Although human fetuses and neonates can desaturate and elongate the chains of 18-carbon precursors to form their respective LCPUFAs, it is uncertain whether the rate of synthesis is sufficient to meet the demands for optimal growth and development. Insufficient activity of fatty acyl desaturase has been reported in human fetuses, especially during the last 2 weeks of gestation. This information has also raised the question of whether preterm infants are at greater risk for LCPUFA insufficiency than term infants because, at birth, preterm infants are deprived of an intrauterine supply of essential fatty acids. Studies have revealed no differences between preterm and term infants in their ability to convert LA and ALA to their long-chain metabolites. Moreover, it has been suggested that LCPUFA formation may be more active at earlier gestational ages. However, this issue has not been definitively clarified.

Because the fetal membranes do not desaturate LA or ALA, a human fetus must acquire most of its fatty acids via placental transfer. Thus, the primary determinant of delivery of a fatty acid to the fetus is the concentration of that fatty acid in the maternal circulation, which is closely related to maternal intake of fatty acids. There is a 3-fold difference in the concentrations of nonesterified DHA between the dam and fetus at the fetal-maternal interface. Investigators have reported preferential transfer of DHA relative to other LCPUFAs in utero and attributed the specificity of the uptake system to a selective retention of AA in the placental tissue.

The fetal liver accumulates DHA and likely plays an integral role in the accretion of DHA in the neural tissues in utero and after birth. Postmortem examination of human fetuses of various gestational ages have revealed that a parabolic increase in DHA concentrations in the fetal liver is paralleled by an increase in acylated DHA concentrations in the fetal brain. After birth, amounts of acylated DHA in the liver and adipose tissue decrease concomitant with a continued increase of acylated DHA in the brain and retinas. Thus, adipose and liver tissues act as sinks that sequester DHA in utero to provide a reservoir of DHA during the early neonatal period.

**DHA and Neurologic Development**

In humans and other animals, brain and retinal functions are dependent on DHA during in utero development and after birth. A period of maximum brain growth in humans begins in the third trimester of gestation, peaks at birth, and continues throughout the first 18 months after birth. During this crucial period, the brain undergoes a 10-fold increase in size, and there is a selective accumulation of AA and DHA within the brain and retinas. This period has been termed the DHA accretion spurt, and accretion of AA and DHA in brain and retinal phospholipids proceeds at a rate 10 times faster than incorporation via chain-elongation and desaturation of their respective precursors, LA and ALA. Thus, both AA and DHA are rapidly incorporated into neural tissues, and high concentrations of DHA in the brain and, in particular, the retinas suggest a functional role in these tissues.

Deficiency of n-3 fatty acids during the developmental accretion phase results in decreased retinal content of DHA, which subsequently is manifested as decreased visual and neural function. Once neural development is complete, a deficiency of n-3 fatty acids does not substantially affect DHA content of the retinas. However, it is important to mention that although patients deficient in n-3 fatty acids are able to achieve concentrations of DHA typical of clinically normal patients upon replenishment of this fatty acid, the functional abnormalities resulting from early deficiencies in n-3 fatty acids are not corrected. Although species differences are likely to exist among mammals, some similarities are apparent between humans and animals.
dogs. As mentioned previously, maximal brain growth in humans begins during the third trimester of gestation and continues throughout the first few months after birth.\textsuperscript{13,14} In dogs, gross appearance and histologic changes observed in brain tissues between 1 and 60 days after birth are similar to those described for brain tissues of humans between 28 and 40 weeks of gestation.\textsuperscript{2} Thus, demands for LCPUFAs during brain development in dogs are likely to be similar to those for humans during gestation and the early neonatal period.

**Accumulation, Function, and Conservation of DHA in Neural Tissues**

The primary site of DHA accretion in neural tissues is the phospholipid fraction of brain and retinal cells.\textsuperscript{22} The ROS contains the highest concentration of DHA within the body; approximately half the fatty acids of the phospholipids in the ROS plasma membrane are DHA.\textsuperscript{23,24} The phospholipids most closely associated with the visual pigment rhodopsin, namely phosphatidyl choline and phosphatidyl serine, have greater amounts of DHA than do other phospholipid fractions.\textsuperscript{25} Furthermore, the interaction of rhodopsin with retinal phospholipid plays a key role in the control of visual function.\textsuperscript{26,27}

The ease with which signaling proteins traverse the ROS plasma membrane dictates the efficiency of the visual transduction cascade. This process is facilitated by a membrane that has more fluidity.\textsuperscript{28} The predominance of DHA within the phospholipids of the ROS suggests that a highly fluid membrane permits rapid enzyme action and ion transport\textsuperscript{29}; this hypothesis has been confirmed in 1 study,\textsuperscript{30} which provides important insights into the relationship between membrane fluidity and signal transduction.

The ROS is a dynamic structure consisting of numerous invaginations in the plasma membrane to form disks of phospholipid bilayers that contain rhodopsin and other enzymes of the visual cascade. The RPE is adjacent to the ROS. The RPE provides metabolic support for the photoreceptors.\textsuperscript{31} Additionally, the RPE plays an integral role in regeneration of visual pigments after photoreceptor bleaching, a process in which 11-cis-retinal is photoisomerized to all-trans-retinal.\textsuperscript{32} Furthermore, and perhaps most importantly, the RPE maintains the supply of DHA to the ROS and aids in retinal conservation of DHA\textsuperscript{33} (Figure 1).

Two possibilities exist for the mechanism by which the ROS becomes enriched with DHA. One possibility is that there is a selective uptake of preformed DHA from the plasma. The other possibility is that the retina synthesizes DHA via the elongation and desaturation of ALA. Conversion of ALA to DHA has been reported\textsuperscript{34} in the RPE but not the retinas of frogs. Orally administered, radiolabeled DHA but not ALA was taken up by retinal tissues of rats.\textsuperscript{35} In other studies,\textsuperscript{36,37} investigators have confirmed that plasma DHA is the preferred substrate for retinal tissues during early development. Enrichment with DHA is evident at the plasma-RPE and RPE-photoreceptor boundaries.\textsuperscript{38} In that study, eicosapentaenoic acid was incorporated into phospholipid in the RPE but not the ROS, which suggests that the RPE-photoreceptor boundary is 1 site of exclusion of 20-carbon PUFAs from the ROS. Furthermore, investigators determined on the basis of chain length and the number of double bonds that the enrichment of 22-carbon PUFAs within retinal tissues is specific.\textsuperscript{39}

Because of the importance of DHA in retinal function, a mechanism must be in place to preserve DHA integrity within neural tissues. This task is accomplished, in part, by the RPE.\textsuperscript{39} Circulating lipoproteins also play a role in establishing and maintaining high amounts of DHA within the photoreceptors.\textsuperscript{40} New photoreceptor disks are continually being assembled at the base of the ROS, which results in a lengthening of the ROS as older disks are pushed apically.\textsuperscript{40} To compensate for this continual elongation, the distal portion of the ROS is shed daily and phagocytized by the RPE; DHA then is shuttled back to the ROS in an efficient recycling loop\textsuperscript{40} (Figure 2). Specifically, the RPE cells are directly involved in short-loop recycling of DHA.
acids. However, investigators in another study raised compared with amplitudes for rats deficient in n-3 fatty acids because rats fed ALA had larger a-wave amplitudes, were affected by dietary content of n-3 fatty acids.

Analyses of data from guinea pigs deficient in n-3 fatty acids revealed complete functional recovery for ERG variables after 10 weeks of feeding a diet that included n-3 fatty acids. However, evaluation of ERGs in guinea pigs reveals a biphasic pattern, compared with the primarily sigmoidal curve observed on an ERG of rats and humans.

Numerous studies have been conducted on DHA and visual development. Analysis of results of these studies has helped to elucidate the role of DHA during development of the visual system in animals.

Rodents and nonhuman primates—Rats and guinea pigs have been used as a proxy for the evaluation of retinal function in humans. In a study of retinal function in rats, amplitudes recorded on an ERG were affected by dietary content of n-3 fatty acids because rats fed ALA had larger a-wave amplitudes, compared with amplitudes for rats deficient in n-3 fatty acids. However, investigators in another study raised rats and guinea pigs to a third generation on diets deficient in n-3 fatty acids and detected no differences in b-wave amplitudes between deficient rats and those fed commercially available diets. Further support for these results was obtained in a study in which investigators observed diminished a- and b-wave amplitudes in young rats fed diets deficient in ALA. In that study, monkeys deficient in n-3 fatty acids did not regain retinal function (as measured by use of an ERG) after DHA was included in the diet. To further confound the issue, analysis of data from guinea pigs deficient in n-3 fatty acids revealed complete functional recovery for ERG variables after 10 weeks of feeding a diet that included n-3 fatty acids. However, evaluation of ERGs in guinea pigs reveals a biphasic pattern, compared with the primarily sigmoidal curve observed on an ERG of rats and humans.

Animals deficient in n-3 fatty acids accumulate low amounts of n-3 fatty acids in the brain and retinas and have impaired visual function, compared with results for control monkeys. Specifically, there was a sharp decrease in the DHA content in the cerebral cortex along with a compensatory increase of n-6 DPA. The total of n-6 and n-3 PUFAs in phospholipid was relatively unchanged, which indicates that a mechanism exists within the brain to preserve the polyunsaturated state of the membrane phospholipid despite n-3 deficiency. In animals deficient in n-3 fatty acids, the impairment of visual function is indicated behaviorally by decreased visual acuity and in the ERG by delayed recovery of a dark-adapted response to a saturating flash. Interestingly, the a- and b-wave amplitudes are unaffected by n-3 deficiency, but the implicit times of both the a- and b-waves are significantly increased in monkeys deficient in n-3 fatty acids. It was reported in another study that despite the inclusion of DHA in the diet, abnormalities in the ERG persisted. These results further underscore the importance of n-3 PUFAs, particularly DHA, during the perinatal period.

In a follow-up study of rhesus monkeys, investigators reported that animals deficient since the intrauterine period but fed a fish oil diet high in eicosapentaenoic acid and DHA as juveniles had rapid accumulation of DHA in the cerebral cortex (amounts of accumulated DHA were equal to or greater than amounts in control monkeys). The recovery of neural DHA concentrations began within 1 week of feeding the fish oil diet and was complete within 12 weeks. Again, the total of n-3 and n-6 fatty acids remained quite similar, and the increase in n-3 PUFAs was accompanied by a reciprocal decrease in n-6 PUFAs.
The effects of supplementation with LCPUFAs on visual acuity and retinal function in infant rhesus monkeys were also investigated. In that study, investigators formulated experimental diets that contained higher quantities of DHA and AA (each represented 1.0% of total fatty acids), compared with quantities contained in diets used in human studies. The investigators measured 23 variables of visual function and found a significant dietary effect for only 2 variables. Failure to detect significant differences between subject groups suggests that an upper limit exists for the developmental and functional benefits of supplementation of LCPUFAs and that high concentrations of DHA and AA do not harm or benefit the development of visual function in 4-month-old animals.

A close, linear correlation between retinal DHA status and a-wave variables has been detected in preterm baboon fetuses. Results for 4-week-old baboons that were born prematurely and then fed nonsupplemented formula or formula supplemented with 0.6% AA and 0.3% DHA had significantly smaller a-wave amplitudes and longer a-wave implicit times, compared with results for full-term, breast-fed neonatal baboons. Although the prematurely born baboons provided the supplemented formula performed better than their counterparts fed the nonsupplemented formula, the degree of retinal function did not match that of the full-term, breast-fed group. Moreover, b-wave amplitudes responded only slightly to increased retinal DHA for the formula groups. In addition, retinal concentrations of AA were unaffected by developmental stage (premature vs full-term) or use of supplemented formula.

Dogs—Information regarding DHA metabolism in dogs is limited, but analysis of studies suggests that DHA is required for neurologic development in neonatal dogs. The retinas of dogs are capable of synthesizing DHA from its 22-carbon precursor, n-3 DPA. Furthermore, DHA is highly conserved in the retinas, and DHA has a role in neurologic function in this tissue. In a study conducted by our laboratory group, DPA but not DHA accumulated in plasma phospholipid when ALA was fed to adult dogs. Although accumulation of DHA was not observed in the plasma fraction of these adult dogs, the accumulated DPA likely serves as an important substrate for uptake by neural tissues and subsequent retinal synthesis of DHA. This notion is consistent with a study in which investigators found that neural tissues of dogs could convert DPA to DHA. Therefore, it is likely that the retinas (and presumably other neural tissues) of dogs synthesize and use DHA in a manner similar to that for other mammalian species and that plasma DPA provides a likely substrate for such synthesis. Thus, a dietary source of DHA, whether provided as its 18-carbon precursor or as a preformed long-chain fatty acid, may be necessary during gestation and suckling for normal neural development in dogs.

The DHA requirement of neural tissues may be met in 1 of 4 possible ways. There can be desaturation and elongation of ALA within the brain and retinas. After hepatic conversion from ALA, DPA can be taken up and further converted within the brain and retinal tissue to form DHA. Circulating DHA synthesized in other tissues, such as hepatic tissues, may be taken up by neural tissues. Finally, DHA can come directly from dietary sources. The inclusion of modest amounts of DHA and other n-3 LCPUFAs in the diet is more effective than high amounts of dietary ALA in improving ERG responses of 12-week-old puppies exposed to those diets during gestation, during lactation, and after weaning. Puppies consuming diets that contain DHA consistently have improvements in rod sensitivity (as measured by a-wave amplitudes, response times, and response thresholds) and elicit the largest increase in amplification of the phosphodiesterase cascade necessary for visual function. Puppies fed a diet rich in the DHA precursor, ALA, had some improvement in rod sensitivity, but it was generally not equivalent to the degree of retinal function evident for the high DHA group. The ALA-fed puppies also accumulated DHA in their plasma phospholipid fraction, although the ability to do so was lost after the puppies were weaned. It should be mentioned that an amount of ALA 10 times greater than the amount of DHA was needed for these effects to be seen. Consequently, results of those studies cannot be used to determine the minimal quantity of ALA, if any, needed to provide some benefit in this regard. Also, because n-6 and n-3 fatty acids both compete for the same enzyme systems, it is unclear as to the role that the dietary LA-to-ALA ratio has on subsequent conversion of ALA to DHA.

Neural development of puppies has also been evaluated by examining learning ability or memory of certain training-related activities. Memory and learning of tasks were improved after providing supplements or feeding diets with LCPUFAs (including DHA) to puppies beginning a few weeks after birth and subsequently testing the puppies when they were between 8 and 16 weeks of age.

Summary

Analysis of data in puppies has revealed that feeding diets enriched in DHA to the dams during gestation and lactation and after weaning results in the accumulation of DHA in plasma lipids, which is associated with improvements in neurologic development, as indicated by the ERG response. Additional evidence has also emerged that indicates diets or supplements containing DHA may also improve memory or learning abilities of young dogs. These findings will help clinicians and researchers understand dietary modifications used to enhance the performance of working and companion dogs, especially those involved in such activities as seeing-eye dogs, sentry dogs, dogs used in field trials, or guard dogs. Improvements in neurologic development may also play a role in enhancing the human-animal bond.


References


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