Prenatal and early postnatal supplementation with long-chain polyunsaturated fatty acids: neurodevelopmental considerations1-4

Mijna Hadders-Algra

ABSTRACT
It takes >20 y before the human brain obtains its complex adult configuration. Most dramatic neurodevelopmental changes occur prenatally and early postnataIly, including a major transformation in cortical organization 3–4 mo after term. The long-lasting changes have practical implications for studies evaluating the effect of prenatal and early postnatal supplementation with long-chain polyunsaturated fatty acids (LC-PUFAs). Whether studies of the effect of supplementation will reveal an effect not only depends on the dosage and duration of supplementation but also on 1) the timing of supplementation, 2) the age at which the outcome is assessed, 3) the application of age-specific sensitive neurodevelopmental tools, and 4) the functional domain evaluated. Studies of the effects of prenatal supplementation with docosahexaenoic acid (DHA) or fish oil have provided inconsistent results. However, maternal and neonatal concentrations of DHA and arachidonic acid are associated with improved outcomes in early infancy, and concentrations of DHA are associated with favorable neurodevelopmental outcome beyond early infancy. Studies of LC-PUFA supplementation in preterm infants have not shown evidence of a positive effect on neurodevelopmental outcome. Similar studies in full-term infants have indicated that supplementation with 0.30% DHA (by wt) promotes neurodevelopmental outcome in early infancy, but positive effects on later outcome have not been shown. However, information on the effects on outcomes at school age or later is virtually absent. This article stresses the need for long-term longitudinal studies that apply age-specific, sensitive neurodevelopmental tools, which also take into account lifestyle habits, maternal prepregnancy nutritional status, and genetic variation in metabolism. Am J Clin Nutr doi: 10.3945/ajcn.110.001065.

INTRODUCTION
Since the mid-1970s, interest in the effect of the early environment on later health and disease has rapidly expanded. In the 1970s, awareness grew that prenatal exposure to chemical substances, such as antiepileptic drugs, was not only associated with an increased risk of gross morphologic changes but also with a higher prevalence of more subtle cognitive or behavioral deficits [“behavioral teratology” (1)]. At the same time, Dörner (2) coined the idea that concentrations of hormones, metabolites, and neurotransmitters during critical periods in early life could preprogram brain and body with an increased vulnerability to disorders in later life (3). The concept of early programming was born. Early programming implies that an insult or stimulus applied at a critical or sensitive period during development may have long-term or lifetime effects on the structure or function of an organism (4). The idea of early programming was largely promoted by the epidemiologic studies of Barker on inverse relations between birth weight and hypertension and mortality due to adult ischemic heart disease (5, 6). Yet, the notion that prenatal, perinatal, or early postnatal conditions may have long-lasting consequences was not new. Already at the end of the 19th century, Little (7) and Freud (8) had indicated that adversities occurring during pregnancy and birth may result in the development of significant motor impairment in later life, ie, cerebral palsy. In the middle of the 20th century, the idea emerged that prenatal and perinatal conditions may also be associated with other developmental problems, such as attention deficit and specific learning disorders (9). It has been well established that certain conditions, such as preterm birth, severe intrauterine growth retardation, or prenatal exposure to maternal smoking (10–12), indeed may have long-lasting negative effects on neurodevelopmental outcome. However, the effect of specific nutritional exposures or deficits during early life on developmental outcome is less clear. In the nutritional domain, much attention is paid to the effect of long-chain polyunsaturated fatty acids (LC-PUFAs). This interest was inspired by the consistent finding that fish consumption during pregnancy and lactation and breastfeeding are associated with a better neurodevelopmental outcome of the child (13, 14) and the knowledge that both fish and human milk are dietary sources of LC-PUFA.

The present article reviewed the effects of early LC-PUFA supplementation on neurodevelopmental outcome in humans. It starts with a concise overview of the ontogeny of the human brain. This section also addresses the consequences of neurodevelopmental mechanisms for nutritional intervention, including the timing of the intervention and the age at which the consequences of the intervention are evaluated. Subsequent sections address fetal and neonatal LC-PUFA supply and LC-PUFA accretion in the...
brain and 2) the effect of early LC-PUFA supplementation on neurodevelopmental outcome.

DEVELOPMENT OF THE HUMAN BRAIN

The development of the human brain spans many years: at about the age of 40 y, the nervous system obtains its full-blown adult configuration (15). The development of the nervous system starts in the fifth week postmenstrual age (PMA) with the development of the neural tube. Shortly after closure of the neural tube, specific areas near the ventricles start to generate neurons. Most neurons are formed between 5 and 25 wk PMA, whereas most glial cells are produced between week 20 and 40 PMA (16). Once neurons have been generated, they move from their place of origin to their final place of destination. Most cortical neurons migrate to their destinations along specialized radial glial fibers, which span the entire thickness of the hemisphere from the ventricular surface to the external pial surface (17). Neuronal migration along the radial glial scaffold most likely is regulated by complex molecular interactions between neuronal and glial cells. Various substances play a role in these cell-cell interactions, such as glycoproteins, membrane lipids containing LC-PUFAs, γ-aminobutyric acid, and glutamate (18). The radial scaffolding guarantees a neat projection of the ventricular neuronal birth map onto the cortical plate. In the human cerebral cortex, migration peaks between the third and fifth months of gestation (19). The point of time when migration stops is still a matter of debate, but recent data indicate that this might well be around 30 wk PMA (18).

Neurons start to differentiate during migration. Neuronal differentiation includes the formation of dendrites and axons, the production of neurotransmitters and synapses, and the elaboration of the intracellular signaling machinery and complex neural membranes. The major part of axon and dendrite sprouting occurs when the cells have reached their final position. In the axonal path to and from the cortex, the subplate plays a major role. The subplate, which emerges during early fetal life, is a transient structure that lies between the intermediate zone, ie, the periventricular white matter, and the developing cortical plate (20). It functions as a “waiting room” and temporary goal of afferent fibers originating from the thalamus, the contra- and ipsilateral hemispheres, the basal forebrain, and the monoaminergic brainstem nuclei heading for a cortical destination. The subplate probably also plays a role in the guidance of some corticofugal pathways (21). The temporary connections in the subplate form functionally active circuitries, which presumably play an important role in the mediation of fetal and neonatal behavior (22, 23). The subplate is particularly present between 24 and 36 wk PMA and regresses thereafter. Approximately 3 mo after term, only minor remnants of the subplate can be distinguished, and by the age of 6 mo the subplate has disappeared (20).

The process of axonal and dendritic differentiation is particularly active in the period spanning the few months before birth to the age of 12–15 mo after term. It is, however, noteworthy that the dendritic growth of cortical neurons continues until about the age of 5 y (24). The period of most active neuronal differentiation coincides with the peak of axonal myelination. After the age of 1 y, myelination continues at a slower pace until the age of about 40 y, when the last intracortical connections complete myelination (25).

A remarkable feature of brain development is that it does not only consist of the creation of components, but also of an elimination of elements. About half of the created neurons die off by means of programmed cell death (apoptosis). Apoptosis is brought about by interactions between genetically determined processes and chemicals and electrical signals induced by experience and occurs particularly during midgestation (26). Similarly, axons and synapses are eliminated, the latter especially between puberty and early adulthood (15).

The complex and protracted course of human brain development has several practical implications for studies on the effect of early LC-PUFA supplementation. First, it is clear that during gestation and in the first months after preterm or term birth a wealth of developmental processes occur. These processes may be affected by nutrition. Most likely the effect of early nutrition depends on the timing of the exposure (or deficit) in a manner similar to the age-dependent effect of hypoxic-ischemic insults to the developing brain. Hypoxic-ischemic insults occurring during the third trimester of gestation usually result in damage of the periventricular regions of the brain, whereas similar insults occurring around term age typically affect the cerebral cortex (27). This means, for instance, that the effect of LC-PUFA supplementation during the first months after birth in preterm infants may differ from that in term infants.

Second, the transient presence of the subplate—a cortical structure that is more prominently present in humans than in other species (21)—means that the cerebral cortex of the fetus and young infant consists of elements other than those of older children and adults. The virtual disappearance of the subplate at 3–4 mo after term coincides with the major transformation in infant behavior, which is reflected by the replacement of predominantly nongoal-directed motility (general movements) with goal-directed movements, such as reaching behavior, underscores the functional significance of the change in cortical structure (28). The transformation at ≈3–4 mo implies that the effects of early LC-PUFA supplementation on neurodevelopmental outcome during the first months after birth may differ from those on later outcomes.

Third, neurodevelopmental instruments to assess the effect of nutritional intervention should be age-specific, ie, they should be tailored to the functional specifics of the nervous system at the age considered. Because the effects of nutritional intervention are relatively subtle (14, 29), it is also important to select sensitive tools. Most studies on LC-PUFA supplementation used the Bayley Scales of Infant Development (BSID; 30), but it is important to realize that the BSID has been standardized and validated as a tool for clinical assessment. This means that it is a reliable instrument for detecting clinically relevant deviations of development, but not the most appropriate for assessing more subtle differences in motor and cognitive outcome. This point can be illustrated by a study from our group (29), in which we were able to show associations between prenatal LC-PUFA status and neurodevelopmental outcome at 18 mo by using the neurologic optimality score of the Hempel neurologic examination (31, 32); however, we failed to find significant associations between prenatal LC-PUFA status and performance on the BSID. The selection of age-appropriate neurodevelopmental assessment techniques should also be guided by the hypotheses tested. For instance, because animal research indicates that LC-PUFA status in early life may affect dopaminergic and serotonergic circuitries (33), it seems prudent to select tests that evaluate functions subserved by these systems, such as the regulation of attention or the modulation of aggression and mood.

Fourth, LC-PUFAs constitute only one aspect of nutrition, and nutrition is only one element in the multitude of factors affecting
neurodevelopmental outcome. Studies aiming at the evaluation of the effect of LC-PUFA supplementation should take this into account. The easiest way to deal with this complexity is to use the design of a randomized controlled trial (RCT). However, the RCT design does not preclude confounding. For instance, background factors may differ despite randomization. In addition attrition at follow-up may be selective (34). Thus, a careful evaluation of the multitude of background factors is not only a prerequisite in observational studies but also in studies with an RCT design.

**LC-PUFA SUPPLY AND ACCRETION IN THE BRAIN IN EARLY LIFE**

Lipids are a major constituent of the brain: $\approx 50\%$ of the dry weight of the adult brain consists of lipids, of which $20–25\%$ is LC-PUFAs. The major LC-PUFA docosahexaenoic acid [DHA, 22:6n-3 (or n-3)] and arachidonic acid (AA, 20:4n-6) may be derived directly from the diet or they may be formed through a series of elongating and desaturating reactions from the fatty acid precursors $\alpha$-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6), respectively (35). ALA and LA are essential fatty acids, which means that these nutrients are needed for healthy survival and that they cannot be synthesized by humans.

The LC-PUFA demand of the developing fetus and young infant is high. This is particularly true during the last 10 wk of gestation, when $\approx 90\%$ of fetal fat deposition occurs (36). To cope with fetal fatty acid demands, maternal fatty acids in plasma phospholipids increase by $50\%$ during the course of pregnancy. The concentrations of all fatty acids increase, but those of AA and DHA increase relatively less (37, 38). The higher concentrations of fatty acids are not brought about by altered dietary behavior, but by an accelerated breakdown of maternal fat depots during the last trimester (39, 40). This suggests that the fetal LC-PUFA supply does not only depend on the LC-PUFA content of the maternal diet during pregnancy but also on the LC-PUFA content of the diet before pregnancy.

Fatty acids cross the placenta by simple diffusion and via the action of fatty acid–binding proteins (41, 42). Because of this selective transplacental transport, LC-PUFA concentrations of the fetus are $300–400$ times those of the mother (36, 43). From about the third trimester onward, the fetus is capable of AA and DHA synthesis, but this capacity is significantly better for AA than for DHA. This implies that from the third trimester of pregnancy onward, the fetus and infant are less dependent on dietary AA than on dietary DHA (36, 41). A point of clinical concern is the finding that maternal smoking is associated with a reduced neonatal DHA and AA status—an association that presumably is brought about by a negative effect of smoking on the capacity to synthesize AA and DHA from the precursor fatty acids LA and ALA or on the preferential LC-PUFA accretion across the placenta (44).

In the brain, LC-PUFAs accumulate mainly in the cortical gray matter, particularly in the synaptic membranes and to a lesser extent in the white matter (35, 45). The baboon study by Diau et al (45) indicated that the highest concentrations of DHA and AA are found in the basal ganglia, hippocampus, thalamus, cerebellum, and precentral, postcentral, prefrontal, and occipital cortices—a finding that suggests that LC-PUFA intake particular affect the circuitries involved in sensorimotor integration, attention-executive function, and memory.

It should be realized that our knowledge on the accretion of LC-PUFAs in the developing human brain is limited. The data available indicate that LC-PUFA accretion in the brain starts at a relatively slow pace during early fetal life (46). However, this does not imply that LC-PUFAs do not matter during the first phases of development, because DHA may promote proliferation and differentiation of cortical neurons (47, 48). It is first in the last trimester of gestation that a spurt in LC-PUFA accretion occurs (49–51). The information available indicates that accretion of AA in the fetal brain exceeds that of DHA, particularly during the first 2 trimesters of gestation (50, 52). As a result, the brain at term contains relatively more AA than DHA (50, 51). LC-PUFA accretion in the nervous system continues after term age. Accretion of DHA gradually surpasses that of AA, so that in the adult brain DHA is the major LC-PUFA (50–54). Postmortem studies indicated that the cerebral LC-PUFA content is affected by postnatal nutrition, because the cerebral DHA content of breastfed infants was higher than that of infants fed standard formula without DHA. The cerebral AA content did not vary with the type of infant nutrition (54, 55). Studies of LC-PUFA supplementation in preterm and term baboons confirmed that DHA accretion in the central nervous system depends on the dietary provision of DHA, ie, on the duration and concentration of DHA supplementation. A similar dietary dependency is absent for AA accretion in the brain, ie, dosage and duration of postnatal AA supplementation do not affect AA accretion in the central nervous system (56).

**EARLY LC-PUFA SUPPLEMENTATION AND NEURODEVELOPMENTAL OUTCOME**

**LC-PUFA supplementation during pregnancy**

RCT studies on LC-PUFA supplementation during pregnancy focus on the effect of $\omega-3$ fatty acids. About half of the RCTs on LC-PUFA or fish-oil supplementation during pregnancy (57–67) reported a better neurodevelopmental outcome in the supplemented groups than in the control groups. A comparison of the studies reporting a positive effect of LC-PUFA supplementation (58, 63–66) with those that reported no effect (57, 59–62) or an adverse effect (67) showed no association between a positive outcome and DHA dosage, the age at which developmental outcome was evaluated, or the functional domain. This is remarkable because these factors showed substantial variation. For instance, doses of DHA varied between 200 and 2200 mg/d, age at evaluation from a few days to 7 y, and functional domain from visual acuity, motor control, and cognitive function to behavioral adaptation.

Additional information on prenatal LC-PUFA status and developmental outcome can be derived from correlations between maternal food consumption, maternal or neonatal LC-PUFA status, and developmental outcome. The studies available suggest that outcome is determined by the age at which outcome is evaluated. Studies that addressed outcomes before the transitional age of 4 mo indicated a positive association between maternal or neonatal DHA (57, 60, 68, 69) and AA (69–71) status and neurodevelopmental conditions. When outcomes were assessed after the age of 4 mo, it usually was associated with DHA status or fish consumption (13, 29, 59, 61, 65, 72–78). Only one study that evaluated outcomes after 4 mo found an association with neonatal AA status (65), whereas 3 others failed to find significant associations between early DHA status and developmental outcome (58, 79, 80).
The abovementioned findings indicate that both DHA and AA status during pregnancy affect neurodevelopmental outcome in early infancy. However, developmental outcome after early infancy seems to depend only on maternal and neonatal DHA concentrations. Yet, the inconsistent findings on the effects of prenatal LC-PUFA or fish-oil supplementation suggest that the achievement of an optimal DHA status does not only depend on the provision of DHA or fish-oil supplements during pregnancy; but most likely also depends on maternal prepregnancy fatty acid status, DHA and AA contents of the material diet during pregnancy, maternal smoking, and genetic variations in metabolic activity (39, 44, 67, 81). The recent study by Van Goor et al (67) illustrates the importance of the delicate balance between DHA and AA. This study indicated that prenatal supplementation with 200 mg DHA/d was associated with a less optimal neuromotor condition at 3 mo, but the addition of 200 mg AA to the daily dose of 200 mg DHA eliminated the slight disadvantage in neuromotor condition.

**LC-PUFA supplementation of infant formula**

Research on the effect of LC-PUFA supplementation of infant formula was inspired by the long-known empirical finding that breastfed children have better cognitive development than do formula-fed children (82). One of the many differences between breastfed and formula-fed groups is exposure to LC-PUFAs: until recently, LC-PUFAs were absent in standard commercially available formulas, whereas LC-PUFAs are present in human milk. However, a fundamental problem with studies evaluating differences in outcome between breastfed and formula-fed infants is the nonrandomized nature of the studies. Mothers who choose to breastfeed their infants differ in many respects from mothers who opt for formula-feeding: eg, mothers who breast-choose to breastfeed their infants differ in many respects from mothers who breast-feed have a better social background, have a higher intelligence quotient (IQ), and smoke less (14, 83). When perinatal and social confounders are taken into account, breastfeeding is associated with an increment of cognitive function of ≈3 IQ points (14). However, when the results are also adjusted for maternal IQ, the cognitive benefit of breastfeeding is reduced to a small and nonsignificant gain of ≈0.5 IQ points (83). Breastfeeding is also associated with a lower prevalence of fine manipulative dysfunction—an effect that remained statistically significant after adjustment for maternal IQ (34). In addition, specific groups of infants may benefit in particular from the favorable effects of breastfeeding, eg, infants who have been prenatally exposed to maternal smoking (84).

The systematic review of Simmer et al (85) indicated that supplementation of formula with LC-PUFAs in preterm infants was not associated with a beneficial effect on developmental outcome. This held true for visual, motor, and cognitive outcomes in early infancy and thereafter. The 3 RCTs (86–88) and 1 individual patient data meta-analysis (89) that were published after the systematic review confirmed the absence of a consistent positive association between LC-PUFA supplementation and neurodevelopmental outcome in preterm infants. Again, it is conceivable that specific groups may profit from LC-PUFA supplementation. For example, Makrides et al (87) found that high doses of DHA may promote cognitive development in girls.

The RCTs on LC-PUFA supplementation in term infants indicated that supplementation of formula with 0.30% DHA (by wt) is associated with beneficial neurodevelopmental outcome until the age of 4 mo (90). However, no consistent beneficial effect of LC-PUFA supplementation on outcomes beyond the age of 4 mo has been shown (34, 91, 92).

**CONCLUDING REMARKS**

Considerable evidence indicates that prenatal and neonatal LC-PUFA status is associated with neurodevelopmental outcome. Prenatal and neonatal DHA and AA concentrations are related to neurodevelopmental outcome in early infancy, but only prenatal and neonatal DHA is associated with outcomes beyond early infancy. The finding that correlational analyses of the effect of LC-PUFAs or fish-oil supplementation during pregnancy provide evidence of consistent associations, whereas RCTs of the same LC-PUFA provide less consistent results, suggests that the association between prenatal DHA status is only partially based on DHA consumption. Other factors that may contribute to the association are lifestyle habits, maternal prepregnancy nutritional status, and genetic variation in metabolic activity (67, 81, 93).

The fact that LC-PUFA accretion is especially abundant during the third trimester of gestation suggests that preterm infants would particularly profit from LC-PUFA supplementation. However, the available literature indicates that the developmental outcome of preterm infants is not promoted by LC-PUFA supplementation. The lack of a beneficial effect suggests that the negative consequences of preterm birth on brain development outweigh the potential positive effects of LC-PUFA supplementation.

LC-PUFA supplementation in term infants is associated with a beneficial effect on short-term neurodevelopmental outcome. The current literature suggests that LC-PUFA supplementation in term infants does not affect outcomes beyond the age of 4 mo. However, it is important to realize that information on long-term outcomes is limited to one study (34), which indicated that LC-PUFA supplementation during the first 2 postnatal months does not result in an improvement in the neurological condition at 9 y.

To improve our understanding of the effects of prenatal and early postnatal LC-PUFA supplementation, long-term longitudinal studies are needed in which age-specific, sensitive neurodevelopmental tools are applied and lifestyle habits, maternal prepregnancy nutritional status, and genetic variation in metabolic activity are taken into account.

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