

ORIGINAL COMMUNICATION

Long-chain polyunsaturated fatty acids at birth and cognitive function at 7 y of age

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Objective: During the central nervous system (CNS) growth spurt, rapid accretion of long chain polyunsaturated fatty acids (LCPUFA) takes place. This particularly concerns docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6), which are thought to play important roles in CNS development and function. The aim of this study was to investigate the relationship between cognitive performance at 7 y of age and LCPUFA levels in umbilical venous plasma phospholipids, representing the prenatal fatty acid availability, and in plasma phospholipids sampled at 7 y.

Design: As part of a follow-up study, the cognitive performance of 306 children, born at term, was assessed at 7 y of age with the Kaufman Assessment Battery for Children. Backward stepwise regression analysis was used to study the relationship between the outcomes and LCPUFA status. Social class, maternal intelligence and parenting skills were included as covariables, among others.

Results: Results show no significant association with either DHA or AA at birth and the cognitive performance at 7 y of age. The LCPUFA levels at 7 y were not associated with these outcomes either. Consistent with the literature, significant relationships were found between cognitive outcome measures and maternal education, maternal intelligence and the child's birthweight.

Conclusions: In conclusion, our results do not provide evidence for a positive association between cognitive performance at 7 y and LCPUFA status at birth or at 7 y of age.

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Introduction

Two fatty acid families, the n-3 and the n-6 family, are essential in humans, because they are necessary as constituents of membrane phospholipids and cannot be synthesized by the human body. The parent fatty acids of these families, alpha-linolenic acid and linoleic acid, respectively, have, therefore, to be supplied by nutrition. Two of their long-chain, highly unsaturated derivatives, docosahexaenoic acid (DHA) and arachidonic acid (AA), account for up to 50% of the total fatty acids in the grey matter of the brain (O'Brien *et al*, 1964). DHA and AA accumulation mainly occurs during the last intrauterine trimester and continues in the first months after birth (Clandinin *et al*, 1980a, b; Martinez & Mougan, 1998).

Indications for the functional importance of these long-chain polyunsaturated fatty acids (LCPUFA) in central nervous system (CNS) development came from studies comparing cognitive functions of children fed either artificial formulas or human milk. Human milk generally contains DHA and AA, whereas standard artificial formulas do not contain these LCPUFA. This difference in fatty acid composition may contribute to the known differences in cognitive function between formula- and breastfed children (Anderson *et al*, 1999). This idea is supported by studies showing a lower brain DHA in infants fed artificial formulas as compared with human milk-fed infants (Farquharson, 1994; Farquharson *et al*, 1992, 1995; Makrides *et al*, 1994).

Some randomized supplementation studies, comparing term infants fed either LCPUFA-enriched formulas or standard artificial formulas, show beneficial effects of LCPUFA supplementation on visual or cognitive development (Agostoni *et al*, 1995; Birch *et al*, 1998, 2000; Carlson, 1996; Carlson *et al*, 1996; Makrides *et al*, 1995; Willatts *et al*, 1998a, b). Other studies, however, did not confirm this (Agostoni *et al*, 1997; Auestad *et al*, 1997, 2001; Lucas *et al*, 1999; Makrides *et al*, 2000). One study even found lower language developmental scores in infants receiving LCPUFA supplementation (Scott *et al*, 1998). In summary, results of these postnatal supplementation studies are not consistent.

Since the absolute accretion rates of the n-3 fatty acids are even greater in the prenatal period compared with the postnatal period (Clandinin *et al*, 1980a, b), it can be hypothesized that prenatal LCPUFA supply might also be important for later cognitive function. We, therefore, investigated the relationship between cognitive function and achievement at 7 y of age and the DHA and AA levels in umbilical venous plasma, representing prenatal LCPUFA availability. In addition, we studied the association between cognitive performance and LCPUFA status at 7 y, as a measure of LCPUFA supply in later life.

Methods

Subjects

The study described in this paper is part of a prospective follow-up study investigating the relationship between essential fatty acid status at birth and cognitive, visual and motor function at 7–8 y of age. The eligible study population consisted of 750 Caucasian children of 7 y old, born between December 1990 and January 1994 in the course of an earlier study on maternal and neonatal LCPUFA status and pregnancy outcome (Al *et al*, 1995). Of these children we traced 728 (97.1%), of which 3 (0.4%) were deceased, and 34 (4.5%) had emigrated. Eventually 691 (92.1%) children were invited to participate in this study. In spite of several letters and repeated phone-calls, we did not receive any response from the parents of 133 children. The parents of 231 children did not give consent. The main reasons were lack of time and the concern of the parents that this study would burden their child too much. Written informed consent was

obtained from 327 of the 691 invited families. During the study, 21 families dropped out, because they repeatedly did not show up for the appointments. Therefore, the ultimate follow-up study population consisted of 306 children. The study was approved by the Ethics Committee of the University Hospital Maastricht/Universiteit Maastricht.

Measurements of cognitive function and achievement

Cognitive function was assessed with the Kaufman Assessment Battery for Children (K-ABC; Kaufman & Kaufman, 1983), a standardized measure of intelligence and achievement. This multi-subtest battery consists of two scales, each measuring a different type of information processing: the 'Sequential processing' scale and the 'Simultaneous processing' scale. Together, these scales form the total score for cognitive performance: the 'Mental Processing Composite'. The K-ABC was administered in a quiet room with blinded windows by a single, well-trained tester (ECB), who was blind for the LCPUFA status of the children.

Analytical procedures and measurements

Fatty acid profiles of umbilical venous plasma phospholipids were determined as described by Al *et al* (1995). Like the umbilical plasma samples, the venous plasma samples taken at 7 y of age were stored at -80°C immediately after sampling until further analyses. The analysis of these samples was slightly different, using the lipid extraction method of Bligh and Dyer (1959) and a CP-sil 88 column (Chrompack[®], 50 m \times 0.25 mm, film thickness 0.2 μm) with 5.0 He as carrier gas (flow rate 0.7 ml/min). The injection temperature was 250 $^{\circ}\text{C}$ and the detection temperature 300 $^{\circ}\text{C}$. The starting temperature of the column was 160 $^{\circ}\text{C}$. After 10 min, the temperature increased up to 190 $^{\circ}\text{C}$ with a rate of 3.2 $^{\circ}\text{C}/\text{min}$ and finally to 230 $^{\circ}\text{C}$ with a rate of 5 $^{\circ}\text{C}/\text{min}$. The split ratio was 5:1. Fatty acid data are presented as relative levels (percentage of total fatty acids, wt/wt).

Measurements of covariables

The following factors are important determinants of intellectual development; therefore, they were included as covariables in the analyses: social class (Andersson *et al*, 1996), maternal intelligence (Bacharach & Baumeister, 1998; Jacobson, 1999), parenting skills (Andersson *et al*, 1996; Jacobson, 1999), maternal smoking (MacArthur *et al*, 1992) and drinking habits during pregnancy (Larroque *et al*, 1995), breastfeeding duration (Anderson *et al*, 1999) and the child's sex (Andersson *et al*, 1998), birth order (Rogan & Gladen, 1993) and birthweight (Richards *et al*, 2001). These data were collected by a questionnaire and an interview with the mother at follow-up, except for maternal smoking and drinking habits during pregnancy, and the child's sex, birth order and birthweight, which were already known. A three-level classification of maternal education was used as an

indicator for the variable social class, as has been done before in the Netherlands (Herngreen *et al*, 1993). In the statistical analyses two dummy variables (maternal education 'high' and maternal education 'low', with maternal education 'middle' as reference) for maternal education were used. Maternal intelligence was tested with Raven's Standard Progressive Matrices (Raven *et al*, 1996). Parenting skills were assessed using mothers' and fathers' self-report scales tapping the degree of attachment, responsiveness, affection, induction, conformity demands and punishment in the parent-child relation (Vermaes *et al*, 1999). Two parenting dimensions, derived using factor analysis on the six scales and averaged across mothers and fathers, were used in the statistical analyses: parental warmth (which included high scores on attachment, responsiveness, and affection) and parental restrictive control (which included high scores on conformity demands and punishment and moderate scores on induction).

Statistical analyses

All data are expressed as means (s.d.). The relation between cognitive function of the children at 7 y of age and their fatty acid status at birth and at 7 y was investigated with backward stepwise multiple linear regression analyses, thereby identifying other significant predictors as well. The scores on the K-ABC (total Mental Processing Composite Standard Score, Simultaneous processing score and Sequential processing

score) were the dependent variables and DHA and AA the (separate) independent variables. Social class (maternal education), maternal intelligence, parenting skills, maternal smoking and drinking habits during pregnancy, duration of breastfeeding, and the child's birth order, gestational age, birthweight, and gender were included as covariables in the initial model. In each step of the analyses and the least significant covariable was removed manually from the regression model (after checking confounding), except for the fatty acid variable, which was always retained. This procedure resulted in a final regression model containing only the fatty acid variable and the significant predicting variables. In the statistical analyses a *P*-value of <0.05 was considered significant, unless mentioned otherwise. The statistical analyses were performed using the statistical computer program StatView version 5.0 (SAS Institute Inc., Cary, NC, USA). No studies on this subject have been published before to base our sample size calculations on. However power calculations indicate that the power to detect a correlation coefficient of 0.20 at the significance level of 0.05 with our sample size of more than 300 children was 90%.

Results

Study population

The study population consisted of 306 children, 170 boys and 136 girls, with a mean age of 7.3 y (range 6.6–8.1 y). The

Table 1 Clinical characteristics of the study population

Variable ^a	Participants (n = 306)	Non-participants (n = 385)
<i>Parental characteristics</i>		
Maternal age at delivery (y)	29.8 (4.1)	28.9 (4.3)
Maternal weight (kg)	65.5 (11.2)	66.2 (10.9)
Maternal intelligence (correct answers on SPM ^b out of 60)	45.6 (7.7)	
Smoking during pregnancy (yes/no, %)	30/70	26/74
Alcohol use during pregnancy (yes/no, %)	23/77	20/80
Maternal education (high/middle/low, %)	60/36/4	57/33/10
Paternal education (high/middle/low, %)	61/36/3	52/27/21
Delivery (spontaneous vaginal/vacuum or forceps extraction/Caesarean section, %)	75/19/6	77/15/8
Parental warmth ^c	0.0 (0.84)	
Parental restrictive control ^c	0.0 (1.0)	
<i>Child characteristics</i>		
Age at measurement	7.3 (0.3)	
Gender (boys/girls, %)	56/44	62/38
Gestational age (weeks)	39.8 (1.6)	39.7 (1.5)
Birth order (first child/other, %)	66/34	73/27
Birth weight (g)	3304 (511)	3247 (518)
Birth length (cm)	49.9 (2.4)	49.8 (2.5)
Head circumference (cm)	34.3 (1.7)	34.2 (2.3)
Apgar score after 5 min	9.6 (0.9)	9.6 (0.8)
Infant feeding habits (human milk/formula, %)	47/53	
Duration of breastfeeding (months, n = 144)	4.6 (4.6)	
Umbilical plasma arachidonic acid (%)	16.6 (1.6)	16.9 (1.5)
Umbilical plasma docosahexaenoic acid (%)	6.1 (1.4)	6.2 (1.4)

^aValues are either mean (s.d.) or percentages of study population.

^bStandard Progressive Matrices of Raven (Raven *et al*, 1996).

^cResults of factor analyses, expressed in s.d. units.

neurological function, as measured with a standard neurological examination, performed in 262 of the children by the child neurologist (JSHV), was within the normal range. Table 1 shows the clinical characteristics (mean \pm s.d.) of both participating and non-participating children. There were no differences in baseline clinical characteristics between the participants and non-participants. However, a small difference in umbilical plasma AA percentages was found (16.6 vs 16.9% for the participants and the non-participants, respectively). We were permitted to take a venous blood sample at follow-up of 261 of the 306 children. The essential fatty acid status at birth (in umbilical plasma phospholipids) and at 7 y of age (in venous plasma phospholipids) are given in Table 2. At 7 y of age the fatty acid values were comparable to adult values.

Table 2 Fatty acid composition of phospholipids of umbilical plasma and of venous plasma at 7 y of age

Fatty acid ^a (%wt/wt of total FA)	Umbilical plasma PL (mean \pm s.d., n = 306)	Plasma PL at 7 y (mean \pm s.d., n = 261)
Total FA (mg/l)	592.4 \pm 120.7	1060.3 \pm 147.8
18:2n-6	7.7 \pm 1.3	23.2 \pm 2.3
20:4n-6	16.6 \pm 1.6	9.2 \pm 1.2
22:5n-6	0.83 \pm 0.27	0.32 \pm 0.08
Σ n-6 LCPUFA	23.4 \pm 1.5	12.9 \pm 1.3
18:3n-3	trace	0.19 \pm 0.07
20:5n-3	0.23 \pm 0.11	0.51 \pm 0.22
22:6n-3	6.1 \pm 1.4	2.8 \pm 0.7
Σ n-3 LCPUFA	6.8 \pm 1.6	4.2 \pm 0.9

^a18:2n-6 = linoleic acid; 20:4n-6 = arachidonic acid; 22:5n-6 = Osbond acid; Σ n-6 LCPUFA = sum of the n-6 long-chain polyunsaturate fatty acids (20:3, 20:4, 22:4, 22:5); 18:3n-3 = alpha-linolenic acid; 20:5n-3 = eicosapentaenoic acid; 22:6n-3 = docosahexaenoic acid; Σ n-3 LCPUFA = sum of the n-3 LCPUFA (20:5, 22:5, 22:6). PL = phospholipids; FA = fatty acids.

Table 3 Cognitive performance, according to quartiles of DHA and AA percentage at birth and at 7 y of age

Cognitive performance (Kaufman ABC)	Mental processing composite mean (s.d.)	Sequential processing mean (s.d.)	Simultaneous processing mean (s.d.)
All children (n = 304)	107.3 (11.9)	101.9 (12.3)	109.4 (11.8)
DHA (%) at birth			
DHA < P25	107.7 (14.0)	102.1 (14.5)	109.7 (13.1)
P25 < DHA < P50	108.2 (11.0)	102.1 (10.7)	110.7 (10.1)
P50 < DHA < P75	107.6 (11.3)	101.9 (11.2)	110.0 (12.2)
DHA > P75	105.5 (10.9)	101.5 (13.0)	107.2 (10.5)
AA (%) at birth			
AA < P25	107.8 (11.1)	101.3 (12.0)	110.7 (10.6)
P25 < AA < P50	108.9 (11.3)	104.8 (11.1)	109.9 (12.3)
P50 < AA < P75	106.7 (12.7)	101.1 (12.8)	109.1 (12.1)
AA > P75	105.5 (12.4)	100.3 (13.2)	108.0 (12.1)
DHA (%) at 7 y of age			
DHA < P25	107.2 (12.7)	101.0 (11.5)	109.9 (13.1)
P25 < DHA < P50	109.8 (10.4)	104.9 (11.3)	111.1 (10.4)
P50 < DHA < P75	105.3 (12.5)	99.2 (14.3)	108.5 (11.1)
DHA > P75	109.0 (11.7)	104.0 (12.1)	110.6 (11.9)
AA (%) at 7 y of age			
AA < P25	108.0 (11.7)	102.5 (11.4)	110.2 (12.2)
P25 < AA < P50	108.6 (11.2)	102.6 (11.6)	110.9 (11.2)
P50 < AA < P75	109.1 (13.6)	103.1 (15.3)	111.2 (12.1)
AA > P75	106.2 (11.0)	101.6 (11.9)	108.2 (10.8)

Cognitive function

Total scores on the Kaufman-Assessment Battery for Children, the Mental Processing Composite, were available for 304 children; two boys refused to co-operate. The cognitive outcomes of all children are shown in Table 3.

Backward stepwise multiple regression analyses for Mental Processing Composite, with either DHA or AA in umbilical plasma as independent variable (retained in the model in each step) and social class (maternal education), maternal intelligence, parenting skills, maternal smoking and drinking habits during pregnancy, duration of breastfeeding, and the child's birth order, gestational age, birthweight and gender as covariables in the initial model, resulted in a model with 'maternal education' and 'maternal intelligence' as the only significant (positive) predictors. In the model with DHA, 'birthweight' was a significant positive predictor as well. The (corrected) associations between DHA or AA and the cognitive outcomes were not significant (partial $P=0.29$ and 0.60 respectively, see Table 4 for regression coefficients).

For the subscale 'Simultaneous processing' comparable regression models were found after backward stepwise multiple regression analyses. Umbilical plasma DHA and AA did not correlate significantly with this outcome (partial $P=0.21$ for DHA, with 'maternal education', 'maternal intelligence' and 'birthweight' as significant covariables, and $P=0.41$ for AA, with 'maternal education' and 'maternal intelligence' as significant covariables).

The same analyses with the subscale 'Sequential processing' resulted in models with 'parental warmth' as significant covariable, in addition to the other significant predictors 'maternal education' and 'maternal intelligence'. Umbilical plasma DHA and AA, forced to remain in the model, were not related to Sequential processing at all (partial $P=0.90$ and 0.94, respectively).

Table 4 Regression coefficients for the relation between LCPUFA status and cognitive performance, corrected for covariables^a

		Mental processing composite		Sequential processing		Simultaneous processing	
		B (s.d.)	95% CI	B (s.d.)	95% CI	B (s.d.)	95% CI
Umbilical plasma	DHA	-0.517 (0.48)	-1.471-0.437	-0.072 (0.52)	-1.104-0.960	-0.610 (0.48)	-1.557-0.337
	AA	-0.223 (0.42)	-1.051-0.605	0.035 (0.47)	-0.886-0.956	-0.340 (0.41)	-1.156-0.476
Plasma at 7y	DHA	-0.156 (1.06)	-2.235-1.922	0.584 (1.17)	-1.713-2.880	-0.727 (1.03)	-2.766-1.312
	AA	-0.136 (0.58)	-1.284-1.011	0.356 (0.64)	-0.902-1.613	-0.210 (0.57)	-1.337-0.917

^aResults of backward stepwise multiple regression analyses; B = corrected regression coefficient, for either docosahexaenoic acid status (DHA) or arachidonic acid status (AA, %, wt/wt).

Comparable regression models, with the same significant predictors, were found for the two LCPUFA at 7y of age. Again, DHA and AA were not associated with the total score on the Kaufman Assessment Battery for Children (partial $P=0.88$ and 0.82 , respectively). Similar analyses with the subscales 'Simultaneous processing' and 'Sequential processing' also resulted in no significant associations between DHA and AA at 7y and these outcomes (partial $P=0.48$ and 0.71 , respectively for Simultaneous processing and 0.62 and 0.58 , respectively for Sequential processing).

Discussion

The main aim of this study was to investigate the relation between cognitive function at 7y of age and the DHA and AA levels in umbilical venous plasma phospholipids, representing the prenatal LCPUFA availability. For this purpose we measured the cognitive function of 304 children at 7y of age using the Kaufman Assessment Battery for Children. Because of the large number of outcome variables and, consequently, the large number of statistical analyses, the chance of a type I error is relatively large. This implies that a significant relation could have been observed by chance and should, therefore, be interpreted with care.

The results showed no relation between umbilical plasma DHA or AA and cognitive performance as measured by the Kaufman Assessment Battery for Children. The significant relationships between this cognitive outcome measure and maternal education, maternal intelligence and the child's birthweight illustrates that we were able to find the same significant results with our methods as described in the literature (Andersson *et al*, 1996; Bacharach & Baumeister, 1998; Richards *et al*, 2001).

As far as we know, publications about the relationships between prenatal LCPUFA availability (measured by proxy) and cognitive performance in childhood are not available yet. The influence of postnatal LCPUFA supply on cognitive function in term infants has been investigated in several supplementation studies (Agostoni *et al*, 1995, 1997; Auestad *et al*, 2001; Birch *et al*, 2000; Carlson, 1996; Lucas *et al*, 1999; Makrides *et al*, 2000; Scott *et al*, 1998; Willatts *et al*, 1998a, b). Some randomized controlled LCPUFA trials in term infants younger than 12 months show a positive influence of dietary

LCPUFA on cognitive function, as measured with different methods (Agostoni *et al*, 1995; Willatts *et al*, 1998a, b). At 12 months of age, an influence of dietary LCPUFA on cognitive function was not observed (Agostoni *et al*, 1996; Auestad *et al*, 2001; Carlson, 1996; Makrides *et al*, 2000; Scott *et al*, 1998). Only one study reported an influence of LCPUFA in infant nutrition on the Mental Development Index (MDI, Bayley Scales of Infant Development) at 18 months of age (Birch *et al*, 2000). Other studies at 6 or 12 months (Auestad *et al*, 2001), 18 months (Lucas *et al*, 1999) or 24 months of age (Agostoni *et al*, 1997; Makrides *et al*, 2000) did not find differences in cognitive function between dietary groups with or without LCPUFA. This summary of the literature suggests that the influence of dietary LCPUFA on cognitive function might be transient. Nonetheless, as stated by Lucas *et al* (1999), it is possible that other effects of early LCPUFA supply can be found in later life.

In long-term development, LCPUFA supply during later life can also play a role. Dietary LCPUFA may be associated to the LCPUFA concentrations in membrane phospholipids. Therefore, we also investigated the association between LCPUFA status at 7y, as a measure of LCPUFA supply in later life, and cognitive performance at this age. With respect to the scores on the Kaufman Assessment Battery for Children there was no significant contribution of DHA or AA at 7y of age. These results are in line with the findings of Birch *et al* (2000), reporting no correlation between DHA at 12 months and developmental outcomes at 18 months.

In conclusion, our results do not provide evidence for a positive association between cognitive performance at 7y and LCPUFA status at birth or at 7y of age. Since there are indications for a transient influence of dietary LCPUFA on cognitive function, there may be an association between prenatal LCPUFA availability and cognitive performance at an earlier age. Moreover, other domains of development, eg visual or motor development, may possibly be associated with prenatal LCPUFA availability.

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