Association between prenatal and current exposure to selected LCPUFAs and school performance at age 7.

ISM van der Wurff\textsuperscript{1*}, EC Bakker\textsuperscript{2}, G Hornstra\textsuperscript{3}, PA Kirschner\textsuperscript{1}, M Gielen\textsuperscript{4}, RWL Godschalk\textsuperscript{4}, S Kremers\textsuperscript{4}, MP Zeegers\textsuperscript{4,5}, RHM de Groot\textsuperscript{1,4}

\textsuperscript{1} Faculty of Psychology and Educational Sciences, Welten Institute, Open University of the Netherlands, Heerlen, the Netherlands
\textsuperscript{2} Faculty of Psychology and Educational Sciences, Open University of the Netherlands, Heerlen, the Netherlands
\textsuperscript{3} Maastricht University (retired) and Nutrisearch, Gronsveld, the Netherlands
\textsuperscript{4} NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands
\textsuperscript{5} CAPHRI School for Public Health and Primary Care Maastricht University, Maastricht, the Netherlands

*Corresponding author.

Inge van der Wurff, MSc.

Welten Institute, Open University of the Netherlands
Valkenburgerweg 177, P.O. Box 2960 6401 DL Heerlen,

inge.vanderwurff@ou.nl

Tel: +31 45 576 2909
Fax: +31 45 576 2800
Abstract

Introduction: Long-chain polyunsaturated fatty acids (LCPUFAs) are important for brain functioning and might, thus, influence cognition and school performance. However, research investigating LCPUFAs relationships with school performance is limited. The objective of this study was to determine the association between levels of the LCPUFAs docosahexaenoic acid (DHA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and n-6 docosapentaenoic acid (Osbond acid, ObA) at study entry, 22 weeks of pregnancy, 32 weeks of pregnancy, at partus, in umbilical cord plasma and child’s plasma at age 7 and school performance scores at age 7.

Methods: Data from the Maastricht Essential Fatty Acid Birth cohort (MEFAB) were used for this study. Fatty acid levels of plasma phospholipids were measured in maternal blood plasma at study entry, 22 weeks of pregnancy, 32 weeks of pregnancy and partus. Childs fatty acid levels of plasma phospholipids were measured in umbilical cord blood plasma, and in blood plasma of the child at age 7. Scores on national standardised tests for spelling, reading and arithmetic at age 7 were obtained via the school (scores were available for 149, 159 and 155 children, respectively). Associations between LCPUFA levels and school performance scores were analysed with categorical regression analyses with correction for covariates (smoking, maternal education, sex, breastfeeding, maternal intelligence, birth weight and BMI at age 7).

Results: Significant (p < 0.001) associations between DHA level at age 7 and both reading (β = 0.158) and spelling (β = 0.146) were found. Consistent significant negative associations were observed between all DHA maternal plasma levels and arithmetic scores at age 7 (all p < 0.001, all β < -0.019). Additional significant negative associations were observed between maternal LCPUFA plasma levels at study entry and both reading and spelling scores at age 7; these associations were less consistent.

Conclusion: Plasma DHA levels at age 7 were positively associated with reading and spelling
scores at age 7. Consistent significant negative associations between maternal plasma DHA levels and arithmetic scores of the child at age 7 were found. Although this is an observational study, which cannot proof causality, the consistent negative associations observed between maternal plasma DHA levels and the arithmetic scores of the children at age 7 calls upon prudence when considering DHA supplementation during pregnancy.

**Keywords:** docosahexaneoic acid, school performance, long-chain polyunsaturated fatty acids, brain function.
Abbreviations

AA, arachidonic acid, 20:4n-6
ALA, \( \alpha \)-linolenic acid, 18:3n-3
CATREG, Categorical regression analysis
DHA, docosahexaenoic acid, 22:5n-3
EPA, eicosapentaenoic acid, 20:5n-3
LCPUFAs, Long-chain polyunsaturated fatty acids
MEFAB, Maastricht Essential Fatty Acid Birth Cohort
ObA, Osbond acid, n-6 docosapentaenoic acid, 22:5n-6
1. Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) are important constituents of all cell membranes. In this way they are involved in, among others, neuronal membrane fluidity, neurotransmission, signal transduction, brain blood flow and blood-brain barrier integrity [1,2]. With respect to brain functioning, four LCPUFAs are of major importance: docosahexaneoic acid (DHA) and arachidonic acid (AA) are important components of the neuronal membrane [3], eicosapentaenoic acid (EPA) influences a large number of brain processes [4], and n-6 docosapentaenoic acid (Osbond acid, ObA) is a deficiency marker of DHA [5]. Because these LCPUFAs seem to be essential for brain functioning they might in turn affect school performance. Therefore, the associations between LCPUFA levels in maternal plasma at study entry (<16 weeks of pregnancy), 22 weeks of pregnancy, 32 weeks of pregnancy and at partus, in umbilical cord blood plasma, and in blood plasma of the child at age 7 and school performance at age 7 were investigated in this study. The maternal plasma levels were used as a proxy for the foetal exposure, as a number of studies have shown that maternal LCPUFA plasma levels correlate highly with the LCPUFA levels of foetuses [6–8].

In earlier studies the association between fish intake (primary source of DHA and EPA) [9–15], LCPUFAs intake calculated based on answers to questions regarding fish consumption [10,12,15] and/or LCPUFA concentrations in blood [12,14–19] and cognition has been studied, but the results of these studies do not show consistent patterns. Similarly, results from randomized controlled trials are mixed. Some supplementation trials studying the influence of maternal n-3 LCPUFA supplementation during pregnancy and/or lactation and/or n-3 supplementation to infants after birth on cognition of the child have shown positive results [20–29], others have found null results [16,23,30] and some found negative results [31–35].
To the best of our knowledge, the association between LCPUFA intake or levels during early life and school performance at age 7 has not yet been addressed. This is unfortunate since children are not judged on their cognition but, rather, on their school performance. Although no studies are available about this association in children, some studies in adolescents have been executed. De Groot et al. showed an inverted u-shaped association in 700 adolescents (age 12-18) between reported fish intake and vocabulary. Thus higher fish consumption was associated with a higher vocabulary score, however fish intake higher than twice a week was associated with a lower vocabulary score. A similar trend (non-significant) trend, was found for the average school grades of Dutch, English and mathematics [36]. Likewise, Kim et al. found a strong positive association between the number of meals containing fish per week and average end-term grade across 16 school subjects in adolescents age 15 years [37]. These studies suggest a possible relation between school performance and LCPUFA intake during adolescence, a period of life which is characterised by development of the prefrontal cortex [38,39].

Brain development is also prominent during pregnancy and childhood. Considering the observed positive association between fish consumption and school grades in adolescents, one could infer that a relationship between LCPUFA levels during pregnancy and childhood and school performance is also possible. Brain development starts very early in pregnancy with neural tube formation and continues throughout pregnancy with in the last trimester and the first 2 years of postnatal life a brain growth spurt. In this growth-spurt DHA rapidly accumulates in the brain [40,41]. DHA availability is in this period therefore very important, as the foetus is largely dependent on supply of DHA and other LCPUFAs via the placenta [42–44]. After birth supply of DHA via breast milk or infant formula is needed to provide sufficient amounts [45]. Lastly, during childhood, DHA might also play a role in brain development and maturation since brain development continues until late adolescence [38].
Overall, the exact influence of LCPUFA exposure during development on school performance remains unclear. Therefore, the objective of the current study is to investigate the associations between pre-peri-, and postnatal exposures to DHA, EPA, AA and ObA and school performance at age 7. Exposure data were inferred from LCPUFA concentrations in phospholipids from maternal plasma collected at study entry, 22 weeks of pregnancy, 32 weeks of pregnancy, at partus, in umbilical cord plasma, and in plasma phospholipids of the children at age 7. It is hypothesised that higher LCPUFA exposure during pregnancy, in umbilical cord plasma and child’s plasma at age 7 are associated with higher school performance scores in children aged 7.
2. Participants and Methods

2.1 Design

Data from the Maastricht Essential Fatty Acid Birth (MEFAB) cohort were used for this study. Founded in 1989, MEFAB was originally set-up to investigate the association between essential fatty acid status during pregnancy and birth outcomes (MEFAB 1, for an overview see [46,47]). Later, the children and their parents were asked to participate in various follow-up studies (MEFAB 2, 3 and 4, for an overview see [48]).

For the current study data from MEFAB 1 and 2 were used to study the association between selected LCPUFA levels (DHA, AA, EPA an ObA) in maternal non-fasted plasma phospholipids collected at study entry (before 16 weeks of pregnancy; μ=10.93 weeks), at 22 weeks and 32 weeks of pregnancy and at delivery and school performance scores at age 7. Furthermore, LCPUFA levels of the children were determined in phospholipids from umbilical cord plasma and from non-fasted venous plasma at age 7, these levels were also used to study the associations between selected LCPUFAs and school performance at age 7.

The study was approved by the Ethics Committee of the University Hospital Maastricht/ Maastricht University.

2.2 Participants

The eligible study population consisted of 750 Caucasian children aged 7-8 years born between December 1990 and January 1994. After 7 years, 728 of these children were traced. Of them 3 were deceased and 34 moved abroad and therefore did not participate in the follow-up. In addition, 133 parents did not reply to the invitation. The parents of 231 children refused to give consent for participation in the follow-up, primarily mentioning lack of time and concerns about the burden on their child as reason for refusal. As a result, the parents of 327
children gave their consent for participation. During the MEFAB 2 study period, 21 children dropped out leaving a final study population of 306 children.

Unfortunately, not all schools attended by the participants administered the standardised school performance tests. In addition, some schools administered the tests at another age or were not willing to provide the results. The school performance scores were obtained for 149, 159 and 155 children for reading, spelling and arithmetic, respectively. For the flow diagram, see Figure 1.

*Insert Figure 1 here*

2.3 Blood and fatty acid analyses

Fatty acid profiles of maternal and umbilical plasma phospholipids were determined by gas-liquid chromatography as described by Al et al. [46]. The analysis of the blood samples at age 7 was slightly different, total lipids were extracted with the method of Blight and Dyer [49]. The gas-chromatographic methodology enabled the quantitative measurement of forty-two fatty acids [46]. Focus of the current paper is on DHA, AA, EPA, and ObA, as previously indicated. Fatty acid data are presented as relative levels (% of weight of total phospholipid-associated fatty acids).

2.4 Measurements of academic achievement

To assess school performance at age 7, scores from the standard Dutch Cito-system (Central institute of test development) for spelling, reading and arithmetic were obtained from the school [50]. Results on the Cito tests are given as a letter (A through E). This letter is based on the scores of a normative group of children on the tests. If the score of a child is in the same range as the 25% best scoring children in the normative group the child will receive an A. The 25% above average is indicated by a B, 25% below average is indicated by a C, the
15% far below average are indicated by a D and the 10% worst scores are indicated by E. To achieve even groups, group D and E have been combined for the current study.

2.5 Covariates

A number of factors known to be important determinants of cognitive development were included as covariates in all the analyses: breastfeeding (yes/no) [51], birth weight in grams [52,53], the sex of the child [54], maternal intelligence [55], maternal education (low/high) [55,56], maternal smoking during pregnancy (yes/no) [57,58], and the child’s BMI at 7 years of age [59]. Maternal intelligence was based on the Raven’s Standard Progressive Matrices (RAVEN) with a maximum score of 60 [60]. Body weight was measured to the nearest 100 gram on a SECA electronic digital scale, while children wore light underwear only. Height was measured to the nearest millimetre using a wall mounted stadiometer (Holtain LTD, Crymych, UK) as described in detail by Gerver and de Bruin [61]. BMI (kg/m²) was calculated and classified for boys as underweight (BMI ≤14.03), healthy weight (BMI 14.04-17.91) or overweight (BMI ≥17.92) and for girls as underweight (BMI ≤13.85), healthy weight (BMI 13.86-17.74) or overweight (BMI ≥17.75).

2.6 Statistical analyses

Because of missing values, multiple imputations (i.e. fully conditional specification technique) were used to analyse the data [62]. Categorical regression analysis [63] was used to estimate the association between DHA, AA, EPA or ObA levels measured at study entry, 22 weeks of pregnancy, 32 weeks of pregnancy, partus, in umbilical cord blood plasma, and in blood plasma of the child at age 7 and school performances at age 7. All covariates were entered in the initial model. When the Pratt-coefficient of relative importance for a given covariate was <.05 or the p-value was>.1 that covariate was excluded from the analysis. The beta and p-value of the fatty acid levels at specific time points in the adjusted model are reported. The beta value as reported is the normal standardized regression coefficient of the
optimally transformed variables. CATREG computes quantifications for each category by optimizing a least squares criterion. For example, if one looks at the association between maternal DHA level at study entry and arithmetic score (A, B, C or D), the categories A, B, C, and D get the values 0.80, -0.50, -1.55 and -2.02, respectively. Here the beta for DHA is -0.170. This implies that an increase of 1 SD in DHA would lower the predicted score of the transformed arithmetic scale with 0.170. For example, a difference between C en D, which is -2.02+1.55=0.47, can be quantified in 0.47/0.170=2.76 standard deviations of DHA. This is 2.76*0.83=2.29 %wt/wt of total FA.

Due to the large number of analyses p <.01 was considered statistically significant, unless noted otherwise. Analyses were carried out using SPSS statistics version 22 (IBM).

Quality check

As check for selection bias characteristics of participants who participated in MEFAB 1 only and those who also participated in MEFAB 2 were compared. Data was first checked for normality. If scale data was normally distributed an independent t-tests was performed, if not a Mann Withney U test was performed. For the nominal and ordinal data a Chi-squared test was performed. These analyses were performed for all fatty acids at all time-points, birthweight, birth length, head circumference, gestational age, age mother, maternal intelligence, sex child and smoking during pregnancy. This procedure was also followed to check for differences between participant of MEFAB 2 of whom school performance scores were available and those whose scores were not available. Lastly the procedure was repeated to check whether there was a difference between mothers who suffered from adverse pregnancy events (intrauterine growth restriction, preeclampsia or pregnancy induced hypertension) and those who did not suffer from adverse pregnancy events.
3. Results

For 170 children, one or more school performance scores were available. The characteristics of these participants and their mothers can be found in Table 1.

*Insert Table 1 here*

3.1 Plasma phospholipid fatty acid levels

Of the 170 children, 140 donated non-fasted blood to determine the plasma phospholipid fatty acid levels at follow-up. The levels of these fatty acids at age 7 can be found in Table 2. This table also contains the maternal fatty acid levels at study entry (mean pregnancy duration 10.93 weeks), at 22 and 32 weeks of pregnancy, and at partus, as well as the umbilical plasma fatty acid levels.

*Insert Table 2 here*

3.2 School performances scores

School performance scores for reading, spelling and arithmetic were available for 149, 159 and 155 children respectively. Table 3 shows the frequency of the available school achievement scores, as well as the percentage of students in this school performance quartile.

*Insert Table 3 about here*

3.3 Associations between fatty acids and school performance

Figure 2 shows the results of the categorical regression analyses for the association between maternal fatty acid levels during pregnancy and at partus, the umbilical plasma fatty acid levels and the child’s fatty acid levels at age 7, and arithmetic, reading and spelling scores at age 7.

For arithmetic scores at age 7 the analyses showed significant negative associations with maternal DHA levels throughout the pregnancy, and with umbilical plasma DHA levels (all
The association with children’s plasma DHA level at age 7 was not significant (p = 0.20).

For reading and spelling, the associations with DHA levels throughout pregnancy and at partus and in umbilical cord blood plasma were non-significant. Significant positive associations were found for spelling (p < 0.001), and reading scores (p < 0.001), and children’s plasma DHA level at age 7.

Maternal AA level at study entry was negatively associated with both arithmetic (p = 0.008) and reading (p = 0.005) scores. In addition, AA level in umbilical cord blood plasma was negatively associated with reading (p = 0.001) as well as spelling (p < 0.001).

Maternal EPA levels at study entry, at 22 weeks of pregnancy, and at partus showed significant negative associations with arithmetic scores (all p < 0.001). The level of EPA at study entry was also negatively associated with spelling scores at age 7 (p < 0.001). Furthermore, there was a positive association between EPA level in umbilical cord plasma and reading scores at age 7 (p = 0.005).

Lastly, maternal ObA levels at 22 and 32 weeks of pregnancy and level in umbilical cord blood plasma were positively associated with arithmetic scores (p = 0.006, p = 0.004 and p = 0.003, respectively). All these associations were positive, and since a higher ObA level reflects a lower DHA status [5], this finding confirms the negative relationship between DHA status and arithmetic scores.

3.4 Quality check results

The group of children whose school performance scores were available and those whose scores were not available were compared, and some group differences were seen. Of special

 Insert Figure 2 about here
interest was the difference in the maternal RAVEN score, which was higher in those with school performance scores available. However, upon further inspection of the data, three extreme outliers were identified in the group without school performances available (RAVEN scores of 8, when the average score was 40). When these outliers were removed the group difference faded out. When the results of children whose mothers suffered adverse pregnancy outcomes were compared to those who had a healthy pregnancy, only a difference on birthweight was seen ($\mu = 3166.9$ vs $\mu = 3383.9$, $p = 0.027$), which is to be expected as this included children whom were small for gestational age. The data-analyses were corrected for birthweight; this difference is thus not expected to influence results.

4. Discussion and conclusion

In line with our hypothesis a positive association between DHA level at age 7 and spelling and reading scores at age 7 was shown. In contrast, mostly significant negative associations were found between maternal DHA levels throughout pregnancy and arithmetic score at age 7. This was supported by ObA, a functional deficiency marker of DHA [5], which showed significant positive associations with arithmetics.

The positive associations between DHA level at age 7 and spelling and reading scores are mostly in line with previous studies in adolescents. The negative associations between DHA levels and arithmetic scores in the current study are mostly in contrast to studies in adolescents that showed a positive association between fish consumption (most important source of DHA) and school grades [36,37]. De Groot et al. did, however, show a negative association when fish consumption was more than twice a week [36]. Though, the studies of De Groot et al. and of Åberg et al., assessed fish intake, which is only a proxy for n-3 LCPUFAs status in the blood [64]. Furthermore, these studies in adolescents looked at grade average over a number of school subjects; this could have led to averaging out negative associations in one school subject with positive associations in another school subject.
While studies investigating the association between LCPUFA concentrations in blood and school performance in children are, to our knowledge, not available, studies that look at the association between LCPUFA blood levels and cognitive measures are available. Some of these studies looking at the association between LCPUFA levels and cognitive measures showed negative associations [31,32,34,35,65,66], while others showed positive [17,19] or no associations [14,16]. Intervention studies also show mixed results: positive [20–29], negative [31–35] or neutral [16,23,30]. These mixed results have been speculated to be due to the large methodologically difference between the studies: differences in cognitive tests used moment, sort and dosage of supplementation [67]. It is however important to keep into mind that cognition and school performance are related, but not the same [68,69]. School performance is dependent on more factors such as motivation, personality and time spend on homework [70]. Thus a positive association between LCPUFA and cognition, does not mean that LCPUFA and school performance are positively associated.

Previous reported results thus remain mixed, with positive, negative en neutral associations being reported between LCPUFAs levels in blood and cognitive measures, and the association with school performance mostly unstudied. In the current study negative associations between maternal plasma levels during pregnancy and arithmetic scores at age 7 are shown, while the association between DHA level at age 7 and reading and spelling scores at age 7 was positive. The opposing associations between LCPUFA levels and arithmetic and LCPUFA levels and spelling/reading are surprising.

Since a positive association for spelling and reading scores at age 7 was found and this was not found for arithmetic scores, one could speculate that this is because these skills are located in different brain region. The brain does not develop uniformly, every regions has its own development curve [38]. Arithmetic is related to executive functioning[71], which is mainly located in the pre-frontal cortex, a region of the brain which develops late in the development.
Language is located mainly in the parietal lobes, a brain region which develops earlier in the development[38].

Though a number of studies observe negative associations between LCPUFA levels and cognitive measurement, most fail to explain these negative associations. No biochemical explanation for the negative associations were found in the literature, with the exception of the possible detrimental effects of high levels of toxic substances present in some fish species (e.g. mercury, cadmium, PCBs and dioxins). However, not all studies that looked at these substances showed unfavourable neurological development (for review see among others Oken [72]). Therefore, it seems time that the research community pays attention to, and further explores the possible unfavourable association between LCPUFA levels and cognition and school performance.

This observational study had a number of limitations. Unfortunately, school performance scores were only available for about 150 children, resulting in 21% of the original sample being analysed on school performance. The non-availability of the school performance scores was due to the fact that some schools did not administer the tests (such tests are not obligatory), administered the tests at another time point than age 7, or the school did not respond to the request for the test scores. These missing data could have influenced the results, especially since the number of children with a score in the lower quartiles was limited. However, no difference between children whose school performance scores were available and children whose school performance scores were not available were seen. Maternal and child plasma LCPUFA levels are furthermore similar to those found in other studies (e.g, [73,74]. Maternal LC n-3 PUFA intake from the women in the current study did not change during pregnancy [75] and the maternal LC n-3 PUFA intake was similar to other pregnant populations [76].

Maternal plasma LCPUFA concentrations were measured frequently during gestation which
is a valuable aspect of this study. Plasma phospholipids were used as a measure of fatty acids level as they are a good indicator of longer term dietary fatty acid intake [77] and as foetal blood values are correlated with maternal blood values[6–8], this is a good measure of foetal fatty acid exposure. Furthermore, participants were followed up until age 7, which allows the study of long-term associations of early life time exposure to LCPUFAs. Finally, even with the more conservative p of 0.01, results were significant, pointing to robust associations. This study is of associative nature and the sample size is rather limited, only randomized-controlled trials can confirm or refute the findings of the current observational study. However, with the negative associations in mind and the fact that such intervention studies, with a long-term follow-up, are difficult to design and covariates might influence the results as well, a randomized controlled trial might not be feasible. Thus, the results of the current study are an useful proxy, which can be used for hypothesis formation.

To summarize, this study is the first, to our knowledge, to investigate the association between prenatal, perinatal and current LCPUFA exposure and school performance scores in children aged 7. Interestingly, consistent significant negative associations were observed between pre- and perinatal exposures to DHA and arithmetic scores at age 7. These results are in line with a few studies regarding cognition that also show negative associations [31–35,66]. In contrast, the association between DHA level at age 7 and spelling and reading scores was positive.

To conclude, although this is an observational study not implying causality, the consistent negative associations observed between maternal plasma DHA levels during pregnancy and the arithmetic scores of the children at age 7 calls upon prudence when considering DHA supplementation during pregnancy.

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**Contribution of authors**

IW was responsible for data analyses and wrote the current paper. EB was responsible for the study design (together with GH) and data collection. All authors contributed to the writing of the paper.
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