Effect of fatty acid status in cord blood serum on children's behavioral difficulties at 10 y of age: results from the LISAplus Study[1–4]

Gabriele Kohlboeck, Claudia Glaser, Carla Tiesler, Hans Demmelmair, Marie Standl, Marcel Romanos, Berthold Koletzko, Irina Lehmann, and Joachim Heinrich for the LISAplus Study Group

ABSTRACT

Background: Little is known about the effect of fatty acid (FA) concentrations in cord blood on long-term behavioral outcomes.

Objective: We assessed the effect of FAs in cord blood serum on children's behavioral difficulties at the age of 10 y.

Design: A longitudinal study of 416 children from the population-based Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood (LISAplus) birth cohort from Munich was conducted. Individual glycerophospholipid FAs in blood were analyzed in venous cord blood. Data on children's behavior were collected with a parent-reported Strength and Difficulties Questionnaire at 10 y of age. Zero-inflated Poisson regression models were applied and adjusted for sex, parental income, smoking during pregnancy, and dietary intake of arachidonic acid (AA) and DHA at 10 y.

Results: A 1% increase in DHA in cord blood serum was found to decrease total difficulties by $(\exp)\beta_{adj} = 0.93$ (SE = 0.02, $P < 0.0001$) and hyperactivity or inattention by $(\exp)\beta_{adj} = 0.94$ (SE = 0.03, $P < 0.04$). Higher long-chain (LC) PUFA concentrations in cord blood serum were associated with fewer emotional symptoms [$(\exp)\beta_{adj} = 0.95$, SE = 0.03, $P = 0.01$], and similarly higher AA concentrations were associated with fewer emotional symptoms [$(\exp)\beta_{adj} = 0.94$, SE = 0.03, $P = 0.03$].

Conclusion: Increased concentrations of DHA, LC-PUFAs, and AA in cord blood serum were associated with lower scores on a parent-completed behavioral screen. An appropriate FA supply to the developing fetus may be essential for optimal long-term behavioral outcomes in children. *Am J Clin Nutr* 2011;94:1592–9.

INTRODUCTION

Some evidence indicates that the supply of LC[5]-PUFAs, DHA (22:6n−3), and AA (20:4n−6) to the developing fetus influences neurodevelopment. LC-PUFAs are biochemically involved in the development of the brain and neuronal structures and are also involved in numerous neuronal processes, ranging from effects on membrane fluidity, signal transduction, to gene expression regulation (1). It is especially important during the last trimester of pregnancy that the fetus receives adequate amounts of DHA, because 70% of brain cells are developing at this time (2). Maternal intake of FA during pregnancy is a major determinant of the FA status of infants at birth (3, 4), and DHA and AA concentrations in neonatal plasma phospholipids of cord blood are related to the maternal dietary lipid supply (5). It has been suggested that interindividual differences in LC-PUFA concentrations in cord blood may serve as a proxy of DHA and AA status of the brain and may be associated with differences in cognitive development (6). Low LC-PUFA concentrations in cord blood were related to preterm delivery and low birth weight (7), small head circumference (8), impaired attention at 12 and 18 mo (9, 10), lower neurologic optimality scores at 18 mo (11), and child's eye and hand coordination at 2.5 y (12). Furthermore, low maternal fish intake during pregnancy, a major source of DHA, has been related to poor cognitive development in infancy (13, 14), at the age of 3 y (15), and during school age (16, 17) and to reduced stereoacuity at 3.5 y (18). To date, little is known about the effect of prenatal FA status on later mental health. Evidence from 2 longitudinal studies investigating maternal fish intake during pregnancy, assessed by a food-frequency questionnaire and behavioral outcomes, were inconsistent. One study found no relation between fish intake and behavioral outcome at the age of 8 y (16), whereas a second study reported that children whose mothers had eaten oily fish in early pregnancy had

[1] From Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Institute of Epidemiology I, Neuherberg, Germany (GK, CT, MS, and JH); the University of Munich Medical Center, Dr von Hauner Children's Hospital, Division of Metabolic and Nutritional Medicine, Munich, Germany (CG, CT, HD, and BK); the Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital Munich, Munich, Germany (MR); and the UFZ–Center for Environmental Research, Leipzig, Germany (IL).

[2] This article does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area.

[3] Supported by grants 01 EG 9732, 01 EG 9705/2, and 01 EE 9401-4 from the Federal Ministry for Education, Science, Research, and Technology; FKZ 20462296 from the Federal Ministry of Environment; Ludwig-Maximilians-Universities' innovative research priority Project MC-Health; the Commission of the European Communities; specific RTD Program NUTRIMENTHE (FP7-212652). BK is the recipient of a Freedom to Discover Award of the Bristol-Myers-Squibb Foundation, New York, NY.

[4] Address correspondence to G Kohlboeck, Helmholtz Zentrum München, Institut für Epidemiologie, Ingolstaedter Landstr. 1, D-85764 Neuherberg. E-mail: gabriele.kohlboeck@helmholtz-muenchen.de.

[5] Abbreviations used: AA, arachidonic acid; ADHD, attention-deficit hyperactivity disorder; ALA, α-linolenic acid; DPA, docosapentaenoic acid; FA, fatty acid; FAMEs, fatty acid methyl esters; LA, linoleic acid; LC, long chain; LISAplus, Influences of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood; SDQ, Strength and Difficulties Questionnaire; ZIP, zero-inflated Poisson.

Received March 15, 2011. Accepted for publication September 9, 2011.

First published online November 9, 2011; doi: 10.3945/ajcn.111.015800.

a reduced risk of hyperactivity at age 9 y compared with those whose mothers did not eat oily fish (19). To our knowledge, only one longitudinal study has investigated cord blood DHA and AA status and mental health at school age (20). In this small interventional study, higher DHA was associated with lower levels of internalizing problems at age 7 y in infants fed with artificial formula, but not in infants fed with human milk. DHA was not significantly associated with externalizing problem behavior. In the current study, the effect of the glycerophospholipid FA composition of cord blood serum on behavior difficulties at 10 y of age was investigated in a population-based birth cohort followed over 10 y.

SUBJECTS AND METHODS

Study population

The LISAplus Study is a population-based birth cohort study investigating the influences of lifestyle-related factors on the immune system and the development of allergies in childhood. Details on the study design are described elsewhere (21, 22). In brief, between November 1997 and January 1999, a total of 3097 newborns were recruited in the 4 German cities Munich, Leipzig, Wesel, and Bad Honnef. Questionnaires on parental social background and lifestyle variables, such as smoking and drinking alcohol, were completed by the parents shortly after delivery. Data on the child's health were collected by repeated parent-completed questionnaires at regular time intervals during the first 10 y (at 0.5, 1, 1.5, 2, 4, 6, and 10 y of age). Fatty acids were measured in cord blood serum only from Munich children. Of 1467 newborns from Munich, 883 children participated in the 10-y follow-up, and 416 parents consented to the assessment of umbilical fatty acid status in their children. Complete data on venous cord blood and from questionnaires were available for 416 participants [221 males (53.1%), 195 females (46.9%)]. Approval from the local Ethics Committees (Bavarian General Medical Council) and written consent from the participant's parents or guardians were obtained.

Measurement of behavioral problems

The SDQ is a brief behavioral screening questionnaire that can be filled out by parents and teachers or self-reported by children aged ≥11 y (23). The German version of the SDQ has been found to be a valid and reliable screening instrument (24). The SDQ comprises 25 items on psychological attributes divided into 5 subscales: emotional symptoms, conduct problems, hyperactivity/inattention, peer relation problems, and prosocial behavior. Each item is reported as 0 = "not true," 1 = "somewhat true," and 2 = "certainly true." The sum of 4 of the 5 subscale scores (the prosocial scale is excluded) was used to calculate a total difficulties score (range: 0–40). The subscale scores of the 4 difficulty dimensions range from 0 to 10, in which higher scores denote more problems. Each subscale and the total difficulties score can be categorized according to the standardization data of the German SDQ version (24) into 3 groups, indicating a "normal," "borderline" (may reflect clinically significant problems), or "abnormal" number of symptoms (substantial risk of clinically significant problems) (25). Cutoff scores for the borderline/abnormal range are ≥14 for total difficulties.

Fatty acid analyses in cord blood serum

In the LISAplus Study, serum fatty acids were analyzed in the autumn of 2010. Cord blood samples were collected in serum separator tubes. Samples were centrifuged, and serum was frozen in plastic vials and stored at −80°C until analyzed. The analysis of serum glycerophospholipid fatty acid composition was performed by a sensitive and precise high-throughput method described recently (26). In brief, 100 μL serum, 100 μL internal standard (1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine dissolved in methanol), and 0.6 mL methanol (precooled to 5°C) were combined in glass tubes and shaken for 30 s. Samples were centrifuged and the supernatant fluid containing phospholipids and nonesterified fatty acids were transferred into additional glass tubes. Triacylglycerols and cholesterol esters were discarded with the precipitated proteins. A total of 25 μL sodium methoxide solution (25% in methanol) was added, tubes were shaken, and base-catalyzed selective synthesis of FAMEs from glycerophospholipids proceeded at room temperature. The reaction was stopped after 3 min by adding 75 μL methanolic HCl (3 mol/L), and FAMEs were extracted twice into 300 μL hexane. Extracts were combined and dried under nitrogen flow at room temperature and taken up in 50 μL hexane (containing 2 g butylhydroxy-toluene/L) for gas chromatographic analysis.

Individual FAMEs were quantified by gas chromatographic with flame ionization detection and identified by comparison with authentic standards: GLC-569B (Nu-Check Prep Inc), *cis*-5,8,11-eicosatrienoic acid methyl ester (Sigma-Aldrich), methyl vaccenate (11*c*), methyl octadecatetraenoate (6*c*, 9*c*, 12*c*, and 15*c*), and methyl brassidate (13*t*) (Larodan Fine Chemicals AB). The response relative to pentadecanoic acid methyl ester (internal standard) was determined by using GLC-85 (Nu-Check Prep Inc) as an external standard. EZChrom Elite version 3.1.7 (Agilent) was used for peak integration. The results are expressed as percentage by weight of all glycerophospholipid FAs detected with a chain length between 14 and 24 carbon atoms. The following fatty acids were derived: total n−3 PUFAs, total n–6 PUFAs, n−3:n−6 ratio, LA (18:2n–6), ALA (18:3n–3), AA (20:4n–6), EPA (20:5n–3), docosapentaenoic acid (22:5n–3), DHA (22:6n–3), and LC-PUFAs (AA + DHA). The total n−6 PUFA value was calculated by summing the concentrations of LA and AA. The total n−3 PUFA value was calculated by summing the concentrations of ALA, 20:3n−3, EPA, DPA, and DHA.

Statistical analysis

Means and SDs were used to describe continuous variables. Wilcoxon's and chi-square tests were used to compare several variables between sexes; a 0.05 level of significance was chosen. The Kolmogorov-Smirnov test and normal probability plots were used to test for normal distribution of fatty acids.

We found a low frequency of higher values of the SDQ behavioral data reported by parents, which indicated that certain behavior problems are rarely shown, such as "easily distracted, concentration wanders" or "restless, overactive, cannot stay still for long." Logistic regression was considered the best option for analysis of the possible predictor variables because the dependent variable showed a skewed distribution, which rendered linear

regression models inappropriate. Because there was little variability in the SDQ subscales (eg, for the subscale "hyperactivity/inattention," about 25% of the sum scores were zero), it is difficult to obtain statistically or practically significant findings with them. The ZIP regression, as compared with logistic regression, is the most appropriate model for the dependent variable because of the overdispersion in the number of zero behavior problems (27). Therefore, we applied ZIP modeling to evaluate the effect of each fatty acid on the SDQ total difficulties scale and SDQ subscales. ZIP uses a Logit model with binomial assumption to determine whether an individual count outcome is from the always-zero or the not-always-zero group and a Poisson model for count data to model outcomes in the not-always-zero group (28). Results of ZIP are presented as (exp)β regression coefficients related to the log count model and their SEs and P values. Multiple testing was performed to reduce the type 1 error and to overcome the problem of false-positive association. The overall number of tests was 27; by applying the Bonferroni correction, a corrected 2-sided α level of 0.19% was applied.

To account for potential confounding factors that might be associated with behavior problems in children at age 10 y, the sociodemographic background of parents, risk factor variables during pregnancy, and actual dietary intake of fatty acids were selected on the basis of information from previous studies and were determined a priori. Potential confounders with high correlations with other confounders or lack of variance across the sample were excluded from analysis. Parental education was determined on the basis of questionnaire-derived information about school education according to the German educational system and was defined by the highest grade completed by either the mother or the father. Thus, children were assigned to a group of low (<10th grade), medium (10th grade), or high (>10th grade) parental education. Net household income per month was reported in the 10-y questionnaire by using a 9-point scale ranging from <€500 to >€3500. The calculation of equivalent income according to the Organization for Economic Cooperation and Development guidelines (29) was carried out by dividing the net household income by an equivalence factor, which gives a weight of 1.0 to the first adult, 0.5 to all other adults and children >14 y of age, and 0.3 to all children ≤14 y of age. Because income was measured categorically, we took the midpoint of each income class to calculate the income level. For the lowest income level (<€500), we calculated two-thirds (€333) of this limit and for the highest income level (>€3500) four-thirds (€4667), as described previously (30). Finally, the new variable was collapsed into 3 groups: low, medium, and high household incomes. To enable comparison of parental education with household income, a similar class size for both variables was needed. Therefore, the classification was based on quintiles so that the percentage of subjects in each group of household income equals the percentage of children in the respective group of parental education. Children's usual food and nutrient intakes over the past year were measured by an 82-item food-frequency questionnaire (31) administered to the parents to estimate fatty acid intake at 10 y of age. The consumption frequencies and portion size estimates were linked to the German Food Code and nutrient database (BLS) version II.3.1 (32). All computations were performed by using the statistical software package SAS for Windows (version 9.1; SAS Institute).

RESULTS

Subjects were assessed ~10 y after the baseline survey at birth. The mean (±SD) age was 10.06 ± 0.2 y (range: 9.8–11.1 y). Characteristics of the LISAplus Study sample are shown in **Table 1**. There were no sex-related differences in these variables.

The SDQ behavioral problem scores of males and females are shown in **Table 2**. Males had significantly higher total scores ($P < 0.001$), more conduct problems ($P < 0.01$), and higher hyperactivity/inattention ($P < 0.0001$) than did females. About 13% of the sample was identified with borderline total difficulties. An abnormal number of total difficulties indicating a likely case of a mental disorder was found in 5.3% of children.

The glycerophospholipid fatty acid composition of cord blood for males, females, and the total sample is shown in **Table 3**. Females had significantly higher concentrations of ALA ($P = 0.01$) and AA ($P = 0.04$) than did males.

Crude ZIP regression models showed that total n−3 PUFA, LC-PUFA, n−3:n−6 ratio, ALA, and DHA values in cord blood serum were significantly associated with parent-reported total SDQ scores in children at age 10 y. All of the n−3 PUFAs were inversely related with total difficulties score (**Table 4**). The parameter estimates for predicting the total difficulties score from DHA in cord blood serum showed that an increase in DHA resulted in the total difficulties score decreasing by (exp)β = 0.94 (SE = 0.02, $P < 0.001$). Thus, children with a 1% increase in DHA had a total difficulties score that was 0.94 lower than that of children with a 1% lower DHA. With regard to n−6 PUFAs, crude ZIP models showed that a 1% increase of LA

TABLE 1
Characteristics of the study population (n = 416) at the 10-y follow-up

	Value (n = 416)
	n (%)
Level of parental education[1]	
High	339 (82.1)
Medium	59 (14.3)
Low	15 (3.6)
Level of parental income[2]	
High	327 (78.6)
Medium	72 (17.3)
Low	17 (4.1)
Maternal smoking during second or third trimester of pregnancy[3]	
No	356 (90.1)
Yes	39 (9.9)
Maternal drinking of any alcohol during second or third trimester of pregnancy[4]	
No	171 (42.5)
Yes	231 (57.5)
Maternal social or psychological strain during pregnancy[5]	
No	394 (96.3)
Yes	15 (3.7)

[1] Educational status based on the highest completed grade in school and vocational training from either parent: 1, high (>10th grade); 2, medium (10th grade); 3, low (<10th grade).
[2] Parental income based on household equivalent income (30): 1, high; 2, medium; 3, low.
[3] Smoking during pregnancy defined as 0 = no, 1 = yes.
[4] Drinking any alcohol defined as 0 = no, 1 = yes.
[5] Social or psychological strain defined as 0 = no, 1 = yes.

TABLE 2
Behavior problems by sex, measured by parent-reported scores on the SDQ at the 10-y follow-up[1]

SDQ scale	Total (n = 416)	Males (n = 221)	Females (n = 195)	P value[2]
Total difficulties	7.03 ± 5.05	7.77 ± 5.14	6.19 ± 4.82	0.0008
Emotional problems	1.90 ± 1.97	1.87 ± 2.03	1.92 ± 1.91	0.3892
Conduct problems	1.57 ± 1.44	1.76 ± 1.42	1.37 ± 1.43	0.0012
Hyperactivity/inattention	2.57 ± 2.28	3.01 ± 2.37	2.08 ± 2.09	<0.0001
Peer relation problems	0.99 ± 1.45	1.14 ± 1.62	0.83 ± 1.21	0.1257

[1] All values are means ± SDs. SDQ, Strengths and Difficulties Questionnaire.
[2] Wilcoxon's test.

would increase total difficulties by (exp)β = 1.05 (SE = 0.02, $P < 0.01$), whereas a 1% increase of n−6 AA would decrease the total difficulties score by (exp)β = 0.98 (SE = 0.01, $P = 0.04$).

We adjusted for a variety of factors that could potentially confound the relation between FAs and mental health. These variables were chosen because previous studies found an association between them and mental health, and they could play a role in predicting behavioral problems (16, 20). Household equivalent income, smoking during the second or third trimester of pregnancy (yes or no), and dietary intake of AA and DHA at age 10 y were included as confounders. Parental education was excluded from the analysis because of high correlation with parental income (r = 0.6963).

The effect of LC-PUFAs, the n−3:n−6 ratio, and DHA on the total difficulties score remained significant after adjustment for confounding factors (sex, household equivalent income, maternal smoking during the second and third trimesters of pregnancy, and dietary intake of AA and DHA at 10 y). In the adjusted model, a 1% increase of DHA resulted in the total difficulties score decreasing by (exp)β = 0.93 (SE = 0.02, $P < 0.001$). LA in cord blood serum significantly predicted SDQ subscale emotional symptoms but did not remain significant after adjustment for confounders. In the adjusted model, a 1% increase of LC-PUFAs resulted in emotional symptoms decreasing by (exp)β = 0.95 (SE = 0.02, P = 0.01). Similarly, a 1% increase of AA resulted in emotional symptoms decreasing by (exp)β = 0.94 (SE = 0.03, P = 0.03).

In the adjusted models, a significant inverse relation between DHA and hyperactivity/inattention indicated that a 1% increase of DHA resulted in hyperactivity/inattention decreasing by (exp)β = 0.94 (SE = 0.03, P = 0.04). Conduct problems showed a significantly positive association (P = 0.025) between n−3 EPA in cord blood serum, whereas a 1% increase of EPA resulted in conduct problems scores increasing by (exp)β = 1.29 (data not shown in Table 4). For peer relation problems, a significantly positive association for ALA was found with a 1% increase of ALA and an increase in peer relation problems by (exp)β = 1.55 (P = 0.03) in the adjusted model (data not shown in Table 4).

DISCUSSION

The current study indicates that n−3 PUFA and n−6 PUFA availability at birth had an important influence on overall behavior difficulties at 10 y of age. Increases of glycerophospholipid DHA and AA concentrations in cord blood serum were associated with lower scores on a parent-reported behavioral screen at 10 y of age, whereas an increase of LA was related with higher scores. Higher concentrations of DHA reduced hyperactivity/inattention scores, higher LC-PUFA and AA concentrations reduced emotional symptoms scores, and higher concentrations of EPA were related with higher scores for conduct problems. These associations could not be explained by confounders, such as parental social background, maternal smoking during pregnancy, and DHA and AA intakes at 10 y of age.

Fatty acids in cord blood serum and overall behavioral difficulties

In our study, we found a strong association between n−3 PUFAs in cord blood, particularly DHA, and lower scores on overall behavior difficulties in children at 10 y of age. To date, there are no findings on total behavioral problems available from a study with a similar design. Our results are not in line with the results from a randomized controlled trial, which reported no

TABLE 3
Mean concentrations of FAs in cord blood serum[1]

FAs	Range	Total (n = 416)	Males (n = 221)	Females (n = 195)	P value[2]
Total n−6 PUFAs (% by wt)	18.52–30.78	26.10 ± 1.77	25.94 ± 1.85	26.23 ± 1.65	0.1978
Total n−3 PUFAs (% by wt)	4.55–13.07	8.12 ± 1.52	8.23 ± 1.49	7.99 ± 1.55	0.1623
Total n−3:total n−6 ratio	15.55–55.87	31.36 ± 6.72	31.94 ± 6.65	30.70 ± 6.75	0.0694
LC-PUFAs (% by wt)	15.03–30.71	25.19 ± 2.03	25.10 ± 2.12	25.29 ± 1.92	0.5755
n−6 LA (% by wt)	5.33–11.36	8.05 ± 1.11	8.07 ± 1.13	8.01 ± 1.09	0.5987
n−3 ALA (% by wt)	0–0.17	0.05 ± 0.03	0.06 ± 0.03	0.05 ± 0.03	0.0068
n−6 AA (% by wt)	11.22–21.81	18.03 ± 1.55	17.87 ± 1.68	18.22 ± 1.37	0.0392
n−3 EPA (% by wt)	0.09–0.84	0.30 ± 0.13	0.30 ± 0.14	0.30 ± 0.13	0.7183
n−3 DHA (% by wt)	3.81–11.44	7.15 ± 1.33	7.24 ± 1.31	7.06 ± 1.36	0.1781

[1] All values are means ± SDs. AA, arachidonic acid; ALA, α-linolenic acid; FAs, fatty acids; LA, linoleic acid; LC, long chain.
[2] Wilcoxon's test.

The American Journal of Clinical Nutrition

TABLE 4
Unadjusted and adjusted regression estimates for the association between FAs in cord blood serum as predictors for SDQ behavior problems at 10 y of age[1]

	Total difficulties score						Emotional symptoms						Hyperactivity/inattention					
Predictors	Crude (exp)β^2	SE	P^3	Adjusted (exp)β^4	SE	P^3	Crude (exp)β^2	SE	P^3	Adjusted (exp)β^4	SE	P^3	Crude (exp)β^2	SE	P^3	Adjusted (exp)β^4	SE	P^3
n−6 PUFAs	1.004	0.010	0.669	1.008	0.013	0.537	0.998	0.023	0.922	0.967	0.027	0.210	1.008	0.018	0.658	1.020	0.023	0.396
n−3 PUFAs	0.979	0.011	0.050	0.977	0.013	0.070	0.998	0.022	0.918	0.980	0.024	0.396	0.992	0.019	0.668	0.986	0.023	0.531
LC-PUFAs	0.964	0.009	0.000	0.958	0.011	0.001	0.968	0.020	0.092	0.946	0.022	0.010	0.984	0.015	0.288	0.981	0.018	0.266
Total n−3:total n−6 ratio	0.995	0.003	0.048	0.993	0.003	0.040	1.000	0.006	0.978	0.996	0.006	0.517	0.998	0.005	0.662	0.996	0.006	0.448
LA (18:2n−6)	1.050	0.017	0.003	1.068	0.020	0.001	1.065	0.032	0.047	1.046	0.044	0.316	1.017	0.029	0.571	1.034	0.035	0.354
ALA (18:3n−3)	1.141	0.055	0.017	1.129	0.066	0.066	1.031	0.115	0.785	1.052	0.130	0.695	1.113	0.097	0.272	1.064	0.114	0.586
AA (20:4n−6)	0.976	0.012	0.041	0.977	0.014	0.102	0.964	0.026	0.155	0.939	0.030	0.035	1.002	0.020	0.930	1.007	0.024	0.790
EPA (20:5n−3)	0.994	0.045	0.898	1.009	0.053	0.863	1.064	0.105	0.555	1.015	0.123	0.904	0.930	0.082	0.377	0.969	0.102	0.762
DHA (22:6n−3)	0.941	0.014	0.000	0.931	0.017	0.001	0.975	0.030	0.413	0.948	0.036	0.143	0.963	0.025	0.127	0.941	0.030	0.044

[1] AA, arachidonic acid; ALA, α-linolenic acid; FAs, fatty acids; LA, linoleic acid; LC, long chain; SDQ, Strengths and Difficulties Questionnaire.
[2] Regression estimate of the fatty acids for the respective outcome (SDQ sum scores) in a zero-inflated Poisson regression model.
[3] Corrected for multiple testing ($P = 0.0019$).
[4] Adjusted for sex, household income (Pearson correlation coefficient with total difficulties score = 0.21), maternal smoking during the second or third trimester of pregnancy (Pearson correlation coefficient with total difficulties score = 0.18), and dietary intakes of AA and DHA at 10 y of age.

significant associations between total behavior problems at age 2.5 y and EPA, DHA, and AA concentrations in cord blood erythrocytes after maternal fish-oil supplementation compared with placebo in pregnancy (12). Because these analyses were based on a small number of cases, sample size power may have been too small to detect differences between groups, n−3 PUFA deficiencies may have affected behavioral problems at a later age than 2.5 y, or that more subtle behavioral difficulties (eg, inattention), although detectable at 10 y of age, may not have been detectable at 2.5 y of age. However, our results also differed from those of 2 observational studies, which found no longitudinal associations between low maternal seafood consumption during pregnancy and total behavior problems at 7 y (16) and 9 y (19). Because these studies estimated prenatal DHA intake by frequency of maternal fish consumption, it is possible that DHA assessed by maternal fish intake at pregnancy reflects other properties than proxies for physiologic measures of DHA. A possible explanation for our observed association is that prenatal DHA concentrations in some children may have been inadequate to support the amount of DHA required for optimal brain development at this age. These prenatal DHA deficiencies may have created a state of vulnerability that contributed to the development of behavior problems, because other gene and environmental risk factors were also present (33, 34). Thus, for the age of 10 y, we suggest that low DHA concentrations in newborns are associated with higher scores on overall behavior difficulties at 10 y. Future longitudinal studies may help to test this hypothesis, and there is also a need to investigate whether prenatal DHA deficiencies are associated with higher scores on behavior difficulties in later childhood or adolescence.

Fatty acids in cord blood serum and emotional symptoms

Our results suggest a beneficial influence of LC-PUFA availability at birth on emotional symptoms scores at 10 y. To some degree, our findings are in line with those of Krabbendam et al (20), a previous longitudinal study that measured DHA and AA in cord blood and internalizing (emotional symptoms) and externalizing symptoms (conduct problems) at age 7 y. We found higher LC-PUFA (ie, the sum of n−3 DHA and n−6 AA) to be associated with lower scores of emotional symptoms, whereas Krabbendam et al found higher DHA concentrations alone to be associated with lower levels of depression at age 7 y. A stratified analysis of their data showed that the association with DHA was markedly present in the infants fed artificial formula, but was absent in the infants fed human milk. Because most of the participants (80%) in our study were breastfed, this finding would suggest that the association between DHA status at birth and emotional symptoms or internalizing is particularly expressed in infants who were fed artificial formula. However, both studies may not be entirely comparable, because we accounted for dietary intake as a potential confounder, whereas Krabbendam et al (20) used plasma concentrations of DHA and AA, which may also have led to differing results. Dietary intervention studies or observational studies on the frequency of fish consumption during pregnancy found no longitudinal relations with emotional symptoms at 2.5 y (12) and 9 y (19). A cross-sectional study investigating cheek cell PUFAs in typically developing children at age 8–10 y (35) found no association between n−3 PUFAs and parent ratings of emotional symptoms,

but found that teacher ratings of emotional symptoms were inversely related with total n−3 PUFAs. Studies are needed to clarify the role of DHA alone and in conjunction with other LC-PUFAs in emotional symptoms during childhood.

Fatty acids in cord blood serum and hyperactivity/inattention score

In the adjusted model, we found a significantly inverse relation with higher DHA concentrations at birth and less hyperactivity/inattention symptoms at 10 y of age. Thus far, only one study has investigated attention in relation to DHA status at birth (9). Children from mothers with higher concentrations of DHA showed accelerated developmental courses in attention across the first year. In line with the results from our study, an observational study investigating fish frequency consumption in pregnancy found that children whose mothers had eaten oily fish in early pregnancy had a lower risk of hyperactivity than did those whose mothers did not eat oily fish (19). However, our results differ from those of another observational study, which found that n−3 PUFAs in pregnancy were not associated with ADHD symptoms at 8 y of age once confounders were taken into consideration (16). Our results, however, are in line with those of several clinical studies that investigated associations between n−3 PUFA status and ADHD symptoms (36–40). Three trials also found no benefits of n−3 PUFA supplementation compared with placebo on ADHD symptoms according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria (41), parental behavioral rating scales (42), and dietary supplement compared with ADHD medication (43). Appleton et al (44) concluded in their review that evidence from clinical studies and trials on the role of n−3 PUFAs for ADHD symptoms is currently very limited and far from conclusive. Our results of a relation between DHA supply at birth and ADHD symptoms should be interpreted with caution, because more evidence of DHA deficiencies measured from dietary intake and cord blood on ADHD is clearly needed.

Fatty acids in cord blood serum and conduct problems

We found a significant association between n−3 EPA and conduct problems after control for potential confounders. In our study, high EPA concentrations did not decrease conduct problems; rather, the opposite tendency was found, ie, higher EPA concentrations were associated with more conduct problems. Our result differs from that of an observational study of a convenient sample of 96 boys, which found an association between low total n−3 PUFA status in blood and high parental ratings of conduct problems (39). However, our results for DHA agree with those of several studies that reported no longitudinal relation at age 7 between DHA in cord blood and conduct problems (20) and no association between maternal seafood intake and conduct problems (16, 45). Our findings are also in line with those of 2 studies of fish-oil supplementation, the findings of which indicate no significant effect on conduct problems in children at 2.5 y (12) and 9 y (19) of age. Waylen et al (45) suggest that associations between n−3 PUFA intake and childhood conduct problems appear to be strongly confounded with sociodemographic factors.

The strengths of our study, apart from its long-term follow-up until 10 y of age, are that it offers the possibility to relate the effect of DHA availability at birth with parent-rated behavioral problems scores in childhood. We measured fatty acids using a sensitive and precise method during a critical period of brain growth and investigated PUFA status at birth directly, independent of the influence of actual dietary intake of AA and DHA. Our results are based on the data of a population-based birth cohort in Munich. Aiming for a homogeneous study population, we included only healthy newborns of German nationality; subjects with low birth weight or preterm birth were excluded. We used a standardized and well-validated, brief behavioral screening questionnaire that has been especially developed for use in epidemiologic studies. We used continuous SDQ scales as the outcome of interest rather than dichotomized SDQ scales, which did not result in a loss of statistical power.

A limitation of our study was that we did not assess all children eligible in Munich. Only children whose parents consented to the assessment of umbilical fatty acid status took part in this study; thus, the results may be associated with potential bias. Because most parents in the LISAplus Study sample were relatively highly educated and we adjusted the ZIP models for household income, we likely eliminated group-specific drop-out due to different sociodemographic backgrounds of the parents. Bonferroni correction was considered as second exploratory analysis; therefore, the *P* values should be evaluated cautiously in light of multiple testing.

The long storage time of the FA probes (10 y) may have affected FA composition. Although studies indicate that the FA composition of plasma and erythrocyte lipids is stable for 4 y (46, 47), we cannot exclude an effect of long-storage exposure on FA composition.

Because this study was designed to explore a broad spectrum of lifestyle factors, environmental exposure and genetics on the immune system, we have no data on aspects of family management, family history of behavioral problems, and parental style that are likely to have an effect on mental health in children and might have an effect on our results. In addition, unlike previous studies on neurodevelopment (48), the effects of FAs on behavioral problems were not controlled for maternal intelligence quotient and the amount of stimulation in the home environment. A source of possible underreporting was that we had to rely on the information provided by the parents. It may be more meaningful to make use of a questionnaire completed by children and teachers and to confirm the status of children's behavioral problems. However, results on SDQ criterion validity showed that total difficulties reported by mothers could discriminate significantly between children attending psychiatric clinics and those attending a dental clinic (23, 49). Although SDQ scales were not specifically based on the *Diagnostic and Statistical Manual of Mental Disorders* or International Classification of Diseases diagnostic criteria, the convergent designation of scales as Conduct Problems and Hyperactivity implies links to the diagnostic categories of Conduct Disorder and ADHD (50).

Conclusions

Our study indicates that availability of fatty acids in newborns correlate with later scores on a parent-completed behavioral screen, independent of the family's socioeconomic status, adverse factors in pregnancy, or actual dietary intake. Perinatal DHA availability may play a critical role in the pathogenesis of a clinically significant behavior problem. Thus, adequate DHA

requirements for mothers during pregnancy may be important to the optimal long-term development of children.

We thank all the families who participated in the LISAplus Study. Furthermore, we thank all members of the LISAplus Study group for their excellent work. We also acknowledge Larissa Piniek-Tzimourtas (University of Munich Medical Center, Dr von Hauner Children's Hospital, Division of Metabolic and Nutritional Medicine, Munich, Germany) for her excellent contribution to the measurements of fatty acid composition. The LISAplus Study group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Munich (J Heinrich, HE Wichmann, S Sausenthaler, CM Chen, and M Schnappinger); Department of Pediatrics, Municipal Hospital "St. Georg," Leipzig (M Borte and U Diez); Marien-Hospital Wesel, Department of Pediatrics, Wesel (A von Berg, C Beckmann, and I Groß); Pediatric Practice, Bad Honnef (B Schaaf); Helmholtz Centre for Environmental Research–UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (I Lehmann, M Bauer, C Gräbsch, S Röder, and M Schilde); University of Leipzig, Institute of Hygiene and Environmental Medicine, Leipzig (O Herbarth, C Dick, and J Magnus); IUF–Institut für Umweltmedizinische Forschung, Düsseldorf (U Krämer, E Link, and C Cramer); Technical University Munich, Department of Pediatrics, Munich (CP Bauer and U Hoffmann); and ZAUM–Center for Allergy and Environment, Technical University, Munich (H Behrendt, J Grosch, and F Martin).

The authors' responsibilities were as follows—GK: analyzed the data and drafted the manuscript; CG and HD: measured the FAs and contributed to the interpretation of the results; CT, MS, MR, IL, and BK: contributed to the statistical analysis and interpretation of the data; and JH: designed the study and contributed to the writing and interpretation of the study. None of the authors declared a conflict of interest.

REFERENCES

1. Schuchardt JP, Huss M, Stauss-Grabo M, Hahn A. Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. Eur J Pediatr 2010;169:149–64.
2. Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n−3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:e39–44.
3. Al MD, Badart-Smook A, von Houwelingen AC, Hasaart TH, Hornstra G. Fat intake of women during normal pregnancy: relationship with maternal and neonatal essential fatty acid status. J Am Coll Nutr 1996; 15:49–55.
4. Matorras R, Perteagudo L, Sanjurjo P, Ruiz JI. Intake of long chain ω3 polyunsaturated fatty acids during pregnancy and the influence of levels in the mother on newborn levels. Eur J Obstet Gynecol Reprod Biol 1999;83:179–84.
5. Rum P, Hornstra G. The n−3 and n−6 polyunsaturated fatty acid composition of plasma phospholipids in pregnant women and their infants. relationship with maternal linoleic acid intake. Clin Chem Lab Med 2002;40:32–9.
6. Ghys A, Bakker E, Hornstra G, van den Hout M. Red blood cell and plasma phospholipid arachidonic and docosahexaenoic acid levels at birth and cognitive development at 4 years of age. Early Hum Dev 2002;69:83–90.
7. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. BMJ 2002;324:447.
8. Al MD, van Houwelingen AC, Badart-Smook A, Hasaart TH, Roumen FJ, Hornstra G. The essential fatty acid status of mother and child in pregnancy-induced hypertension: a prospective longitudinal study. Am J Obstet Gynecol 1995;172:1605–14.
9. Colombo J, Kannass KN, Shaddy DJ, Kundurthi S, Maikranz JM, Anderson CJ, Blaga OM, Carlson SE. Maternal DHA and the development of attention in infancy and toddlerhood. Child Dev 2004;75: 1254–67.
10. Kannass KN, Colombo J, Carlson SE. Maternal DHA levels and toddler free-play attention. Dev Neuropsychol 2009;34:159–74.
11. Bouwstra H, Dijck-Brouwer J, Decsi T, Boehm G, Boersma ER, Muskiet FA, Hadders-Algra M. Neurologic condition of healthy term infants at 18 months: positive association with venous umbilical DHA status and negative association with umbilical trans-fatty acids. Pediatr Res 2006;60:334–9.
12. Dunstan JA, Simmer K, Dixon G, Prescott SL. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 2008;93:F45–50.
13. Jacobson JL, Jacobson SW, Muckle G, Kaplan-Estrin M, Ayotte P, Dewailly E. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the Inuit of arctic Quebec. J Pediatr 2008; 152:356–64
14. Daniels JL, Longnecker MP, Rowland AS, Golding J. Fish intake during pregnancy and early cognitive development of offspring. Epidemiology 2004;15:394–402.
15. Oken E, Radesky JS, Wright RO, Bellinger DC, Amarasiriwardena CJ, Kleinman KP, Hu H, Gillman MW. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. Am J Epidemiol 2008;167:1171–81.
16. Hibbeln JR, Davis JM, Steer C, Emmett P, Rogers I, Williams C, Golding J. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578–85.
17. Boucher O, Burden MJ, Muckle G, Saint-Amour D, Ayotte P, Dewailly E, Nelson CA, Jacobson SW, Jacobson JL. Neurophysiologic and neurobehavioral evidence of beneficial effects of prenatal omega-3 fatty acid intake on memory function at school age. Am J Clin Nutr 2011;93:1025–37.
18. Williams C, Birch EE, Emmett PM, Northstone K. Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. Am J Clin Nutr 2001;73:316–22.
19. Gale CR, Robinson SM, Godfrey KM, Law CM, Schlotz W, O'Callaghan FJ. Oily fish intake during pregnancy–association with lower hyperactivity but not with higher full-scale IQ in offspring. J Child Psychol Psychiatry 2008;49:1061–8.
20. Krabbendam L, Bakker E, Hornstra G, van Os J. Relationship between DHA status at birth and child problem behaviour at 7 years of age. Prostaglandins Leukot Essent Fatty Acids 2007;76:29–34.
21. Schnabel E, Sausenthaler S, Schaaf B, Schafer T, Lehmann I, Behrendt H, Herbarth O, Borte M, Kramer U, von Berg A, et al. Prospective association between food sensitization and food allergy: results of the LISA birth cohort study. Clin Exp Allergy 2010;40:450–7.
22. Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I, Fahlbusch B, Bischof W, Weiss M, Borte M, Wichmann HE. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. Eur Respir J 2002;20:617–23.
23. Goodman R. The Strengths and Difficulties Questionnaire: a research note. J Child Psychol Psychiatry 1997;38:581–6.
24. Woerner W, Becker A, Rothenberger A. Normative data and scale properties of the German parent SDQ. Eur Child Adolesc Psychiatry 2004;13(suppl 2):II3–10.
25. Goodman R. Using the Strengths and Difficulties Questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. Br J Psychiatry 2000;177:534–9.
26. Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. J Lipid Res 2010;51:216–21.
27. Atkins DC, Gallop RJ. Rethinking how family researchers model infrequent outcomes: a tutorial on count regression and zero-inflated models. J Fam Psychol 2007;21:726–35.
28. Liu W, Cela J. Count data models in SAS. Statistics and data analysis 2008:1–12. Available from: http://www2.sas.com/proceedings/forum2008/371-2008.pdf (cited 21 February 2011)|.
29. Hauser R. Adequacy and poverty among the retired. In: Ageing Working Papers. AWP 3.2. Available from: http://www.oecd.org/dataoecd/22/7/2428615.pdf (cited 21 February 2011).
30. Sausenthaler S, Kompauer I, Mielck A, Borte M, Herbarth O, Schaaf B, von Berg A, Heinrich J. Impact of parental education and income inequality on children's food intake. Public Health Nutr 2007;10:24–33.
31. Stiegler P, Sausenthaler S, Buyken AE, Rzehak P, Czech D, Linseisen J, Kroke A, Gedrich K, Robertson C, Heinrich J. A new FFQ designed to measure the intake of fatty acids and antioxidants in children. Public Health Nutr 2010;13:38–46.

32. Dehne LI, Klemm C, Henseler G, Hermann-Kunz E. The German food code and nutrient data base (BLS II.2). Eur J Epidemiol 1999;15: 355–9.
33. Levant B. N-3 (omega-3) Fatty acids in postpartum depression: implications for prevention and treatment. Depress Res Treat 2011;2011: 467349.
34. Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 2006;7:583–90.
35. Kirby A, Woodward A, Jackson S, Wang Y, Crawford MA. Childrens' learning and behaviour and the association with cheek cell polyunsaturated fatty acid levels. Res Dev Disabil 2010;31:731–42.
36. Mitchell EA, Aman MG, Turbott SH, Manku M. Clinical characteristics and serum essential fatty acid levels in hyperactive children. Clin Pediatr (Phila) 1987;26:406–11.
37. Stevens LJ, Zentall SS, Deck JL, Abate ML, Watkins BA, Lipp SR, Burgess JR. Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. Am J Clin Nutr 1995;62:761–8.
38. Stevens LJ, Zhang W, Peck L, Kuczek T, Grevstad N, Mahon A, Zentall S, Arnold LE, Burgess JR. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 2003;38:1007–21.
39. Stevens LJ, Zentall SS, Abate ML, Kuczek T, Burgess JR. Omega-3 fatty acids in boys with behavior, learning, and health problems. Physiol Behav 1996;59:915–20.
40. Antalis CJ, Stevens LJ, Campbell M, Pazdro R, Ericson K, Burgess JR. Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. Prostaglandins Leukot Essent Fatty Acids 2006;75:299–308.
41. Hirayama S, Hamazaki T, Terasawa K. Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder–a placebo-controlled double-blind study. Eur J Clin Nutr 2004;58:467–73.
42. Voigt RG, Llorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC. A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. J Pediatr 2001;139:189–96.
43. Harding KL, Judah RD, Gant C. Outcome-based comparison of Ritalin versus food-supplement treated children with AD/HD. Altern Med Rev 2003;8:319–30.
44. Appleton KM, Rogers PJ, Ness AR. Is there a role for n−3 long-chain polyunsaturated fatty acids in the regulation of mood and behaviour? A review of the evidence to date from epidemiological studies, clinical studies and intervention trials. Nutr Res Rev 2008;21:13–41.
45. Waylen A, Ford T, Goodman R, Samara M, Wolke D. Can early intake of dietary omega-3 predict childhood externalizing behaviour? Acta Paediatr 2009;98:1805–8.
46. Hodson L, Skeaff CM, Wallace AJ, Arribas GLB. Stability of plasma and erythrocyte fatty acid composition during cold storage. Clin Chim Acta 2002;321:63–7.
47. Matthan NR, Ip B, Resteghini N, Ausman LM, Lichtenstein AH. Long-term fatty acid stability in human serum cholesteryl ester, triglyceride, and phospholipid fractions. J Lipid Res 2010;51:2826–32.
48. Jacobson SW, Chiodo LM, Jacobson JL. Breastfeeding effects on intelligence quotient in 4- and 11-year-old children. Pediatrics 1999;103: e71.
49. Goodman R, Scott S. Comparing the Strengths and Difficulties Questionnaire and the Child Behavior Checklist: is small beautiful? J Abnorm Child Psychol 1999;27:17–24.
50. Achenbach TM, Becker A, Dopfner M, Heiervang E, Roessner V, Steinhausen HC, Rothenberger A. Multicultural assessment of child and adolescent psychopathology with ASEBA and SDQ instruments: research findings, applications, and future directions. J Child Psychol Psychiatry 2008;49:251–75.