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Original article

Maternal long-chain polyunsaturated fatty acid status during early pregnancy: Association with child behavioral problems and the role of autonomic nervous system activity

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SUMMARY

Background & aims: The prenatal environment, including availability of critical nutrients, has a profound impact on offspring development. The present study examined the association between maternal long-chain polyunsaturated fatty acid (LC-PUFA) status during pregnancy and later child behavioral problems at the age of 5–6 years. In light of evidence of autonomic nervous system (ANS) dysregulation in some behavioral problems, study further tested if the above association is statistically mediated by cardiac ANS activity.

Methods: Data was collected as part of the Amsterdam Born Children and their Development-study and complete data were available for 1717 mothers and their offspring. Maternal LC-PUFA status was assessed during early pregnancy (mean gestation = 12.7, SD = 2.5 weeks) and quantified as levels of docosahexenoic acid (DHA), arachidonic acid (AA), eicosapentaenoic acid (EPA), as well as the ratio of n-6:n-3 fatty acids. Child emotional problems and peer problems (internalizing problems), as well as conduct problems and inattention/hyperactivity (externalizing problems), were assessed using the Strengths and Difficulties Questionnaire as rated by the mother and teacher at 5–6 years. Child cardiac respiratory sinus arrhythmia (RSA), pre-ejection period (PEP), and heart rate (HR) were utilized as measures of ANS activity at 5–6 years.

Results: The results confirmed an association between maternal LC-PUFA status and internalizing behavioral problems as rated by the mother, as shown for DHA ($\beta = -0.11; p < 0.01$), EPA ($\beta = -0.22; p < 0.05$), and n-6:n-3 LC-PUFA ($\beta = 0.17; p < 0.01$). Statistical mediation was only demonstrated for HR. No associations were observed between LC-PUFA status and externalizing behavioral problems.

Conclusions: The present results are consistent with a role of maternal LC-PUFA status in internalizing behavioral problems as rated by the mother. These results were not observed when problem behavior was rated by the teacher. Analyses did not yield strong evidence supporting ANS activity as a possible mediator in this relationship.

1. Introduction

Experimental and epidemiological studies have implicated perinatal nutritional status of the mother as a determinant of child development and health. Many studies in this area have focused on the role of maternal long-chain polyunsaturated fatty acids (LC-PUFAs) in neuronal and cognitive development of the offspring [1,2]. LC-PUFAs include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which both belong to the omega-3 (*n*-3)

Abbreviations: AA, arachidonic acid; ABCD, Amsterdam Born Children and their Development; ANS, autonomic nervous system; DHA, docosahexenoic acid; EPA, eicosapentaenoic acid; HR, heart rate; LC-PUFA, long-chain polyunsaturated fatty acid; PEP, pre-ejection period; RSA, respiratory sinus arrhythmia; SDQ, Strengths and Difficulties Questionnaire.

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family, as well as arachidonic acid (AA), which belongs to the omega-6 (*n*-6) family. For example, epidemiological studies show a link between higher maternal LC-PUFA status during pregnancy and higher memory performance, and learning abilities in the offspring, as measured during infancy and childhood [3,4] Trial data further show that perinatal LC-PUFAs supplementation of the mother is associated with improvements on tests of global neurodevelopment in their children [5] and with reduced learning disabilities or behavioral problems [6]. In line with the latter results, we and others have also observed a link between maternal LC-PUFA status and the development of behavioral problems in the offspring, such as attention deficit hyperactivity disorder (ADHD) [7–9] and emotional problems [8,10].

Studies on the role of LC-PUFAs in neurodevelopment have been extended beyond their effects on higher central nervous system functions and also include their role in autonomic nervous system (ANS) functioning [11,12]. The ANS consists of two peripheral branches, i.e., the sympathetic system, which has typically been associated with physiological activation (e.g., effort, stress), and the parasympathetic system, which is mostly associated with regulation of metabolic and resting-state activities [13,14]. Dysregulation of the ANS has primarily been linked to aberrant cardiovascular functioning [15,16]. In line, higher maternal plasma n-3 PUFA and lower n-6 PUFA during pregnancy has been related to altered cardiovascular regulation (lower systolic blood pressure) in childhood [17]. Two studies reported a positive effect of postnatal DHA supplementation by formula feeding on the development of the ANS as quantified by an increased parasympathetic tone [11,12]. Colombo and colleagues (2011) additionally found that when infants received DHA supplementation by bottle feeding, they performed better on an attention task, suggestive of a link between LC-PUFAs and central and peripheral neuronal development [12].

In addition to its role in cardiovascular functioning, there is compelling evidence showing that aberrant ANS functioning is linked with impairments in higher cognitive functions [13]. Hence, the two separate literatures reviewed above, i.e., linking maternal LC-PUFAs with infant cognition/behavior and with cardiac-autonomic function, may have a relevant connection. For example, a study on cardiac health in children found that unmedicated children diagnosed with ADHD have a decreased parasympathetic/vagal tone (assessed as heart rate variability (HRV)) and elevated heart rate (HR) compared to healthy controls [18]. Such data are in line with a much larger literature base suggesting that altered activity of the ANS is a marker of vulnerability to psychopathology [13].

In summary, availability of LC-PUFAs during gestation is related to both offspring behavioral problems and altered ANS functioning. The latter has been speculated to contribute to, or at least correlate with, behavioral problems. While these links have each been demonstrated separately, they have yet to be tested in a single model. Therefore, the present study examined a possible mediating role of the ANS in the association between maternal LC-PUFA status during early gestation and behavioral problems in the child at pre-school age. First, we expected an association between maternal LC-PUFA status and behavioral problems in the child at age 5–6 years. Second, we presumed that ANS activity in the child is likewise predicted by maternal LC-PUFA status. Finally, it was expected that ANS activity would act as a mediator in the association between maternal LC-PUFA status and child behavioral problems. If confirmed, the study would be the first to identify ANS activity as a possible marker, and possibly a mechanism, linking maternal LC-PUFA status and behavioral problems in the offspring.

2. Method

2.1. Participants

Participants in this study were enrolled as part of the Amsterdam Born Children and their Development (ABCD) study. In 2003 and 2004 all pregnant women living in Amsterdam were contacted to participate in this study. As shown in Fig. 1, of the 12,373 women invited to participate, 8266 filled out the pregnancy questionnaire. Of these, 7050 gave their permission for a follow-up, and 4389 participated in the biomarker study; 7043 gave permission for follow-up and access to their child's medical files. Included in the present analyses were participants for whom information on maternal LC-PUFA status, the Strengths and Difficulties Questionnaire (SDQ) scored by either mother or teacher, and ANS measures were available. Twins and children with congenital malformations of the nervous system, cardiovascular system, chromosomal deviations, and multiple malformations were excluded from the study. Congenital malformations were defined following the EUROCAT definition [19] and cases were excluded because of their frequent neurological implications and possible concomitant influences on the development of the autonomic nervous system [20]. After applying these criteria, the total number of participants available for analyses was 1717. Additional information on inclusion criteria and the procedure of this study is presented in Fig. 1. Detailed information on the cohort and procedures for data collection is provided elsewhere [21].

Approximately two weeks after their child's 5th birthday all mothers were sent a follow-up questionnaire. This questionnaire included items on the child's health, development and behavior, as well as items on family socio-demographics, maternal lifestyle and psychosocial conditions and family history of medical conditions. The questionnaire also included an informed consent sheet for the child's participation in the ABCD health check. The physical measurements included measurements of ANS functioning, among other measurements.

The study protocol was approved by the medical ethical committees of all Amsterdam hospitals and the Registration Committee of Amsterdam. All participating mothers provided written informed consent for themselves and their children.

2.2. Maternal LC-PUFA

All mothers were approached for the biomarker study, but only those who gave consent for this part were eligible for the current study. They provided an additional blood sample during the prenatal screening at a mean of 12.7 ± 2.5 weeks of gestation. The absolute amounts of omega-6 AA, omega-3 DHA, and omega-3 EPA were quantified on the basis of the recovery of an internal standard and expressed as a relative value (percentage of total amount of phospholipid-associated fatty acids). An extensive description of this procedure has been given elsewhere [22]. The ratio omega-6:omega-3 was calculated by dividing the concentration of AA (n-6) by the concentrations of DHA (n-3) and EPA (n-3) added together.

2.3. Child behavior

A Dutch version of the SDQ was completed by the mother and primary/secondary school teacher when the child was at the age of 5/6 years. The SDQ is a short behavioral screening questionnaire suitable for 4-16-year-old children [23]. The SDQ consists of 5 subscales: emotional problems, conduct problems, hyperactivity/inattention problems, peer relationship problems, and prosocial behavior. Each subscale contains 5 questions with three response

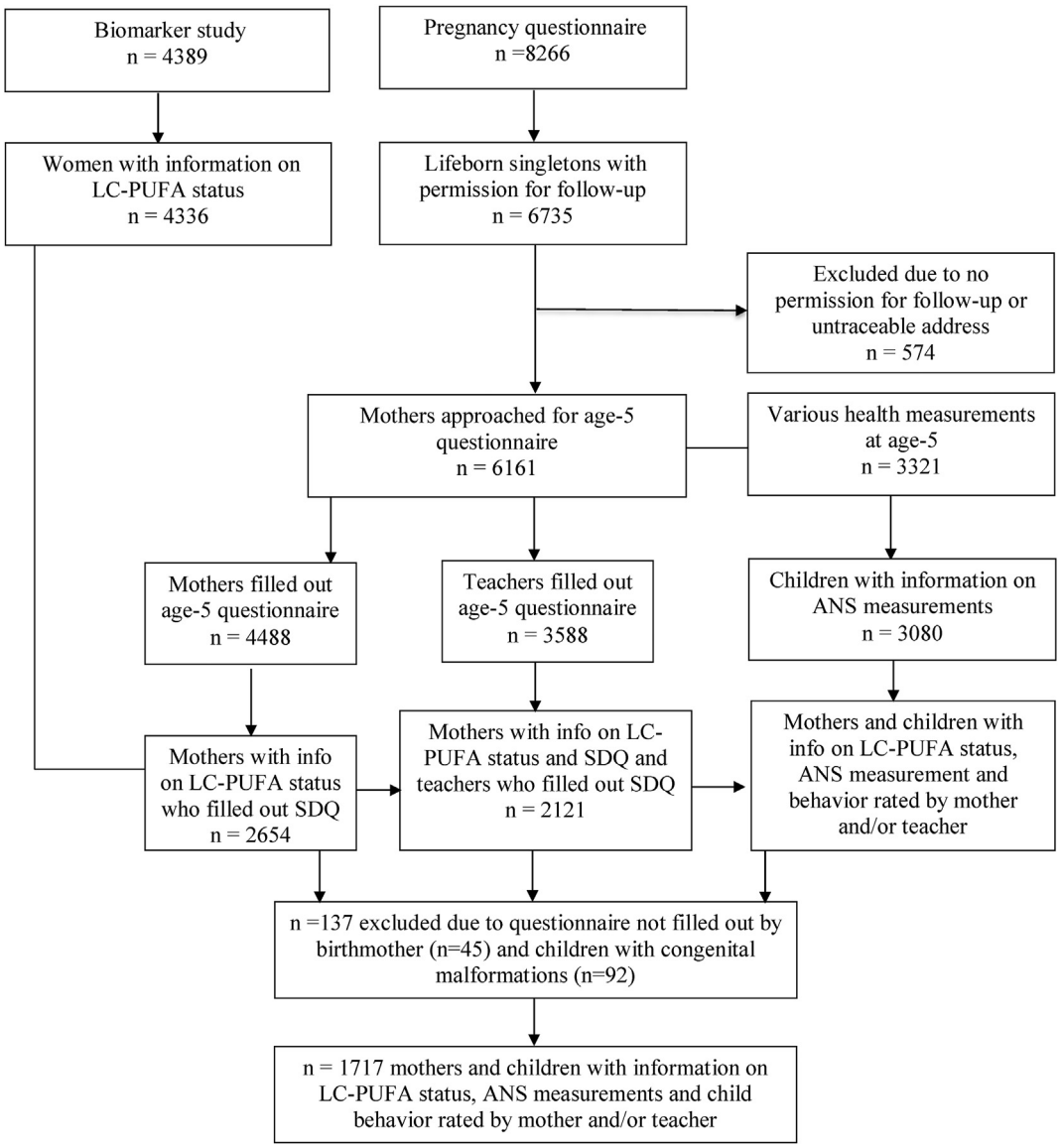

Fig. 1. Flowchart of participants in this study.

categories (0 = not true, 1 = somewhat true, 2 = certainly true). To calculate the score for each subscale, the five item values were summed. The subscale scores for emotional problems and peer relationship problems were further summed into an internalizing behavior problem score; the scores for conduct problems and hyperactivity/inattention problems were summed to generate the externalizing behavioral problem score [24]. As the prosocial subscale is not part of internalizing behavior problem nor externalizing problem, this subscale was not analyzed for this study. The reliability and validity of the Dutch SDQ has been established in a Dutch population with satisfactory psychometric properties [25], albeit inter-informant correlation between parent and teacher tends to be modest. In this study the Pearson's *r* for the internalizing behavioral problem score and the externalizing behavioral problem score were $r = 0.35$ and $r = 0.43$, respectively (both *p*'s < 0.01).

2.4. Autonomic nervous system activity

ANS activity was assessed using the VU University Ambulatory Monitoring System (VU-AMS; Amsterdam, the Netherlands, http://www.vu-ams.nl/) [26]. The procedure of this measurement during the 5–6 year health check-up has been described in detail previously [27]. In short, to start, the child was assessed in supine position after stabilization of 1 min, and registration lasted between 4 and 6.5 min. Next, the child was seated at a table for one minute of stabilization followed by another 6 min of registration. For the present study, only the recordings during 4 min supine position were used as these revealed the most reliable measurements. The following outcome measures of ANS activity were assessed: HR, pre-ejection period (PEP) and respiratory sinus arrhythmia (RSA). All R peaks in the ECG, scored by the software, were manually checked, and were moved, inserted, or deleted when necessary. The software also automatically marked inspirations and expirations in the respiratory signals. These were also manually checked and edited if necessary. RSA was obtained from the resulting data as derivate of parasympathetic nervous system activity. RSA was established using the peak to valley estimation which was obtained automatically by subtracting the shortest inter beat interval during heart rate acceleration in the inspirational phase from the longest beat interval during deceleration in the expirational phase. Pre-ejection period (PEP) was used as a derivate of sympathetic nervous system activity. For PEP, we used the time interval between

the onset of ventricular depolarization (the Q wave onset in the ECG) and the opening of the aortic waves (B-point in ICG) [28]. In summary, the ANS outcome measures were HR, PEP and RSA which were obtained from the resting period in supine position.

2.5. Covariates

Potential confounds/covariates included in the present analyses were: maternal ethnicity (Dutch, other Western country, non-Western country) defined by country of birth of the pregnant woman and her mother [22], maternal age during pregnancy (years), parity (0 or $\geq$ 1), pre-pregnancy body mass index (kg/m^2) based on self-reported height and weight, smoking during pregnancy (no, <5 per day, $\geq$ 5 per day) and alcohol consumption during pregnancy (yes or no), maternal state anxiety during pregnancy (continuous) [29], maternal education (years of education after primary school, continuous), and child's sex and age at SDQ administration (years). Birth weight (grams) and gestational age (weeks) were available from Youth Health Care Registration and the Dutch Perinatal Registration [30]. Information on infant feeding (no, 1–3 months, >3 months of exclusive breastfeeding) was obtained from questionnaires (administered during infancy and retrospectively when the child was 5 years of age) [31].

2.6. Data analysis

Descriptive statistics were used to explore the association between maternal and child characteristics and maternal LC-PUFA status during pregnancy and tested using analysis of variance (ANOVA).

A non-response analysis was performed to compare maternal ethnicity, maternal age, parity, pre-pregnancy body mass index, smoking and alcohol consumption, maternal state anxiety and maternal education, and child's sex, birth weight and gestational age at delivery between the group included in the present study and the non-response group. The non-response group consisted of all participants who were eligible for the present study (approached for the 5-year measurement round without congenital malformations and participation in the biomarker study), but were not included. Statistical differences were tested with ANOVA for continuous variables and chi-squared tests for categorical data.

Linearity was tested using four splines and likelihood ratio tests for each separate path of the model. No departure from linearity was found between continuous outcomes and the maternal LC-PUFA status, nor between continuous outcomes and ANS activity.

Thus, multiple linear regression analysis was performed to test for the association between maternal LC-PUFA status and behavioral problems, as well as for the association between maternal LC-PUFA status and ANS activity, and between ANS activity and behavioral problems. Each of these associations was first examined by a multiple linear regression that accounted for gestational age at blood sampling [32] and the sex and age of the child at the time of SDQ administration (Model 1). Second, multiple linear regression analyses that included all covariates listed above (Model 2) were performed for the three separate associations. The scores on the SDQ as reported by the mother and teacher were analyzed separately. This resulted in separate outcomes for the association between maternal LC-PUFA status and behavioral problems (mother/teacher) and for the association between the ANS and behavioral problems (mother/teacher).

To test for the hypothesized mediation effect, mediation analysis was performed in Model 2. Mediation analysis was tested using the PROCESS tool for SPSS [33]. The proportion of the association between maternal LC-PUFA status and behavioral problems mediated by the ANS measures was calculated by dividing the indirect effect by the absolute total effect (the sum of the indirect effect and the direct effect) [34]. The mediation proportion represents the percentage change of regression coefficient when the mediator is added to the model.

Data were analysed using SPSS 25.0 and the statistical package *R*. The 95% confidence intervals (CI) of the direct and indirect effects were approximated with a bootstrap procedure. The significance level was set at 0.05.

3. Results

3.1. Participants

Table 1 presents summary statistics of the analytical sample. Of the 1717 women included in the analyses, 57.5% were nullipara, 21.2% were overweight or obese according to the WHO criteria [35] at the start of pregnancy, 70.2% were of Dutch origin, and 8.9% experienced a high amount of anxiety during pregnancy defined as a STAI score >51. Non-response analysis revealed that the included mothers consumed more alcohol, more often had a Dutch background, were on average older, scored lower on anxiety symptoms and were higher educated compared to the non-response group (Supplementary Table 1).

Maternal age, pre-pregnancy BMI, educational level, ethnic background, alcohol consumption during pregnancy and parity were significantly associated with most LC-PUFAs. All other associations with LC-PUFA concentrations are presented in Table 1.

As shown in Table 2, children's SDQ score was rated at a mean age of 5.1 years. Mothers scored their children on average lower on internalizing problem behavior and higher on externalizing problem behavior compared to teachers.

3.2. Maternal LC-PUFA status and child's problem behavior

Table 3 shows the results of the minimally adjusted (Model 1) and the fully adjusted model (Model 2) for the association between maternal LC-PUFA status and problem behavior of the child as reported by the mother. After full adjustment, maternal DHA (n-3) and EPA (n-3) status were negatively associated with internalizing problem behavior (β:-0.11; 95%CI: −0.19 to −0.03 and β:-0.22; 95% CI: −0.42 to −0.04, respectively). Analyses of the associations between maternal AA (n-6) status and behavioral problems showed no significant associations. A positive association was found between maternal n-6:n-3 LC-PUFA ratio and internalizing problem behavior (β:0.17; 95%CI: 0.05 to 0.29). None of the maternal LC-PUFA components were associated with externalizing problem behavior after full adjustment. The same held for the associations with the teacher reported problem behavior (Supplementary Table 2).

3.3. Maternal LC-PUFA status and child's ANS activity

Table 4 presents the results of Models 1 and 2 for the association between maternal LC-PUFA status and measures of ANS activity in the child. After full adjustment, higher maternal DHA (n-3) status was associated with lower HR (β: −0.40; 95%CI: −0.83 to 0.00). No associations were found between maternal LC-PUFA status and other ANS measures.

3.4. Child's ANS activity and problem behavior

Table 5 shows the results of the analyses on measures of ANS activity and problem behavior reported by the mother. After full adjustment, a positive association was found between HR and internalizing problem behavior (β: 0.11; 95%CI: 0.03 to 0.20). A

Table 1
Maternal n-3 and n-6 fatty acid concentrations in plasma phospholipids according to characteristics of the study population (n = 1717).

Characteristics	n (%)	% DHA (n-3) Mean ± SD	% EPA (n-3) Mean ± SD	% AA (n-6) Mean ± SD	n-6:n-3 Mean ± SD
Maternal age					
<25	101 (5.9)	4.07 ± 1.02***	0.41 ± 0.20***	9.94 ± 1.54***	2.37 ± 0.78***
25–35 (reference)	1301 (75.8)	4.78 ± 1.12	0.67 ± 0.42	9.18 ± 1.52	1.81 ± 0.74
>35	315 (18.3)	4.69 ± 1.11	0.73 ± 0.56	9.21 ± 1.61	1.83 ± 0.61
Parity					
0	988 (57.5)	4.85 ± 1.11	0.66 ± 0.43	9.10 ± 1.50	1.78 ± 0.80
≥1	729 (42.5)	4.54 ± 1.27***	0.66 ± 0.46	9.39 ± 1.60***	1.94 ± 0.62***
Pre-pregnancy BMI (kg/m2)					
<18.5	59 (3.4)	4.57 ± 1.09	0.55 ± 0.31	9.07 ± 1.82	1.88 ± 0.58
18.5–24.9 (reference)	1293 (75.4)	4.76 ± 1.14	0.69 ± 0.46	9.08 ± 1.48	1.80 ± 0.77
25–29.9	281 (16.4)	4.72 ± 1.13	0.60 ± 0.34*	9.69 ± 1.55***	1.94 ± 0.59*
≥30	83 (4.8)	4.26 ± 0.82***	0.63 ± 0.53	10.13 ± 1.80***	2.16 ± 0.54***
Education after primary school (years)					
0-5	182 (10.6)	4.31 ± 1.23***	0.53 ± 0.46***	9.93 ± 1.79***	2.23 ± 0.75***
5-10	612 (35.8)	4.63 ± 1.15***	0.64 ± 0.47**	9.33 ± 1.61**	1.94 ± 0.94***
>10 (reference)	917 (53.6)	4.86 ± 1.07	0.71 ± 0.41	9.03 ± 1.40	1.71 ± 0.50
Ethnicity					
Dutch (reference)	1205 (70.2)	4.79 ± 1.09	0.70 ± 0.41	9.05 ± 1.43	1.75 ± 0.52
Other Western country	224 (13)	4.78 ± 1.23	0.70 ± 0.52	8.95 ± 1.44	1.86 ± 1.35
Other non-Western country	288 (16.8)	4.40 ± 1.17***	0.51 ± 0.48***	10.17 ± 1.74***	2.23 ± 0.68***
Anxiety during pregnancy (STAI)					
≤35 (reference)	887 (51.9)	4.79 ± 1.12	0.70 ± 0.42	9.14 ± 1.45	1.80 ± 0.82
36-51	670 (39.2)	4.68 ± 1.11	0.63 ± 0.43**	9.27 ± 1.59	1.86 ± 0.59
>51	153 (8.9)	4.52 ± 1.19*	0.60 ± 0.58*	9.53 ± 1.81*	2.02 ± 0.73**
Exclusive breastfeeding (months)					
No	378 (22.2)	4.67 ± 1.11	0.66 ± 0.52	9.47 ± 1.56***	1.91 ± 0.63**
1-3	459 (26.9)	4.66 ± 1.14	0.65 ± 0.43	9.26 ± 1.55	1.91 ± 1.02
>3 (reference)	868 (50.9)	4.76 ± 1.13	0.67 ± 0.41	9.11 ± 1.53	1.79 ± 0.57
Alcohol during pregnancy					
No	1201 (69.9)	4.64 ± 1.16	0.63 ± 0.43	9.33 ± 1.60	1.92 ± 0.81
Yes	516 (30.1)	4.92 ± 1.03***	0.75 ± 0.45***	9.00 ± 1.38***	1.67 ± 0.46***
Smoking during pregnancy					
No (reference)	1566 (91.2)	4.76 ± 1.12	0.67 ± 0.45	9.23 ± 1.55	1.83 ± 0.74
<5 per day	89 (5.2)	4.43 ± 1.08*	0.65 ± 0.37	8.94 ± 1.49	1.90 ± 0.63
≥5 per day	62 (3.6)	4.27 ± 1.26**	0.57 ± 0.38	9.63 ± 1.50	2.15 ± 0.65**

Fatty acid concentrations were standardized at median gestational age at blood sampling (12.7 weeks) and expressed as % of total fatty acids.
BMI = body mass index, STAI=State-Trait Anxiety Inventory, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6:n-3 = AA/(DHA + EPA).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2
Child characteristics at age 5–6 (n = 1717).

Child characteristics	Mean ± SD/%	Mean ± SD
Age (years)	5.1 ± 0.2	
Sex (% boys)	48.9	
HR (beats/minute)	87.1 ± 10.0	
PEP (msec)	71.3 ± 8.9	
RSA (msec)	123.5 ± 63.3	
Problem behavior (SDQ)	Mother	Teacher
Internalizing problem score	1.6 ± 1.8	2.1 ± 2.3
Peer relationship problem score	0.7 ± 1.1	0.9 ± 1.3
Emotional problem score	0.9 ± 1.2	1.2 ± 1.6
Externalizing problem score	3.4 ± 2.9	3.0 ± 3.3
Conduct problem score	1.0 ± 1.2	0.8 ± 1.2
Hyperactivity/inattention problem score	2.4 ± 2.2	2.2 ± 2.5

SDQ=Strengths and Difficulties Questionnaire, HR = heart rate, PEP = pre-ejection period, RSA = respiratory sinus arrhythmia.

negative association was found between HR and externalizing problem behavior when teacher reports were used (β: −0.25; 95% CI: −0.41 to −0.09, Supplementary Table 3).

3.5. Maternal LC-PUFA status and child's problem behavior: mediation by ANS activity

Results were partly consistent with the idea that maternal LC-PUFA status mediated internalizing problem behavior of the child through its effect on HR. Figure 2 presents the mediation of HR in the association between maternal n-6:n-3 LC-PUFA status and internalizing problem behavior as reported by the mother. A bias-corrected bootstrap 95%CI for the indirect effect ($b = 0.0073$) based on 1000 bootstrap samples was entirely above zero (0.0001–0.0213). HR explained about 4% of the association between maternal n-6:n-3 LC-PUFA status and internalizing problem behavior (0.0073/0.1699*100%). A bias-corrected bootstrap confidence interval for the indirect effect of maternal DHA (n-3) and EPA (n-3) on internalizing problem behavior ($b = -0.0045$; 95% CI: −0.0116 to 0.0005 and $b = -0.0075$; 95%CI:-0.0225 to 0.0039, respectively) were not statistically significant, indicating no mediation.

4. Discussion

The present study examined ANS activity as a possible mediator in the association between maternal LC-PUFA status and problem behavior in the offspring. As shown before in this cohort, the results indicated a link between maternal LC-PUFA status and behavioral problems in the child, adjusting for a wide range of possible confounding factors [10]. Specifically, LC-PUFA status was consistently linked with internalizing problem behavior, with more consistent findings for the n-3 LC-PUFAs. These associations were not significant when teacher ratings of internalizing problem behavior were used as an outcome. Finally, analyses provided moderate support for ANS activity as a potential mediator of maternal LC-PUFA status and child behavioral problems, whereby a mediation effect was

Table 3
Association between maternal fatty acid concentrations in plasma phospholipids during early pregnancy and children's reported problem behavior by the mother (n = 1689).

SDQ	Model 1	Model 2
DHA (22:6n-3) (%)		
Internalizing problem behavior	−0.18 (−0.26; −0.11)***	−0.11 (−0.19; −0.03)**
Externalizing problem behavior	−0.17 (−0.30; −0.06)**	−0.08 (−0.20; 0.04)
EPA (20:5n-3) (%)		
Internalizing problem behavior	−0.46 (−0.66; −0.26)***	−0.22 (−0.42; −0.04)*
Externalizing problem behavior	−0.43 (−0.74; −0.12)**	−0.18 (−0.48; 0.12)
AA (20:4n-6) (%)		
Internalizing problem behavior	0.07 (0.02; 0.13)*	−0.02 (−0.08; 0.04)
Externalizing problem behavior	0.11 (0.02; 0.20)*	0.04 (−0.05; 0.13)
n-6:n-3		
Internalizing problem behavior	0.35 (0.23; 0.47)***	0.17 (0.05; 0.29)**
Externalizing problem behavior	0.33 (0.14; 0.51)***	0.13 (−0.06; 0.33)

Model 1: adjusted for gestational age at blood sampling, sex and child's age at SDQ.
Model 2: Model 1 + ethnicity, parity, pregnancy BMI, smoking and alcohol consumption during pregnancy, maternal state anxiety and maternal education.
Values are unstandardized β coefficient (95% CI).
BMI = body mass index, SDQ=Strengths and Difficulties Questionnaire, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6:n-3 = AA/(DHA + EPA).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

found for HR, as an overall indicator of cardiac ANS activity in both branches. No associations were found when specific markers of PNS and SNS activity were analyzed separately.

Results from the present study were largely consistent with earlier reports showing associations between maternal DHA concentration during pregnancy and internalizing behavioral problems or summary scores of behavioral problems in their children [7–9]. The balance of evidence indicates that, overall, there is more evidence to support an association between higher maternal n3 concentrations and reduced problem behavior than for an association with maternal n6, which is likewise supported by our findings [7].

LC-PUFAs have been linked to neurodevelopmental processes that affect ANS activity as well as behavioral problems in the offspring. In light of a possible shared pathway, the present study sought to combine these links in a mediation model. The results yielded partial to support of this model: HR partly mediated the association between maternal DHA status in pregnancy and internalizing problem behavior in the child. The first path of this association, i.e., the association between maternal DHA and the child's HR, is in line with two randomized controlled trails in which infants who were supplemented with DHA formula feeding showed lower HR compared to infants with unsupplemented formula feeding [11,12]. The ANS has been proposed as a mechanistic link between maternal LC-PUFA status and cognitive functioning in the offspring with altered ANS functioning as a result of LC-PUFA status affecting CNS development and maturity of the brain [36,37]. We found some support for this mechanistic link for HR as an overall measure of ANS activity at young age. Follow-up assessments from the ABCD-study could further explore the potential mediating role of the ANS activity at older ages when the ANS matures.

A link between maternal LC-PUFA status and behavioral problems was established only when rated by the mother, and not when rated by the teacher. Correlations between parent and teacher ratings were moderate [25], which might reflect a difference in child behavior in different contexts and/or inherent variability in perceptions between raters [38]. Currently, there is no consensus on whether to combine these scores (hereby possibly increasing reliability/validity) or to analyze these ratings separately [8,10]. We

Table 4
Association between maternal fatty acid concentrations in plasma phospholipids during early pregnancy and autonomic nervous system activity of the child at age 5–6 (n = 1690).

ANS	Model 1	Model 2
DHA (22:6n-3) (%)		
HR (beats/min)	−0.48 (−0.89; −0.06)*	−0.40 (−0.83; 0.00)*
PEP (msec)	−0.15 (−0.53; 0.22)	−0.18 (−0.57; 0.21)
RSA (msec)	0.32 (−2.36; 2.99)	0.62 (−2.16; 3.40)
EPA (20:5n-3) (%)		
HR (beats/min)	−0.82 (−1.88; 0.24)	−0.55 (−1.64; 0.54)
PEP (msec)	−0.27 (−1.23; 0.69)	−0.39 (−1.38; 0.60)
RSA (msec)	2.16 (−4.65; 8.97)	2.20 (−4.81; 9.20)
AA (20:4n-6) (%)		
HR (beats/min)	0.28 (−0.03; 0.58)	0.22 (−0.11; 0.54)
PEP (msec)	−0.24 (−0.51; 0.04)	−0.20 (−0.49; 0.10)
RSA (msec)	−0.29 (−2.24; 1.67)	−1.10 (−3.19; 0.99)
Omega-6:omega-3		
HR (beats/min)	0.86 (0.21; 1.50)**	0.65 (−0.02; 1.32)
PEP (msec)	−0.26 (−0.84; 0.32)	−0.20 (−0.82; 0.42)
RSA (msec)	−1.22 (−5.34; 2.90)	−1.89 (−6.27; 2.49)

Model 1: adjusted for gestational age at blood sampling, sex and child's age at SDQ.
Model 2: Model 1 + ethnicity, parity, pregnancy BMI, smoking and alcohol consumption during pregnancy, maternal state anxiety and maternal education.
Values are unstandardized β coefficient (95% CI).
BMI = body mass index, SDQ=Strengths and Difficulties Questionnaire, AA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, n-6:n-3 = AA/(DHA + EPA), HR = heart rate, PEP = pre-ejection period, RSA = respiratory sinus arrhythmia.
*$p < 0.05$, **$p < 0.01$.

Table 5
The cross–sectional association between autonomic nervous system activity and children's reported problem behavior by the mother at age 5–6 (n = 1708).

SDQ	Model 1	Model 2
HR (10 beats/min)		
Internalizing problem behavior	0.14 (0.06; 0.23)***	0.11 (0.03; 0.20)**
Externalizing problem behavior	−0.07 (−0.21; 0.07)	−0.09 (−0.23; 0.04)
PEP (10 msec)		
Internalizing problem behavior	−0.09 (−0.19; 0.00)*	−0.07 (−0.16; 0.03)
Externalizing problem behavior	0.03 (−0.12; 0.18)	0.05 (−0.10; 0.19)
RSA (10 msec)		
Internalizing problem behavior	−0.01 (−0.02; 0.01)	−0.01 (−0.02; 0.01)
Externalizing problem behavior	0.01 (−0.01; 0.03)	0.01 (−0.01; 0.03)

Model 1: sex and child's age at SDQ.
Model 2: Model 1 + ethnicity, parity, pregnancy BMI, smoking and alcohol consumption during pregnancy, maternal state anxiety and maternal education.
Values are unstandardized β coefficient (95% CI).
BMI = body mass index, SDQ=Strengths and Difficulties Questionnaire, HR = heart rate, PEP = pre-ejection period, RSA = respiratory sinus arrhythmia.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

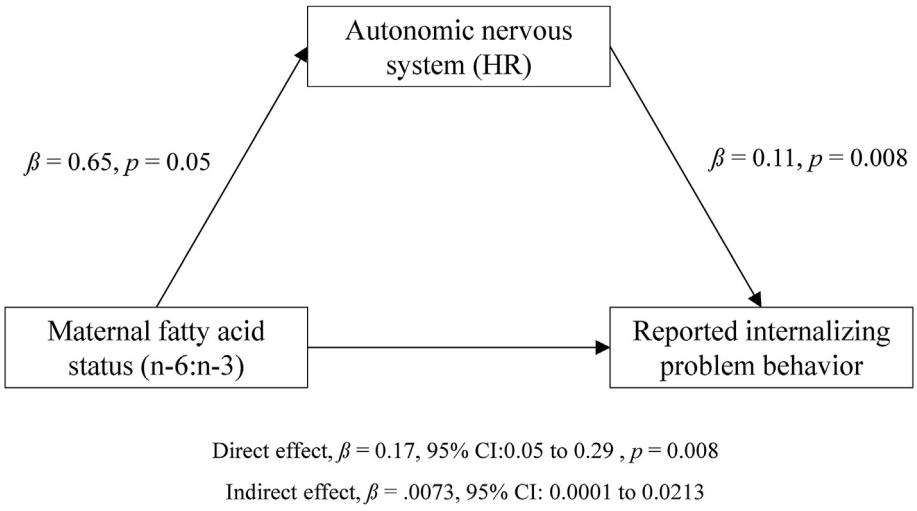

Fig. 2. Mediation effect of heart rate (HR) on the relationship between the ratio of omega-6:omega-3 (n-6:n-3) and reported internalizing problems rated by the mother (Model 2).

did find a negative association between HR and externalizing problem behavior, which was statistically significant when rated by the teacher. The stronger association for teacher scores might be due to the fact that teacher scores generally show better psychometric properties [38]. Moreover the distinct difference in the association between HR and internalizing problem behavior (positive) and externalizing problem behavior (negative) is in agreement with others [39]. Our results might thus indicate that altered ANS function, as evidenced by an increased HR, could be a contributing factor in the association between n3 and internalizing problem behavior (but not for the onset of externalizing problem behavior).

The present study has several strengths. First, this study utilized a large-scale community based multi-ethnic birth cohort, which gives strengths in terms of statistical power and generalizability across ethnic and socio-economic groups. Moreover, the ABCD-study boasts a large catalogue of measures, which allowed adjustment for many potential confounders. Third, the cardiac ANS activity uniquely involved both markers of sympathetic and parasympathetic activity under standardized conditions. Lastly, maternal LC-PUFA status was determined using a blood sample taken during pregnancy, which provides a direct assessment of pregnancy-related LC PUFA status, the biological substrate that reaches the fetus. In light of our research question a direct biological assessment is more pertinent as compared to estimates derived from dietary questionnaires or from a blood sample taken after delivery [7,9,40].

The present study also has limitations. First, although the SDQ has good psychometric properties [25], the SDQ is not a diagnostic instrument and high scores on the SDQ therefore do not necessarily imply the presence of psychopathology. Furthermore, the children assessed in the present study were transferring from kindergarten to elementary school, whereby the latter is more demanding than kindergarten in terms of attention and discipline. This specific context could involve transient perturbations in the child's behavior at home and/or at school and could have confounded ratings by mothers and teachers. Third, nonresponse analysis showed that the included mothers were higher educated, had more often Western or Dutch ethnicity and were on average older than the mothers in the non-response group. This indicates a selection towards participants with a higher socioeconomic status and limits the generalizability of our results. The present study collected only one blood sample during the first half of pregnancy. The developing brain is also vulnerable to nutritional fluctuations between 24 and 42 weeks of gestation, when a high quantity of fatty acids are integrated into the brain and other neural tissues of the fetus [2,41]. In future research, it would be preferable to obtain more blood samples during multiple vulnerable periods. Lastly, the child's current LC-PUFA intake has also been associated with HR and mental functioning [12,42], but this assessment was not included in the present study. However, other research demonstrated that the long-term effect of maternal LC-PUFA status on behavioral problems is not confounded by current LC-PUFA status [7].

In conclusion, in the present study we found some support for ANS activity as a mediator in the association between maternal LC-PUFA status and behavioral problems, although the size of the effect was small and could not be specifically linked to PNS or SNS activity. Further research seems warranted to determine other potential mechanisms underlying the associations between maternal LC-PUFA status and behavioral problems in offspring. Finally, our results stress the importance of an adequate and balanced supply of dietary fatty acids in pregnant women to ensure optimal fetal development and subsequent long-term behavioral outcomes. Additional research is needed to develop effective strategies that can be implemented in the prenatal care setting to reach this goal.

Contribution to authorship

All the authors contributed to the conceptualization and the writing of the manuscript. JS and TV conducted the analyses; JS and JB drafted the manuscript; TV was project manager of the ABCD study and supervised data collection. SdR and JB provided advice on the analyses and interpretation of the data. All authors approved the final version of the manuscript.

Details of ethics approval

Approval for the ABCD study was obtained from the Central Committee on Research involving Human Subjects (CCMO; number P02.0335L, 2002), the Medical Ethical Committees of participating hospitals and the Registration Committee of Amsterdam.

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Conflict of interest

The authors (TV, JS, SdR, JB) declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.11.002.

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