Brain Development and Assessing the Supply of Polyunsaturated Fatty Acid

M.T. Clandinin

Nutrition and Metabolism Research Group, Department of Agricultural, Food and Nutritional Science and Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

ABSTRACT: Membrane lipids are necessary for structure and function of the developing nervous system. Rapid synthesis of brain tissue occurs during the last trimester of development of the human brain and the early postnatal weeks. This synthesis of brain structure involves the formation of complex lipids, many of which contain significant quantities of chain-elongated desaturated homologs of essential fatty acids. The present report discusses the implications of change in nutritional status on processes of brain development and metabolic events that involve lipids.

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EARLY STAGES

In adult vertebrates, the form of nervous system brain development at early stages is remarkably similar. The distinct character of the neural plate and neural tube, from which the nervous system originates in all vertebrates, suggests that development of the central nervous system occurs through similar overall mechanisms. The cells, regions, and various structures of the brain do not develop uniformly, as in other tissues and organs (1–3). Characteristic, well-defined stages of growth occur anatomically and biochemically (4,5) and result in significant growth spurts or critical periods during fetal and neonatal life. The development of any part of the brain occurs in stages: induction of the neural plate, localized proliferation of cells in different regions, migration of cells, formation of identifiable parts of the brain by cell aggregation, differentiation of immature neurons, formation of connections, selective cell death, and modification of connections (6). These changes within the developing nervous system proceed in a caudal (brain stem) to rostral sequence (7). Caudal brain structures include phylogenetically older brain structures, whereas rostral structures are phylogenetically newer (8). Structural changes in the brain during development result in an increase in weight and size. These increases are not necessarily parallel: the greatest growth in size occurs prior to the greatest gain in weight (9). Different parts of the brain grow at different speeds, and not all regions reach their fastest rates at the same time. In this respect, the "growth spurt" and velocity curves as defined by Dobbing (1,10,11) represent rates of change in total brain weight over time and not individual regions of the brain. The early concepts developed by Dobbing did not encompass the developmental processes that are now understood to be critical periods of growth and highly susceptible to insult. As well, they do not reflect the interrelationships of growth occurring in subregions of the developing brain (12).

Neurogenesis. Most neurons are generated in or close to the ventricular zone of the neural tube. A complex set of factors including neuron type, position in the mitotic gradients, and phylogenetic status determines the time of neuronal origin (7). The number of neurons initially formed in any brain region is determined by three factors: the duration of the proliferative period (which may last a few days to several weeks), the duration of the cell cycle (which in a young embryo is a few hours and increases to 4 or 5 d as development progresses), and the number of precursor cells (6).

Depending on the region of the nervous system (13) and neuron type (7), production of neurons occurs over varying lengths of time with different time schedules for formation. Neurons born first in any given region of the mature nervous system are the phylogenetically older neurons and the larger neurons (7). Large neurons apparently become postmitotic early because they have the longest axons and must reach their targets while the embryo is still small. Neurogenesis peaks around the 14th week of gestation and is completed by the 25th week when the adult number of neurons is present (14). In rats these developmental events occur at about 18 and 20 d of gestation, respectively. Known exceptions that continue to proliferate postnatally include some neurons of the hippocampus and the cerebellum. It is important to recognize that the timetable of neuronal differentiation cannot be simply deduced from its time of origin. The type of neuron, its regional situation, and the timing of the arrival of axons with which synapses will form are factors that also affect neuronal differentiation.

Gliogenesis. Glial cells tend to originate after neurons in any particular region of the brain. Formation of glia differs from neuronal formation in three main ways. First, many of the proliferative cells that generate glia lie outside the neuroepithelium, at or near the site where they will be located in the adult (15). Second, the production of glial cells continues throughout

*Address correspondence at Nutrition and Metabolism Research Group, Department of Agricultural, Food and Nutritional Science, 4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.
E-mail: tclandin@gpu.srv.ualberta.ca

adult life (7). Gliogenesis is primarily a postnatal event (16); however, in some brain regions it is detected before birth (16,17). Early gliogenesis is completed by the 15th week of gestation in humans and the 16th day of gestation in rats (12). Third, damage to the glial population is rarely permanent (18–20). However, it is known that a qualitative unbalance or quantitative deficit of food intake produces alterations in the ontogeny and function of the nervous system (21–24).

Making connections with function. Because most nerve cells are generated at or close to the inner, proliferative surface of the neural tube, they subsequently migrate past other cells to their final locations. The temporal origin of neurons may be related to later establishment of anatomical connections (15). There are two spatiotemporal gradients for neuron migration. In nuclear regions of the brain (such as the thalamus and hypothalamus), the oldest neurons produced by the neuroepithelium are pushed farther out as the younger neurons are generated. In these regions, cells accumulate in an "outside-in" or "pushing" gradient (25). The "inside-out" or "passing" gradient occurs in regions of the brain that have a laminar structure (such as the cerebral and cerebellar cortices). Cell accumulation in these regions occurs as younger neurons migrate past the older neurons, which remain closest to the neuroepithelium (26). Despite the heterogeneity of the cell population in the nervous system, the development of the system's complex form is reliable. The developmental program of the nervous system must contain mechanisms for neurons to migrate to their proper destination. This arrangement of neurons must be completed successfully for normal functioning to result.

Functional connections in the nervous system are established through growth of neurites (axons and dendrites) and formation of synapses. Synaptogenesis is the contact between axons and target cells that starts before neurogenesis is completed (27) and follows a cell-specific, region-specific timetable. Most neurons generate and receive many processes, producing many more synaptic connections. A period of programmed cell death and synapse reorganization follows as the final stages of brain morphogenesis. Stages of brain morphogenesis ensue in each brain region in a specifically timed series of events. Each period becomes a critical basis for development of the next (28). Time scales occurring in each brain region are further complicated by migration of cells between regions. Due to the brain's general lack of regeneration potential and its dependence on specialized interactions, any misdirected, mistimed, or absent developmental cues can lead to structural aberrations (29). These structural changes are irreversible and result in functional deficits if future developmental changes are unable to compensate for these structural changes. Thus, insults that alter brain development in the fetus or neonate may permanently alter a myriad of functions in later stages of infant development.

BRAIN LIPIDS AND NUTRITION

Second only to adipose tissue, the brain is the most lipid-concentrated organ in the body. Nervous tissue contains 50% lipid on a dry weight basis, or 10% lipid on a weight/weight basis (30). This lipid plays a role in modifying the structure, fluidity, and function of brain membranes (31–36). A variety of complex lipids exists in the brain, and the composition and metabolism of these lipids change with development and age (37–39).

Fatty acid accretion in the brain. One approach to determining the fatty acid needs of the developing infant is to determine the fatty acid composition during infant growth and quantitate the accretion of fatty acids in body tissues. Although this approach seems simple, it may be hampered by not knowing whether fetal development is the optimal model for the assimilation of fat accretion during the extrauterine growth of infants born prematurely. It should be noted that a difference in the deposition and net absorption of fat exists between perinatal and postnatal growth (40). Accretion of essential fatty acids in adipose tissue during the last trimester of intrauterine development was estimated by using data on net fatty acid accretion and values for adipose tissue fatty acid composition (41). The average accretion rates determined are consistent with the fat deposition estimated by indirect calorimetry for preterm infants fed 120 kcal/kg/d (42). Based on the assumption that tissues not quantitatively analyzed have a total fat and essential fatty acid content similar to that of skeletal muscle (41), minimum values for essential fatty acid utilization in tissue synthesis during the last trimester of intrauterine growth were estimated. Estimates for utilization in *de novo* synthesis of tissues were approximately 522 mg/d of n-6 fatty acids and 67 mg/d of n-3 fatty acids (41). These values are minimal levels, as consideration was not made for the amounts of fatty acids oxidized to meet the energy requirements for tissue accretion. More information about the proportion of fatty acids used in energy metabolism and tissue synthesis is needed before information about body composition can be applied to a practical feeding situation. Proportions of major dietary fatty acids consumed may potentially affect the net contribution of fat oxidation to total energy production (43). This observation was made in adult males; however, it suggests that changing the balance of nonessential to essential fatty acids in the diet affects how fat oxidation is partitioned for energy production.

Analyses of whole-body fat content (44,45) indicate that preterm infants, with an appropriate weight for gestational age of 1300 g at birth, have a total body fat content of about 30 g compared with the term infant of 3500 g with a total body fat content of 340 g. Clandinin *et al.* (46) estimated that approximately 2783 mg of n-6 fatty acids and 387 mg n-3 fatty acids accrue in adipose tissue each week *in utero*. Birth after only a few more weeks of intrauterine development would dramatically increase the potential reserve of fatty acids in adipose tissue both for total fatty acids used for energy production and for essential fatty acids used for synthesis of structural tissues. These estimates also support the body of research by Van Houwelingen *et al.* (47) suggesting that the growing fetus represents a large draw upon maternal essential fatty acid stores and perhaps that a limitation in the size of the maternal essential fatty acid stores may be critical to fetal growth and development.

TABLE 1
Fatty Acid Accretion Rates in Infant Brain and Cerebellum (mg/wk)[a]

	Intra-uterine (26–41 weeks: preterm)	Extra-uterine (0–10 weeks: term)
Total n-6	32.8	82.4
Total n-3	14.6	5.5
Total n-9	31.2	65.5

[a]Data from References 48 and 49.

During the third trimester of human development, n-3 and n-6 fatty acids accrue in fetal tissues as an essential component of structural lipids, and rapid synthesis of brain tissue occurs. This rapid synthesis causes increases in cell size, cell type, and cell number (48). Brain lipid levels increase rapidly during this period. Levels of 18:2n-6 and 18:3n-3 were consistently low in the brain during the last trimester of pregnancy (48). However, accretion of long-chain essential fatty acid desaturation products 20:4n-6 and 22:6n-3 occurred, and the absolute accretion rates of the n-3 fatty acids, specifically 22:6n-3, were greater in the prenatal period compared with the postnatal period (Table 1; 48, 49). It is apparently critical that the developing fetus obtain the correct types and amounts of fatty acids to ensure complete and proper development of the brain. Timing of the availability of these fatty acids is also a factor. Collectively, this quantitative information indicates that large amounts of docosahexaenoic acid (22:6n-3) and arachidonic acid (20:4n-6) are required during development of neural tissue when cellular differentiation and active synaptogenesis are taking place.

Development of the visual system. The entire visual system spans caudal to rostral regions of the brain and includes some lateral areas of the brain. The visual pathway involves a chain of visual processing events that only begin with the retina in the eye. During visual processing, incoming light strikes the photoreceptors and generates electrical signals, which are sent to bipolar cells and ganglion cells. These networks in turn send visual information along the optic nerve to the visual cortex. "Funneling" of information within the eye is the result of an individual neuron receiving, converging, and combining impulses from several incoming nerve fibers. Thus, the separate signals of each nerve fiber are integrated into an entirely new message based on all the inputs. The retinal content of 22:6n-3 normally increases during development, and this change in lipid composition is apparently important to the function of the visual system although it cannot be easily distinguished functionally from the simultaneous increase in 22:6n-3 in the entire visual pathway.

SOURCES OF ESSENTIAL FATTY ACIDS FOR THE FETUS AND NEONATE

The developing fetal brain can synthesize saturated and monounsaturated fatty acids (50,51). High rates of lipogenesis in fetal liver (52) may act as a source of fatty acids for the fetus. Initially the placenta, by controlling the passage of 20:4n-6 and 22:6n-3, determines the amount of these fatty acids available to the fetus, but then the fetus (by its own metabolism) starts to synthesize its own 20:4n-6 and 22:6n-3 (53). The age at which this progression may begin and be completely achieved is not defined. As synthesis of long-chain polyenoic fatty acids by fetal liver has not been clearly demonstrated under physiological conditions, this progression remains hypothetical (53). Thus, the contribution of fatty acid synthesis to 20:4n-6 and 22:6n-3 accumulation by the neonate would be affected by the age of the infant at birth. While *in utero*, it is clear that the fetus relies on the mother for its supply of many fatty acids, particularly the essential fatty acids.

Biomagnification. Crawford *et al.* (54) describe a process in which, compared with parent essential fatty acids, the relative percentage of 20:4n-6 and 22:6n-3 increases in phosphoglycerides progressively from maternal blood to placenta and to fetal blood, liver, and brain. By this process, termed biomagnification, specific mechanisms within the placenta hypothetically result in sequestration and release of specific fatty acids to the fetal circulation. Neuringer *et al.* (55) also reported that, in monkey and human fetuses, the levels of 22:6n-3 and 20:4n-6 are higher in fetal blood compared with maternal blood, whereas the opposite is true for their precursors. Thus, the importance of these long-chain polyunsaturated fatty acids is evident in their preferential, active transfer across the placenta to the fetus in a lipid form normally impermeable to the placental barrier. It has recently been proposed that a more likely mechanism of biomagnification is selective sequestering of long-chain polyunsaturated fatty acids on the fetal side of the placenta (56). King *et al.* (57) found that, when comparing adipose tissue triglyceride between infants and mothers, infants have greater levels of palmitic and palmitoleic acids. The predominance of these two fatty acids in newborns indicates that glucose plays an important role in fetal fat synthesis. Embryonic and fetal lipids in early gestation are derived from maternal fatty acids that cross the placenta, but with advancing gestational age, there is a gradual shift to *de novo* synthesis from glucose in fetal tissue (58).

It is evident that some fatty acids are transferred to the fetus across the placenta. It also appears that the degree of placental and fetal synthesis of fatty acids varies with gestational age (58,59). Clandinin *et al.* (48) reported that 80% of human fetal brain 22:6n-3 accrues between 26 and 40 wk of gestation. These authors also observed that infants born prior to 32 wk gestation have low concentrations of brain 22:6n-3. Early in pregnancy, there is apparently a great dependence on maternal fatty acids to provide the fetus with lipids. This may have important implications for the low-birthweight, premature infant and for the shift of essential fatty acid from maternal stores to fetal tissues during fetal growth.

Altering n-6 to n-3 fatty acid balance in premature infants. Human milk contains both n-6 and n-3 fatty acids. One percent 18:3n-3 and 10 to 15% 18:2n-6 are typical amounts of essential fatty acids in the breast milk fat of North American and European women (41,60). The need to balance n-6 and n-3 fatty acids fed to infants to reflect the overall fatty acid

balance in human milk was proposed by Clandinin *et al.* (61). Moreover, a balance between the levels of very-long-chain C20 and C22 n-6 and n-3 polyunsaturated components was recommended to be 1.4 (61). Based on quantitative analysis of 24-h milk collections, the n-3 long-chain polyenes are present at 0.3–0.6% and n-6 long-chain polyenes at 0.5–1.5% (41,60). Recommended ratios of n-6 to n-3 fatty acids in infant formula have also been suggested to be within the range of 4 to 1 and 10 to 1 (Health and Welfare Canada, 1991) or 5 to 1 and 15 to 1 (62). The concern surrounding an appropriate n-6 to n-3 fatty acid ratio stems from the competition that exists between the n-6 and n-3 series for the Δ-6 and Δ-5 desaturase enzymes. There is also the desire to mimic the composition of fats present in human milk and the effect of feeding this fatty acid balance on the fatty acid composition of various phospholipids in the developing neonatal brain or tissues (61). Studies that have attempted to increase only the n-3 intake by adding marine oil sources to diets have reported a predictable decrease in arachidonate levels in various cellular phospholipids (63–67).

Arachidonic acid status is an important factor in the growth of the fetus and premature infant (41,68). In the fetus and newborn, birthweight is significantly correlated to plasma triglyceride content of arachidonic acid (69). An important consideration in using marine oil sources is that fish oil contains both eicosapentaenoic and docosahexaenoic acid and results in higher levels of n-3 long-chain fatty acids compared with n-6 long-chain polyenes. This produces an unbalanced n-6 to n-3 ratio. Eicosapentaenoic acid may have a greater effect than docosahexaenoic acid on the reduction of arachidonate levels (63,70), which may be due in part to the inhibition of Δ-5 desaturation and competition for insertion into phospholipids. Mohrhauer and Holman (71) reported that the precursor n-3 fatty acid, 18:3n-3, also reduces arachidonate levels. Other groups studying both humans and rats (66,72,73) have since reported this observation. Dyer and Greenwood (32) examined neural long-chain fatty acids in weanling rats in response to a range of 18:2n-6 and 18:3n-3 fatty acid ratios between 1.8 and 165 and concluded that the dietary 18:2n-6 to 18:3n-3 ratio affected membrane fatty acid profile. The level of brain arachidonic acid did not appear to be significantly affected by lower 18:2n-6 to 18:3n-3 ratios.

Effect of dietary 18:2/18:3 ratios on long-chain polyene content of brain. Research on the effect of dietary fat on brain development has generally been limited to undernutrition, malnutrition, or essential fatty acid deficiency and study of animal models. Within what are believed to be essential fatty acid-adequate diets, studies that have focused on altering n-6/n-3 fatty acid ratios have been limited to analyzing the brain as a whole or to considering the response at only one time period. Research in this area has not examined the effect of changes in dietary fat content on lipid composition of different brain regions or different cell types during one of the initial developmental periods. To date, most research has examined the effects of diet on brain composition and inferred effects on neuronal function from behavior. Much less is known about glial cell responsiveness to alterations in nutrient supply in the absence of malnutrition (22). Studies of brain regions have focused primarily on the cerebrum, brainstem, and cerebellum. Few studies have examined the hippocampus, despite the relative ease of isolation and removal of this structure and the fact that it completes development during the postnatal period.

In a recent experiment (74), the fatty acid composition of rat brain cells in different brain regions was examined over time in response to feeding diets varying in fat composition. Diets of similar 18:2n-6 and 18:3n-3 content, with and without small amounts of arachidonic and/or docosahexaenoic acid, were fed to nursing dams. The n-6 to n-3 fatty acid ratios were within the recommended range for infant formulas and varied between 4 to 1 and 7.3 to 1. The cerebellum and the frontal and hippocampal brain regions were excised from rat pups at birth and at 1, 2, 3 and 6 wk of age. Neuronal and glial brain cells were isolated from each brain region. By analysis of the fatty acid composition of ethanolamine-, choline-, serine- and inositolphosphoglycerides, it was apparent that the diets fed altered neuronal and glial cell composition differently and in a region- and time-specific manner. It is thus clear that analysis of whole brain cannot reflect the complexity of changes occurring during development of individual brain regions.

It can be concluded that the temporal development of brain regions is different—the cerebellum and the hippocampus are among two of the regions to complete development postnatally; the timing of development differs between the brain cells—the majority of neurogenesis is complete prior to completion of gliogenesis; and physiological changes in dietary fat affect the fatty acid composition of brain regions and of cell types. The functional implications of these changes in brain structural lipids are still unknown. However, it is certain that feeding small amounts of 20:4n-6 and/or 22:6n-3 will produce remarkable transitions in the fatty acid composition of individual membrane phospholipids during the postnatal growth and development of the brain.

SPECULATIVE RELATIONSHIPS AND COGENT QUESTIONS

The brain is an incredibly complex organ. Connections within the nervous system may exhibit a high degree of variability between individuals and change during the lifetime of each individual (75). These anatomical connections influence learning and memory and ultimately affect how an individual interacts with and adapts to an environment. Confounding variables influencing an individual's interactions are genetics and environmental factors, including nutrition. In addressing possible later behavioral differences resulting from a nutritional insult, such as low 20:4n-6 or 22:6n-3 status during development, consideration of both genetic and environmental factors is relevant. Comparisons of one without the other will not clearly reveal consistent insights, as both factors affect the development of an individual and the expression of traits in later life.

It is difficult to establish conclusive links between the functional and behavioral effects of nutritional insults when many of the methods for testing behavior cannot be clearly or easily related to human functioning (76). Other methods attempt to correlate the appearance of a behavior with the development of specific indices, such as neurotransmitter systems. However, to prove cause-and-effect relationships in this way would be limiting, as no one event occurs independently in the developing brain or whole animal. Thus, the appearance of a behavior, or the lack thereof, may also be correlated with other events, such as postnatal development and maturation of cells, myelination, endocrine system maturation, or skeletal muscle development. Subsequently, modifications produced by behavior or cognition involve not just a single cell and its connections but many cells and their connections. In this regard, it may not be reasonable to expect to identify a clear relationship between n-6 and n-3 fatty acid imbalance and the exhibition, or lack thereof, of a single behavior or functional impairment.

One important challenging question that remains to be addressed is the effect(s) arising from nutritional imbalances or deficiency in availability of long-chain essential fatty acids. If long-chain n-6 and n-3 fatty acids are so important in function and development of the brain, why are specific characteristics not evident in large populations? To answer this question, we need to understand how the lack of or imbalance of 20:4n-6 and 22:6n-3 during development may be manifested in terms of characteristics or behaviors in later life. These "effects" have not been defined, although suggestions have been made that depression, visual development, and perhaps "intelligence" are adversely affected, at least initially. It has been demonstrated that visual function is slow to develop if adequate docosahexaenoic acid is not supplied. However, visual function is improved with the addition of this fatty acid. This does not imply that the individual will never catch up or adapt, nor does it imply that the individual will not be as "smart." Visual function is not related to an individual's intellectual capacity, as evidenced by visually impaired individuals who are not intellectually impaired. Intellect is the "faculty of knowing and reasoning; understanding." Intelligence is acquired through exposure to the environment and experience, which occur in many forms. In this respect, testing intelligence by present methods is limiting and perhaps inappropriate in some situations. These views regarding vision and intellect are no doubt provocative, but until further research is conducted in this area, and until appropriate measures of intelligence are developed and confounding variables carefully analyzed, care should be taken in drawing conclusions and making speculations. Many questions remain unanswered.

Future directions should focus on establishing functional and/or behavioral implications caused by varying the n-6 to n-3 fatty acid ratio within the ranges recommended by experts and by including or omitting long-chain polyunsaturated fatty acids, namely, docosahexaenoic and arachidonic acids. A discrepancy remains regarding the degree to which a newborn human infant is capable of desaturating and elongating 18:2n-6 and 18:3n-3 to their longer-chain homologs. That is, immediately after birth there is no significant accretion of chain elongation–desaturation products. This suggests that the limiting factor may initially exist in liver synthesis of these products or that mobilization of these long-chain polyunsaturated fatty acids from the liver exceeds the capacity of the liver to synthesize them from dietary precursors (61). If the capability for desaturation exists, is it sufficient to support the requirements of the developing nervous system? If it is sufficient, in what instances, diseases, or dietary circumstances may it be compromised? Premature or very-low-birthweight infants are likely candidates in this category. Is the full-term infant also a likely candidate in certain circumstances? What factors are likely to affect this activity? Maternal nutritional status during pregnancy may be one example. Another example is the occurrence of fatty acids in the diet. Fatty acids do not occur individually but with other fatty acids and are associated with vitamins and other nutrients. These clusters of nutrients likely also affect how individual fatty acids are metabolized.

Little quantitative evidence is available to indicate the sources of 20:4n-6, 22:4n-6, 22:5n-3, and 22:6n-3 in the fetus. During intrauterine development, does the fetus rely entirely on placental synthesis and a transfer mechanism to obtain these essential structural long-chain fatty acids or are they synthesized by the fetus or in specific tissues? Because development of the brain is not uniform and many vulnerable periods exist prenatally and postnatally, the use of whole-brain analyses is conceptually quite limiting. In the same regard, because phospholipids vary in fatty acid composition, analyses of total brain phospholipids are also limiting. In terms of correlating functional deficits with morphological alterations, Vitiello and Gombos (21) noted that these can occur only in some cases. These authors point out that relationships between structure and function in brain are not always well defined. Thus, a single morphological structure cannot always be identified as the structure essential for a certain function (21). The need exists to focus our efforts on developing innovative tests to dissect out functions of the central nervous system that are interpretable in terms of brain regions, groups of neurons, or transmitter activity and function. These tests would need to be applicable in some form for use in infants and children and would then lead to the means to develop meaningful markers of essential fatty acid status during fetal and infant development.

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