35</b>	<b>-0.18</b>
LA/ALA	-0.06	0.00	<b>0.10</b>	<b>0.11</b>	<b>0.12</b>	-0.01	0.02	<b>0.08</b>	-0.03	-0.03	-0.03	-0.01	0.04	0.07
AA/DHA	0.05	-0.04	-0.04	0.01	0.01	-0.01	<b>-0.22</b>	-0.01	<b>-0.28</b>	<b>-0.24</b>	<b>-0.33</b>	0.00	<b>0.38</b>	<b>0.20</b>
n-6/n-3	-0.05	0.01	0.07	<b>0.10</b>	<b>0.11</b>	-0.02	<b>-0.07</b>	<b>0.07</b>	<b>-0.14</b>	<b>-0.13</b>	<b>-0.16</b>	-0.01	<b>0.18</b>	<b>0.13</b>

Abbreviations: AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid; DPA, n-3 Docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Bold: low, moderate and highest significant correlations.

<sup>a</sup>Partial Spearman's correlation coefficients adjusted for center and sampling day.

<sup>b</sup>Absolute correlation coefficients under 0.07 did not significantly differ from 0 (P-value > 0.05). Absolute correlation coefficients over 0.13 had corresponding P-values < 0.0001.

**Table 6:** Multivariable models<sup>a</sup> for predictors of ALA or DHA colostrum levels in the EDEN cohort (n = 803).

	Model for standardized <sup>b</sup> ALA colostrum level as the outcome			Model for standardized <sup>b</sup> DHA colostrum level as the outcome		
	$\beta$ (SE)	P	% explained variance	$\beta$ (SE)	P	% explained variance
Fish & seafood intake (times/mo)				0.039 (0.005)	<.0001	6.28
Cheese, Added-Fat, Fresh/dried fruits, (times/mo)	-0.001 (0.001)	0.044	0.45			
MUFA Oil (Yes)	0.113 (0.068)	0.097	0.30			
Coconut butter (Yes)	-0.181 (0.075)	0.016	0.63			
n-6 fatty acids intake (g/d)	0.022 (0.008)	0.006	0.82	-0.002 (0.001)	0.11	0.27
Sampling day (d)	0.091 (0.032)	0.005	0.87	-0.122 (0.032)	0.0001	1.54
Mother's age at delivery (y)	0.015 (0.008)	0.058	0.40	0.028 (0.008)	0.0003	1.41
Body Mass Index		0.123	0.63		0.08	0.73
Underweight	0.07 (0.115)			-0.261 (0.113)		
Normal	Taken as reference			Taken as reference		
Overweight	-0.216 (0.098)			-0.019 (0.096)		
Obese	-0.069 (0.135)			-0.191 (0.132)		
Primiparous (Yes)	0.104 (0.074)	0.16	0.22	0.206 (0.073)	0.005	0.85
Smoker (Yes)				-0.145 (0.078)	0.06	0.37
Total <sup>c</sup> (for explained variance)			13.5			16.3

Abbreviations: ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid; MUFA, monounsaturated fatty acids; SE, standard error. <sup>a</sup>adjusted for center. <sup>b</sup>As ALA and DHA values expressed in % of colostrum total fatty acids were standardized (value-means/standard deviation), the beta coefficients ( $\beta$ ) represent the difference of their respective standard deviation related to one unit variation in the explanatory variable. <sup>c</sup>Total variance explained by the full model.

**Table 7:** Multivariable models<sup>a</sup> for predictors of LA or AA colostrum levels in the EDEN cohort (n = 803).

	Model for standardized <sup>b</sup> LA colostrum level as the outcome			Model for standardized <sup>b</sup> AA colostrum level as the outcome		
	$\beta$ (SE)	P	% explained variance	$\beta$ (SE)	P	% explained variance
Eggs & meats (times/mo)				-0.005 (0.003)	0.12	0.26
Sauces & bread (times/mo)	0.001 (0.001)	0.44	0.07			
n-6 PUFA Oil (Yes)	0.253 (0.073)	0.0005	1.46			
MUFA Oil (Yes)				-0.197 (0.067)	0.004	0.94
Energy intake (Kcal/100/d)	-0.024 (0.09)	0.011	0.79			
n-3 fatty acids intake (g/d)	0.184 (0.141)	0.19	0.21	-0.035 (0.075)	0.64	0.02
Sampling day (d)	0.023 (0.033)	0.49	0.06	-0.283 (0.032)	<.0001	8.50
Mother's age at delivery (y)				0.024 (0.008)	0.002	1.05
Body Mass Index		0.15	0.63		<.0001	2.75
Underweight	-0.092 (0.119)			-0.196 (0.114)		
Normal	Taken as reference			Taken as reference		
Overweight	0.069 (0.102)			0.275 (0.097)		
Obese	0.286 (0.142)			0.501 (0.134)		
Primiparous (Yes)	0.141 (0.072)	0.050	0.46	0.241 (0.074)	0.0011	1.17
Total <sup>c</sup>			5.4			13.5

Abbreviations: AA, arachidonic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SE, standard error. <sup>a</sup>adjusted for center and gestational age at delivery. <sup>b</sup>As LA and AA values expressed in % of colostrum total fatty acids were standardized (value-means/standard deviation), the beta coefficient ( $\beta$ ) represents the difference of their respective standard deviation related to one unit variation in the explanatory variable. <sup>c</sup>Total variance explained by the full model.