Associations between maternal long-chain polyunsaturated fatty acid concentrations and child cognition at 7 years of age

Associations between maternal long-chain polyunsaturated fatty acid concentrations and child cognition at 7 years of age: the MEFAB birth cohort

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Abbreviations: AA, arachidonic acid, 20:4n-6; DHA, docosahexaenoic acid, 22:5n-3; EPA, eicosapentaenoic acid, 20:5n-3; LCPUFAs, long-chain poly unsaturated fatty acids; K-ABC, Kaufman-Assessment Battery; MEFAB, Maastricht Essential Fatty Acid Birth Cohort

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Summary

Dutch women of reproductive age have low concentrations of the fish fatty acids EPA and DHA. As the human brain incorporates high concentrations of these fatty acids in utero, these low EPA and DHA concentrations may adversely affect fetal brain health. We investigated associations between maternal AA, DHA, and EPA and cognitive function with the Kaufman Assessment Battery for Children, including sequential processing, simultaneous processing, and the mental processing composite, at 7 years of age (*n*=292). Only 2% of the children performed more than one SD below the mental processing composite norm score. Fully-adjusted linear regression models did not show associations between maternal AA, DHA, or EPA status during any of the pregnancy trimesters and childhood sequential or simultaneous processing. Concluding, in this population, maternal fatty acid status during pregnancy was not associated with cognitive performance in Dutch children at age 7.

Abstract

Introduction

Concentrations of the fish fatty acids EPA and DHA are low among Dutch women of reproductive age. As the human brain incorporates high concentrations of these fatty acids in utero, particularly during third trimester of gestation, these low EPA and DHA concentrations may have adverse consequences for fetal brain development and functioning.

Methods

Analyses were conducted using longitudinal observational data of 292 mother-child pairs participating in the MEFAB cohort. Maternal AA, DHA, and EPA were determined in plasma phospholipids - obtained in three trimesters - by gas-liquid chromatography. Cognitive function was assessed at 7 years of age, using the Kaufman Assessment Battery for Children, resulting in three main outcome parameters: sequential processing (short-term memory), simultaneous processing (problem-solving skills), and the mental processing composite score. Spline regression and linear regression analyses were used to analyse the data, while adjusting for potential relevant covariates.

Results

Only 2% of the children performed more than one SD below the mental processing composite norm score. Children with lower test scores (<25%) were more likely to have a younger mother with a higher pre-gestational BMI, less likely to be breastfed, and more likely to be born with a lower birth weight, compared to children with higher test scores (≥25%). Fully-adjusted linear regression models did not show associations of maternal AA, DHA, or EPA status during any of the pregnancy trimesters with childhood sequential and simultaneous processing.

Conclusion

Maternal fatty acid status during pregnancy was not associated with cognitive performance in Dutch children at age 7.

Keywords: LCPUFA; cognitive performance; maternal; childhood; offspring.

1. Introduction

Fish consumption in the Dutch population is low [1]. As fish is the predominant source of the long-chain polyunsaturated fatty acids (LCPUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the intake of these fatty acids is low as well. Specifically, Dutch women aged 19-30 have reported a median (25^{th} – 75^{th} percentile) intake of 75 (41-133) mg EPA+DHA/day; those aged 31-51 years have reported an intake of 89 (49-155) mg EPA+DHA/day [1]. To put this into perspective, the European Food Safety Authority (EFSA) currently recommends pregnant women to consume 350-450 mg of EPA and DHA per day [2]. This low intake of these LCPUFAs, particularly DHA, in women of reproductive age is worrisome. Human studies namely indicate that the brain contains high concentrations of DHA [3], of which high quantities are already incorporated during the third trimester of gestation [4]. As the fetus principally depends on the DHA stores/intake of the mother, an adequate and balanced maternal DHA supply during gestation is assumed to be important for the developing fetal brain.

Besides DHA, another predominant LCPUFA in the human brain is arachidonic acid (AA). As AA can be obtained from a more abundant spectrum of food sources than EPA and DHA, including vegetable oils, poultry, eggs, nuts, and whole-grain products, the intake of AA is assumed to be adequate in the Dutch population. Previous literature, however, does indicate an endogenous metabolic competition between n-3 fatty acids (e.g. EPA and DHA) and n-6 fatty acids (e.g. AA) [5]. Hence, not only the quantity of these LCPUFAs, but also their relative proportion may be of importance with respect to fetal brain development.

Studies investigating the impact of prenatal LCPUFA supplementation [6-12], intake [13, 14], or maternal or cord blood concentrations [11, 12, 15-20] on child brain development and function are inconclusive. Whereas a study among 11-year-old Inuit children showed significant associations between higher umbilical cord DHA concentration and a better performance on the digit span forward and California Verbal Learning Test-Children's Version [16], no associations were observed between umbilical cord DHA concentrations and cognitive performance in 7-year-old Norwegian [6] and Dutch children [19]. Beneficial associations were observed for maternal third trimester DHA concentrations and sequential processing scores at age 7 in Norwegian boys and girls [6] and language and verbal ability in 5-year-old children living at the Seychelles [15]. On the contrary, no associations were observed between second or second/third trimester maternal DHA concentrations and cognitive

performance of the child at the age of 3 [13] and 18 months [18]. Clearly, most studies investigated maternal LCPUFA concentrations in late gestation or at delivery in relation to childhood cognition. However, as fetal brain development is a highly complex process that already starts in the first trimester, research on potential LCPUFA effects throughout the whole gestational period is warranted to provide more insight regarding specific LCPUFA requirements during the various critical periods of brain development.

The Maastricht Essential Fatty Acid Birth (MEFAB) cohort provides the unique opportunity to study associations between maternal essential fatty acid status throughout gestation (i.e. <16, 22, 32 gestational weeks) and childhood brain development and functioning. Previous analyses within the MEFAB cohort did not show associations between umbilical cord plasma AA and DHA and sequential and simultaneous processing at age 7 [19], but adverse associations were observed for maternal DHA status across trimesters and school performance based on arithmetic scores at age 7 [21]. Associations between fatty acid status across trimesters and cognitive performance at age 7 have not been explored yet. Therefore, the aim of this study was to examine the associations of maternal LCPUFA concentrations (i.e. AA, DHA, EPA, and DHA:AA) during gestation (i.e. <16, 22, 32 weeks) with childhood cognitive performance at 7 years of age as assessed with the Kaufman-Assessment Battery (K-ABC) in the MEFAB cohort.

2. Patients and Methods

2.1. Study population

This study was performed using data of the MEFAB cohort, a prospective study designed to study relationships of essential fatty acid status during gestation and birth with metabolic health and cognitive, visual and motor function in Dutch children. Recruitment took place from 1989 to 1995. Pregnant women (<16 weeks) without any cardiovascular, neurological, renal or metabolic condition were eligible to participate. In total, *n*=1,334 women were screened; *n*=131 (10%) were either excluded or dropped out before partus. At 7 years of age, *n*=305 participated in the cognitive testing procedures. Excluding those with missing data on maternal fatty acid status in all three trimesters resulted in a sample size of *n*=292 children for the analyses. More detailed information on the design and methods of the MEFAB cohort has been described elsewhere [22]. The Medical Ethics Committee of the University Hospital Maastricht/University Maastricht approved the study protocol and all families gave written informed consent.

2.2. LCPUFA status

Non-fasted blood samples were collected at study entry (<16 gestational weeks), at 22 gestational weeks, 32 gestational weeks, and when the children were 7 years of age. Immediately after sampling, blood samples were stored at -80°C until further analyses were conducted. In total, 41 different maternal fatty acids of plasma phospholipids (PL) were determined by gas-liquid chromatography [23], including C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0, C16:1n-7, C18:1n-7, C20:1n-7, C18:1n-9, C18:2n-9, C20:1n9, C20:3n-9, C22:1n-9, C22:3n-9, C24:1n-9, C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:2n-6, C22:4n-6, C22:5n-6, C24:2n-6, C18:3n-3, C20:3n-3, C20:4n-3, C20:5n-3, C22:3n-3, C22:5n-3, C22:6n-3, C16:0 DMA, C18:0 DMA, C18:1 DMA, C18:2n-6tr, C16:1n-7tr, and C18:1n-9tr. For this study, maternal plasma phospholipid DHA (C22:6n-3), AA (C20:4n-6), and EPA (C20:5n-3) concentrations were selected, providing relative concentrations of DHA, AA, and EPA to total phospholipid-associated fatty acids (% wt/wt).

2.3. Cognitive performance

Cognitive function was assessed with the Kaufman Assessment Battery for Children (K-ABC) [24], which evaluates two different types of information processing: sequential processing (i.e. short-term

memory) and simultaneous processing (i.e. problem-solving skills). The sequential processing score is based on a variety of assignments in which the child arranges items in serial or sequential order, such as reproducing hand taps on a table, recalling numbers, and recalling objects as presented by the researcher. The simultaneous processing score is based on a variety of assignments in which the child completes a facial recognition task, identifies objects or scenes in an unfinished picture, replicates an object using rubber triangles, selects a picture to finalize another picture or complement another picture, has to remember and recall the location of specific pictures, and arranges a variety of pictures in a meaningful order. Together the sequential and simultaneous processing scores form the mental processing composite score, a measure of intelligence. For all three scores, a score of 100±15 points is considered average (i.e. norm score); a score of 85 is one standard deviation below the norm score of 100. Thus, higher scores indicate a better performance. The K-ABC was assessed according to a standard protocol, in a quiet room with blinded windows and by a single well-trained researcher.

2.4. Covariates

Information on child's sex (boy/girl, *n* (%)), gestational age at birth (weeks), birth weight (grams), birth order (first/second/third/fourth/fifth, *n* (%)),breastfeeding (no/yes, *n* (%) and duration), child's age at the time of the cognitive assessment (years), maternal age (years), maternal height (m), maternal pre-pregnancy weight (kg), maternal smoking during gestation (yes/no, *n* (%)), and maternal alcohol consumption during gestation (yes/no, *n* (%)) were collected by means of questionnaires. Bodyweight of the child was measured to the nearest 100g using a digital scale (SECA) while wearing light underwear. Height of the child was measured to the nearest mm using a stadiometer (HoltainLTD, Crymych, UK). BMI was calculated as weight/height^2. APGAR scores 5 minutes after birth were extracted from hospital records. Maternal pre-gestational BMI was calculated as weight/height^2, using the measures of self-reported height and weight. Maternal intelligence was tested with Raven's Standard Progressive Matrices [25].

2.5. Statistical analyses

Participant characteristics are reported as mean with standard deviation (mean±SD), or *n* with percentages (*n*, (%)). Medians with interquartile range (median (IQR)) were used to report skewed variables. Data is shown for the total population, by tertiles of maternal DHA status in the third

trimester, and by cognitive performance score (normal vs. poor performance). Differences between tertiles of maternal DHA status and cognitive performance were analyzed by means of ANOVA in case of continuous variables and chi-square test in case of categorical variables. Correlations between the fatty acids across the trimesters were visualized by means of an ordination plot and quantified using Pearson's correlations. Linearity of the associations of maternal fatty acid status with childhood cognitive performance were investigated using restricted cubic spline regression as well as linear regression analyses by tertile of fatty acid status. As the aforementioned analyses did not point towards non-linearity, multivariable linear regression analyses was used to quantify the strength of the associations between maternal fatty acid status and cognitive performance of the child at age 7. Model 1 was adjusted for child sex, birth weight, gestational age at birth, birth order, duration of breastfeeding, and child BMI at age 7. Model 2 was adjusted for the covariates in model 1 + maternal age, maternal intelligence, maternal pre-gestation BMI, maternal smoking, and maternal alcohol consumption during gestation. Model 3 was adjusted for the covariates in model 2 + fatty acid status of the child at age 7 years. Given the intercorrelatedness between the fatty acids under study no adjustment for multiple testing was applied and hence a two-sided *P*-value of ≤0.05 was considered statistically significant. Restricted cubic spline analyses were performed using R v2.15. The ordination plot was created using Canoco v5. All other statistical analyses were performed using the statistical package SAS, v9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

Participant characteristics are shown in **Table 1**. In this population, the mean±SD maternal age was 29.9±4.2 years and pre-gestational BMI 23.7±4.1 kg/m^2. Smoking was reported by 24% of the pregnant women; 3% reported to consume alcohol during pregnancy. Children were on average born with a gestational age of 40.1±3.3 weeks, weighed 3,302±512 grams, and were breastfed for a median (25-75th percentile) period of 0 (0-3) weeks. 56% of the children were boys. Most children were the first (69%) or second (24%) child of the family. None of the variables displayed in Table 1 differed over tertiles of maternal DHA status. Very few children performed more than one SD below the norm cognitive score, specifically *n*=7 (2%) for the mental processing composite score, *n*=23 (8%) for the sequential processing score, and *n*=4 (1%) for the simultaneous processing score. Nevertheless, children belonging to the group with the lowest cognitive test scores (<25%) were more likely to have a younger mother (28.3±4.4 vs. 30.4±4.0 years at the time of the pregnancy, P<0.05), a mother with a higher pre-gestational BMI (25.2±5.1 vs. 23.3±3.7 kg/m^2, P<0.05), and were less likely to have received breastfeeding (35 vs. 50%, P<0.05) compared to children with higher test scores (≥25%). Children belonging to the group with the lowest cognitive test scores were also more likely to be born with a lower birth weight than children with higher test scores (3,191±571 g vs. 3,338±487 g, P<0.05). Absolute (wt/wt%) concentrations of the fatty acids across trimesters are displayed in **Figure 1**. As shown by the clustering of the arrows in **Figure 2**, the fatty acids under study are generally strongly correlated across the trimesters.

Tests for non-linearity, visualization using restricted cubic splines, as well as linear regression analyses by tertiles (figures and data not shown) disclosed linear associations between the different fatty acids and childhood cognitive performance. Unadjusted linear regression models subsequently showed an inverse association between first trimester maternal AA concentrations and sequential processing scores (β-0.99±0.51, P=0.05) (**Table 2**). Moreover, very modest non-significant inverse trends between first and second trimester maternal AA concentrations and simultaneous processing scores were observed (β-0.83±0.48, P=0.09 and -0.98±0.56, P=0.08). As the sequential and simultaneous processing scores form the mental processing composite score, these trends were also reflected in the results for this overall mental composite score.

The modest associations of first and second trimester AA concentrations with sequential (β-0.68±0.57, P=0.23 and -0.55±0.65, P=0.70) and simultaneous processing (β-0.40±0.52, P=0.44 and -0.19±0.61, P=0.75) fully disappeared after further adjustment for child and maternal characteristics. Neither crude nor adjusted models pointed towards associations between maternal DHA concentrations and childhood cognitive performance. Crude models did show a borderline non-significant positive association between third trimester maternal EPA concentrations and sequential processing scores (β 7.16±3.83, P=0.06). After adjustment for child as well as maternal characteristics a trend towards an association remained (β 7.28±4.05, P=0.07), which somewhat further attenuated after adjustment for EPA status of the child at 7 years of age (β 7.28±4.26, P=0.09). No associations were observed between maternal DHA:AA ratio and cognitive performance of the child at age 7 years.

4. Discussion and Conclusions

This study did not show significant associations of maternal fatty acid status during the different trimesters of gestation with sequential (short-term memory) or simultaneous (problem-solving skills) processing of the children at age 7.

A priori, associations with childhood cognitive performance were particularly hypothesized for third trimester maternal AA and DHA concentrations. Specifically, AA and DHA are considered to be the most important fatty acids for normal brain growth and development, amongst others due to their role in neuronal growth, differentiation, and signaling [26]. These potential effects are particularly expected during the third trimester as this is the period with the highest transfer of fatty acids from the mother to the unborn child [27]. In contrast to these expectations, we did not observe any association between maternal AA, DHA, and EPA status across trimesters and childhood cognitive performance. Our findings are in line with previous analyses within this cohort examining associations of umbilical cord AA and DHA concentrations with cognitive performance at age 7, which also not provided evidence for significant associations between the variables under study [19].

In this study no associations were observed between first trimester fatty acid status and offspring cognition. To the best of our knowledge, this is the first study examining associations between first trimester fatty acid concentrations and childhood cognitive performance and hence this association warrants further verification in other cohorts. We did also not observe associations between second trimester fatty acid status and childhood cognitive performance. These findings are in line with the findings in the Project Viva cohort showing no associations between second trimester maternal erythrocyte DHA concentrations with cognitive performance at 3-years-old [13] and data of an Italian cohort investigating the link between second/third trimester LCPUFAs and child neurodevelopment [18]. Our null-findings with respect to third trimester DHA and AA concentrations and cognitive performance of the child are in contrast to findings of several other studies. After full-adjustment, Strain and colleagues observed associations between higher third trimester maternal DHA concentrations and higher scores on the Preschool Language Scale-Revised for language (β 41.3, SE 19.3, P=0.03) as well as verbal ability (β 24.6, SE 12.2, P=0.04), but not with Kaufman Brief Intelligence Test scores (*n*=225, aged ±5y, the Seychelles) [15]. In addition, this study showed

associations of third trimester maternal AA concentrations with language (β -15.8, SE 6.5, P=0.02), auditory comprehension (β -7.5, SE 3.2, P=0.02), and verbal ability (β -8.3, SE 4.1, P=0.04) [15]. In 7 year old Norwegian children (n=143), third trimester maternal DHA concentrations were positively associated with sequential processing scores of the K-ABC (β 0.06±0.03, P<0.05) [6]. Our null-findings with respect to maternal EPA concentrations and childhood cognitive performance in our study are in line with the null-findings in the Norwegian study [6], Project Viva [13], the Seychelles study [15], as well as the Italian study [18].

Unfortunately, none of the above-summarized studies analyzed data on fatty acid status throughout gestation. Previous analyses within the MEFAB cohort did investigate associations between fatty acid status across trimesters and school performance at age 7 [21]. These analyses pointed towards significant adverse associations between maternal DHA status in all three trimesters and arithmetic scores. Adverse associations were also shown of maternal EPA concentrations in the first and second trimester with arithmetic scores, first trimester EPA with spelling, and first trimester AA with arithmetic and reading scores [21]. However, although cognition and school performance are related, these terms cannot be exchanged, as school performance is probably also affected by other factors such as perseverance and study time. Possible explanations for the inconsistent findings for the studies on maternal fatty acid status and childhood cognition are that they may relate to methodological differences in cognitive assessment (e.g. method lacking sensitivity), power-issues, and limited variation in fatty acid status. It has also been postulated that early life effects of LCPUFA may be transient and that effects are overruled by effects of the LCPUFA supply in postnatal life [18]. However, this last idea is contradicted by the various studies showing significant associations between maternal fatty acid status during gestation and cognitive performance at 5, 7, and 11-years old [6, 15, 16]. Moreover, our models did not substantially change after adjustment for fatty acid status of the child at the age of 7 years.

Finally, in order to put our findings further into perspective, there are several study specific characteristics that warrant some discussion. First of all, cognitive performance was assessed with the K-ABC. Our test results indicate that only very few children in this population performed more than one SD below the norm-score, which may indicate that this test was not sensitive enough to detect robust

associations. However, our test scores and the variation in these scores were relatively similar to the test scores in the study by Helland and colleagues [6], who used the same cognitive test battery and did observe an association between maternal third trimester DHA and sequential processing of the child. Secondly, maternal fatty acids were determined using non-fasted blood samples, which may raise doubts about the long-term representativeness of the measured concentrations. However, as previous work has shown that the incorporation of EPA and DHA in erythrocyte membranes has a half-life of approximately 28 days, where concentrations start to rise after 3 days of fish oil supplementation [28], we do not expect a substantial influence of very recent EPA and DHA intakes on the EPA/DHA concentrations measured. Another limitation of our study may be that only 305 children of the original 1203 mother-offspring pairs completed the cognitive tests at age 7. Though, Bakker and colleagues (2003) compared the data of participating and non-participating children with respect to their clinical baseline characteristics and did not show significant differences between these two groups [19]. Despite aforementioned limitations, a unique feature of this study is that women were included in a very early stage of gestation, providing us with valuable data on fatty acids status from the first to the third trimester. Furthermore, as fatty acids were measured in plasma phospholipids, a generally accepted technique to determine long-term dietary fatty acids intake, it can be confidently stated that the exposure marker studied provided a reliable reflection of long-term fatty acid status. Last but not least, children were followed for on average 7 years of age, which offered the possibility to study potential long-term effects of early-life LCPUFA exposure.

All in all, we conclude that our analyses in the Dutch MEFAB cohort do not provide evidence for a negative nor a positive association between maternal LCPUFA concentrations throughout gestation and cognitive performance at 7-years-old.

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Contribution of authors: EMBB analyzed the data and drafted the manuscript. MG was in charge of data acquisition and data management. All authors contributed to the interpretation of data, revision of the manuscript, and all authors approved the version to be submitted.

Declaration of interest: EMBB, OvdR, RG, MPAZ, MG, and RHMdG have nothing to disclose.

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Figure 1. Fatty acid status (% wt/wt) across trimesters. AA: 9.61±1.47 (T1), 8.58±1.29 (T2), 8.15±1.17 (T3); DHA: 4.02±0.83 (T1), 4.16±0.84 (T2), 4.00±0.74 (T3); EPA: 0.52±0.37 (T1), 0.41±0.36 (T2), 0.35±0.20 (T3); DHA:AA: 0.42±0.09 (T1), 0.49±0.12(T2), 0.50±0.11 (T3).

Figure 2. Ordination plot. Arrows indicate the strength of the correlations between the different fatty acids (% wt/wt) as measured throughout the three trimesters (T1, T2, and T3). In general, arrows for a specific fatty acid are clustered in the same region, indicating strong correlations between the fatty acids across trimester. Specifically, Pearson correlations for AA-T1 vs T2 and T3 were 0.74 ($P<0.0001$) and 0.69 ($P<0.0001$). Pearson correlations for DHA-T1 vs T2 and T3 were 0.58 ($P<0.0001$) and 0.51 ($P<0.0001$). Pearson correlations for EPA-T1 vs T2 and T3 were 0.24 ($P=0.0001$) and 0.19 ($P=0.002$).

Table 1. Population characteristics by tertiles of third trimester DHA status

	Total (*n*=292)	3rd trimester DHA status			Mental processing composite	
		Tertile 1 (*n*=103)	Tertile 2 (*n*=95)	Tertile 3 (*n*=94)	<25% (poor, *n*=71)	≥25% (normal, *n*=221)
Maternal parameters during pregnancy						
Age, years	29.9±4.2	29.4±4.5	30.3±4.3	29.9±3.7	28.3±4.4	30.4±4.0*
Pre-gestational BMI, kg/m^2	23.7±4.1	23.7±3.9	23.6±4.0	23.8±4.5	25.2±5.1	23.3±3.7*
Smoking, *n* (%)	68 (24)	25 (24)	25 (27)	18 (20)	19 (27)	49 (23)
Alcohol consumption, n (%)	9 (3)	5 (5)	2 (2)	2 (2)	2 (3)	7 (3)
Maternal intelligence, score	51±12	52±12	49±13	52±10	50±11	51±12
Child						
Sex, *n* boy (%)	159 (56)	56 (55)	52 (56)	51 (55)	44 (65)	115 (53)
Gestational age, wk	40.1±3.3	40.3±4.9	40.2±1.6	39.9±2.1	40.5±5.8	40.0±1.9
Birth weight, grams	3302±512	3222±539	3389±497	3303±486	3191±571	3338±487*
APGAR score (5 min)	9.6±0.9	9.6±1.0	9.6±0.6	9.5±1.0	9.5±0.9	9.6±0.9
Birth order, *n* (%)						
First	200 (69)	68 (66)	59 (62)	73 (78)	48 (68)	152 (69)
Second	71 (24)	26 (25)	28 (29)	17 (18)	18 (25)	53 (24)
Third	16 (6)	7 (7)	6 (6)	3 (3)	3 (4)	13 (6)
Fourth	4 (1)	2 (2)	1 (1)	1 (1)	1 (1)	3 (1)
Fifth	1 (0)	0	1 (1)	0	1 (1)	0
Breastfeeding, *n* (%)	132 (46)	44 (44)	37 (41)	51 (55)	24 (35)	108 (50)*
Duration breastfeeding, wk	0 (0-3)	0 (0-3)	0 (0-3)	1 (0-4)	0 (0-2)	0 (0-3)
Age at assessment, y	7.3±0.3	7.3±0.2	7.3±0.3	7.3±0.3	7.3±0.3	7.3±0.3
BMI at age 7y	15.6±1.8	15.6±1.7	15.9±2.1	15.2±1.6	15.4±1.7	15.6±1.9
Mental processing composite, score	107±12	108±13	107±12	108±11	92±7	112±9*
Sequential processing, score	102±13	101±12	102±12	104±13	88±10	107±10*
Simultaneous processing, score	109±12	110±12	109±12	109±11	96±7	114±9*

Values are expressed as mean±SD, median (IQR), or n (%). To compare baseline characteristics over tertiles of third trimester DHA status or cognitive performance, chi-squared tests were performed for categorical variables and 1-way analysis of variance for continuous variables. * indicates P<0.05. Missing: Sex child *n*=6, Smoking *n*=4, Alcohol *n*=5, pre-pregnancy BMI *n*=31, gestational age *n*=14, birthweight *n*=1, breastfeeding *n*=8, age at assessment *n*=6, BMI at age 7 *n*=11, APGAR score after 5 minutes *n*=3.

Table 2. Associations of 1st, 2nd, and 3rd AA, DHA, EPA, and DHA:AA status with cognitive performance at age 7 years.

		Crude model		**Model 1**		**Model 2**		**Model 3**	
		(T1 *n*=281; T2 *n*=261; T3 *n*=275)		(T1 *n*=254; T2 *n*=238, T3 *n*=252)		(T1 *n*=229; T2 *n*=212; T3 *n*=225)		(T1 *n*=199; T2 *n*=183; T3 *n*=193)	
		β±SD	*P*	β±SD	*P*	β±SD	*P*	β±SD	*P*
Sequential processing score									
Fatty acid	Trimester								
AA	1	-0.99±0.51	0.05	-1.21±0.55	0.03	-0.68±0.57	0.23	-0.73±0.62	0.24
AA	2	-1.04±0.61	0.09	-0.96±0.64	0.14	-0.25±0.65	0.70	-0.12±0.70	0.87
AA	3	-0.52±0.66	0.43	-0.30±0.69	0.66	0.02±0.72	0.97	-0.10±0.79	0.90
DHA	1	0.55±0.91	0.55	-0.15±0.96	0.88	1.21±1.00	0.23	1.58±1.05	0.14
DHA	2	0.01±0.95	0.99	-0.16±0.98	0.87	0.40±0.97	0.68	0.56±1.04	0.59
DHA	3	0.96±1.04	0.36	0.29±1.10	0.79	1.06±1.15	0.35	1.06±1.23	0.39
EPA	1	-1.39±2.04	0.50	-2.28±2.14	0.29	-0.56±2.21	0.80	0.07±3.22	0.98
EPA	2	-1.27±2.22	0.57	-2.62±2.26	0.25	-2.23±2.13	0.30	-2.53±2.18	0.25
EPA	3	7.16±3.83	0.06	6.05±4.05	0.14	7.28±4.05	0.07	7.28±4.26	0.09
DHA:AA	1	14.14±7.96	0.08	9.73±8.34	0.24	12.96±8.16	0.11	16.34±8.95	0.07
DHA:AA	2	4.24±6.84	0.54	2.00±7.03	0.78	1.10±6.77	0.87	-0.15±7.08	0.98
DHA:AA	3	5.73±7.21	0.43	0.16±7.53	0.98	3.36±7.85	0.67	3.60±8.47	0.67
Simultaneous processing score									
Fatty acid	Trimester								
AA	1	-0.83±0.48	0.09	-0.67±0.50	0.18	-0.40±0.52	0.44	-0.30±0.58	0.61
AA	2	-0.98±0.56	0.08	-0.63±0.58	0.28	-0.19±0.61	0.75	-0.16±0.68	0.81
AA	3	-0.61±0.59	0.30	-0.04±0.61	0.95	0.13±0.65	0.84	0.05±0.72	0.95
DHA	1	-0.33±0.85	0.70	-0.55±0.88	0.53	-0.14±0.91	0.88	0.01±0.98	0.99
DHA	2	-0.19±0.87	0.82	-0.05±0.89	0.95	-0.14±0.91	0.88	-0.22±0.99	0.82
DHA	3	-0.84±0.94	0.37	-1.09±0.98	0.27	-1.54±1.02	0.13	-1.81±1.11	0.10
EPA	1	-0.83±1.91	0.66	-1.39±1.95	0.48	-1.17±2.02	0.56	-1.09±2.96	0.71
EPA	2	-0.67±2.03	0.74	-1.44±2.05	0.48	-0.56±2.00	0.78	-1.32±2.07	0.53
EPA	3	3.61±3.47	0.30	2.69±3.63	0.46	2.82±3.66	0.44	2.51±3.87	0.52
DHA:AA	1	7.20±7.49	0.34	3.70±7.60	0.63	2.69±7.50	0.72	3.10±8.31	0.71
DHA:AA	2	5.44±6.27	0.39	3.40±6.36	0.59	0.13±6.33	0.98	-0.90±6.73	0.89
DHA:AA	3	-1.75±6.50	0.79	-7.96±6.71	0.24	-11.47±7.00	0.10	-12.53±7.60	0.10
Mental processing									

score									
Fatty acid	Trimester								
AA	1	-1.03±0.49	0.04	-1.02±0.52	0.05	-0.59±0.54	0.27	-0.52±0.59	0.38
AA	2	-1.17±0.58	0.05	-0.90±0.61	0.14	-0.19±0.61	0.75	-0.19±0.69	0.79
AA	3	-0.64±0.61	0.29	-0.15±0.64	0.81	0.09±0.66	0.89	0.00±0.74	0.99
DHA	1	-0.01±0.87	0.99	-0.47±0.91	0.61	0.42±0.93	0.65	0.71±2.00	0.48
DHA	2	-0.15±0.90	0.86	-0.14±0.93	0.88	0.09±0.93	0.92	0.13±1.01	0.90
DHA	3	-0.18±0.97	0.86	-0.64±1.02	0.53	-0.61±1.05	0.56	-0.78±1.15	0.50
EPA	1	-1.30±1.96	0.51	-2.06±2.02	0.31	-1.16±2.07	0.58	-0.96±3.04	0.75
EPA	2	-0.97±2.11	0.65	-2.07±2.14	0.33	-1.30±2.04	0.53	-1.96±2.12	0.36
EPA	3	5.73±3.58	0.11	4.63±3.77	0.22	5.21±3.74	0.17	4.90±3.97	0.22
DHA:AA	1	11.22±7.64	0.14	6.95±7.89	0.38	7.64±7.66	0.32	9.32±8.51	0.28
DHA:AA	2	5.59±6.53	0.39	3.22±6.66	0.63	0.73±6.48	0.91	-0.49±6.88	0.94
DHA:AA	3	0.99±6.74	0.88	-5.72±7.00	0.41	-6.59±7.21	0.36	-7.24±7.87	0.36

Associations are adjusted for child sex, birth weight, gestational age at birth, birth order, breastfeeding (yes/no), child BMI at age 7 (model 1) + maternal age, maternal intelligence, maternal pre-pregnancy BMI, maternal smoking (yes/no) + maternal alcohol consumption during pregnancy (yes/no) (model 2) + fatty acid status at age 7 (model 3).