Docosahexaenoic acid supplementation in pregnancy and lactation

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ABSTRACT
The goal of the Experimental Biology symposium on maternal supplementation was to review all available lines of evidence, delineate unanswered questions, and develop, if it seemed reasonable, a research agenda to determine whether maternal supplementation with specific nutrients might be beneficial. In the case of maternal docosahexaenoic acid (DHA) status, the topic addressed in this article, few clinical studies show benefits of maternal DHA supplementation during pregnancy or lactation for the infant or child. However, quite a large number of observational studies link higher intrauterine DHA exposure to a number of positive developmental outcomes. This article reviews the factors known to contribute to DHA status of women and their offspring during the reproductive cycle, relates maternal DHA status to that of the developing fetus and newborn, and reviews the evidence for functional differences in behavior related to DHA status, including the available evidence related to DHA supplementation of women pregnant and lactating and their offspring. Other outcomes for infants and children and for women themselves appear plausible and are also addressed as part of a research agenda for future work. Am J Clin Nutr 2009;89(suppl):678S–84S.

INTRODUCTION
Docosahexaenoic acid (DHA; 22:6n–3) is the major n–3 fatty acid in the central nervous system and retina (1, 2). The most rapid rates of brain DHA accumulation occur during the last intrauterine trimester and the first year of life. These stages of development may be influenced directly by the nutritional status of the mother if the fetus remains in utero to term and the infant is subsequently breastfed for the first year of life, as recommended by the American Academy of Pediatrics (3). Thus, the mother’s DHA status may play a role in the DHA status of her offspring. The body of literature on the effects of DHA supplements on neurodevelopmental outcomes in infants adds to the plausibility that maternal DHA status is important (4–6).

Direct evidence for the importance of DHA to human development comes from studies in animals, particularly nonhuman primates. In these studies, the normally high DHA accumulation that occurs during mammalian brain development was decreased by feeding diets with a high ratio of linoleic acid (18:2n–6) to ω-linolenic acid (18:3n–3), and less mature development of visual acuity and attention was observed (7, 8). More recent animal studies provide evidence that early DHA exposure influences neurotransmitter systems, including dopamine, serotonin, choline, and γ-amino butyric acid (9–16) and may program development of dopaminergic and serotonergic neurotransmitter systems (14–16). Although there are no similar studies of early programming of neurotransmitter function in humans, it is reasonable to suggest that there is also a similar critical window of development for accumulation of DHA during human brain development.

Unlike most animal studies, in which dietary ω-linolenic acid intake was dramatically decreased to reduce brain DHA, studies of human infants have mainly investigated the role of preformed DHA compared with its precursor, ω-linolenic acid. The outcomes measured in the developing infant and child have focused mainly on those that could be related to low brain DHA accumulation during development in animal studies, ie, visual acuity development and evidence of effects on learning or cognition. In the course of those studies, it became clear from reference groups of pregnant and lactating women that large differences in maternal DHA status exist both within and among cultures. These differences have the potential to provide different amounts of DHA to an individual fetus or infant during pregnancy and lactation, respectively. Studies of infant supplementation and of pregnant and lactating women have contributed to the plausibility that differences in DHA status within the population may result in functional differences in development that result from the maternal DHA status during pregnancy and lactation. Ultimately, the need for dietary supplements of DHA will rest on the answer to this question.

ANIMAL STUDIES LINKING CENTRAL NERVOUS SYSTEM DHA ACCUMULATION TO BEHAVIOR
A comprehensive early review on the subject of essential fatty acid and behavior in animals came from Wainwright (17). McCann and Ames (5) have discussed the potential for cognitive benefits of increasing brain DHA based on their analysis of the (mainly) animal literature. Among the behaviors observed in nonhuman primates with lower brain DHA accumulation compared with those with normal DHA accumulation are altered

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electroretinogram (ERG) responses and lower visual acuity (7), changes in attention that suggest slower brain maturation (8), and higher frequency of stereotyped behavior and higher locomotor activity that are evidence of behavioral reactivity (18). The study of Levant et al (14) provides evidence that changes in behavior may be related to altered dopaminergic function, and the study established the principle that factors underlying the programming of neurotransmitter systems could influence behavior well after infancy.

Evidence continues to increase that there are critical windows for development of these neurotransmitter systems, because even modest decreases in brain DHA cause effects on dopaminergic and serotonergic systems that are not reversible after weaning in the rat (14–16). Loss of inhibition of auditory cortical electrophysiology has also been observed and linked to altered \( \gamma \)-amino butyric acidergic function in response to lower cortical DHA accumulation, which likewise is not remedied by increasing DHA in the brain cortex to normal after weaning (13). These animal studies of cortical electrophysiology and neurotransmitter systems raise the possibility that there could be similar critical windows for development of neurotransmitter systems in human fetal or neonatal life. The animal studies suggest the need for timeliness in DHA accumulation, and they support the need for experimental studies to determine whether maternal DHA supplementation can enhance infant and child development.

**ACCUMULATION OF DHA DURING DEVELOPMENT**

It is relevant to the discussion of the need for DHA supplementation during pregnancy and lactation to consider when DHA accumulates in brain. Clandinin et al (19) observed that human brain DHA content was exceedingly low at the beginning of the third trimester of pregnancy, but that it accumulated rapidly during the third trimester as well as after birth (20). Although the early reports were based on a relative small number of brain analyses, the results were confirmed by Martinez (21) that included many more samples of known gestational or postnatal age. Those reports suggested that brain DHA accumulation might be limited by DHA availability during gestation and after birth. This suggestion was strengthened by the first studies showing lower red blood cell (RBC) phospholipid DHA in infants fed formulas without DHA than with human milk (22, 23). Still later, analysis of brain samples obtained at autopsy showed an increase in cortical DHA with duration of human milk feeding (all human milk contains DHA) that was not observed in infants fed formula without DHA (24, 25). Relevant to this review is the work by Clandinin et al (19, 20), suggesting that differences in maternal DHA status could affect variability in DHA accumulation during intrauterine and postnatal life.

**DHA STATUS OF WOMEN WITH PARTICULAR REFERENCE TO WOMEN IN THE UNITED STATES**

Maternal RBC, plasma, and milk DHA phospholipids (either total phospholipid or \( \geq 1 \) individual phospholipid classes) (26, 27) increase with DHA supplementation (28, 29). This supports the use of these measurements as indicators of maternal DHA status. In observational studies, in which the assumption may be made that DHA intakes are relatively constant over time, \( \geq 1 \) of these indicators may be used to estimate usual DHA status and to suggest the amount of fetal and postnatal DHA exposure.

Worldwide, the range in milk DHA content is large, especially when compared with milk arachidonic acid, the main 20–22-carbon \( \omega-6 \) fatty acid in human milk (30). The range in DHA is thought to reflect mainly the variations in maternal DHA intake, because populations with high fish intakes also have the highest milk DHA content. As is well known, a number of ocean fish are excellent sources of dietary DHA, and the amount or frequency of intake from these fish is associated with higher maternal DHA status (31, 32). The highest reported DHA content in milk was 2.8% of total fatty acids among Chinese women consuming a combined mean intake of 4.5 oz/d of fish and shellfish (33). However, even vegan vegetarians, who consume no animal products and therefore no DHA, have a milk DHA content of 0.05% of total fatty acids (34). Accordingly, there is an \( \approx 60 \)-fold difference in the mean milk DHA (percentage of total fatty acids) among groups of women consuming chosen diets. In US women, milk DHA has been reported to be \(< 0.2\% \) of total fatty acids (23, 30). The concentrations are low compared with most groups of European and Asian women with higher intakes of ocean fish. Milk DHA content has been associated with favorable infant outcomes (35).

Maternal RBC phospholipid DHA correlates with DHA in human milk (28); however, compared with human milk, maternal RBC DHA is much less variable among populations. Despite the smaller variability compared with human milk, several studies have associated higher RBC or plasma DHA in the mother (36–38) or infant cord blood (27, 39–41) with favorable outcomes in infants or children. This suggests that both higher maternal and cord blood DHA reflect the relative amount of DHA transferred to the fetus and incorporated into fetal brain or other tissues during intrauterine life.

RBC DHA is influenced by a number of physiologic variables, including pregnancy and lactation. In addition to lower RBC DHA associated with DHA transfer from mother to infant during the last half of pregnancy (26, 31, 42), increases in RBC DHA of \( \approx 50\% \) occur during the first part of pregnancy (31). Birth order and the length of the interval between pregnancies also influence RBC DHA (43, 44). The increases observed in the first trimester may serve to enhance the amount of DHA available for transfer to the fetus during intrauterine life. The maternal RBC DHA returns to a level somewhat lower than before pregnancy within a few weeks after delivery of the infant (42), but this can be mitigated by increasing DHA intake during pregnancy (26).

One area that has received little attention is the variability in RBC phospholipid DHA within groups consuming little or no DHA. The variability appears to be real and stable for persons consuming their usual diet. In a low-DHA–consuming population of US pregnant women, \( \approx 85\% \) of the variance in DHA content of maternal RBCs at the time of delivery was accounted for by DHA content of RBC DHA at \( \approx 20 \) wk gestation (45). Preterm infants fed diets without DHA for long periods of time also show an inherent variability in circulating arachidonic acid and DHA (46). The variability in RBC arachidonic acid and DHA suggest variable maternal biosynthesis or intake, maternal to fetal transfer, variability in status of nutrients required for DHA synthesis, or genetic differences in the ability to synthesize DHA. Burdge and Wootton (47) reported that women converted about twice as much \( \omega-3 \) linolenic acid to DHA than in their similar study of men and women, milk DHA has been reported to be \(< 0.2\% \) of total fatty acids (23, 30). The concentrations are low compared with most groups of European and Asian women with higher intakes of ocean fish. Milk DHA content has been associated with favorable infant outcomes (35).

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noted that women taking a contraceptive with estrogen had more than twice as much conversion as did nonpill users. They suggested that estrogen levels during pregnancy may be responsible for the increase noted during pregnancy in an international comparative study (31). Recently, different alleles for the fatty acid desaturases required for D-5 and D-6 desaturation of fatty acids were linked to the apparent ability to derive cognitive benefit from breastfeeding compared with formula feeding without supplemental DHA (48).

**DHA AND INFANT-CHILD BEHAVIOR**

Most experimental studies of DHA and infant development have involved infants supplemented with DHA by the addition of various amounts and sources of DHA to infant formula. A few studies provided DHA to pregnant or lactating women as a means of increasing DHA exposure during the early stages of brain development and for the purpose of studying such exposure on infant or child behavior.

The outcomes and general conclusions from the studies of infant DHA supplementation support the tenet that developmental outcomes are less mature in children who do not receive supplemental DHA (49, 50). Put another way, there is considerable (although not universal) support from experimental studies for the idea that DHA is a conditionally essential nutrient for the developing infant.

**VISUAL ACUITY**

In the infant clinical studies, visual acuity development is the most frequently studied outcome determined in response to improved infant DHA status, and the results of those studies are mixed. Infants born significantly before term and provided a formula with DHA were shown to have higher visual acuity [51, 52; reviewed in Gibson and Makrides (53) and Cheatham et al (54)]. In term infants provided a formula with DHA, only about half of the studies show higher visual acuity compared with the control group [for reviews, see Morale et al (50), Gibson and Makrides (53), and Cheatham et al (54)].

Visual acuity or ERGs or both have been measured in term infants whose mothers were supplemented with DHA during pregnancy (39, 55, 56) or during supplementation of their lactating mothers (28, 29). Except for the findings by Judge et al (55), no benefits for visual acuity or ERG function were found with maternal DHA supplementation. Although Malcolm et al (39, 56) did not find a benefit of maternal DHA supplementation, they did report significantly higher visual evoked potential acuity and more mature scotopic ERGs associated with higher cord blood DHA status. It was suggested that uncontrolled modifiers of maternal and infant DHA status may influence the ability to observe effects of maternal DHA supplementation in clinical trials (57, 58).

A number of variables among the studies of visual acuity in term infants could underlie the mixed results in experimental studies of DHA supplementation (or increased exposure through human milk), including the amount of DHA provided, the sources of the DHA supplement, the antecedent DHA intake of the population studied (as noted above, there are large cultural differences in DHA intake), and even the sensitivity of the procedures used to measure visual acuity, eg, electrophysiologic measures such as visual evoked potential acuity or subjective measures such as the Teller Acuity Card procedure (59). Studies most likely to show benefits for visual acuity tended to have subjects born before term, provided higher levels of DHA supplementation, used more sensitive (electrophysiologic compared with subjective) measures of visual acuity, or had one or more of these criteria.

**COGNITIVE FUNCTION**

A smaller number of experimental studies have attempted to study the effects of DHA status during development on cognitive function. Results that came from supplementation trials in term and preterm infants were discussed in a review from our laboratory (49) and are not included here. The point to make is that evidence from that body of literature shows that DHA supplementation can influence specific measures of early cognitive function.

A number of studies link higher maternal DHA status or intake to more mature or favorable behaviors in infants and children. Those observational studies and the few experimental studies that exist are reviewed here because they provide the basis for the suggestion that increasing maternal DHA status might enhance these behaviors in infants and children. They cannot be used to conclude, as they frequently have, that maternal DHA supplementation is needed.

Worldwide, DHA intakes are likely the greatest variable in predicting maternal DHA status, but, as noted earlier, there are differences in DHA status that cannot be attributed directly to DHA intake and that may be due to genetic differences in bio-synthesis or other nutrient intake that explain these differences in populations with low DHA intakes. Of potential relevance, most observational studies that show a positive association between DHA status and more mature infant outcomes were conducted in countries with low (or some segment of the population with low) DHA intakes or status, including the United States (37–39, 56, 58).

Results of several observational reports associate accelerated or more mature attentional functions in the first year of life with higher maternal DHA status during pregnancy. For example, duration of looking decreases dramatically between 4 and 8 mo of age, and relatively shorter duration looking at 4 mo of age is associated with higher performance on the Bayley Scales of Mental Development at 18 mo of age (60). Attention is considered an early representation of cognitive development, given that attention in infancy and toddlerhood link to preschool and school-age measures of cognitive function. In a study of 70 infants and children followed from 4 to 18 mo of age, those whose mothers had a DHA status (RBC phospholipid DHA) below the median after birth of their infants compared with those above the median had longer look duration in response to a visual stimulus at 4 and 6 mo of age and lower distractibility at 18 mo of age (36). Longer look duration early in the first year of life has been associated with slower processing and poorer performance on the Bayley Scales of Mental Development at 18 mo of age (60). Overall, the pattern of looking in children of mothers with higher DHA status was similar to that previously associated with accelerated development, ie, shorter look duration early in infancy and longer look duration at 12 and 18 mo of age (36).

Maternal RBC DHA was also the indicator of maternal DHA status used by Willatts et al (37), who found more mature visual
attention at 4 mo of age in children whose mothers had higher DHA status. In both the Colombo et al (36) and Willatts et al (37) studies, early shorter peak look duration is interpreted as an indicator of faster processing of information, an early indicator related to later cognitive ability. Cheruku et al (38) reported more mature sleep behavior in US newborns, once again linked to maternal RBC DHA at the time of delivery. In addition, Helland et al (27) found an association between cord blood DHA and more mature electroencephalography function shortly after birth in Norwegian women supplemented with DHA.

Other indicators of maternal DHA status or fetal DHA exposure were also used and shown to be related to infant development. Jacobson et al (41) reported that higher cord blood DHA was associated with longer gestation and higher visual acuity, novelty preference (an indicator of recognition memory), and 11-mo Bayley Mental and Psychomotor Developmental Indexes. Innis et al (35) found higher maternal milk DHA was associated with higher visual acuity and discrimination of native from foreign sounds, the latter a possible indication of auditory novelty recognition. Bakker et al (40) reported a positive association between newborn DHA status and motor function at 7 y of age in a cohort of 306 children born at term: both total and qualitative scores for movement assessed with the Maastricht Motor Test were associated with higher newborn DHA status.

Observational studies link more mature infant or child development to reported intake of maternal seafood during pregnancy, presumed to increase the DHA status of the mother and fetus. Among these reports, higher seafood intake during pregnancy was associated with higher stereoaucuity in the offspring at 4 mo of lactation (28). This cohort of children was studied again at 7 y of age and is the subject of a report to be published in Pediatrics later this month (66). Like the US study led by Jensen et al (28), the Norwegian study led by Helland et al (27) found no effects of maternal DHA supplementation on early measures of infant development. The absence of observed developmental benefits in the first year of life was not surprising, given that the control groups were also breastfed in both of these studies. The fact that cognitive benefits were found in the experimental groups of both studies when the children reached preschool ages suggests that measures used in infancy were not ideal or that benefits of early DHA exposure were not observable until cognitive function became more complex (49).

The importance of continuing to follow children exposed experimentally to higher DHA during fetal and postnatal life to preschool or school age, when more complex measures of cognitive function can be assessed, is addressed in detail in a review (49). Of course, not all studies of development can be expected to have adequate statistical power for following development to school age. Ideally, studies should be planned that take into account the expected loss in subjects that inevitably occurs with time. Dunstan et al (67) also provided a rather large amount of DHA to women during the last half of pregnancy in the form of a fish-oil supplement that provided 2.2 g of DHA and 1.1 g of eicosapentaenoic acid (EPA) and found higher hand-eye coordination at 1 y of age in infants of women who received the supplement. Another small study that included US women provided 400 mg/d of DHA found higher visual acuity at 4 mo (but not later) and higher problem solving but not recognition memory at 9 mo of age (55, 68).

Other experimental studies of maternal DHA supplementation during pregnancy have not found benefits for the offspring but have found associations with higher maternal DHA status and some functional outcome. For example, Malcolm et al (39, 56) supplemented pregnant women with fish oil to provide ~200 mg DHA during the last 25 wk of pregnancy and saw no effect on the ERG or visual evoked potential acuity but did find an association between DHA status of the infant and more mature function.

OTHER POTENTIAL BENEFITS OF HIGHER MATERNAL DHA STATUS

Prescott et al (69) and Denburg et al (70) have published reports on what appears to be the same cohort of women with a history of atopic disease given fish oil during pregnancy as a supplement of DHA and EPA. Their findings provide some evidence for benefit to the immune function of the children of increasing maternal DHA intake; however, more studies are needed.

Numerous reports associate higher maternal intake of DHA or DHA and EPA with gestation duration and a few experimental studies. A reduction in the incidence of preterm birth would be considered an important clinical finding. At the present time, 2 high-quality studies have looked at the effects of DHA and EPA supplementation in the prevention of preterm birth. A multicenter study in Europe found lower incidence of repeat preterm birth among women who had a prior preterm birth with a supplement of 2.4 g/d of n–3 fatty acids from fish oil (71), whereas a recent
National Institutes of Health–supported US study of a similar group provided a supplement of 2 g/d did not, although women who reported consuming ≥1 fish meal/mo had an adjusted odds ratio for preterm delivery of 0.62 (72).

The potential for DHA exposure to program the developing autonomic nervous system deserves more study. Infants exposed to higher DHA in infant formula in the first 3 mo of life had lower blood pressure at 6 y of age (73).

Other than the cardiovascular benefits for adults consuming more DHA and EPA, which are now well known, the most plausible direct benefit for women in the reproductive cycle is for improved mental health. Various reports have suggested it is plausible that some women could benefit from higher DHA intake (6, 74–76), but properly powered experimental trials of outcomes such as postpartum depression are needed.

Recently, 2 groups have recommended intakes for n–3 long chain polyunsaturated fatty acids (DHA and EPA) that apply to pregnant and lactating women (32, 77). Both groups emphasize consuming seafood. The Perinatal Lipid Intake Working Group has recommended that pregnant and lactating women consume ≥200 mg/d (77). The position of the American Dietetic Association and Dietitians of Canada is that adults, not excepting pregnant and lactating women, should consume a combined intake of 500 mg/d of DHA and EPA, based on an intake of 2000 kcal/d (78). Women following the Institute of Medicine (IOM) guidance for seafood intake during pregnancy and lactation would also consume ≈200–300 mg DHA/d (32).

In one US study, African American women were reported to consume ≈68 mg/d of DHA (79). Some evidence shows that US pregnant women have decreased their DHA intake from seafood (80) following advice from the Food and Drug Administration and Environmental Protection Agency about avoiding specific sources of seafood because of their mercury content (81, 82). In the meantime, several prenatal supplements containing DHA have become available and advertised to pregnant women and their physicians. As a result, DHA intake from supplements may have increased in at least some segments of the US population of pregnant and lactating women. However, there is still a need to educate women about choice of seafood that can be consumed during pregnancy and lactation. As shown in the recent IOM Committee report Seafood: Balancing Benefits and Risks, many kinds of seafood have the potential to provide significant dietary DHA during pregnancy and are low in mercury. That IOM Committee report indicated that pregnant and lactating women could consume ≤12 oz seafood/wk so long as they avoided tilefish, swordfish, shark, and king mackerel and limited their consumption of albacore tuna to 6 oz/wk.

SUMMARY

At present, several lines of evidence suggest that US pregnant and lactating women are among the lowest consumers of DHA in the developed world. A number of observational studies and post hoc analyses of studies in which DHA was provided experimentally find an association between some indicator of maternal DHA status and infant and child behavioral outcomes. Experimental studies of the quality and quantity needed to show that DHA supplementation is important for either infant or child or mother are still few and more work is needed. At present, the studies available do suggest that DHA may be a conditionally essential nutrient for optimal infant or child outcome, at least in some populations and for some outcomes.

RESEARCH AGENDA

- Dose-response studies of maternal DHA supplementation would be helpful (the wide range used in the literature is problematic, especially given the few experimental studies available).
- Other than brain development (visual acuity, cognition, psychomotor development), what physiologic outcomes are influenced by DHA? Some apparently promising candidates for study in infants are autonomic nervous system function and immune function among others; for pregnant and lactating women themselves, studies of the effects of n–3 fatty acid intake on mental health are plausible.
- How do intake of other nutrients and population differences in alleles for the fatty acid desaturase enzymes influence the answers to these questions?
- Studies in preschool and school-age children exposed to higher DHA early in development are needed. Although these studies are labor intensive and require funding beyond the usual funding cycle, they are being done.
- Studies of toddlers supplemented with DHA are still needed to address the question of whether the DHA accumulation shown by Martinez et al (21) to continue during the time of perinatal cortical expansion and maturation is limited if the diet is low in DHA (as in US preschool children (82)).
- Properly powered studies are needed to address the question of whether maternal DHA supplementation can reduce postpartum depression.
- Studies to define the optimal period of DHA supplementation would have been helpful, but they are not likely to be done now that most US infants receive DHA after birth. Although we still do not know the optimal period of maternal DHA supplementation (ie, for the infant to benefit from maternal DHA transfer), Helland et al (65) attributed all of the benefit for 4-y IQ to prenatal DHA exposure to prenatal DHA exposure. However, there are studies, such as those of Jensen et al (28) using maternal supplementation during lactation as well as studies of term infants fed DHA-containing formulas, that show that at least some term infants can still benefit from increasing postnatal DHA exposure. (Other articles in this supplement to the Journal include references 84–88.)

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 supplements for pregnant women and formulas containing DHA for infants. She is the principal investigator on a study in progress (RO1 HD047315) to determine the effects of maternal DHA supplementation on pregnancy duration and infant-toddler development.

REFERENCES

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