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Diffusion tensor imaging and behavior in premature infants at 8 years of age, a randomized controlled trial with long-chain polyunsaturated fatty acids☆,☆☆

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ABSTRACT

Background: Very low birth weight (VLBW, birth weight < 1500 g) children have increased risk of behavioral problems. Diffusion tensor imaging (DTI) of the brain shows reduced white matter maturation. Long-chain polyunsaturated fatty acids are hypothesized to improve both myelination and behavioral outcome.
Aims: To test the hypothesis that postnatal supplementation with docosahexaenoic acid (DHA) and arachidonic acid (AA) to very low birth weight infants would influence cerebral white matter measured by DTI and improve behavioral outcome at 8 years of age.
Study design: Eight-year follow-up of a randomized, double-blinded, placebo-controlled study of postnatal supplementation with DHA and AA to 129 VLBW infants fed human milk.
Subjects: Ninety-eight children (76%) met for follow-up at 8 years.
Outcome measures: Cerebral white matter measured by DTI. Behavioral outcome measured by Strengths and Difficulties questionnaire and selected scales from the Child Behavior Checklist.
Results: No significant differences between the intervention group and the control group were found on white matter microstructure or behavioral data. A non-significant finding of higher fractional anisotropy (FA) in a cluster in the corpus callosum of the intervention group is discussed.
Conclusions: The present study is the first long-term follow-up of a randomized controlled trial with DHA and AA to human milk fed VLBW infants exploring cerebral white matter microstructure measured by DTI and parent-reported behavioral problems. No effects on white matter microstructure or behavioral outcome were observed at 8 years of age.

1. Introduction

Adverse neurological outcome is commonly reported in very low birth weight (VLBW, birth weight < 1500 g) infants [1]. Cognitive impairments are among the most frequently reported sequelae, and there is also a growing interest in the impact of premature birth on mental health; hereunder increased risk of inattention, hyperactivity, behavioral problems and social/emotional difficulties. Approximately 25% of extremely preterm children are later diagnosed with a psychiatric disorder, and up to 50% may have significant difficulties that impact daily life function [2–4].

By use of magnetic resonance imaging (MRI) of the preterm brain, studies of macrostructure have demonstrated smaller total and regional brain volumes compared with children born at term [5–7]. These changes seem to persist into adolescence and correlate with intellectual and motor abilities [8–12]. Diffusion tensor imaging (DTI) is an MRI technique allowing assessment of brain microstructure, hereunder the

Abbreviations: AA, arachidonic acid; AD, axial diffusivity; BW, birth weight; CBCL, Child Behavior Checklist; CC, corpus callosum; DHA, docosahexaenoic acid; DTI, diffusion tensor imaging; FA, fractional anisotropy; GA, gestational age; LCPUFA, long-chain polyunsaturated fatty acid; MD, mean diffusivity; MRI, magnetic resonance imaging; RCT, randomized controlled trial; RD, radial diffusivity; SDQ, Strengths and Difficulties Questionnaire; TBSS, Tract-Based Spatial Statistics; TFCE, Threshold-free cluster enhancement; VLBW, very low birth weight (< 1500 g).

☆ Conflict of interest: The authors report no conflicts of interest.

☆☆ Clinical Trial Registration: The original trial was registered at http://www.clinicaltrials.gov with identifier NCT00226187. The present study is a follow-up of this trial.

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organization of white matter, providing important information about the developing brain of premature children [13]. Several DTI studies have reported lower fractional anisotropy (FA), indicating less mature white matter, both at term and in school-aged children born prematurely. Furthermore, the same studies report that FA may correlate positively with IQ, reading and social skills and negatively with attention deficit hyperactivity disorder (ADHD) [14–18].

Although the effects of prematurity on brain development are well documented using MRI, the pathogenesis is multifactorial and still not fully understood. Nutrition is one of the many factors influencing the developing brain, and nutrient supply has been documented to influence both cognitive function and brain structure measured by MRI [19–23]. Long chain polyunsaturated fatty acids (LCPUFA), in particular docosahexaenoic (DHA) and arachidonic acid (AA), are essential for development of the central nervous system, and influence both cortical metabolic function and cognitive development [24,25]. Premature infants are deprived of the intrauterine cerebral accumulation of DHA and AA in the last trimester. Although human milk is a good natural source of DHA and AA, additional supplementation of these fatty acids has been associated with positive short-term effects on cognition in several studies [25–30], whereas other studies reveal no significant effects [31,32]. Furthermore, the role of LCPUFA on behavior, attention, social/emotional problems and learning is not well described. Some studies suggest positive effects of supplementation on such measures [33–35], whereas others report no benefit [36,37]. LCPUFA, and in particular DHA, is important for myelin development and for the maturation and stability of cortical circuits [38]. DTI assess white matter microstructure and provides indirect measures of structural connectivity. However, only one randomized controlled trial (RCT) employing nutrient supply and DTI in premature infants is published, but this report included enhanced total energy supply with amino acids and vitamins in addition to essential LCPUFA and performed DTI at term-equivalent age [39]. No RCTs reporting long-term effects or focusing exclusively on LCPUFAs and white matter microstructure measured by DTI are found, and further research is required [22].

In our original randomized placebo-controlled trial including 129 VLBW infants, Henriksen et al. tested the effect of postnatal supplementation with DHA and AA, and found positive effects on cognition as measured with event-related potentials (ERP) at 6 months corrected age [28], and at 20 months in terms of attention capacity in free-play sessions [40]. Almaas et al. recently published the first long-term follow-up of this RCT, investigating both cognitive functions and brain macrostructure measured by MRI. No cognitive or brain morphometric effects of the supplementation were detected at 8 years of age [41]. Our present article provides unique and complementary information, as the microstructural DTI- measures are partly independent of macrostructural morphometry [42].

The aim of the present study was to test the hypothesis that postnatal supplementation of DHA and AA to VLBW infants would improve behavioral outcome and influence cerebral white matter measured by DTI at 8 years of age.

2. Methods

2.1. Original trial intervention

The present study is a follow-up of a randomized, double-blinded, placebo-controlled trial of supplementation with DHA and AA to 129 VLBW infants. All infants received human milk (from the mother or donor), from the first or second day after birth. A daily dose of 0.5 mL study oil (DHA/AA or placebo) per 100 mL of human milk was given from the first feeding. The study was designed so that all infants would eventually receive the same amount of supplementation (100 mL), and the intervention lasted on average 9 weeks (until discharge or empty bottle of 100 mL study oil). The intervention group received supplementation of 32 mg (0.86% of total fatty acids) DHA and 31 mg (0.91%) AA per 100 mL of human milk. For a detailed description of the intervention we refer to earlier publications [28]. The supplementation was three-fold higher compared to the level of 0.3% typically added to preterm formula [43]. Exclusion criteria were major congenital abnormalities and cerebral hemorrhage (grade 3 or 4). Written informed consent was obtained from the parents, and the Regional Committee for Medical and Health Research Ethics approved both the original trial and the follow-up study. The trial was registered at http://www.clinicaltrials.gov with identifier NCT00226187.

2.2. Eight-year follow-up study

2.2.1. Participants

The 129 VLBW-infants who completed the original RCT were summoned to participate in the follow-up study. Ninety-eight children met for testing and behavioral questionnaires, at a mean age of 8.6 years (76% follow-up rate; intervention group $n = 45$, control group $n = 53$) [41]. Eighty-four completed MRI, and 82 children (84% of the 98, 64% of the original 129 participants) had DTI of sufficient quality for analysis. Clinically relevant pathology was discovered in four participants on MRI; one with a low-grade glioma in left thalamus; one with volume loss in the left temporal lobe; one with a cystic mass in the cerebellum and one participant with asymmetrical ventricular system (three controls, one intervention). Because all structures beyond these afflicted areas appeared normal, and to allow a more direct comparison with our previous morphological study [41], we included these subjects in the analyses ($n = 82$). In addition, we also performed the analyses with these four subjects removed ($n = 78$).

2.2.2. Behavioral outcomes

Behavioral outcomes were assessed by the Strengths and Difficulties Questionnaire (SDQ) parent-report version, a questionnaire of five scales: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems and prosocial behavior, each consisting of five items [44,45]. The four first scales are combined to generate a total difficulties score, a higher score indicating more severe problems. Additionally, an impact score is produced from separate items focusing on the effects of these difficulties on the child (level of distress; impact on home, friendships, classroom, and leisure activities). SDQ was supplemented with the two scales Social problems and Attention problems from the Child Behavior Checklist (CBCL), a widely used parent-rated checklist to detect emotional and behavioral problems in children and adolescents [46], consisting of 11 and 10 items, respectively.

2.2.3. Statistical analyses of behavioral outcomes

Analyses were performed with SPSS version 22.0. SDQ and CBCL data were tested for group differences with ANCOVAs with sex, age, birth weight (BW) and gestational age (GA) as covariates. Duration of supplementation measured as days of hospitalization was strongly correlated with gestational age ($R - 0.67$, $p < 0.001$) and therefore not included as an independent covariate in the analyses. Statistical significance was defined as $p < 0.05$. Since the number of participants in our follow-up study was already given, a post-hoc power calculation was not conducted. To describe the effect in the two groups we present the 95% confidence interval (CI) of the difference in SDQ total difficulties score between the intervention- and control group.

2.3. DTI

DTI provides indirect measures of tissue microstructure by taking advantage of inherent water diffusion. In the white matter, where the axons are organized into fiber tracts, the water diffusion is anisotropic (greater in one direction). The degree of diffusion and thus anisotropy will differ by degree and direction depending on tissue characteristics (axonal orientation, density, myelin, and properties of the cell membranes) [17,22], and can be measured within each voxel (volume