

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

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

3

4 EM Brouwer-Brolsma <sup>a\*</sup>, O van de Rest <sup>a</sup>, R Godschalk <sup>b</sup>, MPA Zeegers <sup>b</sup>, M Gielen <sup>b</sup>, RHM de Groot  
5 <sup>b,c</sup>

6

7 <sup>a</sup> Division of Human Nutrition, Wageningen University & Research, Wageningen, The Netherlands

8 <sup>b</sup> NUTRIM School for Nutrition and Translational Research in Metabolism, Department of Complex  
9 Genetics, Maastricht University, Maastricht, The Netherlands

10 <sup>c</sup> Faculty of Psychology and Educational Sciences, Welten Institute, Open University of the  
11 Netherlands, Heerlen, The Netherlands

12

13 \*Corresponding author

14 PO Box 8129, 6700 EV Wageningen, The Netherlands

15 Tel: +31 (0)317 481287 Fax: +31 (0)317 482782

16 E-mail: [elske.brouwer-brolsma@wur.nl](mailto:elske.brouwer-brolsma@wur.nl)

17

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

21

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24 Research (NWO, 904 62 186); and The University Hospital of Maastricht (Profilering Fonds).

25 **Summary**

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

36 **Abstract**

37

38 **Introduction**

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

43

44 **Methods**

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

52

53 **Results**

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

60

61 **Conclusion**

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

64

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

66 **1. Introduction**

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

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

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

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

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

113 **2. Patients and Methods**

114 *2.1. Study population*

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

126

127 *2.2. LCPUFA status*

128 Non-fasted blood samples were collected at study entry (<16 gestational weeks), at 22 gestational  
129 weeks, 32 gestational weeks, and when the children were 7 years of age. Immediately after sampling,  
130 blood samples were stored at  $-80^{\circ}\text{C}$  until further analyses were conducted. In total, 41 different  
131 maternal fatty acids of plasma phospholipids (PL) were determined by gas-liquid chromatography [23],  
132 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,  
133 C20:1n-7, C18:1n-9, C18:2n-9, C20:1n-9, C20:3n-9, C22:1n-9, C22:3n-9, C24:1n-9, C18:2n-6, C18:3n-  
134 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,  
135 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-  
136 6tr, C16:1n-7tr, and C18:1n-9tr. For this study, maternal plasma phospholipid DHA (C22:6n-3), AA  
137 (C20:4n-6), and EPA (C20:5n-3) concentrations were selected, providing relative concentrations of  
138 DHA, AA, and EPA to total phospholipid-associated fatty acids (% wt/wt).

139

140 *2.3. Cognitive performance*

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

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

155

#### 156 *2.4. Covariates*

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

168

#### 169 *2.5. Statistical analyses*

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



173 trimester, and by cognitive performance score (normal vs. poor performance). Differences between  
174 tertiles of maternal DHA status and cognitive performance were analyzed by means of ANOVA in case  
175 of continuous variables and chi-square test in case of categorical variables. Correlations between the  
176 fatty acids across the trimesters were visualized by means of an ordination plot and quantified using  
177 Pearson's correlations. Linearity of the associations of maternal fatty acid status with childhood  
178 cognitive performance were investigated using restricted cubic spline regression as well as linear  
179 regression analyses by tertile of fatty acid status. As the aforementioned analyses did not point  
180 towards non-linearity, multivariable linear regression analyses was used to quantify the strength of the  
181 associations between maternal fatty acid status and cognitive performance of the child at age 7. Model  
182 1 was adjusted for child sex, birth weight, gestational age at birth, birth order, duration of  
183 breastfeeding, and child BMI at age 7. Model 2 was adjusted for the covariates in model 1 + maternal  
184 age, maternal intelligence, maternal pre-gestation BMI, maternal smoking, and maternal alcohol  
185 consumption during gestation. Model 3 was adjusted for the covariates in model 2 + fatty acid status of  
186 the child at age 7 years. Given the intercorrelatedness between the fatty acids under study no  
187 adjustment for multiple testing was applied and hence a two-sided *P*-value of  $\leq 0.05$  was considered  
188 statistically significant. Restricted cubic spline analyses were performed using R v2.15. The ordination  
189 plot was created using Canoco v5. All other statistical analyses were performed using the statistical  
190 package SAS, v9.3 (SAS Institute Inc., Cary, NC, USA).

191 **3. Results**

192 Participant characteristics are shown in **Table 1**. In this population, the mean±SD maternal age was  
193 29.9±4.2 years and pre-gestational BMI 23.7±4.1 kg/m<sup>2</sup>. Smoking was reported by 24% of the  
194 pregnant women; 3% reported to consume alcohol during pregnancy. Children were on average born  
195 with a gestational age of 40.1±3.3 weeks, weighed 3,302±512 grams, and were breastfed for a median  
196 (25-75<sup>th</sup> percentile) period of 0 (0-3) weeks. 56% of the children were boys. Most children were the first  
197 (69%) or second (24%) child of the family. None of the variables displayed in Table 1 differed over  
198 tertiles of maternal DHA status. Very few children performed more than one SD below the norm  
199 cognitive score, specifically *n*=7 (2%) for the mental processing composite score, *n*=23 (8%) for the  
200 sequential processing score, and *n*=4 (1%) for the simultaneous processing score. Nevertheless,  
201 children belonging to the group with the lowest cognitive test scores (<25%) were more likely to have a  
202 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  
203 higher pre-gestational BMI (25.2±5.1 vs. 23.3±3.7 kg/m<sup>2</sup>, *P*<0.05), and were less likely to have  
204 received breastfeeding (35 vs. 50%, *P*<0.05) compared to children with higher test scores (≥25%).  
205 Children belonging to the group with the lowest cognitive test scores were also more likely to be born  
206 with a lower birth weight than children with higher test scores (3,191±571 g vs. 3,338±487 g, *P*<0.05).  
207 Absolute (wt/wt%) concentrations of the fatty acids across trimesters are displayed in **Figure 1**. As  
208 shown by the clustering of the arrows in **Figure 2**, the fatty acids under study are generally strongly  
209 correlated across the trimesters.

210

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

220 The modest associations of first and second trimester AA concentrations with sequential ( $\beta$ -0.68 $\pm$ 0.57,  
221  $P=0.23$  and -0.55 $\pm$ 0.65,  $P=0.70$ ) and simultaneous processing ( $\beta$ -0.40 $\pm$ 0.52,  $P=0.44$  and -0.19 $\pm$ 0.61,  
222  $P=0.75$ ) fully disappeared after further adjustment for child and maternal characteristics. Neither crude  
223 nor adjusted models pointed towards associations between maternal DHA concentrations and  
224 childhood cognitive performance. Crude models did show a borderline non-significant positive  
225 association between third trimester maternal EPA concentrations and sequential processing scores ( $\beta$   
226 7.16 $\pm$ 3.83,  $P=0.06$ ). After adjustment for child as well as maternal characteristics a trend towards an  
227 association remained ( $\beta$  7.28 $\pm$ 4.05,  $P=0.07$ ), which somewhat further attenuated after adjustment for  
228 EPA status of the child at 7 years of age ( $\beta$  7.28 $\pm$ 4.26,  $P=0.09$ ). No associations were observed  
229 between maternal DHA:AA ratio and cognitive performance of the child at age 7 years.

230 **4. Discussion and Conclusions**

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

234

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

245

246 In this study no associations were observed between first trimester fatty acid status and offspring  
247 cognition. To the best of our knowledge, this is the first study examining associations between first  
248 trimester fatty acid concentrations and childhood cognitive performance and hence this association  
249 warrants further verification in other cohorts. We did also not observe associations between second  
250 trimester fatty acid status and childhood cognitive performance. These findings are in line with the  
251 findings in the Project Viva cohort showing no associations between second trimester maternal  
252 erythrocyte DHA concentrations with cognitive performance at 3-years-old [13] and data of an Italian  
253 cohort investigating the link between second/third trimester LCPUFAs and child neurodevelopment  
254 [18]. Our null-findings with respect to third trimester DHA and AA concentrations and cognitive  
255 performance of the child are in contrast to findings of several other studies. After full-adjustment,  
256 Strain and colleagues observed associations between higher third trimester maternal DHA  
257 concentrations and higher scores on the Preschool Language Scale-Revised for language ( $\beta$  41.3, SE  
258 19.3,  $P=0.03$ ) as well as verbal ability ( $\beta$  24.6, SE 12.2,  $P=0.04$ ), but not with Kaufman Brief  
259 Intelligence Test scores ( $n=225$ , aged  $\pm 5y$ , the Seychelles) [15]. In addition, this study showed

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

267

268 Unfortunately, none of the above-summarized studies analyzed data on fatty acid status throughout  
269 gestation. Previous analyses within the MEFAB cohort did investigate associations between fatty acid  
270 status across trimesters and school performance at age 7 [21]. These analyses pointed towards  
271 significant adverse associations between maternal DHA status in all three trimesters and arithmetic  
272 scores. Adverse associations were also shown of maternal EPA concentrations in the first and second  
273 trimester with arithmetic scores, first trimester EPA with spelling, and first trimester AA with arithmetic  
274 and reading scores [21]. However, although cognition and school performance are related, these  
275 terms cannot be exchanged, as school performance is probably also affected by other factors such as  
276 perseverance and study time. Possible explanations for the inconsistent findings for the studies on  
277 maternal fatty acid status and childhood cognition are that they may relate to methodological  
278 differences in cognitive assessment (e.g. method lacking sensitivity), power-issues, and limited  
279 variation in fatty acid status. It has also been postulated that early life effects of LCPUFA may be  
280 transient and that effects are overruled by effects of the LCPUFA supply in postnatal life [18].  
281 However, this last idea is contradicted by the various studies showing significant associations between  
282 maternal fatty acid status during gestation and cognitive performance at 5, 7, and 11-years old [6, 15,  
283 16]. Moreover, our models did not substantially change after adjustment for fatty acid status of the  
284 child at the age of 7 years.

285

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

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

309

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

313

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

323 *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:  $9.61 \pm 1.47$  (T1),  $8.58 \pm 1.29$  (T2),  $8.15 \pm 1.17$  (T3); DHA:  $4.02 \pm 0.83$  (T1),  $4.16 \pm 0.84$  (T2),  $4.00 \pm 0.74$  (T3); EPA:  $0.52 \pm 0.37$  (T1),  $0.41 \pm 0.36$  (T2),  $0.35 \pm 0.20$  (T3); DHA:AA:  $0.42 \pm 0.09$  (T1),  $0.49 \pm 0.12$  (T2),  $0.50 \pm 0.11$  (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)	3 <sup>rd</sup> 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)
<b>Maternal parameters during pregnancy</b>						
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 <sup>2</sup>	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
<b>Child</b>						
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 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> 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)	
		$\beta \pm SD$	P	$\beta \pm SD$	P	$\beta \pm SD$	P	$\beta \pm SD$	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

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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).