Early determinants of development: a lipid perspective[1–4]

Susan E Carlson

ABSTRACT

This article results from an International Life Sciences Institute workshop on early nutritional determinants of health and development. The presentation on lipids focused mainly on the longer-chain products of the essential fatty acids, particularly docosahexaenoic acid (22:6n–3), and cognitive development as among the most studied lipids and outcomes, respectively, in early human nutrition. Because there have been several recent reviews on this topic, the present review takes a broader perspective with respect to both early development and lipids: an expanded research agenda is plausible on the basis of observations from some human studies and from animal studies. Other lipids known to be provided in variable amounts to infants through human milk are cholesterol and gangliosides. Short sections address the current state of knowledge and some questions that could be pursued. *Am J Clin Nutr* 2009;89(suppl):1523S–9S.

INTRODUCTION

Humans have enzymes to synthesize choline and lipids from metabolic or nutrient precursors. Consequently, the requirements for choline and lipids, including the longer-chain products of the essential polyunsaturated fatty acids (LCPUFAs), may be influenced not only by individual differences in biosynthesis but also by physiologic conditions that increase their requirement, eg, during early development when tissue growth and development are accelerated. Synthesis of LCPUFAs in humans indeed appears variable and has been related to single nucleotide polymorphisms in the human genome for enzymes that are required for their synthesis (1, 2). A similar influence of single nucleotide polymorphisms influences choline synthesis and, likely, the requirement for this process (3). The period of intrauterine and postnatal nutrition when the brain is growing and developing rapidly is important with regard to the need for both choline (3) and LCPUFAs.

This review has been expanded from the workshop presentation to include other lipids that have been less studied but that are plausibly important during early development, ie, cholesterol and gangliosides. These compounds, like docosahexaenoic acid (DHA; 22:6n–3) and arachidonic acid (AA; 20:4n–6), are in human milk and accumulate rapidly in the brain during the same early stage of development. Unlike DHA and AA, these other lipids have not been added to infant formulas in the United States, which still provide only trace amounts of cholesterol and gangliosides during key periods of brain development. Some animal studies have explored the effects of feeding cholesterol and gangliosides [or the sialic acid, *N*-acetylneuraminic acid (Neu5Ac), which is a component of all gangliosides] during early development, and the results of these studies suggest that it is plausible that they can influence brain development as well as development of other systems. However, human trials with cholesterol and gangliosides (or Neu5Ac) contained in infant formulas have not been reported.

The high concentration of lipids in mammalian brain and their complexity is well known. Brain lipids include cholesterol, phospholipids, cerebrosides, sulfatides, and gangliosides, and all increase during development. The fatty acid pattern of phospholipids also changes during fetal and early neonatal life (4, 5). The specificity of these lipids and their patterns of accumulation during development contribute to the direct and permissive roles that these lipid compounds play in signal transduction in the brain (6).

Human milk, like all mammalian milk, provides a large proportion of energy in the form of triglycerides (≈50% energy) as well as cholesterol and the same complex lipids that accumulate in the newborn brain. As we learn more about the important roles of these compounds, it becomes plausible to suggest that interindividual differences in human milk lipid composition, and the much larger differences in lipid composition between human milk and infant formula, could influence early brain development by influencing the composition of the brain during the time that neuronal cell lineage is developing (eg, the programming of the neuronal cell response to one or more neurotransmitters) and myelination is occurring at a rapid rate.

DOCOSAHEXAENOIC ACID

Although the International Life Sciences Institute workshop focused on the cognitive effects of DHA, it is important to keep in

[1] From the Department of Dietetics and Nutrition, the University of Kansas Medical Center, Kansas City, KS.

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[4] Reprints not available. Address correspondence to SE Carlson, Department of Dietetics and Nutrition, MS 4013, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. E-mail: scarlson@kumc.edu.

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mind that cognitive development is only one functional domain of interest in relation to brain development. Functional outcomes chosen to reflect sensory, motivational, and motor outcomes are also potentially of interest. In fact, visual acuity development is the most studied developmental outcome in clinical trials of infants fed formulas containing DHA; and some studies of motor development have also been done after altering DHA and AA intake in early development.

The choice of age-appropriate measures that specifically target developmental processes that underlie cognition must be emphasized. A lack of understanding of the outcomes in infancy that best reflect later cognition has hampered not only the quality of work done by nutrition scientists attempting such investigations but also the interpretation of the work that has been done by others. Specific measures of attention and memory are viewed within the discipline of child development as more valid assessments of early cognition (7, 8), and these are taken into account by reviewers familiar with child development (9–11). Specific measures are in contrast to global assessments such as the Bayley Scales of Infant Development, the Bayley Mental Developmental Index (MDI), and the Psychomotor Developmental Index. It is ironic that global tests, considered less valid for assessing development, are the only validated assessments available, and, as such, they are administered almost exclusively by scientists without a background in infant development. Reviews that compare studies that use targeted measures in their analysis have been more positive for the potential benefits of DHA for cognition and precognition (9–11) compared with systematic reviews and meta-analyses that include only global measures of development (12–14).

The earliest randomized clinical trials compared very preterm infants ($\leq$28 wk gestation) who were fed formulas with and without added DHA and focused on visual acuity and attention (a measure associated with later cognitive function) in infancy (15–18). Because the last intrauterine trimester is when most fetal brain DHA accumulation occurs, it is not particularly surprising that studies of preterm infants (except one) have shown some benefit of DHA supplementation. Clandinin et al (19) reported that global test scores of development (Bayley MDI and the Psychomotor Developmental Index) were increased in preterm infants fed formulas with 0.3% DHA. A recent report did not find any benefit from formulas with 1% total fatty acids as DHA compared with formulas containing 0.2–0.3% total fatty acids as DHA; however, the subgroup with a birth weight $<$1250 g and female (but not male) preterm infants overall did have higher Bayley MDI scores when fed the formula with the higher, compared with the lower, DHA concentration (20). These studies may have implications for improving maternal DHA nutrition in the United States because the milk of US women has been reported to contain $<$0.3% DHA (*see* reference 21). Two recent publications recommend DHA in the maternal or infant diet (22, 23).

Most studies of postnatal DHA supplementation have evaluated term infants. Of these, most also measured visual acuity, and about half of the published studies that measured visual acuity observed a benefit at some age in infancy or toddlerhood (24, 25). Most of the positive studies come from one group that has shown the benefits of feeding DHA to 12 mo with visually evoked potential acuity (26). In addition, they have grouped several of their studies of different durations of supplementation of LCPUFAs and showed the benefits of a longer duration of feeding of LCPUFAs on visual acuity in infancy (27). A smaller number of studies have measured early or later cognitive function; these studies were about equally divided between global assessments and more targeted assessments that reflect attention (eg, novelty preference, duration of looking, distractibility) or memory (eg, problem solving, A-not-B–type tasks). One study in term infants showed higher Bayley MDI scores at 18 mo (28); however, a pooled meta-analysis, including that trial and 2 other trials that assessed the MDI at 18 mo, did not find any statistically significant differences in MDI scores between the LCPUFA and control groups (12).

As noted above, the systematic reviews have focused on global developmental outcomes and have concluded that there are no benefits from providing DHA to term or preterm infants (12–14). When term infant studies are compared for visual acuity and precognitive outcomes, the studies that showed benefits have some common aspects of design: for example, they are more likely to measure electrophysiologic outcomes, provide greater amounts of DHA in the formula, or use specific rather than global measures to assess cognitive development (8, 29). The optimal dose of DHA for term infants is not known. The first dose-response study of DHA in term infants has been completed, and a preliminary report was made this year (30). The study will likely provide much new information because the children are being followed for development until school age.

As implied above, studies in term and preterm infants have focused attention on the variability in maternal DHA status during pregnancy and lactation, particularly as maternal DHA intakes are related to DHA status at birth and during consumption of human milk in infancy (31, 32). US DHA intake is low relative to most other countries in the world. The body of literature on LCPUFAs and development has been reviewed recently from the perspective of maternal DHA status during pregnancy and lactation and the potential contribution of this variability to the developmental outcomes of the infant or child (21). Most of the data are from observational studies, but a few experimental studies exist. A brief summary of the results of these studies follows.

Under the best dietary circumstances, DHA accumulates rapidly during the last trimester of pregnancy and the first 2 y of life and is shown in high concentrations in neuronal membranes. Animal models link reduced brain DHA accumulation during development to a number of less-than-optimal behaviors (*see* reference 33). These behaviors include lower visual acuity (34), changes in attention that suggest slower brain maturation and slower processing (35), higher impulsivity and increased reactivity and stereotyped behavior (36), as well as alterations in cortical dopaminergic, serotonergic, cholinergic, and γ-aminobutyric acidergic systems (37–44). Animal studies suggest faster learning with higher brain DHA; support a role for DHA in the rate at which information is acquired and the efficiency of storage; and link DHA to synaptic transmission and myelination, a factor in the speed of brain processes. And DHA has been linked to hippocampal long-term potentiation, a factor in memory, which is also linked to speed of processing (*see* reference 8).

Studies in rodents show the irreversible effects of low brain DHA on neurotransmitter-related behaviors. Even modest decreases in brain DHA produce changes in neurotransmitter systems and cortical electrophysiology that are not reversible after early development (41–44). These studies raise the possibility that there

could be similar critical windows for the development of neurotransmitter systems in human fetal/neonatal life.

Five randomized clinical studies have reported benefits of maternal DHA supplementation during pregnancy or lactation (45–50) with findings that included higher cognitive function, hand-eye coordination, visual acuity, and in vitro measures of immune function. Several other experimental studies did not find any benefit of supplementation and yet showed a relation between some measure of higher maternal or infant DHA status and development (51–53), which suggests that the doses provided may have been insufficient or that the inherent variability of DHA status within the population was too great to show a clear benefit of a fixed supplemental increase in DHA intake.

The small number of clinical trials is in contrast to a fair number of observational studies that provide evidence that early programming of some electrophysiologic and behavioral responses occurs in infants/children exposed to higher amounts of DHA during key periods of brain DHA accumulation. Among the observational studies linking maternal DHA status during gestation to benefits for infant and child outcomes, we showed faster processing at 4 mo of age in relation to higher maternal DHA status at the time that the infants were born (54). At 18 mo of age, the children whose mothers had higher-compared-with-lower DHA status showed lower distractibility when provided a target task and presented intermittently with a distracting event in the periphery (54) and more total looking and fewer episodes of inattention in multiple-object free play (55), which was attributed to differences in intrauterine exposure to DHA. Strengthening the suggestion that these findings were related to intrauterine DHA exposure, it should be noted that only 4 of the 70 infants in our study received any human milk and the formula-fed infants consumed only formulas without DHA (unavailable in the United States before 2002).

Other observational studies showed more mature sleep behavior in relation to higher maternal DHA status at the time of birth (56), higher visual acuity and discrimination of native from foreign language sounds at 9 mo of age (57), and vocabulary production at 18 mo of age (58) related to infant red blood cell phospholipid DHA concentration at 2 mo of age. Given that differences in red blood cell phospholipid DHA were related to maternal milk DHA (58), this could reflect either higher intrauterine or higher postnatal DHA exposure or both.

A number of reports representing several different cohorts of children now associate higher cognitive, stereoacuity, or visual acuity in children with higher maternal fish intake, ocean fish being one of the better food sources of DHA. Studies from the Avon Longitudinal Study of Pregnancy and Childhood cohort in the United Kingdom showed higher stereoacuity in children at 3.5 y of age related to higher maternal seafood intake during pregnancy (59), and a recent report using other children from this cohort showed advantages for cognitive function in those children whose mothers consumed >340 g (12 oz) of seafood per week (60), the suggested upper limit for seafood intake during pregnancy and lactation (61, 62). Another cohort of infants monitored by Oken et al (63) in the United States (Boston, MA) also showed evidence of higher cognitive function as children in relation to maternal seafood consumption during pregnancy. A 2008 report from the Danish National Birth Cohort, also led by Oken (64), monitored the development of 25,446 children. The odds ratio for higher overall development at 18 mo was 1.29 in children whose mothers' fish consumption was in the highest compared with the lowest quintile. Use of human milk feeding for >10 mo compared with <1 mo was also a predictor of higher overall development in this cohort but was independent of fish intake.

Differences in fish intake may also be the factor that accounts for much of the variability in maternal DHA status among fish-eating populations. Higher visual acuity and novelty preference were observed in relation to higher cord blood DHA in a traditional high fish-eating group in Canada (65), and Bakker et al (66) showed higher total and qualitative scores for movement on the Maastricht Motor Test at 7 y of age in relation to higher cord blood DHA.

Finally, several studies in which women were supplemented with DHA during pregnancy or lactation did not find an effect of DHA supplementation on infant development (31, 32, 51–53). However, 3 of those studies showed higher visually evoked potential acuity and more mature scotopic electroretinograms and visual acuity (51, 52), more optimal electroencephalogram responses at 2 mo of age (31), or higher visual acuity (53) in infancy in relation to higher DHA status. In 2 of these studies (31, 32), cognitive benefits of maternal DHA supplementation were seen at 4 or 5 y of age, respectively (45, 46), which suggests that much of the research on development after LCPUFA supplementation may have targeted an age for study that was too young. Another cohort from an early multicenter safety study of DHA-supplemented infant formula without early developmental assessment also had lower blood pressure at 6 y of age (67). Children from Helland's study of fish oil (DHA and eicosapentaenoic acid) supplementation during pregnancy (31, 45) were studied at 7 y of age. The authors did not find an effect of early fish oil supplementation on IQ but did find a correlation between maternal DHA status during pregnancy and higher sequential processing, a cognitive outcome (68).

CHOLESTEROL

Human milk provides between 10 and 20 mg cholesterol/dL, whereas US infant formulas contain <10% of this amount of cholesterol. The higher cholesterol intakes of human milk–fed compared with formula-fed infants has been shown to result in higher plasma cholesterol compared with infants fed formulas without DHA (69, 70). The differences in cholesterol intake between nonhuman primate infants fed variable amounts of cholesterol have been studied from the perspective of the possible effects of early cholesterol intake on programming of circulating cholesterol in lipoproteins (*see* reference 71). A recently published systematic review evaluated 17 published studies with 17,498 subjects and concluded that initial breastfeeding was associated with lower blood total cholesterol concentration later in life compared with formula feeding (72).

A perhaps more plausible (and also untested) hypothesis is that cholesterol in human milk may be important for human brain development. Cholesterol is a major component of the brain, accounting for 2–3% by weight and 20–30% of all lipids in the brain (73). Because the brain is ≈75% water, the contribution of cholesterol (and lipids in general) to the dry weight of the brain is obviously great. Cholesterol also accumulates rapidly during

brain development (74), increasing substantially during myelination (75). The neuronal requirement for cholesterol for synaptogenesis and function is recognized from the results of cultured neurons provided with cholesterol from glial cells (76).

Early studies in newborn rats by Dobbing (77) and Jurevics and Morrell (78) provided evidence that exogenous cholesterol was incorporated into the developing brain; however, later work did not support the hypothesis that the developing brain requires exogenous cholesterol such that as supplied by mammalian milk. There is general agreement on the basis of studies in rodents that the developing brain is capable of synthesizing all of the cholesterol needed because studies have shown that little cholesterol in the brain comes from exogenous sources (79, 80). Additionally, Turley et al (81) reported that newly synthesized cholesterol is the major source of brain cholesterol for neonatal lambs. Neurons in cell culture have been shown to acquire exogenous cholesterol by uptake from lipoproteins, and some studies showed increased concentrations of neuronal cholesterol. However, the only suggestion that brain cholesterol uptake could occur from lipoproteins in vivo is related to the regional distribution of lipoprotein receptor expression in the central nervous system (*see* reference 73).

Studies of cholesterol feeding complicate the story further. Neonatal pigs fed diets with 0.5% cholesterol by weight for 4 wk showed an increase in cerebrum weight and cerebrum cholesterol concentration that was observed when the animals were young adults (20–24 wk of age) (82). Similarly, rats raised by dams fed cholesterol and themselves fed diets with 0.5% cholesterol by weight after weaning had significantly more cholesterol and protein per unit weight of brain cortex at 32 d of age compared with rats exposed to cholesterol through the dam or diet (83). Haque and Mozaffar (84) reported that quite high intakes of cholesterol by the dam (1% and 5% of the diet by weight) increased myelin cholesterol in their pups. And Schoknecht et al (85) gave a milk replacer with added cholesterol (200 mg/100 g diet) for 4 wk to pigs and increased cerebral cholesterol among both groups selected for low serum cholesterol and high serum cholesterol. However, only the group selected for low serum cholesterol showed increased exploratory behavior when provided cholesterol in the diet.

It is not possible at this time to reconcile the majority of the studies that find little if any evidence of a discrepancy between cholesterol accumulation and synthesis in the brain with the handful of studies showing that cholesterol feeding in the diet can increase brain cholesterol and/or myelin cholesterol. The human brain is also considerably larger and more sophisticated than that of the other species used as models for study. A comparison of the cholesterol content in autopsy material from human milk–fed and formula-fed infants might prove interesting if it were shown that the 2 groups had different cortical cholesterol content. Analogous studies of cortical DHA (86, 87) and ganglioside sialic acid (88) have lent support to the idea that these components in human milk increase their content in the brain. Now that we have more sophisticated ways of evaluating the electrophysiologic function of the brain and more targeted measures of brain function that can be used in developmental studies, it may be time to conduct clinical studies of formulas with and without cholesterol in the range found in human milk to attempt to determine whether the presence of cholesterol in human milk has a role in the developing brain.

GANGLIOSIDES

Compared with DHA and cholesterol, there are even fewer studies of the role of gangliosides in early diet and development. A few studies have been conducted in animal models, and they provide some evidence that these components (and their metabolic substrate, the sialic acid *N*-acetylneuraminic acid, or Neu5Ac) in human milk could serve as structural components of tissues during development. A short summary of this literature is included here mainly to indicate that intake of these lipids does vary in early life depending on the choice of feeding (human milk or formula). This introduction may lead to more research on this topic.

Human milk does contain gangliosides, and recent work from Clandinin et al (89) suggests that gangliosides could contribute to ganglioside sialic acid content in both the intestine and brain during development. Other recent animal studies have shown the potential for cow-milk–derived glycomacropeptide as a source of sialic acid that increases sialydation in brain glycoproteins and positively influences an outcome analogous to cognition in animal models (90). New data from our laboratory with glycomacropeptide have confirmed these and earlier findings in rodents that indicate that dietary sialic acid intake can increase sialic acid in brain gangliosides during brain development (83).

Morgan and Winick (91) were the first to show that exogenous sialic acid (Neu5Ac) could increase sialic acid concentration in gangliosides and glycoproteins of the brain and improve learning; however, they administered Neu5Ac by intraperitoneal injection. Shortly after their study, we reported high concentrations of sialic acids in human milk oligosaccharides and glyoproteins but low concentrations in infant formulas (92) during the interval of brain development when sialic acid content of gangliosides and glycoproteins increases in the brain (93). Subsequently, we showed that oral Neu5Ac administration was as effective as intraperitoneal administration in increasing the sialic acid content of brain gangliosides and glycoproteins (94). At that time, dietary sources of sialic acid such as cow-milk–derived glycomacropeptide were unavailable for testing the idea that increased sialic acid intake may enhance infant development.

Meanwhile, researchers have reported significantly higher sialic acid in the frontal cortex gangliosides and glycoproteins of human milk–fed compared with formula-fed infants who died in the first year of life (88). Varki et al (95, 96) discovered that a mutation in cytidine monophosphate–sialic acid hydroxylase resulted in an increase in Neu5Ac and a loss in *N*-glycolylneuraminic acid (Neu5Gc) in humans relative to the great apes, that all normal humans studied have antibodies to Neu5Gc, and that humans can incorporate Neu5Gc into tissues. Clinical trials to test the idea that gangliosides or sialic acid may benefit infant development may now be feasible, keeping in mind that sources low or lacking in Neu5Gc would be preferred for such studies.

SUMMARY

The human brain increases dramatically in size and complexity in the first year of life, and some of the lipid components that increase the most are provided by a diet of human milk, specifically LCPUFAs, cholesterol, and gangliosides. Human milk also contains oligosaccharides and glycoproteins with large amounts of Neu5Ac, a key component of brain gangliosides and

glycoproteins, which accumulates in this same period of rapid brain development. Numerous clinical studies have been done to compare the development of infants fed formulas with and without LCPUFAs; other comparisons are available from infants whose mothers were assigned to consume some amount of DHA during pregnancy and/or lactation. Although DHA and ARA were added to US formulas in 2002, work on LCPUFAs continues. The first dose-response studies in preterm and term infants, respectively, were just reported in preliminary form (20, 30). Both reports add to the available evidence that dietary LCPUFAs can optimize the development of human infants.

Like with LCPUFAs, humans have pathways to synthesize cholesterol and gangliosides for the brain and other tissues. Both cholesterol and gangliosides are found in trace amounts in US formulas. Clinical studies have not explored whether preformed dietary sources of these compounds, such as exist in human milk, can make a contribution during early development. In the cases of both cholesterol and gangliosides, some animal work suggests that the idea is plausible. (Other articles in this supplement to the Journal include references 3 and 97–103.)

The author has spoken on the role of DHA for infant and child development and the effect of DHA intake on maternal DHA status for companies that make infant formulas containing DHA and supplements for pregnant women. She is the principal investigator on NIH grant R01 HD047315 to study the effects of maternal DHA supplementation on pregnancy duration and infant/toddler development. The author also has had funding from Mead Johnson Nutritionals to study the effects of cholesterol and sialic acid in animals.

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