Effects of maternal malnutrition and postnatal nutritional rehabilitation on brain fatty acids, learning, and memory

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Undernutrition still affects mothers and children in developing countries and thus remains the major focus of nutritional intervention efforts. Neuronal development, which classically includes neurogenesis, migration, maturation, and synapse refinement, begins in utero and continues into the early postnatal period. These processes are not only genetically regulated but also clearly susceptible to environmental manipulation. Dietary deprivation during early life is known to have adverse effects on brain anatomy, physiology, and biochemistry, and may even lead to permanent brain damage. Although all nutrients are important for the structural development of the central nervous system, lipids such as long-chain polyunsaturated fatty acids, especially docosahexaenoic acid (22:6 n-3) and arachidonic acid (20:4 n-6), are important for normal brain development. The purpose of this literature review is to examine how early undernutrition involving a deficiency in long-chain polyunsaturated fatty acids can affect brain development and function and produce deficits in spatial cognitive learning ability.

INTRODUCTION

Malnutrition is defined as a diet containing insufficient quantities of nutrients or a diet in which one or more essential nutrients is missing or is present in the wrong proportions. Undernutrition refers to an overall deficiency of nutrients (carbohydrates, proteins, fats, vitamins, and minerals) due to an inadequate intake as part of an otherwise well-balanced diet. Undernutrition is a more severe form of malnutrition.

Malnutrition during the first years of life is a major health issue in developing countries. Growth deficits due to malnutrition in childhood are associated with higher mortality, increased incidence of infectious diseases, delayed psychomotor development, academic underachievement, and lower productive capacity in adult life. Infants suffering from severe nutritional deprivation during early postnatal life exhibit neurointegrative disorders and various degrees of mental retardation that may persist for years after nutritional recovery.

However, most of the studies to date report the outcomes of children from developing countries who survive early malnutrition. Flaws in the experimental design of these studies make it difficult to extricate the effects of malnutrition from the concomitant adverse effects of poverty, poor social circumstances, and lack of stimulation.

Even though the worldwide prevalence of severe protein malnutrition in infancy and childhood has decreased over the past 25 years, high birth rates in some countries have actually led to an increase in the number of severely malnourished children in some geographical areas, such as East Africa.

In Brazil, a substantial decline in the prevalence of childhood undernutrition (13.5% in 1996 to 6.8% in 2006/7) has been documented by Monteiro et al. Two-thirds of this reduction can be attributed to the favorable evolution of four factors: 25.7% of the reduction is due to increased maternal schooling; 21.7% of the reduction is due to increased purchasing power of
families; 11.6% of the reduction is due to the expansion of healthcare; and 4.3% of the reduction is due to improvements in sanitation. However, regional and social gaps in malnutrition, which are generally larger in less-developed regions and among lower-income individuals, often remained unchanged or even increased. Simultaneous with the observed decline of childhood undernutrition is increasing evidence of concomitant obesity and malnutrition in Latin American countries, including Brazil. As reviewed by Sawaya and Roberts, undernutrition in Brazil and in developing countries lasts throughout gestation and childhood and can even be followed from one generation to another. The individuals who survive malnutrition are likely the ones who have physiological adaptations and live in environmental conditions that minimize the effects of malnutrition. More recently, high levels of micronutrient deficiencies have also been observed in developing countries, and anemia continues to be highly prevalent and may trend toward an epidemic condition. Thus, despite improvement in several social indicators in Brazil, the gap between the poor and rich is still extremely large, and the fight against hunger is still important. Action must be taken to transform this current scenario and produce a genuine improvement in the living conditions of the poor in terms of health and intelligence indicators.

Childhood nutrition is important for performance based on intelligence indicators, since the formation of the brain begins at the period of conception and spans to the fifth year of life. This developmental period is extremely important for central nervous system (CNS) development. In the 1990s, Colombo revised IQs at different ages (i.e., in infants up to 2 years of age, in preschool-age children, and in school-age children) and related them to the nutritional states of the children. He observed that growth delay and low IQ were associated with poverty. The few studies carried out in Brazil confirm these findings. In a study conducted in São Paulo, Brazil, a significant association between abnormal height at 7 years of age and harmed school performance was observed. These studies involved children with deficiencies in a range of macro- and micronutrients. The issue of whether deficiency of a nutrient or a group of nutrients could impact neurodevelopment is a matter of broad biological importance.

Currently, it is recognized that all nutrients are important for neuronal cell growth and development, but some nutrients appear to be more important than others during the late fetal and neonatal time periods. These nutrients include protein, iron, zinc, selenium, iodine, folate, vitamin A, choline, and long-chain polyunsaturated fatty acids (LC-PUFAs), especially docosahexaenoic acid (DHA) and arachidonic acid (AA). In humans, protein and zinc deficiency in pregnancy has been linked to impaired DNA, RNA, and protein synthesis during brain development and to hereditary brain abnormalities.

One important question has always been whether vulnerable periods for early nutrition can influence or program later cognition or related mental functions. Experimental studies have shown that prenatal or postnatal nutritional deficits may program adult size, metabolism, blood lipids, diabetes, obesity, blood pressure, glomerular hypertrophy, atherosclerosis, behavior, and learning. Thus, perinatal malnutrition may contribute to an increased risk of chronic diseases years later and may lead to alterations in CNS function.

Brain plasticity provides protection for this organ from external influences and allows it to adapt to environmental influences (e.g., malnutrition). However, this adaptation depends on the timing, duration, and severity of the insult experienced. The greatest effect of malnutrition on brain development occurs during times of rapid brain growth and/or vulnerable or sensitive time periods. Rapid brain growth, known as a “brain growth spurt,” occurs from the third trimester of pregnancy to up to 24 months after birth in humans and from gestation day 15 to up to 21 days after birth in rats. An accumulating body of evidence indicates that the earlier the dietary insult, the more severe and permanent its effects. Thus, it is plausible that early malnutrition in humans during a sensitive period of early brain development could result in delayed development and have long-lasting effects on cognitive function. If such effects are due to deficiencies in specific nutrients, such as omega-3 fatty acids, the causal relationships may be hard to prove. Despite the complexity of human cognitive function and interspecies differences in the timing of the brain growth spurt, the possible influence of nutritional factors on the functional properties of the CNS may affect cognitive learning ability.

The principal focus of this review is to obtain better insights into the effects of malnutrition in early life on neurodevelopment by answering the following questions: What are the effects of malnutrition in early life and of rehabilitation from malnutrition on brain fatty acids (FAs) composition? Are the effects of malnutrition irreversible? Will the offspring of mothers who consume a multideficient diet during the perinatal period present behavioral changes, and do these changes persist after nutritional rehabilitation?

**THE NEED FOR DIETARY LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN THE BRAIN**

Two types of PUFAs, omega-6 (n-6) and omega-3 (n-3), are essential for mammals. These PUFAs cannot be synthesized de novo by mammals and need to be acquired.
through the diet. Linoleic acid (LA; 18:2 n-6) is the pre-
cursor of long-chain n-6 PUFA (arachidonic acid [AA];
20:4 n-6), and alpha-linolenic acid (ALA; 18:3 n-3) is the
precursor of long-chain n-3 PUFA (docosahexaenoic acid
[DHA]; 22:6 n-3). AA and DHA are derived from their
respective dietary essential precursors, LA and ALA,
through a series of desaturations and subsequent chain
elongations. In addition, eicosapentaenoic acid (EPA; 20:5 n-3)
and DHA can act as competitors for AA in metabolic
pathways. In human studies, analyses of the composition
of FAs in both blood phospholipid and adipose tissue
have shown a similar competitive relationship between
n-3 LC-PUFAs and AA. The general scientific consensus
is that the increased consumption of n-3 FAs and the
reduced intake of n-6 FAs promote good health.
However, specific quantitative recommendations vary
widely for n-3 FA intake. The extent to which ALA is
converted to EPA and DHA in humans remains unclear,
as does the extent to which the high intake of n-6 FAs
compromises the benefits of n-3 FA consumption.
During pregnancy, the bulk of FA delivery and storage
in the fetus occurs in the last trimester. The developing
human fetus accumulates as much as 400 mg of DHA per
week during the last trimester,17 most of which is incor-
porated into the structural lipids of the developing
brain.19-21 At this time, preformed DHA is preferentially
porated into the structural lipids of the developing
ids.22 ids strongly correlate with maternal plasma phospholip-
ids.22-24 Phospholipids are the main reservoir of n-3 and n-6
LC-PUFAs in the brain. The FA content of various neu-
ronal membranes is subject to dietary modifications, i.e.,
the brain FA composition reflects the composition of
dietary FAs.27,28 Phospholipids consist of a glycerol back-
bone with a hydrophilic phosphate-containing head at
sn-3. Various saturated and unsaturated FAs occupy the
sn-1 and sn-2 positions, respectively. LC-PUFAs n-3 and
n-6 usually occupy the sn-2 position of brain phospho-
lipid molecules. This position is targeted by a family of
acylhydrolases known as phospholipase A2 (PLA2),
which liberate the PUFA situated in the backbone. A
massive influx of Ca2+ triggers PLA2 activation and leads
to the rapid accumulation of n-6 and n-3 PUFAs, specifi-
cally AA and DHA.29 PLA2 activation may also be trig-
ged by the proinflammatory cytokines interleukin-1β
(IL-1β) and tumor necrosis factor-alpha30 or in response
to neurotransmitters such as glutamate. These lipid
messengers may act intracellularly in an autocrine and/or paracrine fashion to regulate
other signaling cascades.31 IL-1β is a potent proinflam-
atory cytokine produced by macrophages and micro-
glia. IL-1 receptors are widely distributed in the brain and
are concentrated in the hippocampus, amygdala, and
hypothalamus. In rats, intracerebroventricular adminis-
tration of IL-1 induces stress, anxiety-like behavior,33 and
memory impairment34 and can alter neurotransmission
properties.35 Rats fed an n-3 FA-deficient diet exhibited
memory impairment and stress/aggressive behavior.36
Spatial memory deficits induced by IL-1 were attenuated
in rats fed 1% EPA for 7 weeks.37 These studies indicate
EPA and AA differentially modulate IL-1-induced
changes in neurotransmitter synthesis and metabolism
and in brain inflammatory responses, which may corre-
late with changes in stress-related behavior.34,37 However,
rats fed diets in which the only lipid source was flaxseed
(which consists of 57% ALA and only 16% LA) displayed
growth deficits but improved learning and memory,
despite low levels of brain AA concentrations, compared
to rats fed a diet with a balanced FA content (Fernandes,
Souza, and Tavares do Carmo, unpublished data).
Furthermore, evidence suggests precursors or
derivatives of n-3 FAs may have different functions from
their end products. For example, EPA, a precursor of
DHA, can effectively treat depression and schizophrenia,
whereas DHA does not have the same effect.38 DHA plays a crucial role in diverse functions at multiple levels. At the membrane level, it has the following effects: changes in blood-brain barrier function; alteration of membrane receptors such as rhodopsin; regulation of the activity of membrane-bound enzymes (Na/K-dependent ATPase), ion channels, and dopaminergic and serotonergic neurotransmission, most likely by changing membrane fluidity; and alteration of signal transduction by means of its effects on inositol phosphates, diacylglycerol, and protein kinase C. At the cellular level, DHA can protect neural cells from apoptotic death, stimulate neurite outgrowth in PC12 cells, induce synaptic growth cone formation during neuronal development, enhance synaptic functions, regulate nerve growth factor, and influence neuron size.31,39–42 Several studies have confirmed the modulatory action of PUFA on gene expression in the brain.36,43,44 It has been reported that brain expression of c-fos, Gir, Glut1 and neuropeptide Y mRNA are decreased in n-3 PUFA-depleted rats.45,46 Furthermore, feeding rats with high-PUFA diets increased pro-opiomelanocortin and galanin-like peptide mRNA expression in the arcuate nucleus compared to the corresponding low-fat diet and the high-saturated-fat diet. It has been observed that high n-3 PUFA feeding halved melanin-concentrating hormone and prepro-orexin mRNA expression in the lateral hypothalamus compared to high-fat and low n-3 PUFA diets.46

In summary, LC-PUFAs are important in the transfer of signals across the synapse between two nerve cells,17 although how they are involved remains unclear. Nevertheless, it is known that human infants and animals with low levels of brain DHA exhibit poor memory and slow learning.47–49

**MALNUTRITION, COGNITIVE FUNCTION, AND NEUROTRANSMITTERS**

Cognition is defined as “the act or process of knowing, including both awareness and judgment,” whereas cognitive function is defined as encompassing learning, memory, and attention processes.50 “Learning” is classically defined as a relatively permanent behavioral change as a result of practice or experience. When an infant or young animal responds in an adaptive way to a stimulus, learning (or information processing) has occurred. “Memory” is then defined as the persistence of a learned behavior over time. “Attention” refers to a global behavioral construct that includes numerous response classes such as impulsivity, sensitivity to delay, activity level, sustained attention, and ability to manage delay of reward.51,52 In infants, attention research has focused on four areas of visual attention: alertness, spatial orienting, attention to object features, and endogenous or internally directed attentional functions (e.g., attention span, perseverence, and distractibility).11

Where are memories stored in the brain, and which structures and areas of the brain are involved in memory and cognitive process? It seems that brain areas involved in the neuroanatomy of memory, such as the frontal and temporal cortex, cerebellum, hippocampus, amygdala, striatum, and mammillary bodies, are involved in specific types of memory. For example, the cerebral cortex appears to play an important role in complex learning, such as spatial and declarative learning. Its main function appears to be involvement in the integration and consolidation of separate sensory information. The amygdala also appears to play an important role in memory consolidation, especially when emotional experience is involved. Furthermore, the cerebellum seems to play a key role in memory for classically conditioned responses and it contributes to many cognitive tasks in general. Learning and memory are attributed to changes in neuronal synapses and seem to be mediated by long-term potentiation (LTP) and long-term depression.52–56 The neurotransmitters serotonin, glutamate, and acetylcholine also appear to be vital to memory function. Other physiological chemicals, structures, and processes also play important roles, although further investigation is required to identify these roles.56

Many studies have shown that malnutrition early in life affects the morphology,57,58 neurochemistry,59 and neurophysiology60 of the hippocampal formation, the main brain region associated with spatial learning and memory.61–63 Given the limitations of experimental research on malnutrition in human subjects, much of what is known about the effects of malnutrition on cognitive function comes from animal research, mostly conducted in rodents. As recognized by McCann and Ames,64 the study of animals is essential for estimating the potentially harmful effects of malnutrition on humans. Experiments in animals have the potential to reveal relationships between early malnutrition, alterations in brain structures, and the resulting behavioral and cognitive consequences because the experimental variables can be precisely controlled. This issue has been discussed in a 2005 review by McCann and Ames.64

Among all types of learning, spatial learning has been particularly well studied using tests such as the radial maze,65 spatial alternation,65,66 the spatial navigation task,66 and, most commonly, the water maze.66,67 In this realm, brain FA composition may be correlated with cognitive behavioral changes in human and nonhuman mammals.68

The role of brain cholinergic activity in learning and memory was first recognized more than 30 years ago.
Damage or abnormalities in forebrain cholinergic projections, which are important to memory structures (e.g., cortex, hippocampus), correlate well with the level of cognitive decline. Acetylcholine is the best-characterized neurotransmitter related to cognitive function, and both muscarinic and nicotinic cholinergic receptors have been found to play important roles. Glutamate systems, particularly those involving N-methyl-D-aspartate (NMDA) receptors, have also been shown to be involved in cognition. Both animal and human studies indicate these receptors play a role in the processes of spatial learning and memory. In addition, M₁ muscarinic cholinergic receptors actively participate in higher cognitive processes, including learning and memory. Muscarinic cholinergic receptors may also have roles in learning and memory during the aging process, as cognitive deficits were observed in aged and memory-impaired rats in a Morris water maze.

There have been several reports that LTP occurs in the hippocampus and leads to a prolonged increased responsiveness of the affected neurons; NMDA receptors are necessary for triggering this process. The NMDA-receptor system is involved in spatial learning and supports the hypothesis that LTP is involved in some forms of learning. Other G-protein-coupled receptors, such as metabotropic glutamate receptors, can contribute to LTP in the hippocampus. According to the assessment of spatial reference memory, M₁ muscarinic cholinergic receptors are important in cortical memory function and in the interaction between the cortex and the hippocampus.

MALNUTRITION, OMEGA-3 POLYUNSATURATED FATTY ACID-DEFICIENT DIET, AND THE BRAIN

Biochemical evidence may exist to support essential FA deficiency in protein-energy malnutrition. Essential FA deficiency is characterized by low levels of LA, often in combination with low levels of AA and DHA, and high levels of 18:1 n-9 and 20:3 n-9, with subsequent cognitive deficit. However, it cannot be extrapolated that deficiencies of some FAs alone cause cognitive deficit. Several studies have been published showing that diets high in saturated fat and simple carbohydrates may affect memory in both humans and rodents, possibly through brain-derived neurotrophic factor (BDNF)-mediated effects on dendritic spines. A deficit in the availability of PUFAs, such as DHA, markedly impairs neuronal signal transmission. Chronic dietary deficiency of n-3 FAs in animals is associated not only with changes in retinal and visual function but also with alterations in performance on various tests of learning and memory. A study conducted by Lukoyanov and Andrade showed that a diet low in protein (8% casein diet) has little effect on motor function and activity but leads to a marked impairment in learning. The inferior learning performance of the protein-deprived group was associated with a nearly 30% reduction in the average density of synaptic vesicles in the terminals of the hippocampal CA1 region.

Studies have shown that a dietary deficiency of ALA in developing animals results in decreased DHA levels, with a reciprocal increase in n-6 FAs, especially docosapentaenoic acid. These alterations in DHA and docosapentaenoic acid levels can be observed in the retina, whole brain, isolated brain membranes, and specific brain regions. In fact, a DHA deficit is associated with attention deficit disorder with or without hyperactivity in children.

DHA deficiency can lead to cognitive impairment and various neurological disorders. Learning and behavior are affected by PUFA deficits, especially deficits in DHA. Rats fed a diet deficient in n-3 FAs demonstrate longer latencies in the Morris water maze compared to rats fed a normal diet. Mice exposed to a diet deficient in ALA both pre- and postnatally require more time to learn the Morris water maze compared to control animals. Deficits in spatial learning and memory in underfed animals were correlated with physiological and neuroanatomical changes during hippocampal formation, mainly in the CA3 and CA4 regions. Several mechanisms may explain how these FAs are involved in learning and memory.

Within the mammalian brain, 22:6 n-3-containing phospholipids are concentrated in synaptic membranes, while the accumulation of esterified 22:6 n-3 in neuronal membranes seems to correlate with development of synapses. There have been several reports on the modulation of bioaminergic neurotransmission in relation to changes in the n-3 FA content of the neuronal membrane, with possible effects on receptor properties. Additionally, the n-3 FA content of membranes can affect receptor activation of the signal transduction pathway. It has been suggested that DHA becomes incorporated into the hippocampus, altering the fluidity of the neuronal membrane, facilitating neurotransmission, and possibly improving learning and memory.

Deficiency in n-3 PUFA results in changes in the membrane phospholipid composition of the hippocampus and the frontal cortex, with a dramatic loss of DHA. However, the cholinergic pathway is modified only in the hippocampus but not in the frontal cortex. Basal acetylcholine (ACh) release in the hippocampus of n-3 PUFA-deficient rats is significantly higher than that in controls, whereas the potassium-chloride-induced release of ACh is lower. Deprivation of n-3 PUFA also causes a reduction in muscarinic receptor binding compared to controls. In contrast, the activity of acetylcholinesterase
and the vesicular ACh transporter in both brain regions is unchanged.\textsuperscript{91,92} Thus, an n-3 PUFA-deficient diet can affect cholinergic neurotransmission, probably via changes in the phospholipid PUFA composition. The increased release of ACh could be due to changes in synthesis, storage, or exocytosis. Diet-induced changes in neuronal lipid composition could result in an increased spontaneous release of ACh (without neuronal depolarization), which would, in turn, deplete the vesicular store of ACh. Thus, the pool of newly synthesized ACh, which is mainly mobilized during neuronal activation, would be reduced in the hippocampus of the n-3 PUFA-deficient rats, leading to a lower stimulated outflow. It has been shown that potassium chloride produces an 11-fold increase in extracellular ACh in control rats, while this effect is reduced by 34% in the hippocampus of n-3 PUFA-deficient rats.\textsuperscript{91}

The concentration gradient between extra- and intracellular Ca\textsuperscript{2+} is maintained by Ca-ATPase in neuronal membranes. Na/K-ATPase activity, which is important for nerve conductance, has been shown to be modulated by dietary n-3 FAs.\textsuperscript{93} LC-PUFAs, like free FAs, affect the frequency of action potentials in mouse hippocampal neurons.\textsuperscript{94} The mechanism of this action appears to be through the inhibition of Na\textsuperscript{+} and Ca\textsuperscript{2+} currents. A recent study reported Na/K-ATPase activity is inhibited by both DHA and EPA.\textsuperscript{95} Unesterified 22:6 n-3 has also been shown to increase the Na\textsuperscript{+} and Ca\textsuperscript{2+} currents of the NMDA receptor in rat pyramidal neurons ex vivo.\textsuperscript{96}

Perinatal dietary n-3 PUFA deficiency also leads to the overexpression of a zinc transporter, zinc transporter 3, which sequesters zinc from extracellular fluid into areas of the brain involved in learning and memory. Overexpression of zinc transporter 3 leads to poor cognitive performance.\textsuperscript{97} Notably, only partial recovery of both brain DHA levels and spatial performance occurs after 2 weeks of dietary repletion; full recovery occurs on the probe trial and partial recovery for the learning task occurs after 6 weeks of diet repletion.\textsuperscript{58}

Nishizaki et al.\textsuperscript{98} suggested AA is produced by the activation of ionotropic glutamate receptors and is involved in LTP. AA has been shown to exert a long-lasting facilitation of synaptic transmission in the CA1 region of rat hippocampal slices, and this facilitation occludes tetanic LTP. Therefore, AA appears to be a significant factor in the expression of LTP; it may act by binding to a site on the NMDA receptor or by modifying the receptor’s lipid environment. Miller et al.\textsuperscript{99} suggested AA released by the activation of NMDA receptors may potentiate NMDA receptor currents and thus amplify the increases in intracellular calcium concentration caused by glutamate. This amplification may explain why inhibition of PLA\textsubscript{2} blocks the induction of LTP.

**PERSPECTIVES OF NUTRITIONAL REHABILITATION AFTER MALNUTRITION**

Data concerning the effects of maternal malnutrition on birth outcome in rural India have been reported in a prospective study of 797 rural Indian mothers, where birth size was strongly associated with consumption of green leafy vegetables and fruits.\textsuperscript{108} In the early 1990s and more recently, a sharp negative association of fish intake during pregnancy with the risk of both low birth weight and preterm delivery in Danish communities was demonstrated.\textsuperscript{101-103} In addition, there is strong evidence that malnutrition is associated with delayed mental development, poor school performance, and reduced intellectual capacity in developing countries and in several geographic regions of the world.\textsuperscript{3,104-108} It is important to note that, despite recent improvements in the indicators, malnutrition still prevails in several regions, although at lower rates. Malnutrition has been assessed by means of the weight/height and height/age indicators, as recommended by the World Health Organization.\textsuperscript{109} The rate of chronic malnutrition was 51.3% among Mexican migrant infants and was attributed to a monotonous and nutrient-poor diet, justifying a nutritional intervention, since this constitutes a specific group at risk.\textsuperscript{110}

In humans, multinutrient supplementation that includes zinc, iodine, choline, and LC-PUFAs, especially n-3 PUFAs, may have advantages over single-nutrient supplements, for example, iron or folate. Future nutritional care programming for healthy subjects in utero may require individual assessment and follow-up, including preconceptional nutritional preparation, appropriate weight control, metabolic balance assessment, and food-based regimens enhanced by key nutrient fortification and/or supplementation, leading to further research on nutritional optimization of pregnancy outcomes.\textsuperscript{111} For example, in a recent study, participants with moderate or intensive use of olive oil compared to those who never used olive oil showed lower odds of cognitive deficit for verbal fluency and visual memory.\textsuperscript{112}

For obvious reasons, it is not possible to obtain direct information regarding the brain composition of rehabilitated human subjects who survive malnutrition in early life. Follow-up studies show that children who are malnourished during their first years maintain a reduced head circumference throughout their lives, even if they undergo the best possible nutritional rehabilitation until 5 years of age.\textsuperscript{113} A wide range of cognitive deficits has been observed in malnourished children in India.\textsuperscript{114} The literature indicates the end result is cognitive deficit; however, studies are inconclusive regarding a partial or total reversal by means of nutritional rehabilitation after malnutrition.
Table 1  Fatty acid composition of total lipids in experimental diets.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Commercial lab chow (n = 5 diets)</th>
<th>Regional Basic Diet (RBD) (n = 6 diets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣSFA</td>
<td>27.41 ± 0.85</td>
<td>39.37 ± 0.78**</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>22.52 ± 1.28</td>
<td>25.96 ± 0.78*</td>
</tr>
<tr>
<td>18:2n-6 cis linoleic</td>
<td>45.48 ± 1.15</td>
<td>19.66 ± 0.85**</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>0.13 ± 0.01</td>
<td>0.23 ± 0.02**</td>
</tr>
<tr>
<td>20:2n-6</td>
<td>2.87 ± 0.20</td>
<td>nd</td>
</tr>
<tr>
<td>20:4n-6 AA</td>
<td>0.31 ± 0.03</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Σ(n-6) PUFA</td>
<td>46.39 ± 1.36</td>
<td>20.38 ± 0.90**</td>
</tr>
<tr>
<td>18:3n-3 α-linolenic</td>
<td>2.87 ± 0.09</td>
<td>11.44 ± 0.57**</td>
</tr>
<tr>
<td>20:5n-3 EPA</td>
<td>0.16 ± 0.01</td>
<td>0.48 ± 0.05**</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.21 ± 0.02</td>
<td>0.16 ± 0.01**</td>
</tr>
<tr>
<td>22:6n-3 DHA</td>
<td>0.31 ± 0.03</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Σ(n-3) PUFA</td>
<td>3.44 ± 0.07</td>
<td>12.25 ± 0.57**</td>
</tr>
<tr>
<td>18:2n-6/18:3n-3</td>
<td>15.91 ± 0.39</td>
<td>1.75 ± 0.13**</td>
</tr>
<tr>
<td>18:2n-6 + 18:3n-3</td>
<td>48.35 ± 0.94</td>
<td>31.10 ± 0.62**</td>
</tr>
<tr>
<td>AA/DHA</td>
<td>1.00 ± 0.03</td>
<td>2.20 ± 0.03**</td>
</tr>
<tr>
<td>(n-6)/(n-3)</td>
<td>13.49 ± 0.19</td>
<td>1.69 ± 0.11**</td>
</tr>
<tr>
<td>(n-6) + (n-3)</td>
<td>49.82 ± 1.43</td>
<td>32.63 ± 1.09**</td>
</tr>
</tbody>
</table>

*P < 0.05 and **P < 0.001 in relation to commercial lab chow according to t test.

The total lipids were extracted according to the method of Bligh and Dyer (1959). Fatty acids from the experimental diets were chromatographed as methyl esters according to Lepage and Roy (1986). Analysis was performed on a Perkin-Elmer Autosystem XL gas chromatograph equipped with a flame ionization detector and Turbochrom software. The column was wall-coated with 0.20 mm SP-2560. Hydrogen was used as the carrier gas, and synthetic air was used as the make-up gas. The split ratio was 1:70. The injection port temperature was 260°C, and the detector temperature was 280°C. The column temperature was held at 135°C for 5 min and then increased in a stepwise fashion until it reached a plateau of 240°C. The gas chromatograph was calibrated using a standard mixture of fatty acids (Sigma, Supelco).

The results are expressed as percentage of weights (mg/100 g all fatty acid). Values represent means ± SEM.

Abbreviations: nd, not determined; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Σ(n-6) PUFA, sum of linoleic series; Σ(n-3) PUFA, sum of linolenic series; (n-6) + (n-3), sum of linoleic and linolenic series; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

In northeastern Brazil, malnutrition is still a serious problem. A laboratory-prepared Regional Basic Diet (RBD), simulating what is normally consumed by people living in a specific region of this area, the Zona da Mata de Pernambuco, known for sugar-cane cultivation, was devised. The RBD lacks protein, vitamins, and minerals in quality or quantity, being very poor in lipids, relatively rich in n-3 FAs and poor in n-6 FAs (Table 1). The n-6 to n-3 FA ratio is 1.69 to 1 and produced in rats a type of balanced diet was presented after the lactation period, female offspring showed a complete reversal in the growth rate deficit and attained the same absolute and relative body and brain weights as the control rats. However, males who underwent nutritional rehabilitation were not able to fully recover (see Table 2). These results are consistent with the literature, which shows that the deficit of absolute body weight induced by protein restriction during the rapid growth phase is not completely re-established by a normal diet. However, the body weight gain (%) was fully restored in male rats.

The RBD diet caused severe loss of body and brain weight in malnourished male and female rats. When a balanced diet was presented after the lactation period, female offspring showed a complete reversal in the growth rate deficit and attained the same absolute and relative body and brain weights as the control rats. However, males who underwent nutritional rehabilitation were not able to fully recover (see Table 2). These results are consistent with the literature, which shows that the deficit of absolute body weight induced by protein restriction during the rapid growth phase is not completely re-established by a normal diet. However, the body weight gain (%) was fully restored in male rats.
Table 2  Effect of experimental diets on the absolute and relative body and brain weights of male and female rats at 70 days of life.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Malnourished</th>
<th>Rehabilitated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number tested</td>
<td>17</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>263.59 ± 13.12a</td>
<td>34.49 ± 1.65b</td>
<td>187.42 ± 19.82c</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>1.716 ± 0.038a</td>
<td>1.193 ± 0.037b</td>
<td>1.566 ± 0.032c</td>
</tr>
<tr>
<td>Brain (g/100 gBW)</td>
<td>0.65 ± 0.010a</td>
<td>3.46 ± 0.009b</td>
<td>0.84 ± 0.014c</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number tested</td>
<td>14</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>204.15 ± 8.42a</td>
<td>34.54 ± 1.62b</td>
<td>192.83 ± 10.83a</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>1.512 ± 0.045a</td>
<td>1.139 ± 0.035b</td>
<td>1.470 ± 0.021a</td>
</tr>
<tr>
<td>Brain (g/100 gBW)</td>
<td>0.74 ± 0.019a</td>
<td>3.30 ± 0.014b</td>
<td>0.76 ± 0.011a</td>
</tr>
</tbody>
</table>

Wistar rats originating from the colony at the Instituto de Nutrição Josué de Castro of Universidade Federal do Rio de Janeiro (Brazil) were used. Virgin mature female rats were mated with males and kept in single cages, maintained under standard laboratory conditions (24 ± 2°C and a 12:12-h light-dark cycle) and provided with water and chow ad libitum. Pregnancy was assumed to have been established when sperm was found in vaginal secretions. The day following the night of mating was considered day zero of pregnancy and, at this moment, the female rats were divided into two groups according to the diet employed during pregnancy and lactation: 1) nourished group (control group), where the rats received the control diet with 23% protein and 5% lipids (Purina of Brazil Ltda.); and 2) malnourished group, where the rats received the Regional Basic Diet (RBD). Given its higher carbohydrate content, the RBD is considered to be an isocaloric diet because its caloric adequacy has been found to be comparable with that of the commercial control diet. The fatty acid compositions of the diets are summarized in Table 1. After weaning (22 days after birth), female and male offspring from each group were subdivided according to nutritional treatment: the control offspring continued on a nutritionally adequate diet (control group) until the day of experimentation, while the RBD offspring either continued on the multideficient diet (malnourished group) or were switched to the control diet (rehabilitated group). Absolute body and brain weights and relative brain weights were determined using a precision digital scale.

Values are expressed as mean ± SEM. Different letters in the same row represent statistically different values, with P < 0.05 determined by the Bonferroni post-hoc test.

Abbreviations: g, grams; g/100 g BW, grams of tissue per 100 g body weight.

larger brain weight and larger heads relative to the body, confirming previous studies that suggest the existence of mechanisms that protect important tissues such as the brain.124 Evolutionary perspectives on FA contributions to selection pressures suggest the n-3 LC-PUFAs may have played a role in the enlargement of the human brain,24 improving cognition to the detriment of amelioration of behavioral impairments. These data support the view that the mature CNS displays a remarkable potential to recover both structurally and functionally after nutritional insult.81 This observation is of great importance, especially if the results can be extrapolated to humans. In a model of malnutrition using the multideficient RDB diet, nutritional rehabilitation resulted in reduced behavioral impairments of female offspring. Spatial memory was evaluated using the Morris water maze and aversive memory was tested using the passive avoidance task. The data suggest fetal brain development is affected in a negative and possibly irreversible fashion when dams are fed the RBD during pregnancy and lactation and the offspring consume the same diet (RBD) throughout life. When a standard rodent diet was introduced (Labina® lab chow), the animals’ spatial memory was recovered, showing that cognitive behavior induced by malnutrition, at least of this type, can be reversed.116

In Galler’s studies125,126 in which female rats underwent rehabilitation for one or two generations on an adequate level of dietary protein following a history of intergenerational malnutrition, no recovery occurred after two generations of dietary rehabilitation for most measures of maternal behavior, including active nursing, passive nursing, pup-oriented behavior, time spent in the nest, or time spent in contact with the young. In contrast, nest quality improved to normal levels after rehabilita-
tion. In addition, the growth of pups born to females rehabilitated for one generation was similar to the growth of control pups, and in the case of mothers rehabilitated for two generations, the growth of offspring exceeded that of the control pups. Consequently, some of these abnormalities in maternal behavior may result in persistent abnormalities in the behavior of offspring over several generations.

There are many controversial results in the literature with respect to complete reversal of cognitive deficits after nutritional rehabilitation, both in animal studies and in studies with humans.

CONCLUSION

Malnutrition imposed by a multideficient diet during critical periods of development affects the spatial learning capability of offspring. However, nutritional intervention can reverse these deficits. Furthermore, there is an association between these alterations and changes in LC-PUFAs of 20- and 22-carbon atoms in the brain. LC-PUFAs play important roles in the development of the CNS. Increased or decreased levels of LC-PUFAs may reflect an attempt to preserve brain function by altering the absorption, turnover, and utilization of these nutrients. Since several brain regions develop during the perinatal period, the full genetic brain potential may never be achieved due to early malnutrition, despite the great adaptability of the brain and later reversal of nutrient deficiencies. The degree of recovery following malnutrition may depend on the duration, quality, and intensity of deprivation.

Members of the n-3 FA family are mostly present in cerebral structures and may exert a positive influence on animal performance in cognitive testing. However, supplementation with n-3 FAs aimed at improving neurological performance must be undertaken with caution. The levels of n-6 FAs must also be taken into account, as deviations from a normal ratio of AA to DHA can lead not only to growth deficits but also to changes in the synthesis of prostaglandins, leukotrienes, and thromboxanes; changes in this ratio may interfere with hormone production, fertility, immunity, and inflammatory response.

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Declaration of interest. The authors have no relevant interests to declare.

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