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Review Article

Has an aquatic diet been necessary for hominin brain evolution and functional development?

John H. Langdon*

Departments of Biology and Anthropology, University of Indianapolis, 1400 East Hanna Avenue, Indianapolis, IN 46227, USA

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A number of authors have argued that only an aquatic-based diet can provide the necessary quantity of DHA to support the human brain, and that a switch to such a diet early in hominin evolution was critical to human brain evolution. This paper identifies the premises behind this hypothesis and critiques them on the basis of clinical literature. Both tissue levels and certain functions of the developing infant brain are sensitive to extreme variations in the supply of DHA in artificial feeding, and it can be shown that levels in human milk reflect maternal diet. However, both the maternal and infant bodies have mechanisms to store and buffer the supply of DHA, so that functional deficits are generally resolved without compensatory diets. There is no evidence that human diets based on terrestrial food chains with traditional nursing practices fail to provide adequate levels of DHA or other *n*-3 fatty acids. Consequently, the hypothesis that DHA has been a limiting resource in human brain evolution must be considered to be unsupported.

Brain evolution: Brain development: DHA: Fatty acids: Nutrition

The n-3 hypothesis of brain evolution

Long-chain PUFA (LCPUFA) are present in membrane phospholipids in body tissues. DHA (22 : 6*n*-3), a member of the *n*-3 family of fatty acids, represents only a small percentage in most membranes, but is especially concentrated in the central nervous system (CNS), where it accounts for a third of all fatty acids (Neuringer *et al.* 1988). DHA has been identified as the only *n*-3 lipid of functional significance in the brain (Martinez, 1992). Other dietary *n*-3 fatty acids, including α-linolenic acid (ALA; C18: 3*n*-3), are thought to be important primarily as precursors for synthesizing DHA or eicosanoid hormones. Along with other LCPUFA, DHA participates in a number of important functions, for example as a part of membrane structure, in ion-channel formation, in cell signalling systems, as a mediator of the response of rhodopsin to light, as a regulator of gene expression and as a precursor to eicosanoids (Innis, 2003).

Crawford and his colleagues (Crawford 1992, 2002; Broadhurst *et al.* 2002) proposed that the availability of preformed dietary LCPUFA played a critical limiting role in the evolutionary expansion of the human brain. Because the marine food chain provides the best sources of these nutrients, fish and shellfish were an essential part of the ancestral hominin diet. 'On land, these fatty acids [i.e. arachidonic acid and DHA] can only be obtained by carnivores consuming meat, and then only in low concentrations. Until sufficient brain evolution had occurred, we suggest that early small hominins would not have been able to compete as a carnivorous species' (Cunnane *et al.* 1993). This argument has been modified to identify a food chain involving Rift Valley lake fish and shellfish as the essential dietary niche (Broadhurst *et al.* 1998). Chamberlain (1996) has offered a similar hypothesis by which early hominins utilized both fish and meat to increase their supply of fatty acids over the supply from leaves available to other primates. I will refer to this set of ideas as the *n*-3 hypothesis of brain evolution. The core hypothesis is that a marine or aquatic diet rich in DHA and other *n*-3 fatty acids was essential in the evolution of the human brain.

The *n*-3 hypothesis depends upon a number of premises: premise 1, that relative reproductive success correlates with variations in brain function; this premise is the starting point for all models of human brain evolution and cannot be challenged in this general form; premise 2, that these brain functions are sensitive to variations in DHA supply; premise 3, that the DHA supply to the brain is sensitive to variations in dietary intake; premise 4, that an aquatic food chain is the only effective dietary source for DHA and its precursors in the required quantity; premise 5, that the dietary supply of DHA has been a limiting resource for brain evolution in the terrestrial niche.

Abbreviations: ALA, α-linolenic acid; CNS, central nervous system; LCPUFA, long-chain PUFA.

* **Corresponding author:** Dr John H. Langdon, fax +1 317 788 3546, email langdon@uindy.edu

From these premises, it would follow that a shift to a DHA-rich aquatic diet was an essential step to increased fitness in a population with larger brains. The purpose of this paper is to examine these premises critically on the basis of published clinical and cross-cultural research. It does not presume to challenge the importance of nutritional balance in individual development. Rather, it critiques the evidence for an argument about our evolutionary past.

In this paper, I offer the following alternative interpretation: that cross-cultural evidence supports clinical findings that normal human diet and traditional breast-feeding practices provide an adequate supply of essential fatty acids for brain development. A woman transfers these nutrients to her infant both through the placenta and through her milk. Her body draws upon stores representing a lifetime accumulation of DHA through dietary intake and synthesis. Uncertainties in the transfer of DHA from mother to infant are tolerated through a combination of buffering mechanisms and synthesis within the infant's body. Because the pre-agricultural diet was generally as healthy as, if not nutritionally better than, modern human diets, DHA supply has not been a limiting factor in human brain evolution.

Premise 2: Are critical brain functions sensitive to variations in DHA supply?

Changes in dietary intake affect tissue levels of DHA in both mothers and infants. Because the fetus and nursing infant are dependent on the mother for nutrition, her nutritional status and diet, reflected in her bloodstream or milk, directly affect the offspring. Breast milk is generally understood to supply a sufficient balance and quantity of essential fatty acids and has been used as the dietary standard for most studies of DHA nutrition in human infants. Most of the published research on *n*-3 and DHA deficiencies has focused on the consequences of replacing breast milk with formula for human infants, or of manipulating the diets of laboratory animals. Term infants that were breast-fed had a higher level of DHA than did infants fed formula lacking DHA when measured as a percentage of total fatty acid in plasma or erythrocytes (Innis, 1992; Carlson, 2001; Gil *et al.* 2003) or in the brain itself (Farquharson *et al.* 1992). These observations are consistent with results from experiments with laboratory animals, including rats, cats, pigs and rhesus monkeys, described later.

The late fetal and neonatal periods are critical for constructing the DHA-rich nervous tissues. The rate of DHA accumulation in the human forebrain increases rapidly before birth as the brain itself is expanding, but it then slows for the first 2 years after birth (Ballabriga, 1994; Clandinin, 1999). The rate of accretion of DHA has been calculated to be 14·6 mg/week in the 15 weeks before birth and 5·5 mg/week in the 10 weeks after (Clandinin, 1999). In contrast, the level of DHA in brown and white fat declines by about 10 % from the third trimester to the first 112 weeks post partum (Clandinin *et al.* 1981). The amount of ALA declined 20 % in brown fat, but increased by 100 % in white. The implication is that DHA deposition in fat is less critical than that in the brain, and that adipose tissue is less discriminating in its uptake of fatty acids. Furthermore, DHA in the adipose tissue serves primarily as a reserve for the still-growing brain (Haggarty, 2004).

The developing brain is mildly sensitive to *n*-3 insufficiency. Numerous studies now have documented slowed or impaired nervous system development in humans and laboratory animals fed no DHA and widely varying amounts of other *n*-3 fatty acids. Ambiguities remain, however, in part because different methodologies have been employed in different laboratories (Wainwright, 1991; Gibson & Makrides, 1998, 2000; Morris, 2003). Most of the earlier studies addressed visual development, especially evoked potentials and visual acuity, because vision is one of the more accessible means of assessing the CNS and it may indicate more widespread but less observable problems.

Preterm infants fed on formula very low in *n*-3 fatty acids show lower retinal performance, including reduced acuity, higher sensitivity thresholds and altered evoked potentials, compared with breast-fed infants (Uauy-Dagach & Mena, 1995; Gibson & Makrides, 1998, 2000). Similar results were obtained by Carlson *et al.* (1993, 1996) when adequate levels of ALA but no *n*-3 LCPUFA were present. Supplementing the formula with ALA or DHA can improve visual acuity; however, these performance differences tend to resolve within months without intervention (Uauy-Dagach & Valenzuela, 1992; Carlson *et al.* 1996; Birch *et al.* 1998).

Full-term infants showed similar differences and inconsistencies. Infants fed formula lacking DHA displayed some deficiences, largely temporary, compared with breast-fed infants or those fed formula supplemented with DHA at levels of at least 0·35 % total fatty acids. Absence of DHA in formula may result in reduced DHA levels, as a percentage of fatty acids by weight, in the CNS during the first few months (Farquarson *et al.* 1992). It may depress plasma levels of DHA up to a year (Hoffman *et al.* 2003), but not at age 4 years (Ghys *et al.* 2002). Visual acuity may be affected during the first year (Makrides *et al.* 1995; Birch *et al.* 1998; Gibson & Makrides, 1998; Jorgensen *et al.* 1998; Carlson, 2001; Uauy *et al.* 2003; Morale *et al.* 2005), and comparable deficits have been noted in auditory and somatosensory thresholds (Khedr *et al.* 2004; Unay *et al.* 2004). DHA supplementation of less than 0·35 % total fatty acids had no discernable effect on acuity (Jorgensen *et al.* 1998; Auestad *et al.* 2003), visual evoked potentials (Auestad *et al.* 2003) or intelligence (Helland *et al.* 2003).

Many studies, however, found no differences resulting from unsupplemented compared with breast mik or supplemented formula (0·35 % DHA or higher) diets: for example in visual acuity or cognitive tasks with breast milk compared with unsupplemented formulas at 9 months (Innis *et al.* 1996); in visual acuity or visual evoked potentials with DHA-supplemented compared with unsupplemented formulas (Gibson & Makrides, 1998); in visual acuity with milk of varying DHA levels at 2 months (Krasevec *et al.* 2002); in blood levels and retinal function in supplemented maternal diet at 1 week (Malcolm *et al.* 2003). In a review of published studies, Koo (2003) found that DHA supplementation of formula did not offer consistent benefits to neural development or to retinal function. Such inconsistent results support the suggestion by Gibson & Makrides (1998) that multiple nutritional factors and deficits may be involved when impaired function is observed.

Only recently have trials investigated the effects of supplying DHA supplements to normal breast-fed infants or to their

mothers. Hoffman *et al.* (2004) found that DHA supplementation around weaning may accelerate the development of visual acuity. Helland *et al.* (2003) observed superior cognitive skills in children at age 4 whose mothers had received DHA-rich supplements before and after delivery. Other studies of cognition and intelligence show no correlation between DHA status at birth and performance at age 4 (Ghys *et al.* 2002) or 7 (Bakker *et al.* 2003) years. Buffering mechanisms in the mother's body to ensure a consistent availability of DHA may negate most benefits that a fetus might receive from supplements to her diet (Montgomery *et al.* 2003; Haggarty, 2004).

Animal studies permit more extreme manipulation of diet and show clearer functional deficits. In particular, because animal milk may be a more important source of DHA than prenatal transfer through the placenta (in rats, for example; Carlson, 2001), manipulated formulas may have a greater impact on laboratory animals than on humans. Diets deficient in *n*-3 fatty acids produced biochemical and functional abnormalities in the retina of a variety of mammalian species (Neuringer *et al.* 1988; Simopoulos, 1989; Wainwright, 1991; Innis, 2000). In these tests, the animals were subjected to an artificial deprivation of DHA or of *n*-3 acids, sometimes from before conception (for example, in cats (Pawlosky *et al.* 1997) and in rats (Garcia-Calatayud *et al.* 2005)) or even over several generations (for example, in rats; Weisinger *et al.* 2002). Diets low in *n*-3 acids also interfered with spatial tasks such as exploration and maze performance by rats (Lamptey & Walker, 1976; Bourre *et al.* 1989; Tanabe *et al.* 2004; Garcia-Calatayud *et al.* 2005). Spatial task impairment was sometimes reversible, even after three generations of an ALA-deficient diet in rats (Moriguchi & Salem, 2003). However, as animal studies may amplify the effects of variation in diet quality, they become less relevant to non-clinical human populations.

In summary, the CNS may show subtle and generally reversible effects following DHA deprivation with artificial formulas. In most of the studies tracking deficits over time, the problems appear to resolve on their own within a period of months as the infant matures. Thus, deficits may be better understood as retardation of development than as permanent impairments. The issues relating to formula-feeding deserve consideration and further study. Nonetheless, with few long-term data available and in the absence of evidence for the persistence of functional neural deficits, the premise that variations in levels of DHA in the diet would significantly affect evolutionary fitness has little support.

Premise 4: Is an aquatic food chain the only effective dietary source for DHA and its precursors in the required quantity?

Within the conventional Western diet, foods from the marine food chain (specifically marine fish and cold-water shellfish) are the only good source of preformed DHA. However, some plants, including mosses, liverworts and ferns, often have high levels of *n*-3 LCPUFA. ALA is the predominant fatty acid in green leafy vegetables (Simopoulos, 1988; Jones, 1994). Additional dietary sources of ALA include eggs, walnuts, legumes, rapeseed oil, soyabeans and soyabean oils, rapeseed, flaxseed and mustard. ALA also comprises a high proportion of lipids in many fruits and cereals, although those foods have low lipid content overall (Kris-Etherton *et al.* 2000).

n-3 Nutrients are also present in the animals that eat these, including grazers and browsers, and in other potential food animals, including insects, amphibians, reptiles, and birds, according to the food chain to which they belong (Tashima & Cahill, 1965). Horse milk, for example, is very high in ALA, perhaps because of its concentration in leafy grasses (Tinoco, 1982). Because brains concentrate DHA and other LCPUFA, they naturally represent a good dietary source of these. The level of DHA has been found to be roughly equivalent in all mammal brains. For example, Crawford *et al.* (1976*a*) measured the content in the brain of a herbivore (kob) as the same as that in a carnivore (hyena). The liver, however, differed. A hyena liver preferentially accumulated DHA, whereas a deer liver concentrated docosapentaenoic acid. A broader comparative sample confirmed this pattern of consistent brain composition and variable liver composition.

How well can we assess the fatty acid composition of the potential hominin diet in the relevant ancestral environment? Because the fatty acid content of animals depends on the food chain on which they feed, lipid analyses of laboratory-reared animals or of domestic animals are not very informative about ancestral nutrient sources. Today's domesticated plants often have fewer unsaturated fatty acids than wild plants (Simopoulos, 1990). Our dependence on agricultural grains and grain-fed animals skews fatty acids in favour of the *n*-6 rather than the *n*-3 series. Domestic animals fed on grains and domesticated plants pass this skewed distribution up the food chain to us. Chicken eggs, for example, may vary in the ratio of *n*-6:*n*-3 fatty acids between 1·3 and 19·3 depending on whether they were free-range or factory-raised (Simopoulos, 1990). Therefore, the modern Western diet is a poor approximation and substantial underestimation of the nutrients available to hunter-gatherers.

Extrapolations from wild plants and animals indicate that the paleolithic diet would have had a much higher proportion of polyunsaturated to saturated fats. Eaton & Konner (1985) estimated such a ratio to be 1·41 compared with 0·44 for the contemporary American diet. This ancestral diet contained a rough balance between *n*-3 and *n*-6 fatty acids (Simopoulos, 1990, 1991, 2002; Eaton, 1992). Both a decrease in saturated fatty acids and a relative increase in the proportion of *n*-3 to *n*-6 fatty acids would increase the rate of DHA synthesis.

In a survey of the literature pertaining to 123 modern hunter-gatherer groups, Kelly (1995) found that twenty-three did not produce a measurable part of their diet from fishing. Cordain *et al.* (2000) published a similar survey for 229 hunter-gatherer cultures. Of these, thirty obtained 0–5 % of their diet by fishing. Such surveys are hardly representative of past humanity as modern hunter-gatherers include a disproportionate representation from both the Arctic and the desert. They do, however, indicate that aquatic foods are commonly incorporated into hunting and gathering, but are not an essential part of the human diet. Moreover, most freshwater food chains do not accumulate *n*-3 fatty acids as effectively as marine ecosystems; thus, an even greater proportion of hunter-gatherer societies are living independently of DHA-rich aquatic foods than the figures suggest.

By all estimates, hunter-gatherer and ancestral diets are healthier and more nutritionally balanced than our own (Eaton & Konner, 1985; Haenel, 1989; Simopoulos, 1990, 1991; Milton, 1991; O'Dea, 1991; Southgate, 1991). Although such populations undoubtedly faced more restrictions in total calorie and protein availability than we do today, pregnant and nursing women have a great ability to buffer their infants from temporary shortages, as discussed later. Only gross starvation or the separation of an infant from its mother is likely to generate functionally significant DHA shortages; in those circumstances, infant development and survival are already jeopardized. We must conclude that a terrestrial hunting-gathering diet can provide healthy quantities of essential fatty acids.

Premise 3: Why does the brain not appear to be sensitive to variations in dietary DHA intake?

The developing brain is supplied by a direct transfer of maternal stores through the placenta before birth and via milk afterwards (Fig. 1). Adequacy of the supply of DHA is assured by additional synthesis from shorter-chain *n*-3 fatty acids and by other buffering mechanisms. Acquisition of DHA continues by dietary intake and synthesis in the post-weaning period.

Human milk provides sufficient DHA

The functional deficiencies reported earlier pertain to a near-total absence of DHA in an artificial infant diet or, in some animal studies, in the maternal diet as well. From an evolutionary perspective, these are artificial and extreme conditions. Human milk is the critical source of essential fatty acids, including LCPUFA during the postnatal period. Human milk fatty acid composition reflects the mother's diet (Widdowson *et al.* 1975; Lammi-Keefe & Jensen, 1984; Sarda *et al.* 1987; Chulei *et al.* 1995; Sanders, 1999; Kuipers *et al.* 2005; see also Vernon & Pond, 1997, for other mammals). Some studies report that supplementing the mothers' diets with DHA resulted in elevated levels of DHA in milk and maternal plasma (Jensen *et al.* 2000; Boris *et al.* 2004; Lauritzen *et al.* 2004), although supplements of ALA had less effect on milk levels of DHA (Gibson & Rassias, 1990). Others, however, found the correlation of DHA in the diet and in milk to be limited (Marangoni *et al.* 2002; for a review, see Koletzko, 1992). Lauritzen *et al.* (2002) document substantial day-to-day fluctuations in DHA content, rather than sustained levels, in association with consuming fish or fish oil.

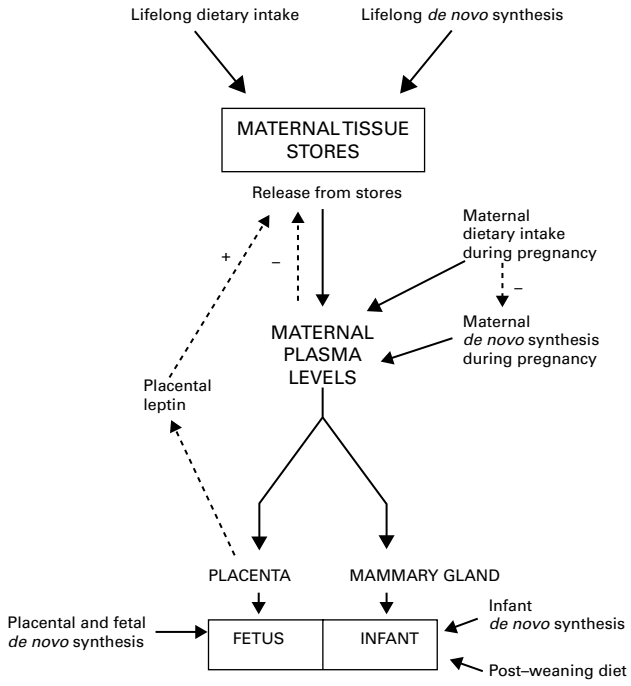

Fig. 1. Sources of docosahexaenoic acid (DHA) for the developing brain. Both before and immediately after birth, the primary source is a transfer of preformed DHA from the mother. Previously stored DHA, continued dietary intake and synthesis by both mother and offspring are all significant. Dotted lines represent regulatory interactions.

Changes in the DHA content of milk during the course of lactation provide evidence that the mother is drawing upon her body's stores. Colostrum is higher in its concentration of LCPUFA than is mature milk from the same women (Gibson & Kneebone, 1981; Lammi-Keefe & Jensen, 1984; Koletzko *et al.* 2001), although absolute quantities within colostrum fall within the range of variation for mature milk. The early milk of mothers with premature infants may contain more DHA (as a percentage of total fatty acid weight) than milk for term infants (Beijers & Schaafsma, 1996), and mature milk a week after birth contains more DHA than milk produced later (Lammi-Keefe & Jensen, 1984). A few weeks after birth, the level of DHA has stabilized. These observations suggest that ready reserves in the mother's tissues may sustain milk levels until those reserves are depleted; after this point, milk content depends heavily on newly synthesized or dietary DHA. It should be noted, however, that published observations reflect the concentration of fatty acids during a period when quantity of milk production and consumption is increasing. Patterns of total maternal DHA production or total infant consumption have not been reported.

Variations in the human diet during pregnancy, including supplementation with fish oils, did not significantly change the plasma levels of DHA in newborns (De Vriese *et al.* 2002; Francois *et al.* 2003; Malcolm *et al.* 2003; De Groot *et al.* 2004; Haggarty, 2004). Although the infants of mothers with a fish-oil-supplemented diet showed slightly enhanced retinal development in some measures, both the control and experimental groups of infants tested within a normal range of function (Malcolm *et al.* 2003). The lack of expected increase in DHA transfer may be explained by mechanisms that buffer the levels of DHA in maternal plasma. For example, fish oil is rich in both *n*-3 and *n*-6 LCPUFA, and both of these may inhibit the *de novo* synthesis of DHA, as noted below. Furthermore, the intake of preformed dietary DHA may slow the release of stored DHA from maternal tissues.

Published measures of the concentration of *n*-3 fatty acids in human milk have utilized different methods and are difficult to compare directly. The level of *n*-3 fatty acids in Western milk is typically about 1·0–1·5 % of total milk fat by dry weight (Lammi-Keefe & Jensen, 1984; Koletzko, 1992; Lanting & Boersma, 1996; Koletzko *et al.* 2001). Non-Western populations

have higher values, as much as 3–4 % *n*-3 (Chulei *et al.* 1995; Kuipers *et al.* 2005). The lowest levels of DHA in human breast milk, less than 0·2 % of total milk fatty acids, occur in some Western individuals (Lammi-Keefe & Jensen, 1984), including vegans (Sanders, 1999), and in one African sample (Kuipers *et al.* 2005).

Although clinical studies consistently assume that human milk provides an appropriate level of DHA and other fatty acids to infants, those needs are not well established. Some estimates have been extrapolated on the basis of observed levels of DHA in the body tissues of infants, others apparently on the milk content of Western women. During the last trimester of gestation, human fetuses require about 522 mg *n*-6 fatty acids/d and 67 mg *n*-3 fatty acids/d to maximize tissue levels of LCPUFA (Clandinin *et al.* 1981). Connor & Neuringer, (1988) suggest that ALA should be about 1 % of total fat intake. Uauy (1990) offers 0·5 % for infants and 0·5 % as other *n*-3 LCPUFA. *n*-3 and *n*-6 fatty acids, both short and long chain, are competitive inhibitors in one other's metabolism; thus, recommendations must also address the ratio of *n*-6:*n*-3 fatty acids.

It should be noted that these standards are higher than those used in clinical studies and have not been correlated with function or clinical signs. Fat constitutes approximately 50–60 % (Friedman, 1987; Kohn, 1992) of the calories in human milk. Therefore, if *n*-3 fatty acids represent about 1 % of fat (by dry weight) in Western milk, this may be very roughly compared with about half of the amount recommended by authors cited above. Likewise, as DHA accounts for 0·30 % of milk lipid, it represents approximately 0·15 % of total milk calories. The higher rates cited for non-Western women mostly do meet the proposed standards. Although human milk may or may not meet standards, no culture-wide deficiencies have been identified, even when a large number of Western infants were being raised on DHA-free formula; and that milk has been regarded as the healthy standard in clinical studies. Given the apparent clinical sufficiency of Western milk, it is doubtful that traditional (non-Western or non-modern) breast-feeding practices would result in fatty acid deficiencies as long as the mothers themselves were not malnourished.

DHA is synthesized by tissues of both mother and offspring

A woman can increase her stores of DHA by synthesis from other *n*-3 fatty acids. Adult vegan men with a diet containing effectively no DHA were able to maintain a stable lipid level of DHA, albeit lower than that of omnivores, through synthesis (Rosell *et al.* 2005). The rate of synthesis may be higher in women of child-bearing age than in males (Burdge & Wootton, 2002; Burdge *et al.* 2002; Pawlosky *et al.* 2003). This capacity is enhanced by estrogens (Giltay *et al.* 2004) and appears to be elevated during pregnancy and lactation (Smit *et al.* 2003; Stark *et al.* 2005). This appears to be an adaptive strategy to construct reserves of DHA for the next generation and to protect the mother against depleting her own nutrients during pregnancy and lactation.

Infants can also synthesize DHA. Studies using stable isotope tracers have detected the conversion to DHA of precursors supplied in formula to preterm infants with a gestational age of 27–35 weeks at the time of study (Carnielli *et al.* 1996; Gibson & Makrides, 1998). The synthesis of DHA occurs within the placenta, fetal liver and fetal brain (Crawford *et al.* 1976*b*; Ballabriga, 1994; Clandinin, 1999), and its rate increases with fetal age. The absolute rates of synthesis by fetuses and newborns has not been calculated and appears to vary substantially (Jensen & Heird, 2002). It is known to be inhibited by excessive quantities of linoleic acid, as *n*-6 and *n*-3 fatty acids compete for some of the same enzymes in desaturation and elongation pathways (Horrobin & Manku, 1990; Bezard *et al.* 1994; Gerster, 1997; Crawford, 2000). That rate may also be sensitive to the intake of preformed DHA: synthesis was elevated in infant baboons fed a formula lacking in DHA (Sarkadi-Nagy *et al.* 2004) and in rats with a fat-free diet (Bezard *et al.* 1994).

Some researchers have argued that synthesis may enable the term infant to be independent of preformed dietary DHA (Guesry, 1998): 'Presently no other theories explain how vegetarians obtain enough DHA for the neural tissues of their offspring except through consumption of foods containing [ALA]' (Nettleton, 1991). Others have disagreed, observing that most studies show DHA levels in erythrocytes, brain, adipose and other tissues to be lower in infants fed with ALA-enriched formulas than in breast-fed infants (Farquharson *et al.* 1992; Nettleton, 1993; Neuringer, 1993; Innis, 1994; Lanting & Boersma, 1996; Gibson & Makrides, 1998; Cunnane, 1999). These are not necessarily contradictory findings. If the ability to synthesize DHA continues to increase in the period after birth, we can better understand how infants deprived of dietary DHA can resolve early deficits.

Importantly, although the sufficiency of the rate of synthesis has been questioned, sufficiency is not a relevant question for a normal nursing infant, as the great majority of DHA will be delivered from the mother via the placenta or milk. From an evolutionary perspective, the issue is whether a nursing infant can buffer any shortfall during the development of the brain. Synthesis is only one of several buffering mechanisms.

The n*-3 fatty acid supply is buffered*

Buffering of the nutrient supply may occur in different ways, either between tissues within the body or across the lifespan. Buffering of the DHA supply for the brain, specifically, may be effected by shifting DHA in and out of other body tissues. Although most DHA deposited *in utero* is in adipose tissue, the needs of the nervous system have priority over other tissues when DHA is in limited supply (Haggarty, 2004). After birth, stores in adipose tissue decline as those in the brain increase. The mobilization of fat begins within a few hours after birth (Haggarty, 2004). In a study by Lefkowitz *et al.* (2004) involving the synthesis of new DHA from labelled ALA, 40 % of the DHA added to the brain came from pre-existing body stores. Haggarty (2004) estimated the stores of DHA in the adipose tissue of a term infant at birth to represent the needs of the infant for the next 2 months if no other source of DHA was available.

An overall body deficiency permits DHA to be substituted by other fatty acids within the membranes, including docosapentaenoic acid (22 : 5*n*-6) or arachidonic acid (20 : 4*n*-6), so that body tissue composition can shift fairly quickly as diet changes (Neuringer *et al.* 1988; Uauy, 1990; Neuringer, 1993). This replacement may be temporary, until body

levels of DHA are restored, and it implies that temporary shortages of DHA need not interfere with the rate of overall brain growth.

A pregnant or nursing mother may buffer against nutritional shortfall in her diet by storing nutrients in her body when they are more readily available and releasing them during reproduction (Haggarty, 2004). Adolescent girls tend to increase fat stores, and the ability to maintain those stored nutrients is among the important determinants of their reproductive potential (Ellison, 1990; Frisch, 2002). Moreover, women typically create extra fat stores during the first two trimesters of pregnancy and then release those to support the fetus and newborn during the third trimester and during lactation. The fetus may have some ability to regulate the release of fatty acids from maternal stores by the secretion of placental leptin (Haggarty, 2004). Once fatty acids are circulating in the maternal blood, the placenta preferentially transports LCPUFA to the fetus. Maternal stores decline over the course of a pregnancy, but are built up again gradually in the months following delivery (Singh, 2005). Predictably, the maternal stores of DHA decline over the course of multiple pregnancies (van Gool *et al.* 2004).

These data provide support for a buffering mechanism in which DHA can be borrowed from non-neural tissues to supply a developing brain. Therefore, a short-term deficit of *n*-3 in the diet is not likely to cause permanent impairment of CNS function. Unlike many nutritional requirements, the need for DHA appears to be at least partly cumulative, rather than daily. The fetal period is particularly well buffered, as the DHA available from the mother reflects her lifetime accumulation through diet and synthesis (Koletzko *et al.* 2001).

Catch-up provisioning is possible

Because DHA is readily incorporated into cell membranes, it appears that early deficits may be compensated by later nutrients without special supplementation. Brain tissue deficits were reversed in rats at the level of individual neurones by later provisioning (Bourre, 1992). Tissue levels recovered in monkeys even when supplementation occurred after brain growth was complete (Wainwright, 1991). The functional deficits observed in the human and animal studies usually resolved themselves in early childhood with or without dietary supplementation.

A substantial return toward normality was reported in rhesus infants whose mother was deprived of nearly all *n*-3 intake during pregnancy (Anderson *et al.* 2005). When the infants were fed a diet rich in ALA from birth, the DHA levels in the brain matched those of controls (raised on a formula containing ALA) within 15 weeks, whereas the overall fatty acid profile did so within 3 years, although recovery of retinal composition was less complete. Visual acuity thresholds were comparable in both deprived and control animals, whereas electroretinograms differed in some measures. In this experiment, any recovery of DHA levels must be attributed to new synthesis from the ALA in the diet. Catch-up development from moderate deficiency thus appears to be normal and is probably due in part to the increasing ability of the infant to synthesize DHA from other *n*-3 fatty acids.

To summarise, the third premise stated that DHA supply to the brain is sensitive to variations in dietary intake. Although this appears to be true in an absolute sense, buffering mechanisms and the possibility of catch-up accumulation allow only unnatural and prolonged deprivation of DHA to have measurable functional consequences for the CNS. Any link between functional variation and fitness occurring under natural nursing practices remains speculative, untested and unsupported.

Premise 5: Has the dietary supply of DHA has been a limiting resource for brain evolution in the terrestrial niche?

The proposed limiting role of DHA in brain evolution must be posed in terms of absolute brain size, reflecting the absolute quantities of DHA accumulated in the brain. Hominin brain expansion can be traced through the past 2 million years (Table 1). Early species of *Homo*, including *H. erectus sensu latu*, have a brain size starting about 800 cm^3 about 1·7 million years ago. Shortly after 1·0 million years ago, brain size exceeded 1000 cm^3 in Java and Europe. It reached its modern size of about 1400 cm^3 in European Neanderthals and earliest *H. sapiens* in Africa in the past 200 000 years.

Our ancestors faced a continuing and increasing demand to supply those brains with energy and nutrients. Archaeological evidence shows that stone tools appeared about 2·5 million years ago, about the same time as cutmarks on animal bones, evidence for the processing of carcasses obtained through either hunting or scavenging (de Heinzelin *et al.* 1999; Bunn, 2001). Additional evidence for competition with savanna carnivores at this time comes from the molecular phylogeny of human tapeworms (Hoberg *et al.* 2000). Evidence for hunting by *H. erectus* grows stronger after 1·0

Table 1. Evolution of cranial capacity over time in the hominin fossil record

Species and sample size	Mean cranial capacity (cm^3)	Range (cm^3)	Age (Mya)
Earliest *Homo* (*n* 8) (*H. habilis* and *H. rudolfensis*)	650	510–810	1·5–1·9
Homo erectus sensu latu			
H. ergaster (*n* 6)	772	600–909	1·6–1·75
H. erectus (*n* 31)	1035	727–1231	0·3–1·2
H. heidelbergensis (*n* 22)	1197	775–1450	0·1–0·8
Homo neanderthalensis (*n* 21)	1438	1200–1748	0·04–0·16
Earliest *H. sapiens* (*n* 19)	1380	1120–1585	0·13–0·25
Modern *H. sapiens*	1400	900–2000	

Mya, million years ago.

million years ago on the basis of both tools and weapons (Thieme, 1997; Pitts & Roberts, 2000). Isotopic analyses of C and N in Neanderthal bones have revealed a diet heavily dependent on meat (Dorozynski & Anderson, 1991; Bocherens *et al.* 1991, 2001, 2005), whereas Sr isotopes suggest a diet of up to 90 % meat (Balter *et al.* 2001). Evidence for a significant exploitation of aquatic resources appears with anatomically modern humans in Africa and Europe, in the context of increasing cultural complexity, population pressure and dietary diversification over the last 100 000 years (Klein 1983, 1999; Sealy & van der Merwe, 1985; Grine *et al.* 1991; McBrearty & Brooks, 2000; Stiner *et al.* 1999, 2000; Richards *et al.* 2001; Mannino & Thomas, 2002). Modern behaviour and modern anatomy both emerged very gradually in Africa, well after brain size was overlapping modern figures. (McBrearty & Brooks, 2000).

In order to demonstrate the fifth premise, one of the following arguments must be asserted. Either the absence of DHA from a terrestrial diet makes the development of a human brain impossible, or DHA somehow played a direct role in causing evolutionary change. The first of these suggestions is demonstrably false. Many hunter-gatherer and agricultural populations thrive on diets containing few if any DHA-rich aquatic foods and suffer no apparent deficiencies of brain function. After surveying the nutritional value of a variety of African terrestrial animal and plant foods, both aquatic and terrestrial, Cordain *et al.* (2001) concluded that the best source for both the energy and lipid requirements of the brain is a combination of mammalian brain and marrow fat, consistent with a scavenging model for early hominins (Blumenschine, 1991). Both of these substances are available to hunters able to bring down large game. Humans do not even have the largest terrestrial brains: those of African elephants, which today subsist on a great variety of terrestrial plant foods, are about $3000–4000\,cm^3$, compared with $1400\,cm^3$ for average humans.

The second possibility, that an increase in dietary DHA itself causes evolutionary change, has no basis in evolutionary theory. No mechanism has been proposed to explain whereby the brain would evolve in the presence of superfluous levels of nutrients but not in the presence of an adequate supply.

The hypothesis of an evolutionary dependency of the human brain on aquatic or marine resources or on any other single food source is unnecessary and unsupported. Current data do not suggest that an ancestral population modelled after modern terrestrial hunter-gathers would have encountered fatty acid deficiencies that would limit the evolution of the brain.

Conclusion: adaptive strategies for large brains

We can now evaluate the premises behind the *n*-3 hypothesis. Premise 1, that relative reproductive success correlates with variations in brain function, can be accepted. Premise 2, that these brain functions are sensitive to variations in DHA supply, is supported only for extreme or artificial diets. Premise 3 is that DHA supply to the brain is sensitive to variations in dietary intake. This premise is unsupported for breast-feeding populations: buffering mechanisms, *de novo* synthesis and long-term accumulations protect the developing brain. Premise 4, that an aquatic food chain is the only effective dietary source for DHA and its precursors in the required quantity, is contradicted by cultures living on a terrestrial food base without signs of deficiency. Premise 5 says that the dietary supply of DHA has been a limiting resource for brain evolution in the terrestrial niche. This premise has no theoretical basis, and there is no correlation between the exploitation of aquatic resources and the expansion of the hominin brain.

Clinical research has revealed the importance of specific nutrients in the human diet and helps us to identify optimal levels of those nutrients. These are very different questions from those asked by evolutionary biologists. Optimal nutrition is not normal for our species; and as we attempt to construct dietary recommendations we discover how difficult, perhaps impossible, it is to define optimality. Goals of optimizing nutrition may be defined in terms of growth patterns, resistance to disease, postponement of ageing, neural performance or longevity, but these different goals yield different recommendations. For example, elevated caloric intake is associated with secular trends of increasing height and earlier maturation, but restricted caloric intake corresponds to increased longevity. In evolutionary thought, optimization is defined only in terms of reproductive success. Different strategies of reproduction have different implications for ageing and longevity. Natural selection favours individuals who can find appropriate compromises for dealing with limited resources and balancing the allocation of those resources between growth and body maintenance on the one hand and reproduction on the other. Thus, clinical goals and evolutionary goals may be substantially different.

The limits to supporting a large brain include, among others, the costs of specific nutrients for the mother and infant. The niche in which the brain evolved must have offered a generally adequate supply of calories and nutrients, and the advantages of larger brains must have outweighed the associated risk of shortfall and malnutrition. Although there are other constraints to be considered, if greater brain size confers a proportionate selective advantage and if nutrients are the primary limitation on brain size, all other thing being equal, brain size may track an expected long-term availability of limiting nutrients. This is the logic of the *n*-3 hypothesis. It has, however, failed to demonstrate the limiting role of DHA.

Environmental quality and food availability fluctuate over periods ranging from days to intergenerational lengths. Individuals poised to take advantage of unusually good conditions will be at a selective advantage over competitors when nutrients are plentiful. Individuals that are more sensitive to poorer conditions will be at a selective disadvantage in bad times. The risk of insufficiency may be reduced if the deficiency is buffered by stored reserves, catch-up growth is possible when nutrients become available, and even a larger brain with suboptimal nutrition functions as well as a well-nourished but smaller brain. We observe all three of these adaptations in human infants. Although plentiful quantities of dietary DHA are quickly reflected in tissue levels, a lesser but significant source of DHA is the slow synthesis from ALA within the body. An infant begins life with a rapid transfer of *n*-3 fatty acids from maternal stores. Shortages in the supply for the brain can be made up by borrowing DHA from other tissues and by temporarily substituting other LCPUFA in the neural membranes. Both mother and child can therefore compensate for any shortfall and build or replenish reserves in subsequent years.

This strategy is effective for modern humans across the widest range of environments of any known vertebrate. It is reasonable to conclude that the evolutionary solution to the LCPUFA requirements of hominin brains did not require a significant departure from the omnivorous hunting-gathering diet that is already a part of conventional scenarios of human evolution.

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