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# *Fatty acids in red blood cells in infants and their mothers in relation to allergy development.*

***Analysis of erythrocyte fatty acids in mothers and infants in the NICE cohort at four- and twelve-months post-partum, maternal diet and the influence on allergy development.***


CHALMERS

Master's thesis in Biotechnology

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CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2019

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# Abstract

The prevalence of asthma, atopic eczema and IgE-mediated allergies has significantly increased in recent years, along with a general decrease of fish consumption. Findings from previous studies point to a connection between lower levels of dietary intake of n-3 long-chain polyunsaturated fatty acids (LCPUFA) and allergy development.

This study analyzed 694 samples of erythrocyte fractions from mothers and children in the Nutritional Impact on Immunological maturation in Childhood in relation to the Environment (NICE) cohort, with the aim to investigate the relationship between fatty acid proportions in mothers and children, diet, and allergy development.

Children diagnosed with allergy (n=27) primarily had lower proportions of α-linolenic acid (ALA) compared to non-allergic children (n=120) at 4 months after birth, while mothers to children diagnosed with allergy had a lower total n-3 LCPUFA proportion and lower proportion of docosahexaenoic acid (DHA). Blood proportions of dairy biomarkers C15:0 and C17:0 were found to be lower for allergic children than non-allergic at 4 months post-partum, and C17:0 lower for allergic children (n=39) also at 12 months post-partum when compared to non-allergic children (n=203) at 12 months post-partum.

This study also found several connections between the mothers' diet, red blood cell fatty acid levels among both mothers and their children, and the prevalence of hypersensitivity conditions among the children. Higher maternal intake of fish and seafood as well as n-3 LCPUFAs was positively correlated to higher n-3 proportions in red blood cells primarily among the mothers at 4 months, but seemed to also positively influence n-3 to n-6 ratio among the children at 4 months.

When comparing the diets of mothers to 4-month-old children without allergies (n=241) to those with children with diagnosed allergies (n=60) the total daily intake of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids as well as daily intakes of individual fatty acids C4:0-C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C16:1, C18:1, C18:2 (LA) and C18:3 (ALA) were significantly higher among mothers to non-allergic children.

For 4-month-old children with asthma (n=5) and without asthma (n=123), the diet of mothers with asthmatic children was found to include significantly lower ($p<0.05$) amounts of C16:0, AA, EPA, DPA and DHA.

The results from this thesis generally agree with current research, indicating a negative correlation of fish consumption and subsequent increased n-3 LCPUFA proportions and development of allergy and asthma. Intake of fish and n-3 LCPUFAs was shown to correlate with erythrocyte proportions both for mothers and their children, and erythrocyte fatty acid proportions generally reflected dietary intake of the corresponding fatty acids. A general conclusion of this thesis is that maternal fish consumption during pregnancy and breast-feeding may prevent or reduce the prevalence of allergy and other hypersensitivity conditions in infants through increased n-3 fatty acid levels in mothers and children, although the underlying mechanisms and/or the genetic interplay are not fully understood. Proposed areas of future research would include the correlation between altered fatty acid metabolism and allergy development, and further investigation of other factors affecting allergy development.

Keywords: Allergy, asthma, NICE, infants, LCPUFA, n-3 PUFA, n-6 PUFA, diet, fish intake, red blood cells

# Acknowledgements

I would like to direct a warm thank you to my supervisor Malin Barman, who agreed to take me on at short notice, and has provided valuable guidance and feedback throughout the project. Thank you Assi for taking the time to review my work and act as my examiner.

I would also like to thank Karin, Cornelia and Johanna for all the help and introduction to the laboratory process, as well as for the pleasant coffee-breaks. Thanks to Jacob for the help with the GC. To everyone at the food and nutrition division, and especially to my office-colleagues: Thank you for making me feel welcome and for making my time with you really enjoyable!

A special thank you to my wonderful and patient girlfriend Matilda. I love you and look forward to new adventures with you and our soon-to-be-born little Molly.

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# 1. Introduction

During the past decades, food allergy and other IgE-mediated allergies have increased in Sweden and other Western societies. The consumption of fish in these societies has meanwhile decreased, while red meat consumption is higher than it has ever been (1).

It has been hypothesized that the dietary intake of long chain polyunsaturated fatty acids (LCPUFAs) such as omega-3 (n-3) fatty acids found primarily in fish and seafood, and omega-6 (n-6) fatty acids found in for example red meat can affect the development of allergy. So far, however, the evidence is rather inconclusive, and results from different studies on the matter are somewhat contradictory (2).

# 2. Background

## 2.1. Fatty acid metabolism

Polyunsaturated fatty acids, particularly long chain varieties of at least 20 carbon atoms are important for a multitude of functions in humans, and especially developing fetuses and infants. The two most impactful classes of PUFAs are the omega 3- and omega 6 fatty acids; also referred to as n-3- and n-6 PUFAs. The position of a carbon atom in a fatty acid can be indicated from either the carboxy end, or from the methyl end, with the last position counting from the carboxy end is indicated as omega as shown in figure 1 below. An omega-3 fatty acid is named after the position of the first occurring double bond in the carbon chain counting from the omega, namely from the third carbon (3).

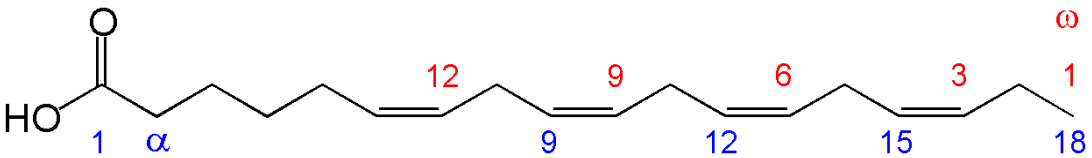


*Figure 1: Numbering of carbons in fatty acids; figure showing C18:4n3 Stearidonic acid with 18 carbon atoms, 4 double bonds – the first on the omega-3-position. Image created for the public domain by Wikipedia user Edgar181 and retrieved from Wikipedia.com.*

Unlike humans and animals, plants are equipped with the enzymes necessary for synthesis of the omega-3 fatty acid alpha-linolenic acid (ALA), and the omega-6 fatty acid linoleic acid (LA), used as precursors for longer chain fatty acids important in human physiology. Therefore, ALA and LA are said to be essential and must be supplied through dietary intake (4).

Humans do however have enzymes for conversion of LA and ALA into different LCPUFAs. Three different fatty acid desaturases (Δ-3-, Δ-6- and Δ-9-desaturase) and elongases act competitively on n-3 and n-6 fatty acids producing longer fatty acids of the same class, leading to that the ratio of n-3 and n-6 LCPUFAs is affected by the ratio of LA to ALA in the diet (4).

The pathways for conversion of LA and ALA into longer fatty acids are shown in figure 2 below, used with permission from Malin Barman, Chalmers University of Technology.

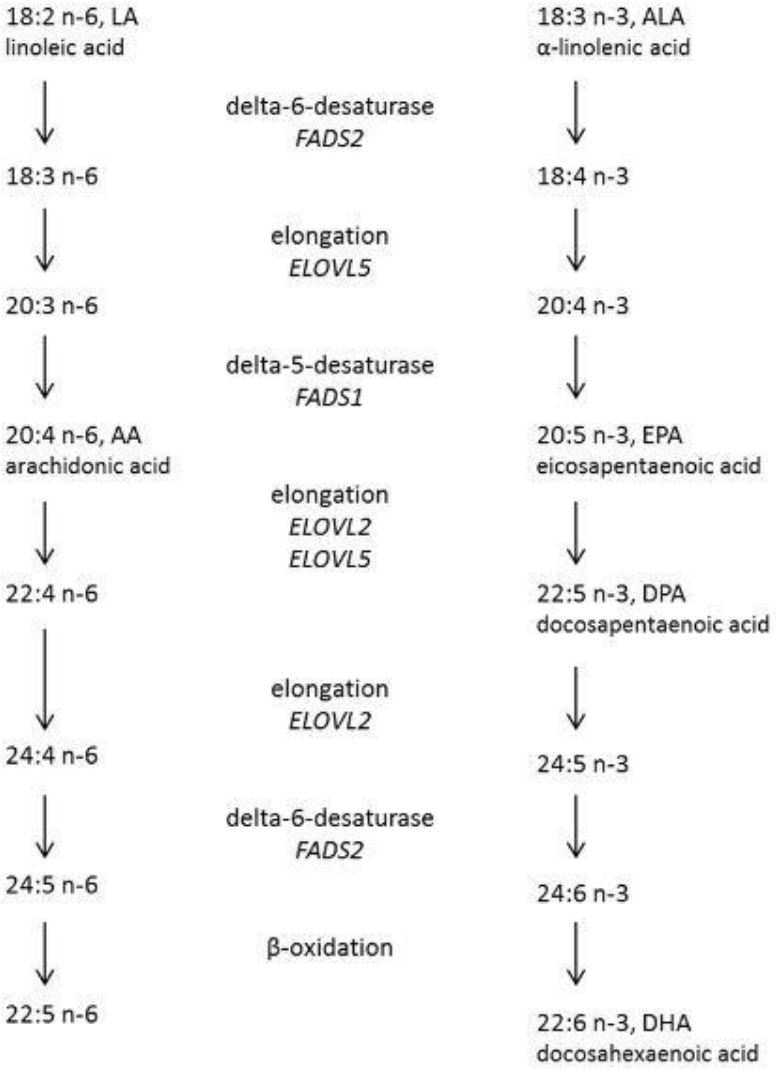


*Figure 2: Enzymatic pathways for elongation of LA and ALA.*

Fatty acids from the diet of pregnant women have been shown to be incorporated into erythrocyte membranes in their babies, reflecting the mothers' long-term fatty acid intake (5).

A balanced intake of various fatty acids is required for maintaining good health, as fatty acids have a variety of functions in the body, for example as an energy source, building blocks and precursors for lipid membranes and other cell constituents, and intracellular signalling (4)(6).

LCPUFAs have found to be important for normal fetal development, and some LCPUFAs including DHA and AA have been hypothesized to be transported actively and selectively from the mother to the fetus, across the placenta (7).

The relation between dietary intake, blood proportions of both n-3 and n-6 and prevalence of different allergies and inflammatory conditions have been reviewed in several studies, with somewhat inconclusive or contradictory results; a brief summary of a selection of such studies is found in section 2.3.

## 2.2. Allergy

An antigen, (antibody generator) is a substance foreign to the body that causes antibody production by the immune system. Antigens can be different kinds of molecules, for example chemical compounds, proteins, peptides and polysaccharides among others. Different antigens may also have widely divergent origin, such as bacteria, pollen, viruses or synthetic materials (8).

When antigens, or a larger entity containing an antibody generating peptide sequence enter the body, they are captured through phagocytosis and processed by some sort of antigen presenting cell (APC), for example a macrophage or a dendritic cell. These APCs contain a type of protein complex called major histocompatibility complex class II (MHC II), that bind peptide sequences of the foreign entity and migrates to present them on the surface of the APC. APCs interact with T cells that upon exposure to the peptide bound to the major histocompatibility complex II differentiate into type 1 T helper cells ($T_h1$). The $T_h1$ cells in turn interact with B cells through surface protein expression and cytokine signalling, promoting B cell proliferation and immunoglobulin class switching to produce protective IgG antibodies, which upon later introduction of the specific allergen will find and destroy or immobilize said allergen without causing an allergic reaction. $T_h1$ differentiation is primarily promoted by the cytokine interferon gamma (IFN-γ) and inhibited by interleukins IL-4 and IL-10 (9)(10).

A different cytokine environment surrounding the T cell that is being presented the antigen can however lead to differentiation into a $T_h2$ cell, and the antibodies formed will be of the IgE type. IgE antibodies bind to mast cells containing inflammatory substances such as histamine and heparin. This process is called sensitization, and when an allergen that has caused this is re-introduced to the body, the response will be an allergic reaction or anaphylaxis. The most important cytokine driving $T_h2$ production is IL-4, while IFN-γ inhibits it (9)(5).

Allergy is believed to at least partly be a consequence of insufficient or incorrect immune regulation during infancy, and perhaps also during the fetal development (4)(11).

Many mediators and signalling molecules that regulate immune response and inflammation are derived from LCPUFAs. Eicosanoids are a large family of 20-carbon signalling molecules derived from AA (20:4), DGLA (20:3) and EPA (20:5). AA and DGLA are omega-6 fatty acids metabolized from the essential fatty acid LA, and EPA is an omega-3 fatty acid metabolized from the other essential fatty acid ALA.

The eicosanoids include several classes of signalling molecules, some of the most important being leukotrienes, prostaglandins, thromboxanes, lipoxins, resolvins and eoxins. Each of these classes consist of different subclasses, and have different roles in the immune system and inflammatory process, both pro-inflammatory and anti-inflammatory. Generally, eicosaonoid metabolites derived from the omega-6 precursors display more pro-inflammatory effects, and omega-3 derived ones anti-inflammatory or inflammatory-resolving effects, but their interaction is complex and not fully-understood; balanced levels are desirable for proper function.

## 2.3 Immune system regulation

It is believed that proper regulation and modulation of the immune system prevents the formation of IgE antibodies and subsequent allergy, but which environmental factors affect these processes negatively, or positively, is still unclear (11). However, several factors have in multiple previous studies been linked to reduced sensitization and allergy development: mainly early exposure to different microbes and other allergens (7).

Several recent studies have investigated the link between allergy development and dietary intake of LCPUFAs, both by infants, children and expectant mothers.

The collective research on LCPUFA levels and allergy suggests that LCPUFAs plays a part in immune system maturation and may help avoid development of allergy, but the results are far from conclusive, and in many studies a general trend or tendency can be determined. However, statistically significant result from randomized well-controlled studies are scarce. A selection of studies on the subjects of LCPUFAs and infant allergy development is found below. Notably, many of the referenced studies are focusing on different sample types, e.g. phospholipids in serum or breast milk, and not membrane-bound fatty acids in erythrocytes. The assumption is however made that the proportions of relevant fatty acids and the implications for allergy development somewhat correlates between different sample types.

In a systematic review and meta-analysis study from 2009 by Anandan et. al.(2) the conclusion was made that “supplementation with omega 3 and/or 6 oils is unlikely to be associated with a marked reduction in risk of developing sensitization or indeed allergic disease”.

However, maternal consumption fish oil during pregnancy, and subsequent increased levels of n-3 LCPUFAs in the erythrocyte membranes in cord blood at birth was correlated to a decrease of the prevalence of allergy, and a delayed onset compared to control groups in a study by Dunstan et. al.(11) from 2003.

In a study by Kull et. al. (12), the fish consumption of infants during their first year of life was studied, and fish consumption was found to reduce the risk of allergic disease and sensitization at four years of age.

A position paper by the European Academy of Allergy and Clinical Immunology (EAACI) conveyed that higher maternal ALA proportions, along with total n-3 and C16:0 proportions were linked to decreased risk to develop asthma (13), and higher maternal AA proportions were linked to higher risk.

Low n-3 LCPUFA levels in breastmilk has been reported in multiple studies to be positively correlated with higher risk of development of atopic eczema in children, (14)(15)(16) while high levels were also positively correlated to atopic eczema in another study by Thijs et. al.(17).

A study from 2013 by Barman et. al.(7) showed that increased levels of either n-3 or n-6 LCPUFAs in cord serum phospholipids were found to be positively associated with allergy development. One of the proposed explanations for this is that the immunosuppressive effects of n-6 and n-3 derived metabolites impedes maturation of the immune system and tolerization to non-harmful antigens.

The positive correlation between both n-3 and n-6 PUFAs and allergy development was also shown in a study from 2017 by Mikkelsen et. al. (18).

Several mechanisms whereby PUFAs affect immune functions are presented and referenced in a doctoral thesis by Malin Barman (4); PUFA constitution in cellular membranes affect membrane fluidity and receptor signalling, act as ligands for certain receptors as well as bind to other receptors, and are also precursors of different mediators affecting immune response and inflammation.

A hypothesis is also that metabolism of LCPUFAs differ between individuals who develop allergy and those who do not, and that this may to unknown extent be affected by LCPUFA levels in blood, or vice versa (7).

In summary, the details regarding the links between dietary intake of LCPUFAs and allergy development are all in all not entirely known, and results from different studies in the area are in many cases inconclusive or contradictory. This study, and other studies performed on the NICE-birth cohort (19) is hoped to further investigate the connections between dietary LCPUFA intake and allergy, and corroborate previous conclusions from other studies.

# 3. Method and materials

## 3.1. Sample and data collection, handling and analysis

All analyzed samples were from the NICE (Nutritional Impact on Immunological maturation in Childhood in relation to the Environment) cohort, a prospective birth cohort study aiming to evaluate the impact of diet and other environmental factors on children's immune system maturation, allergy development and neurological development.

Recruitment of study participants and sample collection, and also further information on the study is found in (19). Briefly, study participants were recruited among all families planned to give birth at Sunderby hospital in Norrbotten, Sweden, from February 2015 to March 2018. 655 families agreed to participate, and were asked to fill out questionnaires about diet, living environment, history of allergies and other information. Samples consisting of blood, urine, saliva, breast milk and placenta were taken from the mothers at different time points from gestational week 28 up to 12 months after birth. The same samples, and also feces, meconium and hair were taken from the children from birth and up to 48 months after birth. From the blood samples, the red blood cell fraction was collected separately, and this type of blood sample taken from mothers and children at 4 months post-partum, and only from children at 12 months post-partum were used in the present study. Blood samples were stored 15-30 min in room temperature after sampling, at 4° C until aliquoting, at -20° C for a maximum of 12 weeks, and at -80° C for long-term storage.

Sample preparation procedure was based on an article from 2005 by Masood et. al.(20) and includes transesterification with methanol to produce volatile fatty acid methyl esters (FAME), which are extracted by n-hexane. Samples were subsequently analyzed by gas chromatography-flame ionization detector (GC-FID) utilizing fast GC principle with hydrogen as carrier gas and narrow bore capillary GC column.

The sample preparation procedure is described briefly below:
Samples were thawed and vortexed before 50µl of RBC was pipetted into a glass tube, and 100µl internal standard solution (100µg/ml) was added along with 1.8 ml acetyl chloride-MeOH solution (10 % v/v) fortified with BHT. Samples were incubated in water bath at 70 °C for 60 min, and vortexed every 30 min. To the tubes 1.5 ml n-hexane was added before the tubes were vortexed and centrifuged (3000 rpm, 3 min). Approximately 1 ml of the upper organic phase was extracted with a glass Pasteur pipette and added to GC vials before the solvent was subsequently evaporated using a vacuum evaporator at 30° C for about 30 min. 200µl of n-hexane was added to the vials, which were vortexed and analyzed with GC-FID.

All sample batches were run along with an internal standard (Methyl tricosanoate - C23:0 methyl ester as internal standard, Nu-Chek Prep. MN, USA), an external standard dilution series (GLC-462 mixed FAME external standards (Nu-Chek Prep. MN, USA) containing 28 fatty acids from C10:0 – C24:1), two water blanks, one n-hexane blank and in-house pooled RWBC samples as quality control (QC) making up ~10 % of the total sample size. Samples from the same family were run in sequence, but the order of families and single samples was randomized.

## 3.2. Dietary intake assessment

Dietary intake information used for data analysis was obtained from web-based food-frequency questionnaires (Meal-Q) answered by the mothers at 4 months. Further information about dietary intake for mothers, fathers and infants were available, but were not used in this study.

## 3.3. Allergy diagnosis

The diagnosis of allergic diseases was done by a pediatrician specialized in allergy at 12 months after birth. Sensitization of common allergens (egg, milk, birch, timothy, cat, dog) was assessed by performing a standard skin prick test.

Atopic eczema was diagnosed in accordance by criteria proposed by Williams et. al.(21)(22)(23).

Food allergy diagnosis was based on medical history of allergic reaction to the specified allergen on at least two consecutive occasions, and an elimination-provocation procedure carried out in the home or at the hospital if required. Pet and pollen allergy diagnoses were based on medical history of allergic reactions upon exposure to the allergen, and subsequent disappearance of symptoms upon removal of the allergen.

The prevalence of asthma was established if there was documented wheeze between infections, there was a persistent wheeze for at least 4 weeks, there was a period of wheeze during an infectious disease with concomitant allergic disease or three episodes of wheeze during infectious disease, with no concomitant allergic disease.

## 3.4. Data processing and analysis

GC-data was processed using Xcalibur™ software from Thermo Fisher Scientific, where chromatograms were manually inspected and curated, and subsequently fatty acid concentration in samples were computed and exported.

## 3.5 Data analysis

Several statistical tests were used to examine different aspects of blood and dietary data.

To investigate the correlation between the blood fatty acid proportions of a mother and her own child at 4 months post-partum, Spearman's rank correlation test was performed. This test was also used to test the correlation of blood fatty acid proportions between a child at 4 months post-partum and 12 months post-partum, as well as the correlation between dietary intake and blood fatty acid proportions.

Spearman's rank correlation test is a non-parametric test which requires two paired groups of data, such as mothers and their children. The groups are sorted individually, and each

observation is given a rank corresponding to its value, from lowest to highest. The Spearman's rank correlation coefficient (Spearman's rho, $r_s$) describing the monotonic relationship between the groups is then calculated from:

$$r_s = 1 - \frac{6 \sum d_i^2}{n(n^2 - 1)}$$

where $d_i^2$ is the squared difference of the ranks and $n$ is the number of observations(24).

To investigate the differences in distribution between the mothers and children in regards to blood fatty acid proportions, a Wilcoxon signed rank test was used. The Wilcoxon signed rank test is a non-parametric hypothesis test, analogue to the paired Student's t-test, and does not require normally distributed populations. The null hypothesis is generally formulated such as the difference between the pairs follow a symmetric distribution around zero. The absolute value of the difference between paired observations from the two populations are calculated, and given a rank from lowest to highest. Pairs where the difference is equal to zero are excluded, and the reduced sample size is denoted as $N_r$. The test statistic $W = \sum_{i=1}^{N_r} [sgn(x_{2,i} - x_{1,i}) \cdot R_i$, where $R_i$ is the rank of the $i^{th}$ *pair*, is then compared to a reference critical value to see whether to reject the null hypothesis or not(24).

The Mann-Whitney U test is similar to the Wilcoxon signed rank test, but can be used to assess independent samples at small sample sizes. Once again, observed numerical values in both groups are ranked from lowest to highest, the test statistics $U_1 = R_1 - \frac{n_1(n_1+1)}{2}$ and $U_2 = R_2 - \frac{n_2(n_2+1)}{2}$ are calculated, and the smaller value of $U_1$ and $U_2$ is compared to a reference table to decide whether or not to reject the null hypothesis that the distributions of both tested populations are equal(24).

# 4. Results

## 4.1. Overview

Children were examined at 12 months after birth and diagnosed with different types of allergy as described in (19). Statistical analyses were performed using IBM SPSS Statistics version 19 on 301 samples taken from mothers at 4 months after birth, 150 children at 4 months after birth, and 242 children at 12 months after birth.
For practical reasons, blood samples from children were not available for all families for all time-points.

Table 1 shows the number of samples for each group that correlated with a child diagnosed at 12 months as non-allergic or with different allergies. Individuals categorized as having *any allergy* have been diagnosed either with eczema, food allergy, asthma, various other allergies including pollen and pets, or a combination of these. Since allergic diagnoses can overlap (e.g. one individual can be diagnosed with both asthma and food allergy) the number of individuals with *any allergy* is not equal to the sum of all the other allergy groups.

*Table 1: Number of samples from mothers and children from the NICE-study for which statistical data is included in this report, with children diagnosed at 12 months after birth as non-allergic, or with different types of allergy.*

| | **Mothers, 4 months** | **Children, 4 months** | **Children, 12 months** |
|---|---|---|---|
| Non-allergic | 241 | 123 | 204 |
| Eczema | 20 | 13 | 10 |
| Food allergy | 30 | 16 | 25 |
| Asthma | 22 | 5 | 8 |
| Any allergy | 60 | 27 | 39 |
| Total | 301 | 150 | 243 |

Data are presented as number of individuals diagnosed as free from allergies or diagnosed with eczema, food allergy, asthma or any combination of allergic conditions.

## 4.2. Assessment of variation in and between batches

To examine the variation within and between batches run in the GC-FID, one QC-sample for every ten study samples was included in an analysis run.

To examine intra-batch variation, the coefficient of variation (CV) was calculated for each fatty acid of interest for all the QC-samples in a batch, and a total mean CV for a batch was obtained by taking the average of these CVs. Generally, the variation within a batch was around 10 % or lower, but for some batches considerably higher. For all these batches except two however, the mean CV was found to be below 10 % if one QC-sample deemed as an outlier (for example because the total fatty acid concentration was found to differ

significantly from other QC-samples in the same batch) was excluded from the calculations. For one batch, the CV was above 20 %, even after redoing the sample preparation and GC-analysis and was therefore not included in any statistical analysis. The mean CV for all other batches together, with no outliers removed was 13.05 %.

In table 2 below, CV-values from four arbitrarily selected batches are shown for two fatty acids with relatively large chromatogram peaks (C16:0 & C18:2-n6), two fatty acids with relatively small peaks (C16:1 & C18:3n-3), as well as the mean CV for the whole batch.

*Table 2: Coefficient of variation (CV) for four selected fatty acids and mean CV in four of the analyzed batches.*

| **CV (%)** | ***C16:0*** | ***C16:1*** | ***C18:2n-6*** | ***C18:3n-3*** | ***Batch mean*** |
|---|---|---|---|---|---|
| **Batch 2** | 2.69 | 8.30 | 2.69 | 29.75 | 7.83 |
| **Batch 5** | 2.55 | 13.63 | 2.60 | 25.67 | 6.35 |
| **Batch 10** | 2.13 | 28.57 | 2.60 | 16.73 | 6.65 |
| **Batch 15** | 8.0 | 4.61 | 9.25 | 20.64 | 10.33 |

Data is given in %.
CV-values presented for two fatty acids with relatively large chromatogram peaks (C16:0 & C18:2-n6), two fatty acids with relatively small peaks (C16:1 & C18:3n-3), as well as the mean CV for the whole batch. The batches are arbitrarily picked to represent different time points in the analytical process.

The mean CV for the main fatty acids of interest across all 17 batches used for statistical data processing are listed in table 3 below.

*Table 3: CV in % for the main fatty acids of interest across all batches.*

| **Fatty acid** | **CV (%)** |
|---|---|
| *C16:0* | 7.90 |
| *C16:1n7* | 19.07 |
| *C18:0* | 7.60 |
| *C18:1n9* | 7.30 |
| *C18:1n7* | 9.07 |
| *C18:2n6 LA* | 7.43 |
| *C18:3n3 ALA* | 32.35 |
| *C20:0* | 19.32 |
| *C20:1n9* | 14.36 |
| *C20:2n6* | 21.70 |
| *C20:3n6* | 9.74 |
| *C20:4n6* | 8.93 |
| *C22:0* | 11.96 |
| *C20:5n3* | 12.73 |
| *C22:4n6* | 10.78 |
| *C24:0* | 11.36 |
| *C24:1n9* | 9.84 |
| *C22:5n3* | 13.07 |
| *C22:6n3* | 14.31 |

Mean CV-values given for the 19 most central fatty acids in all QC-samples in the 17 batches that are the basis for statistical data analysis.

## 4.3. Relation between FA proportions in mothers and children

The relation of fatty acid proportions between mothers and children at the two different time-points was examined to establish a background for further investigation. Generally, the proportions of different fatty acids was relatively strongly correlated between both mothers and children at 4 months, as well as between children at 4 months and children at 12 months.

Table 4 and table 5 show the median for the relative proportion of each individual fatty acid, and medians of total n-3 PUFA and LCPUFA proportions, total-n6 PUFA and LCPUFA proportions, as well as the n-3 to n-6 fatty acid ratio. Data is given as percentage of total fatty acid concentration. $P_1$ indicates the p-values for a Wilcoxon signed rank test assessing the statistical significance of the difference in population distributions between mothers and children. The null hypothesis was that the difference between pairs follows a symmetric distribution around zero. Significant p-values are indicated by an asterisk.

A Spearman's rank correlation was performed assessing the rank correlation for fatty acid proportions between mothers and children; $r_s$ is the Spearman's rank correlation coefficient (Spearman's rho), and $p_2$ is the corresponding p-value. For $p_2$, significant values are indicated by an asterisk.

*Table 4: Median proportions of each individual fatty acid and some groupings of n-3 and n-6 fatty acids found in red blood cells for children at 4 months, and mothers at 4 months, as well as the range of the 25th percentile to the 75th percentile.*

| **Fatty acid** | **Child, 4 months, (%) (n=193)** | **Mother, 4 months, (%) (n=388)** | **$p_1$** | **$r_s$** | **$p_2$** |
|---|---|---|---|---|---|
| *C14:0* | 0.76 (0.45-1.02) | 0.32 (0.26-0.40) | 3.81E-26* | 0.074 | 0.317 |
| *C15:0* | 0.14 (0.09-0.18) | 0.16 (0.13-0.19) | 1.09E-03* | 0.166 | 0.023* |
| *C16:0* | 22.60 (20.22-25.83) | 20.26 (19.53-20.89) | 1.77E-24* | 0.267 | <0.001* |
| *C16.1n7* | 0.41 (0.24-0.66) | 0.44 (0.30-0.60) | 0.199 | 0.249 | 0.001* |
| *C17:0* | 0.29 (0.22-0.35) | 0.27 (0.24-0.32) | 2.93E-02* | 0.324 | <0.001* |
| *C18:0* | 18.37 (16.68-20.71) | 16.37 (15.51-17.09) | 2.24E-15* | 0.211 | 0.004* |
| *C18:1n9* | 19.26 (16.41-21.56) | 15.51 (14.66-16.47) | 2.56E-27* | 0.173 | 0.018* |
| *C18:1n7* | 1.36 (1.18-1.54) | 1.11 (1.01-1.20) | 3.74E-22* | 0.213 | 0.004* |
| *C18:2n6 (LA)* | 10.64 (9.04-12.20) | 12.94 (11.67-14.09) | 9.93E-14* | -0.062 | 0.402 |
| *C18:3n3 (ALA)* | 0.16 (0.09-0.28) | 0.16 (0.12-0.23) | 4.89E-02* | 0.109 | 0.14 |
| *C20:0* | 0.35 (0.29-0.42) | 0.19 (0.13-0.23) | 3.77E-29* | 0.175 | 0.017* |
| *C20:1n9* | 0.34 (0.29-0.39) | 0.24 (0.19-0.28) | 1.79E-23* | 0.258 | <0.001* |

| | | | | | |
|---|---|---|---|---|---|
| *C20:2n6* | 0.19 (0.11-0.24) | 0.15 (0.11-0.18) | 2.34E-10* | 0.372 | <0.001* |
| *C20:3n6 (DGLA)* | 1.04 (0.76-1.37) | 1.47 (1.29-1.68) | 2.25E-19* | 0.128 | 0.082 |
| *C20:4n6 (AA)* | 9.35 (5.82-12.85) | 13.08 (12.28-14.00) | 3.70E-22* | 0.09 | 0.222 |
| *C22:0* | 0.95 (0.80-1.12) | 0.99 (0.89-1.13) | 0.947 | 0.239 | 0.001* |
| *C20:5n3 (EPA)* | 0.30 (0.17-0.49) | 0.97 (0.78-1.18) | 4.06E-30* | 0.414 | <0.001* |
| *C22:4n6* | 1.36 (0.55-2.07) | 2.07 (1.77-2.39) | 3.13E-18* | 0.163 | 0.027* |
| *C24:0* | 2.13 (1.93-2.53) | 2.38 (2.16-2.65) | 2.56E-03* | 0.244 | 0.001* |
| *C24:1n9* | 3.04 (2.64-3.39) | 3.17 (2.85-3.50) | 0.130 | 0.304 | <0.001* |
| *C22:5n3* | 0.95 (0.51-1.68) | 2.48 (2.22-2.83) | 9.73E-31* | 0.264 | <0.001* |
| *C22:6n3 (DHA)* | 3.26 (1.46-5.00) | 4.14 (3.45-4.80) | 3.47E-10* | 0.18 | 0.014* |
| *Total n-3 PUFA* | 4.94 (2.44-7.35) | 7.77 (6.93-8.76) | 1.97E-25* | 0.212 | 0.004* |
| *Total n-6 PUFA* | 23.8 (17.69-27.51) | 29.86 (28.84-30.95) | 2.05E-27* | -0.095 | 0.196 |
| *n-6/n-3 ratio* | 4.52 (3.44-6.93) | 3.49 (3.13-4.05) | 3.24E-18* | 0.23 | 0.002* |
| *Total n-3 LCPUFA* | 4.57 (2.25-7.1) | 7.61 (6.71-8.59) | 1.55E-25* | 0.202 | 0.006* |
| *Total n-6 LCPUFA* | 12.07 (7.2-16.57) | 16.66 (15.69-17.87) | 4.11E-22* | 0.122 | 0.098 |

Data are presented as median value (% of total fatty acid concentration), and range: ($25^{th}$- and $75^{th}$ percentiles, % of total fatty acid concentration). Wilcoxon's signed rank test was used to test the population distribution differences between mothers and children, and the corresponding p-values are shown as $p_1$, with non-significant values indicated by †. A Spearman's rank correlation test was performed assessing the rank correlation for fatty acid proportions between mothers and children; $r_s$ is the Spearman's rank correlation coefficient (Spearman's rho), and $p_2$ is the corresponding p-value. For $p_2$, significant values are indicated by an asterisk.

*Table 5: Median proportions of each individual fatty acid and some groupings of n-3 and n-6 fatty acids found in red blood cells for children at 4 months, and children at 12 months, as well as the range of the 25th percentile to the 75th percentile.*

| Fatty acid | Child, 4 months, (%) (n=193) | Child, 12 months, (%) (n=257) | $p_1$ | r | $p_2$ |
|---|---|---|---|---|---|
| *C14:0* | 0.76 (0.45-1.02) | 0.36 (0.25-0.51) | 6.42E-09 | 0.065 | 0.531 |
| *C15:0* | 0.14 (0.09-0.18) | 0.13 (0.10-0.16) | 0.664† | 0.347 | 0.001* |
| *C16:0* | 22.60 (20.22-25.83) | 19.28 (18.54-20.31) | 1.52E-13 | 0.2 | 0.053 |
| *C16.1n7* | 0.41 (0.24-0.66) | 0.28 (0.20-0.46) | 9.75E-04 | 0.326 | 0.001* |
| *C17:0* | 0.29 (0.22-0.35) | 0.27 (0.23-0.32) | 0.837† | 0.156 | 0.134 |
| *C18:0* | 18.37 (16.68-20.71) | 15.95 (14.88-16.82) | 4.54E-13 | 0.186 | 0.073 |
| *C18:1n9* | 19.26 (16.41-21.56) | 16.98 (15.57-18.36) | 6.78E-05 | 0.216 | 0.037* |
| *C18:1n7* | 1.36 (1.18-1.54) | 1.20 (1.08-1.32) | 2.08E-06 | 0.396 | <0.001 |
| *C18:2n6 (LA)* | 10.64 (9.04-12.20) | 13.91 (12.69-15.48) | 1.07E-15 | 0.093 | 0.375 |
| *C18:3n3 (ALA)* | 0.16 (0.09-0.28) | 0.25 (0.16-0.41) | 1.21E-05 | 0.161 | 0.122 |
| *C20:0* | 0.35 (0.29-0.42) | 0.22 (0.18-0.27) | 4.22E-16 | 0.411 | <0.001* |
| *C20:1n9* | 0.34 (0.29-0.39) | 0.38 (0.31-0.43) | 1.90E-04 | 0.195 | 0.06 |
| *C20:2n6* | 0.19 (0.11-0.24) | 0.23 (0.18-0.28) | 1.52E-04 | 0.344 | 0.001* |
| *C20:3n6 (DGLA)* | 1.04 (0.76-1.37) | 1.29 (1.13-1.50) | 2.31E-05 | 0.159 | 0.125 |
| *C20:4n6 (AA)* | 9.35 (5.82-12.85) | 12.92 (11.87-13.83) | 2.23E-07 | 0.095 | 0.36 |
| *C22:0* | 0.95 (0.80-1.12) | 0.84 (0.74-0.94) | 2.14E-07 | 0.354 | <0.001* |
| *C20:5n3 (EPA)* | 0.30 (0.17-0.49) | 0.46 (0.35-0.59) | 6.37E-07 | 0.287 | 0.005* |
| *C22:4n6* | 1.36 (0.55-2.07) | 2.17 (1.86-2.50) | 6.62E-10 | 0.121 | 0.245 |
| *C24:0* | 2.13 (1.93-2.53) | 2.03 (1.75-2.29) | 3.67E-05 | 0.376 | <0.001* |
| *C24:1n9* | 3.04 (2.64-3.39) | 3.01 (2.66-3.42) | 0.353† | 0.238 | 0.021* |
| *C22:5n3* | 0.95 (0.51-1.68) | 1.90 (1.65-2.18) | 4.85E-10 | 0.149 | 0.151 |
| *C22:6n3 (DHA)* | 3.26 (1.46-5.00) | 4.06 (3.42-4.75) | 1.92E-03 | 0.23 | 0.026* |
| *Total n-3 PUFA* | 4.94 (2.44-7.35) | 6.78 (5.99-7.71) | 3.36E-06 | 0.232 | 0.024* |
| *Total n-6 PUFA* | 23.8 (17.69-27.51) | 30.66 (29.55-31.73) | 8.09E-16 | -0.006 | 0.958 |
| *n-6/n-3 ratio* | 4.52 (3.44-6.93) | 4.14 (3.61-4.76) | 2.74E-03 | 0.378 | <0.001* |
| *Total n-3 LCPUFA* | 4.57 (2.25-7.1) | 6.44 (5.64-7.43) | 1.92E-05 | 0.225 | 0.029* |
| *Total n-6 LCPUFA* | 12.07 (7.2-16.57) | 16.4 (15.16-17.75) | 9.03E-08 | 0.048 | 0.643 |

Data are presented as median value (% of total fatty acid concentration), and range: (25th- and 75th percentiles, % of total fatty acid concentration). Wilcoxon's signed rank test was used to test the population distribution differences between mothers and children, and the corresponding p-values are shown as $p_1$, with non-significant values indicated by †. A Spearman's rank correlation test was performed assessing the rank correlation for fatty acid proportions between mothers and children; $r_s$ is the Spearman's rank correlation coefficient (Spearman's rho), and $p_2$ is the corresponding p-value. For $p_2$, significant values are indicated by an asterisk.

## 4.4. Relation between FA proportions and allergy

Differences in fatty acid proportions were compared for non-allergic and allergic children and their mothers using a non-parametric analogue to the Student's t-test called the Mann-Whitney U-test, not dependent on normally distributed data.

Table 6 shows the relative proportions of individual fatty acids found in red blood cells of children at 4 months of age without and with any allergy diagnosis established 12 months after birth. Notably, alpha-linolenic acid (18:3n3) was found to make up a statistically significantly smaller proportion of the total fatty acid concentration in children with any type of allergy diagnosis.

*Table 6: Relative proportions of fatty acids in red blood cells in non-allergic and allergic children at 4 months.*

**Any allergy**
Child, 4 months

| **Fatty acid** | **Non-allergic, (n=120)** | **Allergic (n=27)** | **p** |
|---|---|---|---|
| *C14:0* | 0.78 (0.5-1.12) | 0.51 (0.4-0.9) | 0.061 |
| *C15:0* | 0.15 (0.1-0.19) | 0.1 (0.05-0.16) | 0.009* |
| *C16:0* | 22.99 (20.31-25.96) | 23.64 (19.8-25.46) | 0.928 |
| *C16.1n7* | 0.42 (0.29-0.64) | 0.29 (0.13-0.74) | 0.116 |
| *C17:0* | 0.31 (0.23-0.36) | 0.23 (0.14-0.35) | 0.033* |
| *C18:0* | 18.43 (16.73-20.64) | 19.36 (17.59-21.66) | 0.271 |
| *C18:1n9* | 19.69 (17.19-22.01) | 18.48 (16.26-21.69) | 0.387 |
| *C18:1n7* | 1.38 (1.19-1.56) | 1.31 (1.09-1.54) | 0.496 |
| *C18:2n6 (LA)* | 10.72 (8.89-12.26) | 10.15 (9.49-11.5) | 0.656 |
| *C18:3n3 (ALA)* | 0.17 (0.1-0.29) | 0.13 (0-0.19) | 0.044* |
| *C20:0* | 0.36 (0.29-0.43) | 0.33 (0.29-0.39) | 0.371 |
| *C20:1n9* | 0.34 (0.29-0.39) | 0.37 (0.29-0.42) | 0.163 |
| *C20:2n6* | 0.18 (0.12-0.22) | 0.21 (0-0.28) | 0.233 |
| *C20:3n6 (DGLA)* | 1 (0.68-1.32) | 1.03 (0.78-1.43) | 0.401 |
| *C20:4n6 (AA)* | 8.65 (5.03-12.93) | 8.95 (6.12-12.51) | 0.722 |
| *C22:0* | 0.96 (0.81-1.12) | 0.97 (0.79-1.19) | 0.81 |
| *C20:5n3 (EPA)* | 0.3 (0.15-0.51) | 0.25 (0.14-0.4) | 0.471 |
| *C22:4n6* | 1.12 (0.49-2.08) | 1.16 (0.63-1.98) | 0.484 |
| *C24:0* | 2.13 (1.93-2.46) | 2.52 (1.86-2.69) | 0.153 |
| *C24:1n9* | 3.01 (2.68-3.31) | 3.17 (2.7-3.71) | 0.192 |
| *C22:5n3* | 0.93 (0.5-1.73) | 0.91 (0.45-1.53) | 0.495 |
| *C22:6n3 (DHA)* | 2.96 (1.34-4.98) | 2.3 (1.55-4.82) | 0.9 |
| *Total n-3 PUFA* | 4.66 (2.34-7.48) | 3.8 (2.35-6.47) | 0.592 |
| *Total n-6 PUFA* | 23.65 (17.05-27.43) | 20.67 (18.4-27.24) | 0.924 |
| *n-6/n-3 ratio* | 4.74 (3.47-7.08) | 4.98 (3.56-8.1) | 0.467 |
| *Total n-3 LCPUFA* | 4.39 (2.08-7.23) | 3.6 (2.07-6.4) | 0.700 |
| *Total n-6 LCPUFA* | 11.08 (6.19-16.59) | 10.99 (7.58-15.72) | 0.592 |

Data are presented as median value (% of total fatty acid concentration), and range: ($25^{th}$- and $75^{th}$ percentiles, % of total fatty acid concentration). Mann-Whitney U-test was used to compare each individual fatty acid between the two groups. Significant p-values ($p<0.05$) are highlighted with an asterisk.

Table 7 shows the relative proportions of individual fatty acids found in red blood cells at 4 months after birth of mothers to children without and with any allergy diagnosis, established at 12 months after birth. Two monounsaturated 18-carbon fatty acids, 18:1n9 oleic acid and 18:1n7 vaccenic acid,

were found to be present at higher proportions in mothers to children diagnosed with any allergy than mothers to non-allergic children. The omega-3 fatty acid 22:6n3 DHA was found to be present at lower proportions in mothers to children diagnosed with allergy than mothers to healthy children. The three remaining omega-3 fatty acids ALA, EPA and DPA were found in lower proportions in mothers to allergic children, although not statistically significant. However, the total proportions of n-3 PUFA and n-3 LCPUFA were found to be statistically significantly lower in mothers to children with allergy than for mothers to non-allergic children.

*Table 7: Relative proportions of fatty acids in red blood cells at 4 months in mothers to non-allergic and allergic children.*

**Any allergy**
Mother, 4 months (%)

| **Fatty acid** | **Non-allergic (n=241)** | **Allergic (n=60)** | **p** |
|---|---|---|---|
| *C14:0* | 0.32 (0.25-0.4) | 0.31 (0.26-0.4) | 0.934 |
| *C15:0* | 0.16 (0.13-0.19) | 0.15 (0.12-0.18) | 0.131 |
| *C16:0* | 20.27 (19.5-20.83) | 20.41 (19.71-21.23) | 0.179 |
| *C16.1n7* | 0.43 (0.3-0.57) | 0.44 (0.31-0.74) | 0.257 |
| *C17:0* | 0.27 (0.25-0.32) | 0.27 (0.23-0.31) | 0.394 |
| *C18:0* | 16.36 (15.52-17.12) | 16.41 (15.23-17.06) | 0.679 |
| *C18:1n9* | 15.41 (14.56-16.26) | 15.81 (15.06-16.65) | 0.025* |
| *C18:1n7* | 1.09 (1-1.18) | 1.15 (1.01-1.25) | 0.05* |
| *C18:2n6 (LA)* | 12.95 (11.88-14.07) | 12.6 (11.26-14.3) | 0.412 |
| *C18:3n3 (ALA)* | 0.16 (0.12-0.22) | 0.16 (0.11-0.22) | 0.959 |
| *C20:0* | 0.19 (0.13-0.24) | 0.19 (0.1-0.25) | 0.922 |
| *C20:1n9* | 0.24 (0.19-0.28) | 0.23 (0.18-0.28) | 0.718 |
| *C20:2n6* | 0.14 (0.11-0.18) | 0.15 (0.11-0.19) | 0.456 |
| *C20:3n6 (DGLA)* | 1.46 (1.29-1.68) | 1.51 (1.3-1.67) | 0.855 |
| *C20:4n6 (AA)* | 13.13 (12.38-13.9) | 12.92 (12.01-13.91) | 0.426 |
| *C22:0* | 1 (0.9-1.15) | 0.97 (0.87-1.15) | 0.524 |
| *C20:5n3 (EPA)* | 0.99 (0.82-1.21) | 0.91 (0.73-1.13) | 0.092 |
| *C22:4n6* | 2.03 (1.77-2.38) | 2.06 (1.69-2.37) | 0.976 |
| *C24:0* | 2.43 (2.22-2.69) | 2.33 (2.09-2.73) | 0.118 |
| *C24:1n9* | 3.18 (2.85-3.51) | 3.17 (2.92-3.61) | 0.77 |
| *C22:5n3* | 2.48 (2.21-2.85) | 2.41 (2.19-2.72) | 0.122 |
| *C22:6n3 (DHA)* | 4.21 (3.5-4.87) | 3.88 (3.33-4.68) | 0.09* |
| *Total n-3 PUFA* | 7.87 (6.94-8.94) | 7.4 (6.88-8.09) | 0.035* |
| *Total n-6 PUFA* | 29.89 (28.86-30.96) | 29.53 (28.36-30.84) | 0.286 |
| *n-6/n-3 ratio* | 3.44 (3.09-4.02) | 3.62 (3.22-4.16) | 0.089 |
| *Total n-3 LCPUFA* | 7.69 (6.72-8.8) | 7.2 (6.7-7.98) | 0.035* |
| *Total n-6 LCPUFA* | 16.66 (15.76-17.76) | 16.75 (15.46-17.68) | 0.659 |

Data are presented as median value (% of total fatty acid concentration), and range: ($25^{th}$- and $75^{th}$ percentiles, % of total fatty acid concentration). Mann-Whitney U-test was used to compare each individual fatty acid between the two groups. Significant p-values ($p \leq 0.05$) are highlighted with an asterisk.

Table 8 shows the relative proportions of individual fatty acids found in red blood cells of children at 12 months after birth, with and without established allergy diagnoses. Only one fatty acid, C17:0 margaric acid was found to be statistically significantly lower proportionally in allergic children than in non-allergic children.

*Table 8: Relative proportions of fatty acids in red blood cells in non-allergic and allergic children at 12 months.*

**Any allergy**
Child, 12 months (%)

| Fatty acid | Non-allergic (n=203) | Allergic (n=39) | p |
|---|---|---|---|
| *C14:0* | 0.37 (0.25-0.52) | 0.31 (0.24-0.49) | 0.355 |
| *C15:0* | 0.13 (0.1-0.16) | 0.11 (0.07-0.16) | 0.064 |
| *C16:0* | 19.29 (18.56-20.31) | 19.22 (18.45-20.23) | 0.884 |
| *C16.1n7* | 0.29 (0.2-0.47) | 0.26 (0.21-0.37) | 0.596 |
| *C17:0* | 0.28 (0.24-0.32) | 0.25 (0.22-0.3) | 0.033* |
| *C18:0* | 15.83 (14.78-16.79) | 16.21 (15.13-16.92) | 0.322 |
| *C18:1n9* | 16.78 (15.44-18.35) | 17.12 (15.83-18.7) | 0.421 |
| *C18:1n7* | 1.2 (1.07-1.31) | 1.21 (1.09-1.38) | 0.165 |
| *C18:2n6 (LA)* | 14 (12.83-15.59) | 13.56 (11.98-14.95) | 0.171 |
| *C18:3n3 (ALA)* | 0.25 (0.16-0.41) | 0.26 (0.17-0.39) | 0.898 |
| *C20:0* | 0.22 (0.18-0.27) | 0.21 (0.16-0.25) | 0.171 |
| *C20:1n9* | 0.37 (0.31-0.43) | 0.38 (0.33-0.45) | 0.476 |
| *C20:2n6* | 0.23 (0.17-0.27) | 0.25 (0.21-0.3) | 0.067 |
| *C20:3n6 (DGLA)* | 1.3 (1.14-1.53) | 1.22 (1.04-1.49) | 0.216 |
| *C20:4n6 (AA)* | 12.91 (11.88-13.89) | 12.86 (11.78-13.53) | 0.504 |
| *C22:0* | 0.85 (0.74-0.95) | 0.8 (0.7-0.91) | 0.124 |
| *C20:5n3 (EPA)* | 0.47 (0.36-0.62) | 0.42 (0.34-0.59) | 0.217 |
| *C22:4n6* | 2.17 (1.87-2.48) | 2.29 (1.94-2.64) | 0.451 |
| *C24:0* | 2.06 (1.75-2.29) | 1.99 (1.74-2.29) | 0.643 |
| *C24:1n9* | 3 (2.64-3.41) | 3.15 (2.84-3.56) | 0.095 |
| *C22:5n3* | 1.89 (1.65-2.16) | 1.91 (1.61-2.18) | 0.765 |
| *C22:6n3 (DHA)* | 4.06 (3.44-4.78) | 4.07 (3.22-5.04) | 0.641 |
| *Total n-3 PUFA* | 6.78 (5.98-7.71) | 6.64 (5.76-7.9) | 0.551 |
| *Total n-6 PUFA* | 30.83 (29.62-31.88) | 30.43 (29.03-31.38) | 0.134 |
| *n-6/n-3 ratio* | 4.16 (3.66-4.79) | 4.08 (3.42-4.76) | 0.743 |
| *Total n-3 LCPUFA* | 6.44 (5.65-7.42) | 6.39 (5.38-7.46) | 0.629 |
| *Total n-6 LCPUFA* | 16.44 (15.15-17.79) | 16.25 (15.26-17.46) | 0.585 |

Data are presented as median value (% of total fatty acid concentration), and range: ($25^{th}$- and $75^{th}$ percentiles, % of total fatty acid concentration). Mann-Whitney U-test was used to compare each individual fatty acid between the two groups. Significant p-values ($p \leq 0.05$) are highlighted with an asterisk.

Differences in fatty acid proportions were examined for all groups using Mann-Whitney U-test, sorting on individual diagnoses eczema, asthma and food allergy. Significant differences were fewer than for comparisons between non-allergic and children with any allergy, and significant differences are shown below.

Figures 3-8 show the relative proportions of fatty acids exhibiting statistically significant difference between children with any type of food allergy and non-allergic children (and mothers to allergic and non-allergic children), within the age-groups (mothers, children - 4 months and children - 12 months).

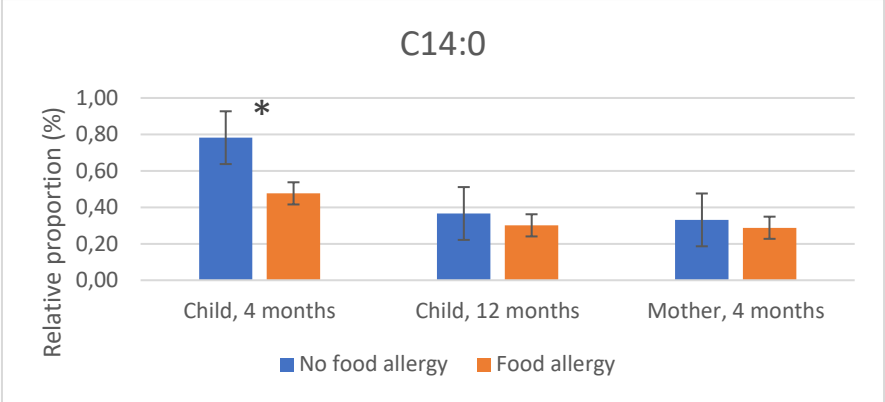


*Figure 3: Relative proportions of fatty acids 14:0 (Myristic acid) in mothers at 4 months, children at 4 months, and children at 12 months, with and without diagnosed food allergy, respectively. Statistically significant differences are indicated with an asterisk.*

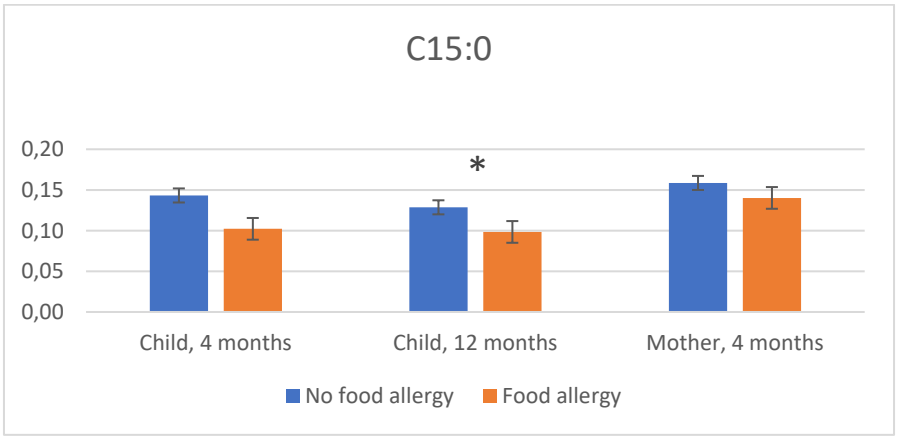


*Figure 4: Relative proportions of fatty acid C15:0 (Pentadecylic acid) in mothers at 4 months, children at 4 months, and children at 12 months, with and without diagnosed food allergy, respectively. Statistically significant differences are indicated with an asterisk.*

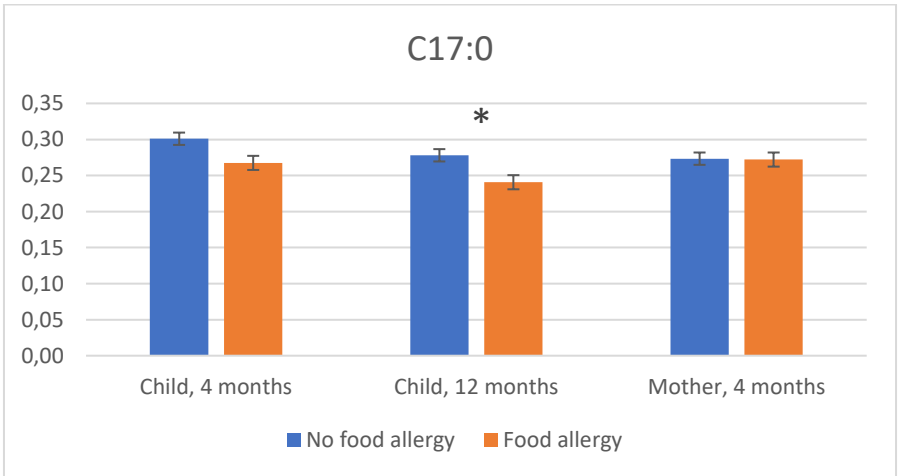


*Figure 5: Relative proportions of fatty acid C17:0 (Margaric acid) in mothers at 4 months, children at 4 months, and children at 12 months, with and without diagnosed food allergy, respectively. Statistically significant differences are indicated with an asterisk.*

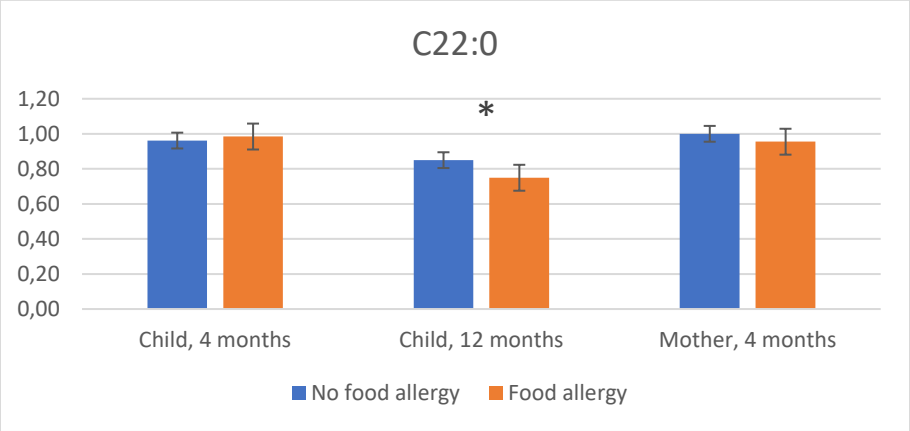


*Figure 6: Relative proportions of fatty acid C22:0 (Behenic acid) in mothers at 4 months, children at 4 months, and children at 12 months, with and without diagnosed food allergy, respectively. Statistically significant differences are indicated with an asterisk.*

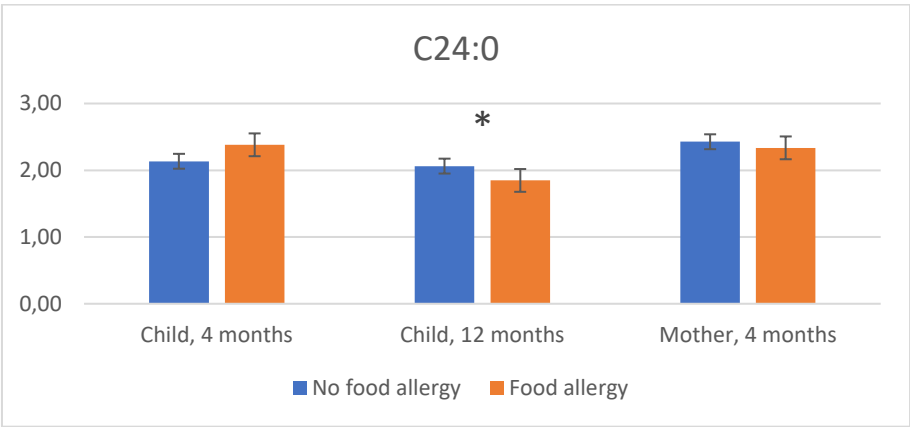


*Figure 7: Relative proportions of fatty acid C24:0 (Lignoceric acid) in mothers at 4 months, children at 4 months, and children at 12 months, with and without diagnosed food allergy, respectively. Statistically significant differences are indicated with an asterisk.*

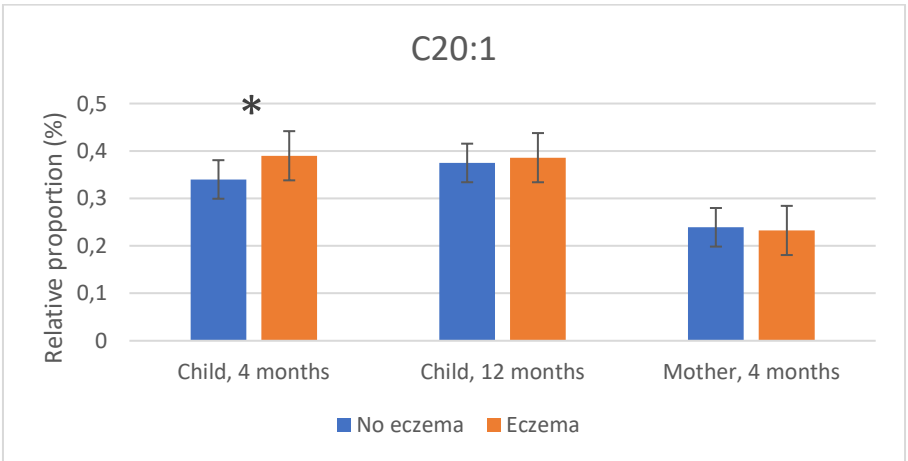


*Figure 8: Relative proportions of fatty acid 20:1 (Gondoic acid) in mothers at 4 months, children at 4 months, and children at 12 months, with and without diagnosed eczema, respectively. Statistically significant differences are indicated with an asterisk.*

## 4.5. Relation between diet and allergy

Maternal dietary intake was assessed using food-frequency-questionnaires (FFQs), answered 4 months after birth. Based on stated food consumption, daily intake of different fatty acids was calculated, and correlated to red blood cell fatty acid proportions for both mother and child. Weekly fish intake was reported in discrete intervals (e.g. 0-50 g/week) and not continuous values (e.g. 38 g/week). This led to that most median intake values of different kinds of fish were identical between allergy and non-allergy groups, even when the difference in intake between groups was found to be statistically significant, and so a graphical representation of intake values for fish and seafood is not included below.

Table 9 shows Spearman's rank correlation coefficient (Spearman's rho) for maternal dietary intake of fatty acids, and the proportion found in blood of the mothers, and corresponding p-values.
The correlation between the mothers' intake and blood proportions for children at 4 months was also assessed, and showed significant correlation only for C18:2n6 (r=-0.206, p=0.006), and C20:5n3 (r=0.183, p=0.014). The maternal dietary intake of individual fatty acids did not seem to be correlated with the proportions of those FAs found in the children's blood at 4 months, although a division of exclusively breast-fed infants and non-exclusively breast-fed infants was not done.

*Table 9: Correlation between the mothers' dietary intake and relative proportions in red blood cells for mothers, at 4 months after child birth.*

| **Spearman correlation between intake (g/week) & proportion in blood (%)** | **$r_s$** | **p** |
|---|---|---|
| ***C14:0*** | 0.227 | <0.001* |
| ***C16:0*** | 0.008 | 0.872 |
| ***C16:1n7*** | 0.015 | 0.782 |
| ***C18:0*** | 0.113 | 0.030* |
| ***C18:2n6 LA*** | 0.178 | <0.001* |
| ***C18:3n3 ALA*** | 0.077 | 0.139 |
| ***C20:0*** | -0.05 | 0.344 |
| ***C20:4n6 AA*** | 0.058 | 0.264 |
| ***20:5n3 EPA*** | 0.238 | <0.001* |
| ***22:5n3 DPA*** | 0.064 | 0.222 |
| ***22:6n3 DHA*** | 0.326 | <0.001* |

$r_s$ is the Spearman rank correlation coefficient. Significant p-values are indicated with an asterisk.

Table 10 shows the correlation between the dietary intake of fish and seafood and blood fatty acid proportions for the mothers at 4 months. The only significant correlation between maternal fish and seafood intake at 4 months and blood fatty acid proportions in the children at 4 months was for fatty fish and C20:5n3 (r=0.166, p=0.027).

*Table 10: Intake of fish for the mothers at 4 months after child birth, and the Spearman correlation coefficients and p-values for proportions of collections and individual n-3 and n-6 fatty acids.*

| | Mothers, 4 months | Total n-3 PUFA | Total n-6 PUFA | n-6/n-3 ratio | Total n-3 LCPUFA | Total n-6 LCPUFA | C18:3n-3 ALA | C20:4n-6 AA | C20:5n-3 EPA | C22:4n-6 AdA | C226n-3 DHA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Intake of lean fish (g/week)** | *Corr. coeff.* | - | - | - | - | - | - | - | - | - | - |
| | *p-value* | - | - | - | - | - | - | - | - | - | - |
| **Intake of fatty fish (g/week)** | *Corr. coeff.* | 0.266 | -0.146 | -0.256 | 0.268 | -0.122 | - | - | 0.207 | -0.179 | 0.303 |
| | *p-value* | <0.001* | 0.005* | <0.001* | <0.001* | 0.002* | - | - | <0.001* | <0.001* | <0.001* |
| **Intake of tuna(g/week)** | *Corr. coeff.* | - | -0.125 | - | - | - | - | - | - | -0.123 | 0.117 |
| | *p-value* | - | 0.017* | - | - | - | - | - | - | 0.019* | 0.025* |
| **Intake of seafood (g/week)** | *Corr. coeff.* | 0.155 | -0.115 | -0.162 | 0.152 | - | - | - | - | - | 0.168 |
| | *p-value* | 0.003* | 0.028* | 0.002* | 0.003* | - | - | - | - | - | 0.001* |

The diets of mothers of non-allergic children and mothers of children with any allergy diagnose was found to differ in several respects; overall the intake of SFAs, PUFAs and LCPUFAs in grams per day was higher for mothers to non-allergic children than for mothers to allergic children. This group of mothers also had a higher intake of lean fish in grams per week compared to the mothers with allergic children.

Mothers to non-allergic children notably had significantly higher intake of both essential fatty acids LA and ALA, precursors to the n-3 and n-6 LCPUFAs associated with lower prevalence of allergy.

No significant differences between aforementioned groups were found however for dietary intake of the LCPUFAs AA, EPA, DPA or DHA.

The pattern mentioned above held true also when comparing mothers to children with food allergy, and mothers to non-allergic children, with the exception that there was no significant difference in the weekly intake of lean fish. Median intakes, 25$^{th}$ and 75$^{th}$ percentile values and p-values correlating to a Mann-Whitney U-test assessing the differences in dietary intakes between groups are shown in table 11.

*Table 11: Daily intake of FAs at 4 months for mothers of children with any type of allergy, food allergy, and their non-allergic counterparts.*

| | **Any allergy** | | | **Food allergy** | | |
|---|---|---|---|---|---|---|
| | **Non-allergic (n=241)** | **Allergic (n=60)** | **p** | **Non-allergic (n=271)** | **Allergic (n=30)** | **p** |
| **ΣSFA (g/day)** | 28.25 (21.68-38.95) | 22.96 (16.65-31.71) | <0.001* | 28.11 (21.58-38.07) | 19.93 (15.64-31.07) | <0.001* |
| **SCFA (C4:0-10:0) (g/day)** | 2.32 (1.57-3.2) | 1.68 (1.17-2.73) | <0.001* | 2.27 (1.54-3.19) | 1.63 (1.03-2.11) | <0.001* |
| **C12:0 (g/day)** | 1.04 (0.74-1.38) | 0.79 (0.61-1.17) | <0.001* | 1.03 (0.72-1.36) | 0.75 (0.49-0.99) | <0.001* |
| **C14:0 (g/day)** | 3.08 (2.24-4.12) | 2.3 (1.72-3.32) | <0.001* | 3.06 (2.16-4.11) | 2.15 (1.53-2.97) | <0.001* |
| **C16:0 (g/day)** | 14.45 (11.28-19.17) | 11.39 (8.76-16.14) | <0.001* | 14.21 (11.14-18.9) | 10.73 (8.67-15.55) | <0.001* |

| | | | | | | |
|---|---|---|---|---|---|---|
| **C18:0 (g/day)** | 6.44 (4.77-8.78) | 5.04 (3.61-8.08) | <0.001* | 6.3 (4.73-8.59) | 4.11 (3.46-7.76) | 0.001* |
| **C20:0 (g/day)** | 0.21 (0.16-0.29) | 0.16 (0.12-0.25) | 0.0001* | 0.21 (0.15-0.29) | 0.14 (0.11-0.24) | 0.001* |
| **ΣMUFA (g/day)** | 24.58 (18.98-29.81) | 19.25 (15.86-27.02) | <0.001* | 24.17 (18.79-29.69) | 18.54 (15.88-25.08) | 0.002* |
| **C16:1 (g/day)** | 1.19 (0.97-1.47) | 1.06 (0.82-1.33) | 0.023* | 1.18 (0.95-1.47) | 1.05 (0.83-1.31) | 0.079* |
| **C18:1 (g/day)** | 22.48 (17.24-27.63) | 17.72 (14.33-24.86) | <0.001* | 22.01 (17.03-27.33) | 17.1 (14.29-22.78) | 0.002* |
| **ΣPUFA (g/day)** | 8.43 (6.37-11.04) | 7.24 (5.36-9.77) | 0.011* | 8.42 (6.29-10.97) | 6.52 (5.29-9.63) | 0.013* |
| **LA (C18:2) (g/day)** | 6.49 (4.72-8.58) | 5.57 (4.01-7.66) | 0.014* | 6.48 (4.67-8.5) | 4.74 (3.92-7.53) | 0.012* |
| **AA (C20:4) (g/day)** | 0.09 (0.07-0.12) | 0.09 (0.06-0.12) | 0.677 | 0.09 (0.07-0.12) | 0.09 (0.07-0.13) | 0.871 |
| **ALA (C18:3) (g/day)** | 1.21 (0.87-1.67) | 1.04 (0.77-1.43) | 0.008* | 1.2 (0.87-1.65) | 0.94 (0.74-1.38) | 0.005* |
| **EPA(C 20:5) (g/day)** | 0.08 (0.05-0.15) | 0.07 (0.05-0.15) | 0.354 | 0.08 (0.05-0.15) | 0.07 (0.04-0.16) | 0.725 |
| **DPA (C22:5) (g/day)** | 0.05 (0.03-0.08) | 0.04 (0.03-0.08) | 0.632 | 0.05 (0.03-0.08) | 0.04 (0.03-0.08) | 0.882 |
| **DHA (C22:6) (g/day)** | 0.18 (0.12-0.3) | 0.16 (0.1-0.31) | 0.542 | 0.18 (0.12-0.3) | 0.16 (0.1-0.33) | 0.968 |

Conversely, weekly intake of lean fish was the only statistically significant dietary difference between mothers to children with eczema and mothers to children with no eczema diagnosed at 12 months, with the intake being higher for mothers to children without eczema.
Regarding the dietary differences of mothers to children with or without asthma, yet another pattern in dietary differences were found to be statistically significant; weekly intake of fatty fish, daily intake of palmitic acid (16:0), AA, EPA, DPA and DHA were all higher among mothers to children without asthma.

Median intakes, 25$^{th}$ and 75$^{th}$ percentile values and p-values corresponding to mothers to children with and without asthma are presented in table 12.

*Table 12: Daily intake of selected FAs at 4 months for mothers of children with asthma, and children without asthma.*

| | **No asthma (n=123)** | **Asthma (n=5)** | **p** |
|---|---|---|---|
| **C16:0 (g/day)** | 14.11 (10.94-18.9) | 11.73 (8.43-16.14) | 0.045* |
| **AA (C20:4) (g/day)** | 0.09 (0.07-0.12) | 0.08 (0.05-0.09) | 0.014* |
| **EPA(C 20:5) (g/day)** | 0.08 (0.05-0.15) | 0.06 (0.03-0.08) | 0.018* |
| **DPA (C22:5) (g/day)** | 0.05 (0.03-0.08) | 0.04 (0.03-0.05) | 0.027* |
| **DHA (C22:6) (g/day)** | 0.18 (0.11-0.3) | 0.13 (0.09-0.19) | 0.021* |

# 5. Discussion

Although the mechanisms behind allergy development, and the relation between fatty acid proportions in red blood cells are not fully understood, the general results from the present study do not contradict commonly occurring observations or hypotheses.

As seen in section 4.2, proportions of a majority of the studied LCPUFAs were strongly correlated between mothers and their children, as well as between the children at 4 months and at 12 months after birth. Proportional differences between the groups are most likely explained by natural fluctuations due to growth and development.

Previous studies and review articles have described proportions of n-3 PUFA/LCPUFAs to correlate negatively with general allergy development, although the cause-and-effect-relationship has not been established (11)(12)(13).

ALA proportions were significantly lower at 4 months for children who were diagnosed with any allergy than for non-allergic children, possibly indicating higher demand for ALA-derived n-3 LCPUFAs and metabolites of these LCPUFAs, causing ALA levels to decrease.

The proportions of DHA were found to be significantly lower at 4 months for mothers of children diagnosed with any allergy than for mothers to non-allergic children, as well as proportions of n-3 PUFA. This corroborates findings from previous studies (6)(25)(26)(5) , while the question of causality still remains unanswered; is allergy the cause of an altered n-3 fatty acid metabolic state, or is development of allergy causing an altered metabolism?

Regarding only food allergies, all significant findings in this study pointed towards proportions of saturated fatty acids being negatively correlated with allergy. The roles of saturated fatty acids and MUFA in allergy development have not been as extensively studied as the LCPUFA, but reviewed studies have shown both equivalent proportions of saturated fatty acids between allergic and non-allergic individuals (18) as well as higher among the allergic group (27).

The mothers' diets at 4 months seems to influence their own blood fatty acid proportions in more or less the expected manner; intake of fish and seafood, and n-3 LCPUFAs in the diet was correlated with higher n-3 proportions in the blood, and higher n-3 to n-6 ratio. The correlations between the mothers dietary fatty acid intake and their breast-fed 4-month infants were less pronounced, but seemed to influence the ratio of n-3 to n-6 FAs in the babies' erythrocytes. However, the fatty acid proportions found in blood might not only be affected by dietary intake and allergic disease. Environmental factors might lead to higher or lower levels, as well as the metabolic state of the individual; it is for example hypothesized that women in fertile age and especially while pregnant may be able to upregulate conversion of DHA (28)(29). Some studies have also proposed that the placental transport of fatty acids to the fetus can be both selective and regulated (30)(31) and that infants and maybe also fetuses themselves convert LA and ALA to LCPUFAs (32), therefore blood proportions in infants may not reflect maternal dietary intake.

The dietary differences at 4 months between mothers to allergic and non-allergic children were quite significant regarding intake of both saturated and unsaturated fatty acids up to 18 carbons, while the difference for intake of AA, EPA, DPA and DHA was not found to be significant between the allergy and non-allergy group. Importantly, the intake of LA and ALA, precursors for the LCPUFAs were significantly higher for mothers of children without allergy, possibly indicating that the

conversion of these shorter-chain FAs to the longer varieties is indeed lower in individuals who develop allergy.

Conversely, the intake of AA, EPA, DPA and DHA (along with C16:0), but not the shorter FAs was found to be significantly different between mothers of children who developed asthma at 12 months, and children who did not. This is in line with findings from previous studies (33)(34), but the mechanisms are still not unveiled.

This study found that proportions of C15:0 and C17:0 were significantly lower for allergic children at 4 months, and C17:0 lower for allergic children at 12 months. C15:0 and C17:0 have been described as biomarkers for dairy consumption (35). Whether particular saturated fatty acids might be protective against allergy development, or early introduction of common allergens such as milk may prevent allergy is however unclear.

Worth to note when examining the part that diet plays in the development of allergy, is that dietary data is often, as in this case, self-reported frequencies, and may not accurately or precisely reflect actual intake. Also, in this study the 4-month-old children were assumed to have breast-milk from their mothers as a primary diet. Their fatty acid proportions might certainly be affected by any food items or supplementary milk formulas etc., but corrections and exclusions based on dietary data for the 4-month-olds were not feasible for the time-frame of this study.

As pointed out in many other studies, diet may often be connected with other factors that can influence the matter of examination; for example, higher fish intake is often viewed as a proxy for a healthier lifestyle, which may in turn impact the development of allergy etc.

Other components that have been suggested to affect allergy development is the maternal and paternal heredity, which was not accounted for in this study, mainly due to time constraints, and the same is true for several other factors such as the occurrence of older siblings or pets.

Ongoing and future studies on the NICE cohort will hopefully help to validate results of this study, and make it possible to draw conclusion regarding the relationship between fatty acid proportions in different sample types of the same individuals. Since the NICE study is prospective, it is possible to investigate the fatty acid proportions in individuals before the outbreak of allergic symptoms, and hopefully along with genotype studies shed light on the relation and causality of allergy and fatty acid metabolism.

# 6. Conclusions

Intake of fish was shown to be significantly correlated to increased levels of n-3 in erythrocytes, and the proportions of fatty acids was shown to generally reflect the dietary intake. Proportions of many fatty acids in erythrocytes were also shown to be related between mothers and their children 4 months, and also between the children at 4 and 12 months.

Although not conclusive, this study has generally found significant negative correlations between higher erythrocyte proportions of n-3 LCPUFA proportions and development of allergy and asthma. Based on these results and previous findings, intake of fish seems to be protective against development of allergy and hypersensitivity conditions, and eating fish could be recommended for the population in general, and especially pregnant and breast-feeding women.

Mothers of children diagnosed with asthma were also found to have significantly lower dietary intake of fatty fish, palmitic acid (C16:0), AA, EPA, DPA and DHA, while the erythrocyte fatty acid proportions did not show the same strong correlation specifically for asthma. As stated earlier, fish, and especially fattier fish seems to be protective against both allergy and asthma development, and increased consumption ought to be encouraged. It is unclear if the n-3 LCPUFAs are the only components in fish that helps prevent allergy development and asthma, but the fact that lower dietary intake of LCPUFAs AA, EPA, DPA and DHA was connected to higher prevalence of asthma indicates that LCPUFAs are indeed the primary protective factors of fish consumption.

Children diagnosed with any allergy were found to have lower proportions of ALA than non-allergic children at 4 months, while mothers of allergic children had lower proportions of the ALA-derived LCPUFA DHA, and lower total n-3 LCPUFA proportions than mothers of non-allergic children. The dietary intake of ALA was however also lower for mothers with allergic children, so whether lower ALA levels depend on altered fatty acid metabolism as a result of allergy development as proposed by some, or solely on the dietary intake cannot be distinguished.

In a similar manner, the higher proportions of dairy biomarkers C15:0 and C17:0 in non-allergic children could indicate that they act as a protection against allergy. However, it could also simply indicate a higher dairy consumption and allergen exposure, which in turn could reduce the development of allergy.

Generally, higher fish consumption seems to prevent development of allergy and hypersensitivity conditions, but the mechanisms are not fully understood. Future research may shed light on the relationship between erythrocyte fatty acid proportions and allergy development, as well as establish if altered fatty acid metabolism causes allergy, or vice versa.

# References


1. Miljöhälsorapport 2013 Institutet för Miljömedicin [Internet]. [cited 2019 Feb 25]. Available from: http://www.imm.ki.se/MHR2013.pdf

2. Anandan C, Nurmatov U, Sheikh A. Omega 3 and 6 oils for primary prevention of allergic disease: Systematic review and meta-analysis. Allergy: European Journal of Allergy and Clinical Immunology. 2009.

3. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, et al. Molecular Biology of the Cell. 5th ed. New York, NY: Garland Science; 2008.

4. Barman M. THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY Long chain polyunsaturated fatty acids in serum phospholipids Relation to genetic polymorphisms, diet and allergy development in children [Internet]. [cited 2019 Feb 3]. Available from: http://publications.lib.chalmers.se/records/fulltext/214957/214957.pdf

5. Dunstan JA, Mori TA, Barden A, Beilin LJ, Holt PG, Calder PC, et al. Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition. Eur J Clin Nutr [Internet]. 2004 Mar 26 [cited 2019 Jun 7];58(3):429–37. Available from: http://www.nature.com/articles/1601825

6. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta - Mol Cell Biol Lipids [Internet]. 2015 Apr 1 [cited 2019 Jun 18];1851(4):469–84. Available from: https://www.sciencedirect.com/science/article/pii/S1388198114001656?via%3Dihub

7. Barman M, Johansson S, Hesselmar B, Wold AE, Sandberg A-S, Sandin A. High Levels of Both n-3 and n-6 Long-Chain Polyunsaturated Fatty Acids in Cord Serum Phospholipids Predict Allergy Development. Krauss-Etschmann S, editor. PLoS One [Internet]. 2013 Jul 10 [cited 2019 Feb 3];8(7):e67920. Available from: https://dx.plos.org/10.1371/journal.pone.0067920

8. Vorvick LJ. Antigen. In: US National Library of Medicine Medical Encyclopedia [Internet]. 2017. Available from: https://medlineplus.gov/ency/article/002224.htm

9. Desentum. Allergic sensitization [Internet]. [cited 2019 Mar 2]. Available from: https://www.desentum.fi/en/how-allergies-work/allergic-sensitization

10. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature [Internet]. 1998 Mar [cited 2019 Apr 28];392(6673):245–52. Available from: http://www.nature.com/articles/32588

11. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, et al. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: A randomized, controlled trial. J Allergy Clin Immunol [Internet]. 2003 Dec 1 [cited 2019 Feb 24];112(6):1178–84. Available from: https://www.sciencedirect.com/science/article/pii/S0091674903022711?via%3Dihub

12. Kull I, Bergstrom A, Lilja G, Pershagen G, Wickman M. Fish consumption during the first year of life and development of allergic diseases during childhood. Allergy [Internet]. 2006 Aug 1 [cited 2019 Feb 24];61(8):1009–15. Available from: http://doi.wiley.com/10.1111/j.1398-9995.2006.01115.x

13. Venter C, Meyer RW, Nwaru BI, Roduit C, Untersmayr E, Adel-Patient K, et al. EAACI position paper: Influence of dietary fatty acids on asthma, food allergy, and atopic dermatitis. Allergy [Internet]. 2019 May 3 [cited 2019 May 29];all.13764. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31032983

14. Hoppu U, Rinne M, Lampi A-M, Isolauri E. Breast Milk Fatty Acid Composition Is Associated with Development of Atopic Dermatitis in the Infant. J Pediatr Gastroenterol Nutr [Internet]. 2005 Sep [cited 2019 Jun 7];41(3):335–8. Available from: https://insights.ovid.com/crossref?an=00005176-200509000-00012

15. Duchén K, Casas R, Fagerås-Böttcher M, Yu G, Björkstén B. Human milk polyunsaturated long-chain fatty acids and secretory immunoglobulin A antibodies and early childhood allergy. Pediatr Allergy Immunol [Internet]. 2000 Feb [cited 2019 Jun 7];11(1):29–39. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10768733

16. Oddy WH, Pal S, Kusel MMH, Vine D, Klerk NH, Hartmann P, et al. Atopy, eczema and breast milk fatty acids in a high-risk cohort of children followed from birth to 5 yr. Pediatr Allergy Immunol [Internet]. 2006 Feb [cited 2019 Jun 7];17(1):4–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16426248

17. Thijs C, Müller A, Rist L, Kummeling I, Snijders BEP, Huber M, et al. Fatty acids in breast milk and development of atopic eczema and allergic sensitisation in infancy. Allergy [Internet]. 2011 Jan [cited 2019 Jun 7];66(1):58–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20659079

18. Mikkelsen A, Galli C, Eiben G, Ahrens W, Iacoviello L, Molnár D, et al. Blood fatty acid composition in relation to allergy in children aged 2–9 years: results from the European IDEFICS study. Eur J Clin Nutr [Internet]. 2017 Jan 21 [cited 2019 Jun 8];71(1):39–44. Available from: http://www.nature.com/articles/ejcn2016158

19. Barman M, Murray F, Bernardi AI, Broberg K, Bölte S, Hesselmar B, et al. Nutritional impact on Immunological maturation during Childhood in relation to the Environment (NICE): a prospective birth cohort in northern Sweden. BMJ Open [Internet]. 2018 Oct 21 [cited 2019 Feb 5];8(10):e022013. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30344169

20. Masood A, Stark KD, Salem N. A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. J Lipid Res [Internet]. 2005 Oct [cited 2019 Apr 27];46(10):2299–305. Available from: http://www.jlr.org/lookup/doi/10.1194/jlr.D500022-JLR200

21. Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ, et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. Br J Dermatol [Internet]. 1994 Sep [cited 2019 Jun

18];131(3):383–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7918015

22. Williams HC, Burney PG, Strachan D, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. II. Observer variation of clinical diagnosis and signs of atopic dermatitis. Br J Dermatol [Internet]. 1994 Sep [cited 2019 Jun 18];131(3):397–405. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7918016

23. WILLIAMS HC, JBURNEY PG, PEMBROKE AC, HAY RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. Br J Dermatol [Internet]. 1994 Sep 1 [cited 2019 Jun 18];131(3):406–16. Available from: http://doi.wiley.com/10.1111/j.1365-2133.1994.tb08532.x

24. Ejlertsson G. Statistik för hälsovetenskaperna. 2nd ed. Lund: Studentlitteratur AB; 2012.

25. Stratakis N, Gielen M, Margetaki K, de Groot RHM, Apostolaki M, Chalkiadaki G, et al. PUFA status at birth and allergy-related phenotypes in childhood: a pooled analysis of the Maastricht Essential Fatty Acid Birth (MEFAB) and RHEA birth cohorts. Br J Nutr [Internet]. 2018 Jan 23 [cited 2019 Feb 23];119(02):202–10. Available from: https://www.cambridge.org/core/product/identifier/S0007114517003348/type/journal_article

26. Willemsen LEM. Dietary n-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment. Eur J Pharmacol [Internet]. 2016 Aug 15 [cited 2019 Jun 18];785:174–86. Available from: https://www.sciencedirect.com/science/article/pii/S0014299916301984?via%3Dihub

27. Harris C, Demmelmair H, von Berg A, Lehmann I, Flexeder C, Koletzko B, et al. Associations between fatty acids and low-grade inflammation in children from the LISAplus birth cohort study. Eur J Clin Nutr [Internet]. 2017 Nov 7 [cited 2019 Jun 19];71(11):1303–11. Available from: http://www.nature.com/articles/ejcn201773

28. Burdge GC, Wootton SA. Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr [Internet]. 2002 Oct 9 [cited 2019 Jun 19];88(4):411–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12323090

29. Burdge GC, Calder PC. Conversion of **α** -linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev [Internet]. 2005 Sep [cited 2019 Jun 19];45(5):581–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16188209

30. Haggarty P. Fatty Acid Supply to the Human Fetus. Annu Rev Nutr [Internet]. 2010 Jul 20 [cited 2019 Jun 19];30(1):237–55. Available from: http://www.annualreviews.org/doi/10.1146/annurev.nutr.012809.104742

31. Haggarty P, Ashton J, Joynson M, Abramovich DR, Page K. Effect of Maternal Polyunsaturated Fatty Acid Concentration on Transport by the Human Placenta. Biol Neonate [Internet]. 1999 [cited 2019 Jun 19];75(6):350–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10325438

32. Carnielli VP, Wattimena DJL, Luijendijk IHT, Boerlage A, Degenhart HJ, Sauer PJJ. The Very Low Birth Weight Premature Infant Is Capable of Synthesizing Arachidonic and Docosahexaenoic Acids from Linoleic and Linolenic Acids. Pediatr Res [Internet]. 1996 Jul [cited 2019 Jun 19];40(1):169–74. Available from: http://www.nature.com/doifinder/10.1203/00006450-199607000-00029

33. Romieu I, Torrent M, Garcia-Esteban R, Ferrer C, Ribas-Fitó N, Antó JM, et al. Maternal fish intake during pregnancy and atopy and asthma in infancy. Clin Exp Allergy [Internet]. 2007 Apr [cited 2019 Jun 19];37(4):518–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17430348

34. Lumia M, Luukkainen P, Tapanainen H, Kaila M, Erkkola M, Uusitalo L, et al. Dietary fatty acid composition during pregnancy and the risk of asthma in the offspring. Pediatr Allergy Immunol [Internet]. 2011 Dec [cited 2019 Jun 19];22(8):827–35. Available from: http://doi.wiley.com/10.1111/j.1399-3038.2011.01202.x

35. Jenkins B, West J, Koulman A. A Review of Odd-Chain Fatty Acid Metabolism and the Role of Pentadecanoic Acid (C15:0) and Heptadecanoic Acid (C17:0) in Health and Disease. Molecules [Internet]. 2015 Jan 30 [cited 2019 Jun 19];20(2):2425–44. Available from: http://www.mdpi.com/1420-3049/20/2/2425