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Prenatal Omega-6:Omega-3 Ratio and Attention Deficit and Hyperactivity Disorder Symptoms

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Objective To evaluate whether higher omega-6:omega-3 (n-6:n-3) long-chain polyunsaturated fatty acid ratio in cord plasma is associated with more symptoms of attention deficit and hyperactivity disorder (ADHD) at 4 and 7 years of age.

Study design This study was based on a population-based birth cohort in Spain. N-6 arachidonic acid and n-3 eicosapentaenoic and docosahexaenoic acid concentrations were measured in cord plasma. At 4 years old, ADHD symptoms were reported by teachers through the ADHD *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed checklist (n = 580). At 7 years old, ADHD symptoms were reported by parents through the Conners' Rating Scale-Revised (short form; n = 642). The ADHD variable was treated as continuous (score) and as dichotomous (symptom diagnostic criteria). Child and family general characteristics were prospectively collected through questionnaires. We applied pooled zero-inflated negative binomial and logistic regressions adjusted for covariates.

Results A higher omega-6:omega-3 long-chain polyunsaturated fatty acid ratio in cord plasma was associated with a higher ADHD index (incidence rate ratio, 1.13; 95% CI, 1.03, 1.23) at 7 years old. The association was not observed at 4 years old (incidence rate ratio, 1.04; 95% CI, 0.92-1.18). No associations were found using ADHD symptom diagnostic criteria.

Conclusions High prenatal omega-6:omega-3 long-chain polyunsaturated fatty acid ratio preceded the appearance of subclinical ADHD symptoms during mid-childhood. Our findings suggest that maternal diet during pregnancy may modulate the risk to develop long-term ADHD symptoms in the offspring. *(J Pediatr 2019;■:1-8)*.

Omega-3 (n-3) and omega-6 (n-6) long-chain polyunsaturated fatty acids (LCPUFA) are among the main components of cell membranes.[1] The main source of these LCPUFA in humans is diet, which is estimated to contain a n-6:n-3 ratio of 15-20:1 in Western countries.[2,3] A balanced intake of both series of omega is particularly important, because n-3 and n-6 compete for incorporation into cell membranes[4] and have opposing physiological functions; n-6 promotes systemic proinflammatory states, and n-3 promotes anti-inflammatory states.[3]

n-3 and n-6 LCPUFA comprise 15%-30% of the dry weight of the brain.[5] Three of these LCPUFA play a crucial role in the function and architecture of the central nervous system,[6–8] particularly during the later stages of gestation and early postnatal life[9]: the n-3 docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids,

AA	Arachidonic acid (C20:4n-6)
ADHD	Attention deficit and hyperactivity disorder
CPRS-R:S	Revised Conners' Parent Rating Scale Short Form
DHA	docosahexaenoic acid (C22:6n-3)
DSM-IV	*Diagnostic and Statistical Manual of Mental Disorders*, 4th edition
EPA	Eicosapentaenoic acid (C20:5n-3)
IPW	Inverse probability weighting
IRR	Incidence rate ratio
LCPUFA	Long-chain polyunsaturated fatty acids
n-6	Omega-6
n-3	Omega-3

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and the n-6 arachidonic acid (AA). During these periods, the main source of n-3 and n-6 LCPUFA are through placental transfer of these substances and breastfeeding.[9] An inadequate maternal nutritional pattern during this period could be related to child attention deficit and hyperactivity disorder (ADHD), a neurodevelopmental disorder that is estimated to affect 5.3% of children globally.[10] Children with ADHD symptoms have higher n-6:n-3 ratios compared with children without symptoms, which could be due to dietary patterns, altered gut microbiota, or abnormal LCPUFA metabolism.[7,11–13] Whether a high prenatal n-6:n-3 LCPUFA ratio precedes the appearance of ADHD symptoms during childhood, which may suggest a role in the development of the symptoms, remains unknown.

Following developmental origins of health and disease theory,[14] our study aimed to analyze the association between n-6(AA):n-3(DHA+EPA) LCPUFA ratio concentration in cord plasma, a proxy of fetal LCPUFA availability during late gestation,[15] and child ADHD symptoms at 4 and 7 years of age. We hypothesized that higher n-6:n-3 ratios were associated with more child ADHD symptoms.

Methods

This study was based on a Spanish population-based birth cohort, including 4 Spanish regions: Asturias (n = 494), Gipuzkoa (Basque Country; n = 638), Sabadell (Catalonia; n = 657), and Valencia (n = 855). Between November 2003 and January 2008, pregnant women who visited the public health centers for their first trimester ultrasound examination were invited to participate in the project if they fulfilled the following inclusion criteria: age ≥16 years, singleton pregnancy, no use of assisted reproductive techniques, intention to deliver at the reference hospital, and ability to speak and understand Spanish or a local language. A baseline survey was performed at enrollment (approximately 12 weeks of pregnancy), and follow-up surveys were performed at 20 and 32 weeks of pregnancy, at birth, and when children were 6 months, and 1, 2, 4, or 5, and 7 years old. LCPUFA were assayed in 953 cord plasma samples based on availability. In total, 580 and 642 children in the 4-year-old and 7-year-old assessment periods, respectively, had data both on LCPUFA and ADHD and were included in the present analyses (Figure 1). All parents signed the informed consent form approved by the Clinical Research Ethical Committees of the Asturias, Donostia (Gipuzkoa), La Fe (Valencia) Hospitals, and the Medical Assistance Municipal Institute (Barcelona).

LCPUFA Levels

Whole blood samples were collected by using venipuncture of cord vessels before the placenta was delivered. Samples were processed, separated into aliquots of 1 mL, and then frozen to −80°C until the time of analysis. In a subsample of cord plasma, LCPUFA methyl esters were prepared and extracted following the method developed and validated by Moltó-Puigmartí et al.[16] LCPUFA were then separated and quantified by using fast-gas chromatography with flame ionization detection. The measurement of LCPUFA in plasma reflects the dietary availability of these components during the last 2-3 days.[17] The relative amount of each LCPUFA quantified was expressed as the percentage of the total fatty acids. The n-6:n-3 LCPUFA ratio was calculated by dividing AA percentage by the sum of the DHA and EPA percentages.[3,18] A higher ratio reflects a greater imbalance between both series of omega.

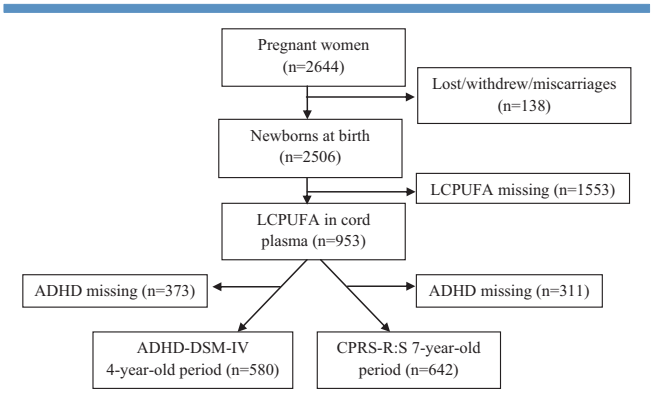

Figure 1. Flowchart of the study population.

ADHD Characterization

At 4 years of age, teachers reported the ADHD symptoms of the participants by using the ADHD *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed (DSM-IV) form list,[19] which consists of 18 symptom items categorized under 2 symptom subscales: the inattention subscale (9 symptoms) and the hyperactivity-impulsivity subscale (9 symptoms). Each ADHD symptom item was rated on a 4-point scale (0 [never or rarely], 1 [sometimes], 2 [often], or 3 [very often]), so that the total ADHD symptoms score ranges between 0 and 54, and the inattention and the hyperactivity-impulsivity subscales scores range from 0 to 27. We recorded the options 0 and 1 as 0 (symptom absent), and ratings of 2 and 3 as 1 (symptom present).[20] We generated a dichotomous variable of ADHD symptom diagnostic criteria, using 6 symptoms as a cut off point. The ADHD-DSM-IV form list showed good internal consistency, with Cronbach alpha coefficients of 0.90 for the total symptoms score, 0.88 for the inattention subscale, and 0.87 for the hyperactivity-impulsivity subscale.

At 7 years of age, parents reported the ADHD symptoms by using the Revised Conners' Parent Rating Scale Short Form (CPRS-R:S),[21] which consists of 27 items summarized on 4 subscales: ADHD index (12 items), oppositional (6 items), cognitive problems/inattention (6 items), and hyperactivity (6 items). Each item was rated on a 4-point scale (0 [never or rarely], 1 [sometimes], 2 [often], 3 [very often]). Therefore, the ADHD index score ranges between 0 and 36, and the other subscales range between 0 and 18. We generated a dichotomous variable of ADHD symptom diagnostic

Table III. Description of the LCPUFA concentrations in cord plasma and the ADHD variables at the 4- and 7-year-old assessment periods for the final sample

LCPUFA and ADHD variables	Minimum	25th percentile	50th percentile	75th percentile	Maximum
Cord plasma (n = 715)					
AA among total fatty acids, %	0.32	12.74	14.33	15.94	25.02
DHA among total fatty acids, %	1.68	3.89	4.71	5.95	11.42
EPA among total fatty acids, %	0	0.13	0.19	0.30	2.09
n-6:n-3 ratio*	0.07	2.33	2.86	3.47	6.28
4-year-old period (n = 580)					
ADHD symptom score†	0	1	4	10	52
Inattention	0	0	2	5	25
Hyperactivity-impulsivity	0	0	2	5	27
7-year-old period (n = 642)					
ADHD index‡	0	3	6	11	36
Oppositional	0	1	3	5	18
Cognitive problems/inattention	0	1	2	5	18
Hyperactivity	0	1	3	6	18

*AA:(DHA+EPA).
†ADHD-DSM-IV symptom score based on 18 items (teachers).
‡Conners' Rating Scale-Revised (short form) ADHD index based on 12 items (parents).

criteria, using T score of 66 as a cut off point.[21] The CPRS-R:S showed good internal consistency, with Cronbach alpha coefficients of 0.91 for the ADHD index, 0.85 for the oppositional subscale, 0.89 for the cognitive problems/inattention subscale, and 0.82 for the hyperactivity subscale. Higher scores in ADHD-DSM-IV and CPRS-R:S indicates more ADHD symptoms.

Covariates

We used face-to-face questionnaires to collect data on maternal education (primary or lower, secondary, and university), occupation, maternal age, and parity during the first trimester of pregnancy (zero, 1, and ≥2). Data on maternal tobacco use were collected at the third trimester of pregnancy. Child's date of birth, sex, and birth weight were obtained from clinical records. Gestational age at delivery was based on the self-reported date of last menstrual period and it was corrected using the crown-rump length measurement from an early ultrasound examination. We collected data on breastfeeding at 6 months and 1.5 years through a questionnaire. We calculated child's age based on birth date and ADHD assessment date. We used the Food Frequency Questionnaire[22] to collect information on maternal diet between weeks 12 and 32 of pregnancy and on child diet at 4 years of age. We calculated the relative Mediterranean Diet Score, an indicator of adherence to the Mediterranean diet, for the mothers based on the consumption of vegetables, fruits and nuts, cereals, legumes, fish, olive oil, meat, and dairy products.[23] Maternal mental health was measured through the Global Severity Index of the Symptom Checklist-90-R[24] and their verbal IQ proxy was tested through the Similarities subtest of the Wechsler Adult Intelligence, 3rd edition[25] when children were 4 years old.

Statistical Analyses

We evaluated the differences in the n-6:n-3 ratio and ADHD symptom scores by sociodemographic characteristics using Kruskal-Wallis test. The continuous variables of child age, maternal mental health, and maternal IQ proxy were split in categories for these tests. We used the median for splitting child age and tertiles for maternal variables. We evaluated sample differences between complete cases and incomplete cases and between cohort regions using the Fisher exact test for categorical variables and Kruskal-Wallis test for continuous variables.

We evaluated crude and adjusted associations between the n-6:n-3 ratio and ADHD symptoms at 4 (n = 580) and 7 (n = 642) years of age. To avoid an underestimation of the outcome owing to missing values in scale items, we multiplied the obtained raw score by the total number of items on the scale, divided by the total number of items that had responses, and then rounded to the nearest whole number.[21] Because ADHD symptoms occur as a continuum in the general population,[26] this variable was primarily treated as continuous (score) and as dichotomous (symptom diagnostic criteria) to follow a more clinical approach. For the continuous outcomes, we applied zero-inflated negative binomial regression models, previously shown to improve the statistical modelling of ADHD studies.[27] This method combines and simultaneously estimates 2 separate regression models. The excess of zeros is modelled under a logistic distribution and the count data with the standard negative binomial distribution. For the dichotomous outcome, we performed logistic regression models. We adjusted the models for maternal education, sex, age, and region. The confounders were selected a priori based on previous studies[28,29] and after building a directed acyclic graph (**Figure 2**; available at www.jpeds.com). We also tested the heterogeneity of the associations between regions using the I^2 statistic, as well as the interaction terms between the n-6:n-3 ratio and sex in the association with ADHD symptoms.

We applied inverse probability weighting (IPW) to control the potential selection bias induced by restricting the analysis to complete cases.[30] Only individuals with observed data

Table IV. Median n-6:n-3 ratio in cord plasma and ADHD symptom scores at 4- and 7-year-old periods by sociodemographic characteristics

Sociodemographic characteristics	n-6:n-3 ratio in cord plasma (n = 715)	*P* value*	ADHD† 4 years old (n = 580)	*P* value*	ADHD‡ 7 years old (n = 642)	*P* value*
Child age (4 years old)		.844		.029		
<4.5	2.83		4			
≥4.5	2.90		5			
Child age (7 years old)		<.001				1.000
<7.5	3.13				6	
≥7.5	2.71				6	
Child sex		.269		<.001		<.001
Females	2.93		3		5	
Males	2.83		6		8	
Maternal mental health§		.360		.458		<.001
First tertile (35.5-44.2)	2.95		4		4.5	
Second tertile (44.3-52.5)	2.83		5		6	
Third tertile (52.6-96.6)	2.95		5		9	
Maternal IQ¶		.466		.184		.046
First tertile (0-9.0)	2.95		4		8	
Second tertile (9.1-11.2)	2.84		5		6	
Third tertile (11.3-18.6)	2.89		4		6	
Maternal education		<.001		.178		.001
Primary	3.03		5		8	
Secondary	3.00		5		7	
University	2.66		4		5	

*Kruskal-Wallis test.
†ADHD-DSM-IV symptom score (teachers).
‡Conners' Rating Scale-Revised (short form) (parents).
§Global Severity Index the Symptom Checklist-90-R, T score.
¶Similarities subtest of the Wechsler Adult Intelligence, 3rd edition, (mean, 10; SD, 3).

were analyzed, but we used weights to rebalance the set of complete cases so that it is representative of the whole sample. We predicted the probability of being a complete case using logistic regression to generate the inverse probability weights (**Table I** and **Table II**; available at www.jpeds.com). Breastfeeding, maternal social class, prematurity, and smoking during pregnancy contained missing values (<2%) that were singly imputed as the variable median value.

We performed some sensitivity analyses. First, we ran the model without including IPW. Second, we additionally adjusted the models for maternal mental health and IQ proxy, maternal and child fish intake (together and separately), and maternal adherence to a Mediterranean diet. Although these variables were not considered confounders and the sample size was decreased by ≤16%, we tested whether the inclusion of these variables in the models modified the effect estimates of the main analyses. Third, we repeated the models for each specific LCPUFA (AA, EPA, and DHA).

We used R (3.0.2; R Foundation for Statistical Computing, Vienna, Austria) and Stata 12.1 (Stata Corporation, College Station, Texas) to perform the statistical analyses.

Results

Participants had a mean age of 4.85 years (SD, 0.72 years) and 7.35 years (SD, 0.63 years) at each of the assessment periods. The sample was equally distributed by sex (48% of the participants were females). The mean of maternal age at delivery was 32 years old (SD, 4 years), 45% of the mothers were manual workers, and 40% had secondary education levels. Three percent of the participants were born preterm and the breastfeeding duration mean was of 27 weeks (SD, 22 weeks).

The concentrations of LCPUFA in cord plasma and the ADHD scores at both assessment periods are described in **Table III**. **Table IV** presents the median values of the n-6:n-3 LCPUFA ratio concentrations and the ADHD scores according to sociodemographic characteristics. The ADHD symptom reports were associated with child's age and sex, maternal education, mental health and IQ. The n-6:n-3 ratio was associated with child's age and maternal education. **Table V** and **Table VI** (available at www.jpeds.com) show that the main variables were differently distributed across the Spanish cohort regions included in this study.

Table VII describes the weighted crude and adjusted associations between the n-6:n-3 ratio and ADHD symptoms, treated as dichotomous and continuous variable, at 4 and 7 years old. We observed that the n-6:n-3 LCPUFA ratio in cord plasma did not predict the risk of presenting ADHD symptom diagnostic criteria at 4 years old (OR, 1.11; 95% CI, 0.73-1.71). Similarly, no association was observed with the continuous ADHD symptoms score (incidence rate ratio [IRR], 1.04; 95% CI, 0.92-1.18), without differences between subscales. We did not observe substantial heterogeneity among cohorts (I^2 = 5.7%; *P* = .365) or interaction by child sex (*P* for interaction = .124). At 7 years old, we did not obtain a statistically significant association using the outcome

Table VII. Crude and adjusted associations* between n-6:n-3 ratio in cord plasma and ADHD symptoms

Subscales (range)	Crude		Adjusted†		Between-regions heterogeneity (I^2), %‡
	Estimate	95% CI	Estimate	95% CI	
4-year-old period§					
ADHD diagnostic criteria (≥6 symptoms)	1.13	0.77-1.64	1.11	0.73-1.71	0
ADHD (0-52)	1.09	0.95-1.25	1.04	0.92-1.18	5.70
Inattention (0-27)	1.09	0.96-1.25	1.05	0.93-1.18	0
Hyperactivity-impulsivity (0-27)	1.10	0.93-1.30	1.04	0.89-1.22	0
7-year-old period¶					
ADHD diagnostic criteria (T score >66)	1.52	1.10-2.11	1.40	0.98-2.00	35.20
ADHD (0-36)	1.15	1.06-1.25	1.13	1.03-1.23	23.40
Oppositional (0-18)	1.04	0.95-1.15	1.04	0.95-1.15	56.50**
Cognitive problems/inattention (0-18)	1.16	1.05-1.28	1.12	1.01-1.25	0
Hyperactivity (0-18)	1.16	1.04-1.28	1.13	1.02-1.25	59.20**

*The IRR is estimated by zero-inflated negative binomial regression models for the continuous outcomes and the OR is estimated by logistic regression models for the dichotomous outcome. We applied IPW in all models.
†Models were adjusted for maternal education, child sex, child age, and region.
‡Percentage of the total variability in the estimates that is attributable to heterogeneity between regions.
§ADHD-DSM-IV symptom score (teachers).
¶Conners' Rating Scale-Revised (short form) (parents).
**Test for heterogeneity with $P < .10$.

variable as dichotomous (OR, 1.40; 95% CI, 0.98-2.00); however, we found that the ADHD index increased by 13% per each n-6:n-3 LCPUFA ratio unit (IRR, 1.13; 95% CI, 1.03-1.23). The subscales that showed statistically significant associations were the cognitive problems/inattention (IRR, 1.12; 95% CI, 1.01-1.25) and the hyperactivity subscales (IRR, 1.13; 95% CI, 1.02-1.25). There was a significant heterogeneity between cohort regions in some subscale outcomes, specifically in the oppositional and the hyperactivity subscales, causing almost 60% of the total variability in the estimates. In those cases, the associations were strong in Asturias, and they were weak in the other regions (data not shown). The interaction term between the n-6:n-3 ratio and sex in the association with ADHD index was not significant (P for interaction = .418).

The results were similar but weaker when IPW was not applied in the models (**Table VIII**; available at www.jpeds.com). The inclusion of maternal mental health and IQ proxy scores, maternal and child fish intake (together and separately), and maternal Mediterranean diet in the final weighted models did not change the results meaningfully (**Table IX**; available at www.jpeds.com). In further analyses using each LCPUFA component separately as exposure in weighted and adjusted models, we observed that DHA was negatively associated with ADHD symptoms at 7 years old, EPA was positively associated with ADHD symptoms at 4 years old, and AA was not associated with ADHD symptoms (**Table X**; available at www.jpeds.com).

Discussion

In this study, we observed an association between the n-6:n-3 ratio (AA/[EPA+DHA]) concentration in cord plasma and subclinical ADHD symptom scores during childhood. We found that ADHD symptom scores increased about 13% per each n-6:n-3 ratio unit at 7 years old, although this association was not observed at 4 years old. In separated association analyses, DHA seemed to present a major role in this association. The longitudinal approach of this study allowed us to demonstrate that a higher prenatal n-6:n-3 LCPUFA ratio preceded subclinical ADHD symptoms during childhood.

The present findings are consistent with previous studies that have linked the prenatal and perinatal n-6:n-3 ratio intake or in maternal fluids with early neurodevelopment.[31–34] The n-6:n-3 ratio intake during pregnancy showed negative associations with language, psychomotor, cognitive, and social development at early ages.[31,32] A higher ratio measured in the maternal serum during pregnancy was related to slower psychomotor development at 9 months of age.[33] The n-6:n-3 ratio in breast milk and in plasma phospholipids at 44 weeks of gestational age was negatively associated with mental and motor development in premature infants.[34]

Both series of omega acids have important physiological functions in the brain; n-3 modulates the synthesis, transport, and release of neurotransmitters,[8] and n-6 is involved in signal activation and reception.[2] When n-6 ingestion is higher than n-3 LCPUFA, the first one replaces the last in the neuron membrane, altering its function and promoting a proinflammatory state.[4] According to our findings, a high prenatal n-6:n-3 ratio concentration in the cord plasma is associated with ADHD symptoms during childhood and this association may be driven by low DHA concentrations. The high ratio during development could be related to the appearance of ADHD symptoms through early fetal programming. The nutrient supply during early stages of life programs the structure and the function of the organs, which has an impact on the individual's health.[35] The brain is particularly vulnerable to misprograming owing to its long period of development.[36,37] These alterations could lead to neurodevelopmental disorders, such as ADHD, because monoaminergic systems have been found to be affected by LCPUFA status.[38] It has been demonstrated in rodents that decreased

concentrations of DHA during development alter the neurobiological pathways, which has long-term negative consequences on behavior.[39] A recent study in humans showed an early association of LCPUFA-related genotypes with cognitive performance at school age after correction for current DHA blood concentrations, demonstrating the programming effect of these nutrients on the brain development.[40] Nevertheless, the use of LCPUFA supplements during pregnancy for preventing behavioral problems is not supported by clinical trials.[41–44] We also observed a positive association between EPA and ADHD symptoms at 4 years of age. A similar result was reported in a German birth cohort study that observed that higher EPA concentrations in cord blood serum were associated with more conduct problems at 10 years old.[45]

In the present study, we assessed ADHD symptoms during 2 different periods, at 4 and 7 years of age, but we only observed associations with the n-6:n-3 ratio in the second period. This finding could be explained by the measurement error at early ages, because the ADHD symptoms reported could be originated by a delay in neurodevelopment within normality.[46] Indeed, ADHD symptom phenotype is usually detected at school age.[10] Furthermore, the different instruments and informants used at 4 and 7 years of age could also explain the observed differences, because agreement between parents and teachers regarding ADHD symptoms is usually relatively poor.[47] Our main findings remained significant after adjusting the models for key confounders selected by directed acyclic graph models, and for other variables, identified in the literature, that could modify the associations as well. Such associations were not explained by maternal mental health, IQ proxy, or other LCPUFA-related nutrients obtained from diet during pregnancy, or by child diet. This is probably explained by the fact that the LCPUFA ratio in cord plasma is a valid measurement of the LCPUFA internal concentration and the one that the fetus gets at the end of the pregnancy period. Furthermore, the LCPUFA ratio associations observed herein were partly driven by Asturias, where the fish consumption in mothers during pregnancy and in children was higher than in the rest of the cohort regions. These differential dietary habits, combined with the relatively low prevalence of ADHD symptoms, could explain the heterogeneity detected in the associations between cohort regions.

In this study, the prenatal n-6:n-3 ratio did not influence the risk of presenting ADHD symptom diagnostic criteria during childhood. However, we found significant associations with the continuous score of ADHD symptoms. Despite the relatively low and clinically irrelevant effect estimates obtained, these findings are important at the population level. If the whole population is exposed to a high n-6:n-3 ratio, the distribution for ADHD symptom scores would likely move to the right, and the prevalence of extreme values would increase substantially, which may have a negative impact on the community's health costs and productivity.[48]

This study presents some limitations. The measurement of LCPUFA in cord plasma did not reflect long-term dietary exposure to these components, as with adipose tissue or red blood cells. The missing data owing to the lack of cord plasma samples and loss to follow-up could have biased these results and led to low external validity. This limitation was minimized by applying IPW in all the analyses. Moreover, we measured ADHD symptoms of children indirectly, and the informants and instruments were different at each assessment period. This strategy could decrease the comparability of results between both age periods, limiting our ability of disentangling age from both instrument and informant influence on ADHD symptoms score.

This study encompasses several strengths. We used a large sample size from a population-based birth cohort in which different Spanish regions were represented, which guarantees a relative degree of generalization of the results to the general population. The longitudinal design of this study allowed us to discard the reverse causation that can affect cross-sectional studies. We collected the key LCPUFAs for brain development, namely, AA, DHA, and EPA, from cord plasma, instead of using a single determination collected from maternal biological samples or intake estimations. The use of a dimensional approach for the ADHD variables allowed us to study subtle subclinical dysfunction and increase statistical power. Finally, the models were adjusted for the main confounders identified in the literature after following directed acyclic graph models and other additional important variables that could influence the tested associations.

Our findings suggest that maternal diet during pregnancy may modulate the risk of developing long-term ADHD symptoms in the offspring. Future research should measure ADHD symptoms using the same rating scales across assessment periods to further understand the trajectory patterns of this complex outcome. Longer follow-up times are warranted to explore the stability of this long-term association until adolescence periods, and randomized trials are needed to explore the potential nutritional sources of such LCPUFA ratio to improve nutritional guidelines during pregnancy. ■

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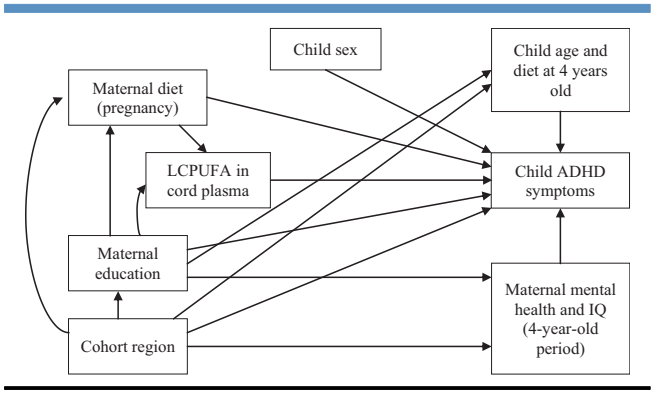

Figure 2. Directed acyclic graph.

Table I. Sociodemographic characteristics of the study participants* and nonparticipants at the 4-year-old period

Sociodemographic characteristics	Complete cases* (n = 580)	Incomplete cases (n = 2064)	*P* value†
Child age, years	4.85 (0.72)	4.94 (0.68)	<.001
Child female sex	48.28	48.57	.469
Region			<.001
Asturias	13.79	20.06	
Gipuzkoa	18.45	25.73	
Sabadell	43.79	19.53	
Valencia	23.97	34.69	
Birth weight, g	3296.39 (439.10)	3240.50 (494.71)	.022
Gestational age, weeks	39.73 (1.39)	39.54 (1.80)	.114
Preterm	2.97	5.19	.015
Any breastfeeding, weeks	27.29 (22.32)	23.80 (21.77)	<.001
Maternal age	31.74 (3.81)	31.27 (4.51)	.017
Maternal occupation			<.001
I/II managers/technicians	22.97	20.87	
III nonmanual	31.26	24.12	
IV/V manual	45.77	55.01	
Maternal education level			.013
Primary	20.69	26.27	
Secondary	42.24	41.22	
University	37.07	32.51	
Parity			.662
0	55.96	56.32	
1	36.27	36.96	
≥2	7.77	6.72	
Smoking at week 32 of pregnancy, cigarettes/day	0.77 (2.38)	1.19 (3.21)	.021
Maternal mental health‡	50.36 (10.37)	49.76 (9.71)	.472

Values are mean (SD) or percent.
*Number of children with cord blood LCPUFA, ADHD-DSM-IV, and potential confounders available.
†Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables.
‡Global Severity Index of the Symptom Checklist-90-R T score.

Table II. Sociodemographic characteristics of the study participants* and nonparticipants at the 7-year-old period

Sociodemographic characteristics	Complete cases* (n = 642)	Incomplete cases (n = 2002)	*P* value†
Child age in years	7.35 (0.63)	7.66 (0.60)	<.001
Child female sex	48.91	48.36	.423
Region			<.001
Asturias	11.37	21.03	
Gipuzkoa	22.12	24.78	
Sabadell	42.37	19.23	
Valencia	24.14	34.97	
Birth weight, g	3291.82 (423.34)	3240.21 (501.27)	.032
Gestational age, weeks	39.74 (1.39)	39.53 (1.81)	.084
Preterm	2.52	5.41	.001
Any breastfeeding, weeks	27.24 (21.73)	23.69 (21.96)	<.001
Maternal age	31.63 (3.89)	31.29 (4.52)	.104
Maternal occupation			<.001
I/II managers/technicians	24.49	20.31	
III nonmanual	30.58	24.12	
IV/V manual	44.93	55.57	
Maternal education level			.003
Primary	21.03	26.34	
Secondary	40.65	41.70	
University	38.32	31.96	
Parity			.551
0	56.01	56.32	
1	37.91	36.44	
≥2	6.08	7.24	
Smoking at week 32 of pregnancy, cigarettes/day	0.73 (2.36)	1.22 (3.24)	.002
Maternal mental health‡	49.97 (10.04)	49.95 (9.88)	.858

Values are mean (SD) or percent.
*Number of children with cord blood LCPUFA, Conners' Rating Scale-Revised, and potential confounders available.
†Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables.
‡Global Severity Index of the Symptom Checklist-90-R, T score.

Table V. Characteristics of the study participants according to cohort region at the 4-year-old period

	Asturias (n = 80)	Gipuzkoa (n = 107)	Sabadell (n = 254)	Valencia (n = 139)	*P* value*
Child age, years	4.77 (0.39)	4.40 (0.21)	4.44 (0.25)	5.99 (0.40)	<.001
Child female sex	43.75	49.53	51.18	44.60	.507
% AA among total fatty acids	13.26 (1.80)	17.58 (2.29)	13.69 (1.76)	13.51 (2.14)	<.001
% EPA among total fatty acids	0.30 (0.15)	0.33 (0.27)	0.19 (0.12)	0.22 (0.18)	<.001
% DHA among total fatty acids	4.85 (1.18)	6.90 (1.65)	4.42 (1.16)	4.60 (1.39)	<.001
n-6:n-3 ratio	2.73 (0.76)	2.58 (0.77)	3.15 (0.81)	3.01 (0.95)	<.001
ADHD symptom score†					
All	9.90 (9.94)	5.22 (7.04)	6.70 (7.84)	7.63 (9.07)	.001
Males	11.93 (10.56)	6.67 (8.47)	8.23 (9.05)	9.22 (9.82)	.014
Females	7.29 (8.51)	3.75 (4.86)	5.25 (6.16)	5.66 (7.66)	.182
ADHD diagnostic criteria‡					
All	16.25	4.67	4.72	17.00	.001
Males	22.22	7.41	7.26	18.18	.014
Females	8.57	1.89	2.31	4.84	.276
Maternal mental health§	50.15 (11.42)	50.35 (9.32)	50.29 (10.51)	50.61 (10.05)	.833
Maternal IQ¶	9.59 (3.09)	9.60 (2.97)	10.62 (2.89)	10.10 (3.11)	.013
Maternal education					<.001
Primary	11.25	13.08	22.05	29.50	
Secondary	40.00	37.38	43.70	44.60	
University	48.75	49.53	34.25	25.90	
Maternal fish intake**	83.64 (52.52)	79.94 (34.13)	64.11 (33.67)	65.36 (37.22)	<.001
Child fish intake††	39.96 (20.37)	34.71 (14.49)	36.78 (17.22)	31.09 (16.90)	.001
Maternal Mediterranean diet‡‡	8.08 (2.36)	9.25 (2.62)	7.84 (2.40)	7.32 (2.35)	<.001

Values are mean (SD) or percent unless otherwise noted.
*Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.
†ADHD-DSM-IV symptom score (teachers).
‡Six or more symptoms.
§Global Severity Index of the Symptom Checklist-90-R, T score.
¶Similarities subtest of the Wechsler Adult Intelligence, 3rd edition (mean, 10; SD, 3).
**Food Frequency Questionnaire, grams per day, between weeks 12 and 32 of pregnancy.
††Food Frequency Questionnaire, grams per day, at 4 years old.
‡‡Relative Mediterranean Diet Score, constructed with Food Frequency Questionnaire data considering the consumption of vegetables, fruits and nuts, cereals, legumes, fish, olive oil, meat, and dairy products.

Table VI. Characteristics of the study participants according to cohort region at the 7-year-old period

	Asturias (n = 73)	Gipuzkoa (n = 142)	Sabadell (n = 272)	Valencia (n = 155)	*P* value*
Child age, years	8.24 (0.33)	7.76 (0.13)	6.73 (0.37)	7.64 (0.19)	<.001
Child female sex	42.47	49.30	51.47	47.10	.547
% AA among total fatty acids	13.34 (1.94)	17.67 (2.21)	13.67 (1.87)	13.54 (2.08)	<.001
% EPA among total fatty acids	0.29 (0.12)	0.32 (0.27)	0.19 (0.13)	0.23 (0.18)	<.001
% DHA among total fatty acids	4.82 (1.18)	6.77 (1.68)	4.44 (1.14)	4.68 (1.44)	<.001
n-6:n-3 ratio	2.76 (0.74)	2.65 (0.75)	3.13 (0.81)	2.96 (0.91)	<.001
ADHD symptom score†					
All	7.40 (6.64)	7.28 (7.46)	7.96 (7.06)	9.43 (7.12)	.003
Males	7.95 (6.42)	8.51 (8.68)	9.50 (7.51)	11.47 (7.51)	.003
Females	6.65 (6.96)	6.01 (5.74)	6.51 (6.29)	7.14 (5.90)	.482
ADHD diagnostic criteria‡					
All	5.48	9.15	8.82	10.32	.692
Males	4.76	11.11	9.09	10.98	.670
Females	6.45	7.14	8.57	9.59	.933
Maternal mental health§	49.40 (10.77)	49.24 (9.01)	50.33 (10.55)	50.01 (9.37)	.721
Maternal IQ¶	9.64 (3.23)	9.85 (2.86)	10.69 (2.94)	10.16 (3.29)	.019
Maternal education					<.001
Primary	13.70	10.56	22.06	32.26	
Secondary	35.62	35.21	44.49	41.29	
University	50.68	54.23	33.46	26.45	
Maternal fish intake**	80.53 (51.17)	78.75 (29.85)	66.61 (40.16)	65.03 (40.43)	<.001
Child fish intake††	39.54 (20.81)	34.57 (14.62)	37.48 (17.30)	31.68 (17.14)	.001
Maternal Mediterranean diet‡‡	7.93 (2.35)	9.24 (2.64)	7.83 (2.33)	7.30 (2.27)	<.001

Values are mean (SD) or percent unless otherwise noted.
*Kruskal-Wallis test for continuous variables and Fisher exact test for categorical variables.
†Conners' Rating Scale-Revised (short form) (parents).
‡T score of >66.
§Global Severity Index of the Symptom Checklist-90-R, T score.
¶Similarities subtest of the Wechsler Adult Intelligence, 3rd edition, (mean, 10; SD, 3).
**Food Frequency Questionnaire, grams per day, between weeks 12 and 32 of pregnancy.
††Food Frequency Questionnaire, grams per day, at 4 years old.
‡‡Relative Mediterranean Diet Score, constructed with Food Frequency Questionnaire data considering the consumption of vegetables, fruits and nuts, cereals, legumes, fish, olive oil, meat, and dairy products.

Table VIII. Adjusted associations* between the n-6:n-3 ratio in cord plasma and ADHD symptoms without applying IPW

Subscale (range)	Estimate	95% CI
4-year-old period†		
ADHD diagnostic criteria (≥6 symptoms)	1.13	0.78-1.66
ADHD (0-52)	1.05	0.94-1.17
Inattention (0-27)	1.04	0.92-1.17
Hyperactivity-impulsivity (0-27)	1.06	0.93-1.22
7-year-old period‡		
ADHD diagnostic criteria (T score >66)	1.27	0.91-1.76
ADHD (0-36)	1.09	1.00-1.18
Oppositional (0-18)	1.02	0.94-1.12
Cognitive problems/inattention (0-18)	1.09	0.99-1.21
Hyperactivity (0-18)	1.09	0.99-1.19

*The IRR is estimated by zero-inflated negative binomial regression models for the continuous outcomes and the OR is estimated by logistic regression models for the dichotomous outcome. Models were adjusted for maternal education, child sex, child age, and region.
†ADHD-DSM-IV symptom score (teachers).
‡Conners' Rating Scale-Revised (short form) (parents).

Table IX. Associations* between the n-6:n-3 ratio in cord plasma and ADHD symptoms adjusted for additional variables

	Maternal mental health and IQ†		Maternal fish intake‡		Child fish intake§		Maternal and child fish intake		Maternal mediterranean diet¶	
Subscales (range)	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**Estimate**	**95% CI**
4-year-old period**										
ADHD diagnostic criteria (≥6 symptoms)	1.12	0.71-1.76	1.15	0.74-1.79	1.23	0.80-1.89	1.27	0.81-2.00	1.12	0.72-1.74
ADHD (0-52)	1.08	0.94-1.24	1.08	0.95-1.23	1.09	0.97-1.23	1.11	0.98-1.27	1.07	0.94-1.21
Inattention (0-27)	1.12	0.99-1.26	1.08	0.96-1.22	1.06	0.94-1.21	1.09	0.96-1.24	1.07	0.95-1.21
Hyperactivity-impulsivity (0-27)	1.06	0.89-1.25	1.09	0.92-1.28	1.12	0.96-1.30	1.14	0.98-1.34	1.07	0.91-1.26
7-year-old period††										
ADHD diagnostic criteria (T score >66)	1.14	0.78-1.65	1.40	0.97-2.02	1.37	0.96-1.97	1.35	0.93-1.97	1.42	0.98-2.04
ADHD (0-36)	1.09	1.00-1.20	1.11	1.02-1.21	1.13	1.03-1.24	1.12	1.02-1.23	1.12	1.02-1.23
Oppositional (0-18)	1.00	0.91-1.11	1.04	0.95-1.15	1.06	0.96-1.18	1.07	0.96-1.18	1.04	0.95-1.14
Cognitive problems/inattention (0-18)	1.09	0.98-1.22	1.12	1.01-1.24	1.12	1.01-1.26	1.12	1.01-1.25	1.13	1.02-1.25
Hyperactivity (0-18)	1.08	0.97-1.20	1.12	1.01-1.24	1.13	1.01-1.26	1.13	1.01-1.26	1.12	1.01-1.24

*The IRR is estimated by zero-inflated negative binomial regression models for the continuous outcomes and the OR is estimated by logistic regression models for the dichotomous outcome. We applied IPW in all models. Models were also adjusted for maternal education, child sex, child age, and region.
†Global Severity Index of the Symptom Checklist-90-R, T score, and Similarities subtest of the Wechsler Adult Intelligence, 3rd edition; mean, 10 and SD, 3, at the 4-year-old wave.
‡Food Frequency Questionnaire, grams per day, between weeks 12 and 32 of pregnancy.
§Food Frequency Questionnaire, grams per day, at 4 years old.
¶Relative Mediterranean Diet Score, constructed with Food Frequency Questionnaire data considering the consumption of vegetables, fruits and nuts, cereals, legumes, fish, olive oil, meat, and dairy products.
**ADHD-DSM-IV symptom score (teachers).
††Conners' Rating Scale-Revised (short form) (parents).

Table X. Adjusted associations* between each LCPUFA (AA, DHA, and EPA) in cord plasma and ADHD symptoms

	AA, C20:4n-6		DHA, C22:6n-3		EPA, C20:5n-3	
Subscales (range)	**Estimate**	**95% CI**	**Estimate**	**95% CI**	**Estimate**	**95% CI**
4-year-old period†						
ADHD diagnostic criteria (≥6 symptoms)	0.97	0.81-1.16	0.92	0.68-1.24	5.72	1.92-17.03
ADHD (0-52)	0.99	0.94-1.04	0.96	0.89-1.04	1.59	1.06-2.38
Inattention (0-27)	0.98	0.93-1.03	0.94	0.88-1.02	1.25	0.83-1.88
Hyperactivity-impulsivity (0-27)	1.00	0.94-1.06	0.95	0.86-1.05	1.75	1.04-2.96
7-year-old period‡						
ADHD diagnostic criteria (T score >66)	1.04	0.91-1.20	0.75	0.57-0.99	2.88	0.78-10.66
ADHD (0-36)	1.02	0.98-1.05	0.93	0.89-0.98	1.31	0.98-1.77
Oppositional (0-18)	1.01	0.98-1.05	0.99	0.93-1.05	1.37	1.00-1.88
Cognitive problems/inattention (0-18)	1.01	0.96-1.05	0.92	0.87-0.98	1.30	0.89-1.92
Hyperactivity (0-18)	1.03	0.99-1.08	0.95	0.89-1.01	1.25	0.89-1.75

*The IRR is estimated by zero-inflated negative binomial regression models for the continuous outcomes and the OR is estimated by logistic regression models for the dichotomous outcome. We applied IPW in all models. Models were adjusted for maternal education, child sex, child age, and region.
†ADHD-DSM-IV symptom score (teachers).
‡Conners' Rating Scale-Revised (short form) (parents).