ELSEVIER

Alpha-linolenic acid supplementation during human pregnancy does not effect cognitive functioning

R.H.M. de Groot[a,b,*], J. Adam[c,d], J. Jolles[d,e], Hornstra[b]

[a] Department of Human Biology, Maastricht University, The Netherlands
[b] Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, The Netherlands
[c] Department of Movement Sciences, Maastricht University, The Netherlands
[d] Brain and Behaviour Institute; Division of Cognitive Disorders, The Netherlands
[e] Department of Psychiatry and Neuropsychology, Maastricht University, The Netherlands

Abstract

Increasing evidence suggests a positive association between docosahexaenoic acid (DHA, 22:6n-3) and cognitive performance. In addition, pregnancy is associated with a reduction of the DHA status and cognitive deficits. In the current study, cognition was assessed in pregnant women receiving a margarine enriched with alpha-linolenic acid (ALA, 18:3n-3, the ultimate dietary precursor of DHA) and some linoleic acid (LA, 18:2n-6, to prevent a possible reduction in n-6 fatty acids). A control group received a margarine enriched with LA only.

ALA supplementation hardly affected the maternal DHA status and no significant differences were found in cognitive performance between the two groups. This indicates that ALA supplementation during pregnancy does not affect cognitive performance during and 32 weeks after gestation. At week 14 of pregnancy and 32 weeks after delivery, higher plasma DHA levels were associated with lower cognitive performance as indicated by longer reaction times on the finger precuing task (partial correlation coefficients 0.3705 and 0.4633, respectively, $P < 0.01$). Since this could imply an unexpected adverse association between DHA and certain aspects of cognitive functioning this certainly needs further investigation.

1. Introduction

Metabolites of the two essential fatty acids (EFAs) alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) are important constituents of all cell membranes, including those of the central nervous system. Especially the long-chain polyunsaturated fatty acids (LCPUFAs) docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA 20:4n-6) are present in high concentrations in the cell membranes of the central nervous system [1,2]. Recently, the vital role of the long-chain polyunsaturated derivative of ALA, DHA, for neural development has been recognized [3,4].

Several studies have shown that the EFA and LCPUFA status of pregnant women decreases during pregnancy [5,6]. During pregnancy, cognitive functioning decreases too [7,8]. These two observations suggest that there may be a link between LCPUFA status and cognitive functioning during pregnancy. This possibility is supported by evidence in children showing that the fatty acid status at birth may affect certain aspects of later brain function [9]. Both in premature [10–19] and term infants [20–27], dietary long chain polyunsaturated fatty acids, especially DHA, have been shown to improve some functional aspects of the cerebral cortex and retina. However, other studies failed to find an effect [28–32], especially in term infants. In addition, Yehuda et al. showed that supplementation of Alzheimer patients with linoleic acid (LA, 18:2n-6) and ALA resulted in improvements in short term memory and improved ability to navigate in the home [33]. This

---

*Corresponding author. Department of Psychiatry and Neuropsychology, Maastricht University, P.O. Box 616, Maastricht 6200MD, The Netherlands. Tel.: +31-433881038; fax: +31-433884092.

E-mail address: rhm.degroot@NP.unimaas.nl (R.H.M. de Groot).

might also suggest that EFA supplementation influence cognition.

As far as we know, there are no studies investigating the effect ALA, the ultimate dietary precursor of DHA, on cognition during human pregnancy. This was the goal of the present study. Hence, 56 pregnant women were randomly allocated to either a control or an experimental group. Both groups consumed a special margarine from week 14 of gestation until the end of pregnancy. Subjects in the experimental group received a margarine enriched with the parent EFA of DHA, ALA, whereas subjects in the control group received a control margarine. Margarines from both groups contained an extra amount of linoleic acid (LA, 18:2n-6), the dietary precursor of n-6 LCPUFAs, in order to prevent a possible decrement in n-6 LCPUFAs, especially AA [34], as this is also a predominant LCPUFA in brain tissue.

The key question was whether the experimental group would show a cognitive performance different from that in the control group. In addition, as DHA and AA are the two most prominent LCPUFAs in brain tissue, correlations between plasma DHA status and AA levels, and cognitive task performance were examined.

2. Material and methods

2.1. Design

The current study was part of a double blind intervention trial, investigating the effects of EFA supplementation on maternal and neonatal LCPUFA status and pregnancy outcome [35]. For this study, pregnant women were followed from week 14 of pregnancy through 32 weeks after delivery. Intervention involved the consumption of at least 25 g of a special margarine per day from week 14 of gestation on until the end of pregnancy. The women were randomly allocated either to the experimental (margarine with ALA + LA) or the control group (margarine with LA). The subjects were visited at their homes every 3 weeks to receive their margarines. The leftovers were collected in order to estimate margarine consumption. At weeks 14, 17, 29, and 36 of pregnancy, and 32 weeks postpartum, cognitive tests were administered. Blood for plasma fatty acid analyses was sampled at weeks 14, 26, and 36 of gestation, at partus, and at 32 weeks after delivery. Correlations between fatty acid levels and cognitive performance were calculated for weeks 14, 26/29, and 36 of pregnancy, and 32 weeks after delivery.

2.2. Study population

Subjects were recruited by midwives in the regions Maastricht, Heerlen and Sittard and by the Department of Obstetrics and Gynecology of hospitals in the same area: University Hospital Maastricht, Atrium Medical Centre in Heerlen and Maasland Hospital in Sittard. Inclusion criteria were a gestational age less than 14 weeks at study entry, and good general health. Exclusion criteria were diastolic blood pressure higher than 90 mmHg, multiple pregnancy, use of medication, use of (LC)PUFA rich supplements, origin other than Caucasian, or fish consumption higher than two times a week.

Each subject gave written consent to participate in the study, which was approved by the Medical Ethics Committees of the University Hospital Maastricht and Maasland Hospital Sittard.

2.3. Supplements

As described in detail before [35] the margarines provided by Unilever Research and Development, Vlaardingen, The Netherlands, contained 79.5% fat, while the remaining consisted of water (20%), vitamins (0.04%), flavor (0.04%), lecithin (0.03%), and BHT (0.12%). The experimental margarine provided 2.82 g ALA and 9.02 g LA per day with the requested consumption of 25 g per day as this margarine contained 14.2% ALA and 45.4% LA of total fatty acids. The margarine of the control group contained 0.17% ALA and 55.02% LA of total fatty acids, resulting in 0.03 g ALA and 10.94 g LA per 25 g margarine per day.

2.4. Cognitive test battery

Cognitive performance was measured, using different neuropsychological tests with proven sensitivity to assess cognitive functions [36–39], as recently described by de Groot et al. [7,8]. In the present study we used the following tests in five parallel versions, which were randomly allocated to the subjects.

2.4.1. The visual verbal word learning task (WLT)

This task evaluates the ability to acquire and retain new verbal information [40]. After presentation of 15 words, participants were asked to recall as many words as possible (repeated three times). Dependent variables were the mean number of words recalled over the first two trials (WLTtot) in order to measure encoding, and the numbers of words recalled after 20 min, the delayed recall (WLTdr), in order to measure retrieval.

2.4.2. The concept shifting test (CST)

The CST was used to measure the capability to shift between concepts [41]. This test consists of three subtasks: the subject had to cross out, in the proper order, 16 circles containing numbers, (1–16; subtask I), letters (A–P; subtask II), or both (1A–2B–3C, etc. subtask III). The variables of interest were the average times to complete subtasks I and II as a measure for

general information processing speed, and the time to complete subtask III as a measure for concept shifting ability.

2.4.3. The Stroop Color-word Interference Test

The Stroop Color-word Interference Test as introduced by Stroop et al. [42] is a test of selective visual attention and interference susceptibility. Subjects had to read aloud a series of colors presented in black, to name the colors of colored patches presented, and to mention the incongruously ink color the color names were printed in. Mean speed for subtasks I and II was used as a measure of information processing speed (Str I + II). Subtask III was used as a measure of color word interference susceptibility (Str III).

2.4.4. The letter-digit-substitution-test (LDST)

The LDST is a measure for coding, including processes such as visual perception, attention, and memory [43]. Subjects were instructed to copy digits in cells indexed by a letter. The letters referred to nine letter/digit combinations at the top of the form. The total number of digits correctly related to a letter was used as the dependent variable.

2.4.5. Finger precuing task

Selective attention was operationalized by means of this test, which involves the preparation and selection of 2 out of 4 possible responses. This task requires subjects to respond to spatial stimuli with discrete responses from index and middle finger from both hands. The subtraction score between the reaction time on the original four choices reaction time tasks (uncued condition) and any of the other two choices conditions is called the preparation benefit. Mean reaction time (RT(ms)) and mean preparation benefit (PB(ms)) were taken as measures of selective attention [44].

2.5. Fatty acid analyses

The fatty acid composition of the plasma phospholipids has been reported before by de Groot et al. [35].

For the present study it was decided to focus on plasma levels of DHA and AA(%wt/wt), which are the predominant LCPUFAs in brain tissue. In addition, Osbond acid (ObA, 22:5n-6), which is generally accepted as the deficiency marker for DHA received attention.

2.6. Questionnaires

At the start of the study (week 14), a questionnaire was filled out in both groups. This questionnaire included items about age, pre-pregnancy weight, height, smoking habits, supplement usage, and medical history, including former pregnancies and medical treatments. In addition, education was measured on an 8-point scale, ranging from primary education to higher vocational training and university [45]. Thirty-two weeks after delivery another questionnaire was filled out, which included items about course and duration of pregnancy and breastfeeding.

2.7. Statistics

Unpaired *t*-tests were used to determine whether the characteristics age, weight, height, and margarine consumption were different between the control and the experimental group. The Mann–Whitney *U* Test was used in order to test whether differences existed between the two groups in education levels, parity, and gravidity. To detect possible differences in alcohol consumption or smoking the Chi-square test was applied.

To investigate whether the supplement was effective in changing the fatty acid levels during pregnancy, repeated measures analyses of variance (ANOVA) was used. Differences in cognitive performance between the experimental group and the control group were analyzed by repeated measures ANOVA, too. Time during pregnancy (weeks 17, 29 and 36) was used as within-subject factor, and group (experimental group or control group) as between-subjects factor. In all analyses, corrections were made for possible differences in starting levels by including test performance on week 14 as a covariable. Education level was included as a covariable, too. The Greenhouse-Geisser corrected significance values were used to adjust the tests involving the within-subject factor heterogeneity of variance and covariances.

For both groups together, partial correlation coefficients were calculated between selected fatty acid levels as independent variables and cognition parameters as dependent variables at weeks 14, 26/29, and 36 of gestation and 32 weeks after delivery. Adjustments were made for level of education. In addition, outliers as indicated by high Cook's distance were deleted from the data set (3 subjects maximum per analysis).

The statistical package SPSS 10.0 was used to analyze the results. Because of multiple testing, *P*-values <0.01 were considered significant, unless otherwise indicated.

3. Results

3.1. Subjects

Only women from whom data were available at all time moments, both on the cognitive tests and the fatty acid analyses, were included in the present study. In total, 57 pregnant women were considered suitable for enrollment in the current study. However, 1 woman in the control group was removed from the data set, because she developed diabetes mellitus gravidae, from which it is known that it influences fatty acid status

[46–48]. Thus, finally 56 women participated in the study, 26 in the control group and 30 in the experimental group.

Table 1 shows the subjects' characteristics. No significant differences were observed between the two groups for any of these variables ($P > 0.05$). Average margarine consumption per day in the control and the experimental group was 27.0 ± 2.9 and 27.7 ± 3.4 g, respectively. Based upon the ALA and LA content of the respective margarines, the daily ALA and LA intakes from margarine in the control group was 0.03 and 11.8 g, respectively, whereas women in the experimental group consumed 3.12 g ALA and 9.99 g LA from the margarines supplied.

3.2. Effect of EFA supplementation on cognition

In Table 2 the results on the respective cognitive tasks are presented. As shown in Table 3, no statistically significant differences were found between the experimental and the control group in cognitive performance ($P > 0.01$). Both between-subjects effects as well as within-subject effects were not statistically significantly different for the two groups. Only for one cognitive test (i.e. the LDST) a trend was observed for an interaction between time and group, indicating better performance in the experimental group over time ($F = 3.199$, $P = 0.048$).

Table 1
Subject characteristics (mean $\pm$ SD)

Characteristics	Control group ($n = 26$)	Experimental group ($n = 30$)
Age (yr)	29.0 ± 3.7	29.9 ± 3.3
Weight (kg)	71.8 ± 12.1	72.7 ± 16.6
Height (cm)	168 ± 6	170 ± 5
Education[a]	3.9 ± 1.5	4.2 ± 1.5
Margarine consumption (g/day)	27.0 ± 2.9	27.7 ± 3.4
Alcohol consumption (%) yes/no	23.1/76.9	36.7/63.3
Smoking (%) yes/no	30.8/69.2	13.3/86.7
Parity (%) 0/1/ > 1	42.3/38.5/19.2	40.0/50.0/10.0
Gravidity (%) 1/2/3/ > 3	34.6/34.6/15.4/15.4	33.3/50.0/6.7/10.0

[a] Education has been measured on an 8-point scale [45].

Table 3
Results of the statistical analyses for the cognitive tests (P-values)

Test	Group effect	Group*time effect
WLTtot (learning)	0.416	0.868
WLTdr (retrieval)	0.411	0.397
CST (I + II/2) (speed of operation in working memory)	0.453	0.957
CST (III) (shifting ability)	0.132	0.132
Str (I + II/2) (information processing speed)	0.113	0.084
Str (III) (color word interference)	0.384	0.222
LDST (coding)	0.146	0.048
RT (selective attention)	0.312	0.377
PB (selective attention)	0.870	0.154

WLT: words learning test, tot: total, dr: delayed recall, CST: concept shifting test, Str: Stroop test, LDST: letter digit substitution test, RT: reaction time on the finger precuing task, PB: preparation benefit on the finger precuing task.

Table 2
Cognitive performance during pregnancy in the control (C) and experimental (E) group (mean $\pm$ SD)

Test		Week 14	Week 17	Week 29	Week 36	32 weeks pp
WLTtot # words	C	10.5 ± 1.8	10.3 ± 2.2	11.1 ± 1.6	11.4 ± 1.5	10.8 ± 1.5
	E	9.9 ± 1.3	9.8 ± 2.0	10.7 ± 2.2	10.9 ± 1.5	10.3 ± 1.1
WLTdr # words	C	12.3 ± 2.0	12.0 ± 2.3	12.6 ± 2.1	13.0 ± 1.9	12.7 ± 1.6
	E	11.5 ± 1.9	11.8 ± 2.5	12.8 ± 2.2	12.8 ± 1.6	12.5 ± 1.7
CST (I + II)/2 (s)	C	13.6 ± 3.3	13.3 ± 3.2	12.6 ± 2.7	13.5 ± 3.8	12.3 ± 3.5
	E	12.6 ± 3.1	12.1 ± 3.4	11.8 ± 2.3	12.2 ± 2.9	12.0 ± 2.9
CST (III) (s)	C	21.7 ± 7.6	22.1 ± 7.2	19.5 ± 6.3	21.5 ± 7.3	21.4 ± 1.6
	E	20.9 ± 7.2	19.6 ± 6.2	19.3 ± 1.1	18.5 ± 0.9	19.1 ± 5.8
Str (I + II)/2 (s)	C	17.5 ± 2.3	17.0 ± 2.0	17.1 ± 2.1	17.4 ± 0.4	16.5 ± 1.6
	E	17.2 ± 3.4	16.6 ± 2.5	16.4 ± 2.7	16.4 ± 2.3	16.0 ± 2.4
Str (III) (s)	C	35.4 ± 6.4	31.9 ± 1.1	32.5 ± 3.8	32.5 ± 6.6	30.4 ± 5.4
	E	33.9 ± 8.0	30.7 ± 8.3	31.2 ± 7.4	29.8 ± 6.1	29.0 ± 7.3
LDST # numbers/min	C	38.7 ± 6.9	39.6 ± 6.4	39.9 ± 1.1	40.2 ± 1.2	39.7 ± 6.7
	E	40.8 ± 5.4	40.9 ± 0.9	42.5 ± 0.9	43.7 ± 1.0	42.9 ± 1.0
RT (ms)	C	4144 ± 764	3974 ± 594	3906 ± 490	3938 ± 541	3746 ± 460
	E	3834 ± 465	3666 ± 426	3731 ± 478	3651 ± 451	3589 ± 442
PB (ms)	C	18.9 ± 33.5	30.2 ± 25.3	17.8 ± 19.5	21.8 ± 20.1	26.0 ± 18.8
	E	32.0 ± 27.5	29.7 ± 25.1	27.9 ± 18.5	27.5 ± 15.1	33.3 ± 21.2

Note: pp: postpartum, WLT: words learning test, tot: total (mean of trial 1 and 2), dr: delayed recall, CST: concept shifting test, Str: Stroop test, LDST: letter digit substitution test, RT: reaction time on the finger precuing task, PB: prebaration benefit on the finger precuing task.

Table 4
Plasma fatty acid levels during and after pregnancy (mean ± SD) (n = 26 in control group (C) and 30 in experimental group (E))

FA		Week 14	Week 26	Week 36	Partus	32 week pp
ALA[a,b]	C	0.22 ± 0.08	0.23 ± 0.07	0.21 ± 0.06	0.20 ± 0.08	0.15 ± 0.06
	E	0.22 ± 0.08	0.43 ± 0.13	0.39 ± 0.13	0.38 ± 0.14	0.20 ± 0.07
AA	C	9.83 ± 1.75	8.74 ± 1.31	8.28 ± 1.23	8.71 ± 1.28	10.13 ± 1.73
	E	9.56 ± 1.70	8.06 ± 1.62	7.80 ± 1.51	8.19 ± 1.36	9.54 ± 1.93
DHA	C	4.04 ± 0.87	3.72 ± 0.94	3.68 ± 0.92	3.57 ± 0.82	3.15 ± 1.03
	E	4.48 ± 1.01	3.89 ± 0.68	3.95 ± 0.87	3.94 ± 0.96	3.10 ± 0.80
ObA[a]	C	0.34 ± 0.13	0.39 ± 0.14	0.44 ± 0.15	0.45 ± 0.19	0.21 ± 0.09
	E	0.30 ± 0.08	0.28 ± 0.08	0.30 ± 0.11	0.33 ± 0.11	0.21 ± 0.08

Note: ALA: alpha-linolenic acid (18:3n-3), AA: arachidonic acid (20:4n-6), DHA: docosahexaenoic acid (22:6n-3), ObA: Osbond acid (22:5n-6).
[a] Significant difference between the two groups at weeks 26 and 36 of pregnancy and at partus ($P < 0.05$).
[b] Significant difference between the two groups 32 weeks after delivery ($P < 0.05$).

3.3. Effect of EFA supplementation on EFA and LCPUFA status

EFA supplementation during pregnancy resulted in statistically significant main group effects for ALA and ObA, indicating significantly higher levels of ALA and lower concentrations of ObA in the experimental group ($P = 0.000$ and $P = 0.003$, respectively) compared to the control group during pregnancy (Table 4). AA and DHA levels were not significantly different between the two groups. No significant group*time effects were found, indicating that none of the investigated fatty acids changed over time.

Thirty-two weeks after delivery, the two groups were not significantly different from each other in terms of fatty acid levels, with the exception of ALA, which was still significantly higher in the experimental group ($P = 0.005$).

3.4. Correlation between plasma fatty acid concentrations and cognitive functioning

DHA concentrations during early pregnancy (week 14) and at 32 weeks after delivery were positively related to the reaction times of the finger precuing task (partial correlation coefficients 0.3705 and 0.4633, respectively, $P < 0.01$ for both). Thus, reaction times were longer (and, consequently, this aspect of cognition lower) with higher plasma DHA concentrations. Other significant correlations were not observed.

Neither AA nor ObA was significantly related to performance on any of the cognitive tests.

4. Discussion

The aim of the current study was to investigate the effect of supplementation with ALA, the parent fatty acid of DHA, on various aspects of cognitive functioning during and 32 weeks after human pregnancy. Additional LA was given in order to prevent a

decrement in AA concentration, which frequently accompanies an increased consumption of n-3 fatty acids [34].

Performance on none of the cognitive tests was different between the two groups. Thus, it can be concluded that we did not find an effect of ALA supplementation on cognitive functioning during pregnancy.

DHA and AA are the predominant LCPUFAs in brain tissue and although ALA + LA supplementation did not increase DHA levels in the plasma phospholipid fatty acids of the experimental group, the lower ObA levels in this group indicated a better functional DHA status indeed [35]. Alterations in brain function are presumed to follow from the biochemical consequences of modifying membrane PUFA content [49]. Known effects include modifications in membrane fluidity, in the activities of membrane-associated functional proteins, such as transporters, enzymes, and receptors, and in the production of important signaling molecules derived from EFA, such as prostaglandins and eicosanoids [50–52]. A possible explanation for the fact that no effect of EFA supplementation on cognition was found in the current study could be that the ALA and LA conversion into DHA and AA, respectively, was too limited, since these processes have been shown to be rather inefficient, indeed [35]. In this study the women were supplemented with ALA and LA, and, as has been shown repeatedly, these parent fatty acids are desaturated and elongated into their longer-chain more-unsaturated fatty acids DHA and AA in low amounts [53–57].

Fatty acids of the n-3 fatty acid family do not necessarily affect all neural domains to the same extent. Thus, it has been shown that deficiency of n-3 fatty acids in monkeys lead to slower visual processing speed [58], but did not influence learning or memory performance [59]. In the current study, we did find correlations between plasma DHA levels and reaction time on the finger precuing task, which measures selective attention. However, these correlations were negative, indicating

that a higher DHA concentration in maternal plasma phospholipids was associated with lower cognitive performance. Whether this finding can be attributed to chance or whether this is a realistic finding, with a plausible biochemical explanation needs to be further elucidated.

The above-mentioned negative correlation was only found at early pregnancy (week 14 of gestation) and at 32 weeks after delivery, but not during advanced pregnancy. However, a potential association between plasma DHA concentrations and cognition during mid- and late-pregnancy might be overruled by other factors influencing cognition, such as hormones [60], mood [61,62], and sleep deprivation [63], which are all striking features of pregnancy. Further research is needed to clarify this issue.

In conclusion it can be said that ALA supplementation during pregnancy does not influence cognitive performance. As it can be expected that supplementation with DHA will affect brain DHA levels more efficiently than ALA supplementation, a supplementation study with DHA (preferably in combination with AA) is warranted to obtain further information about the possible role of LCPUFAs in cognitive performance. However, such a study should be restricted to men and/ or non-pregnant women, as long as the causality of the negative relation observed in the present study between plasma DHA concentration and selective attention has not been refuted.

Acknowledgements

References

[1] P.S. Sastry, Lipids of nervous tissue: composition and metabolism, Prog. Lipid Res. 24 (1985) 69–176.

[2] L. Svennerholm, Distribution and fatty acid composition of phosphoglycerides in normal human brain, J. Lipid Res. 9 (1968) 570–579.

[3] W.E. Connor, M. Neuringer, S. Reisbick, Essential fatty acids: the importance of n-3 fatty acids in the retina and brain, Nutr. Rev. 50 (1992) 21–29.

[4] M. Neuringer, G.J. Anderson, W.E. Connor, The essentiality of n-3 fatty acids for the development and function of the retina and brain, Annu. Rev. Nutr. 8 (1988) 517–541.

[5] M.D. Al, A.C. v. Houwelingen, A.D. Kester, T.H. Hasaart, A.E. de Jong, G. Hornstra, Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status, Br. J. Nutr. 74 (1995) 55–68.

[6] S.J. Otto, A.C. v. Houwelingen, M. Antal, et al., Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study, Eur. J. Clin. Nutr. 51 (1997) 232–242.

[7] R.H.M. de Groot, J.J. Adam, G. Hornstra, Selective attention deficits during human pregnancy, Neurosci. Lett. 340 (2003) 21–24.

[8] R.H.M. de Groot, E.F.P.M. Vuurman, G. Hornstra, J. Jolles, Differences in cognitive performance during pregnancy and early motherhood, submitted for publication.

[9] E.C. Bakker, Long-chain polyunsaturated fatty acids and child development, Human Biology (thesis). Maastricht University, Maastricht, 2002, p. 128.

[10] R.D. Uauy, D.G. Birch, E.E. Birch, J.E. Tyson, D.R. Hoffman, Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates, Pediatr. Res. 28 (1990) 485–492.

[11] K.S. Bjerve, L. Thoresen, K. Bonaa, T. Vik, H. Johnsen, A.M. Brubakk, Clinical studies with alpha-linolenic acid and long-chain n-3 fatty acids, Nutrition 8 (1992) 130–132.

[12] E.E. Birch, D.G. Birch, D.R. Hoffman, R. Uauy, Dietary essential fatty acid supply and visual acuity development, Invest. Ophthalmol. Vis. Sci. 33 (1992) 3242–3253.

[13] K.S. Bjerve, A.M. Brubakk, K.J. Fougner, H. Johnsen, K. Midthjell, T. Vik, Omega-3 fatty acids: essential fatty acids with important biological effects, and serum phospholipid fatty acids as markers of dietary omega 3-fatty acid intake, Am. J. Clin. Nutr. 57 (1993) 801S–805S (discussion 805S–806).

[14] S.E. Carlson, S.H. Werkman, P.G. Rhodes, E.A. Tolley, Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation, Am. J. Clin. Nutr. 58 (1993) 35–42.

[15] S.E. Carlson, S.H. Werkman, J.M. Peeples, W.M. Wilson 3rd., Growth and development of premature infants in relation to omega 3 and omega 6 fatty acid status, World Rev. Nutr. Diet 75 (1994) 63–69.

[16] S.E. Carlson, S.H. Werkman, J.M. Peeples, W.M. Wilson, Long-chain fatty acids and early visual and cognitive development of preterm infants, Eur. J. Clin. Nutr. 48 (Suppl. 2) (1994) S27–30.

[17] S.E. Carlson, S.H. Werkman, E.A. Tolley, Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia, Am. J. Clin. Nutr. 63 (1996) 687–697.

[18] S.H. Werkman, S.E. Carlson, A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until nine months, Lipids 31 (1996) 91–97.

[19] D.L. O'Connor, R. Hall, D. Adamkin, et al., Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial, Pediatrics 108 (2001) 359–371.

[20] C. Agostoni, S. Trojan, R. Bellu, E. Riva, M. Giovannini, Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids, Pediatr. Res. 38 (1995) 262–266.

[21] S.E. Carlson, A.J. Ford, S.H. Werkman, J.M. Peeples, W.W. Koo, Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin, Pediatr. Res. 39 (1996) 882–888.

[22] E.E. Birch, D.R. Hoffman, R. Uauy, D.G. Birch, C. Prestidge, Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants, Pediatr. Res. 44 (1998) 201–209.

[23] P. Willatts, J.S. Forsyth, M.K. DiModugno, S. Varma, M. Colvin, Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age, Lancet 352 (1998) 688–691.

[24] P. Willatts, J.S. Forsyth, M.K. DiModugno, S. Varma, M. Colvin, Influence of long-chain polyunsaturated fatty acids on infant cognitive function, Lipids 33 (1998) 973–980.

[25] E.E. Birch, S. Garfield, D.R. Hoffman, R. Uauy, D.G. Birch, A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants, Dev. Med. Child Neurol. 42 (2000) 174–181.

[26] M. Makrides, M.A. Neumann, K. Simmer, R.A. Gibson, A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial, Pediatrics 105 (2000) 32–38.

[27] I.B. Helland, L. Smith, K. Saarem, O.D. Saugstad, C.A. Drevon, Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age, Pediatrics 111 (2003) e39–e44.

[28] N. Auestad, M.B. Montalto, R.T. Hall, et al., Visual acuity erythrocyte, fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Ross Pediatric Lipid Study, Pediatr. Res. 41 (1997) 1–10.

[29] A. Lucas, M. Stafford, R. Morley, et al., Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomised trial, Lancet 354 (1999) 1948–1954.

[30] M. Makrides, M.A. Neumann, B. Jeffrey, E.L. Lien, R.A. Gibson, A randomized trial of different ratios of linoleic to alpha-linolenic acid in the diet of term infants: effects on visual function and growth, Am. J. Clin. Nutr. 71 (2000) 120–129.

[31] N. Auestad, R. Halter, R.T. Hall, et al., Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study, Pediatrics 108 (2001) 372–381.

[32] I.B. Helland, O.D. Saugstad, L. Smith, et al., Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women, Pediatrics 108 (2001) E82.

[33] S. Yehuda, S. Rabinovtz, R.L. Carasso, D.I. Mostofsky, Essential fatty acids preparation (SR-3) improves Alzheimer's patients quality of life, Int. J. Neurosci. 87 (1996) 141–149.

[34] G. Hornstra, Importance of polyunsaturated fatty acids of the n-6 and n-3 families for early human development, Eur. J. Lipid Sci. Technol. 103 (2001) 379–389.

[35] R.H.M. de Groot, G. Hornstra, A.C. v. Houwelingen, F. Roumen, Effect of a-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome, Am J Clin. Nutr. (accepted for publication).

[36] N. Bohnen, A. Twijnstra, J. Jolles, Performance in the Stroop color word test in relationship to the persistence of symptoms following mild head injury, Acta Neurol. Scand. 85 (1992) 116–121.

[37] P.J. Houx, J. Jolles, Age-related decline of psychomotor speed: effects of age, brain health, sex, and education, Percept. Mot. Skills 76 (1993) 195–211.

[38] M. Klein, P.J. Houx, J. Jolles, Long-term persisting cognitive sequelae of traumatic brain injury and the effect of age, J. Nerv. Ment. Dis. 184 (1996) 459–467.

[39] J.T. Moller, P. Cluitmans, L.S. Rasmussen, et al., Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. International Study of Post-Operative Cognitive Dysfunction, Lancet 351 (1998) 857–861.

[40] N. Brand, J. Jolles, Learning and retrieval rate of words presented auditorily and visually, J. Gen. Psychol. 112 (1985) 201–210.

[41] P. Houx, Cognitive Aging and Health-related Factors, Rijksuniversiteit Limburg, Maastricht, 1991.

[42] J. Stroop, Studies of interference in serial verbal reaction, J. Exp. Psychol. 18 (1935) 643–662.

[43] A. Smith, The symbol Digit Modalities Test: a neuropsychologic test for economic screening of learning and other cerebral disorders, Learning Disorders 3 (1968) 83–91.

[44] J. Miller, Discrete versus continuous stage models of human information processing: in search of partial output, J. Exp. Psychol. Hum. Percept. Perform. 8 (1982) 273–296.

[45] S.E. de Bie, Standaardvragen, 1987, Voorstellen voor uniformering van vraagstellingen naar achtergrondkenmerken en interviews (Standard questions 1987: Proposal for uniformisation of questions regarding background variables and interviews), 2 Edition, Leiden, Leiden University Press, The Netherlands, 1987.

[46] S.C. Couch, E.H. Philipson, R.B. Bendel, V. Wijendran, C.J. Lammi-Keefe, Maternal and cord plasma lipid and lipoprotein concentrations in women with and without gestational diabetes mellitus. Predictors of birth weight?, J. Reprod. Med. 43 (1998) 816–822.

[47] V. Wijendran, R.B. Bendel, S.C. Couch, et al., Maternal plasma phospholipid polyunsaturated fatty acids in pregnancy with and without gestational diabetes mellitus: relations with maternal factors, Am. J. Clin. Nutr. 70 (1999) 53–61.

[48] V. Wijendran, R.B. Bendel, S.C. Couch, E.H. Philipson, S. Cheruku, C.J. Lammi-Keefe, Fetal erythrocyte phospholipid polyunsaturated fatty acids are altered in pregnancy complicated with gestational diabetes mellitus, Lipids 35 (2000) 927–931.

[49] J.D. Fernstrom, Effects of dietary polyunsaturated fatty acids on neuronal function, Lipids 34 (1999) 161–169.

[50] R.R. Brenner, Effect of unsaturated acids on membrane structure and enzyme kinetics, Prog. Lipid Res. 23 (1984) 69–96.

[51] A.A. Spector, M.A. Yorek, Membrane lipid composition, cellular function, J. Lipid Res. 26 (1985) 1015–1035.

[52] R. Uauy, P. Mena, C. Rojas, Essential fatty acids in early life: structural and functional role, Proc. Nutr. Soc. 59 (2000) 3–15.

[53] G.C. Burdge, A.E. Jones, S.A. Wootton, Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men∗, Br. J. Nutr. 88 (2002) 355–363.

[54] G.C. Burdge, S.A. Wootton, Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women, Br. J. Nutr. 88 (2002) 411–420.

[55] S.H. Vermunt, R.P. Mensink, M.M. Simonis, G. Hornstra, Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid, Lipids 35 (2000) 137–142.

[56] H. Gerster, Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)?, Int. J. Vitam. Nutr. Res. 68 (1998) 159–173.

[57] H.M. Su, M.C. Huang, N.M. Saad, P.W. Nathanielsz, J.T. Brenna, Fetal baboons convert 18:3n-3 to 22:6n-3 in vivo. A stable isotope tracer study, J. Lipid Res. 42 (2001) 581–586.

[58] S. Reisbick, M. Neuringer, E. Gohl, R. Wald, G.J. Anderson, Visual attention in infant monkeys: effects of dietary fatty acids and age, Dev. Psychol. 33 (1997) 387–395.

[59] S. Reisbick, M. Neuringer, W.E. Connor, Effect of n-3 fatty acid deficiency in nonhuman primates, in: J.G. Bindels, A.C. Goedhart, H.K.A. Visser (Eds.), Recent Developments in Infant Nutrition, Kluwer Academic Publishers, Dordrecht, 1996, pp. 157–172.

[60] B.B. Sherwin, Estrogenic effects on memory in women, Ann. N. Y. Acad. Sci. 743 (1994) 213–230 (discussion 230–231).

[61] R.M. Cohen, H. Weingartner, S.A. Smallberg, D. Pickar, D.L. Murphy, Effort and cognition in depression, Arch. Gen. Psychiatry 39 (1982) 593–597.

[62] P.A. Lichtenberg, T. Ross, S.R. Millis, C.A. Manning, The relationship between depression and cognition in older adults: a cross-validation study, J. Gerontol. B Psychol. Sci. Soc. Sci. 50 (1995) P25–P32.

[63] L. Dotto, Sleep stages, memory and learning, Cmaj 154 (1996) 1193–1196.