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Alterations in Levels and Ratios of n-3 and n-6 Polyunsaturated Fatty Acids in the Temporal Cortex and Liver of Vervet Monkeys from Birth to Early Adulthood

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Running Title: Developmental changes in PUFAs of temporal cortex and liver

Highlights:

- Levels of the n-3 long chain polyunsaturated fatty acid (PUFA), docosahexaenoic acid, increased dramatically in the brain in the vervet monkey from birth to early adulthood.
- Marked changes in the ratios of n-6 to n-3 PUFAs occurred in the brain during this same time period.

- This period of DHA accretion is concurrent with the symptomatic onset of numerous psychiatric and developmental disorders in humans.
- These data raise the question of whether this is a period where lack of dietary n-3 PUFAs impacts brain development.

Abstract

Deficiencies in omega-3 (n-3) long chain polyunsaturated fatty acids (LC-PUFAs) and increases in the ratio of omega-6 (n-6) to n-3 LC-PUFAs in brain tissues and blood components have been associated with psychiatric and developmental disorders. Most studies have focused on n-3 LC-PUFA accumulation in the brain from birth until 2 years of age, well before the symptomatic onset of such disorders. The current study addresses changes that occur in childhood and adolescence. Postmortem brain (cortical gray matter, inferior temporal lobe; n=50) and liver tissues (n=60) from vervet monkeys fed a uniform diet from birth through young adulthood were collected from a tissue bank. Lipids were extracted and fatty acid levels determined. There was a marked reduction in the ratio of n-6 LC-PUFAs, arachidonic acid (ARA) and adrenic acid (ADR), relative to the n-3 LC-PUFA, docosahexaenoic acid (DHA), in temporal cortex lipids from birth to 7 years of age. This decreased ratio resulted from a 3-fold accumulation of DHA levels while concentrations of ARA remained constant. Early childhood through adolescence appears to be a critical period for DHA accretion in the cortex of vervet monkeys and may represent a vulnerable stage where lack of dietary n-3 LC-PUFAs impacts development in humans.

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INTRODUCTION

Long chain PUFAs (LC-PUFAs) have numerous roles that include biophysical properties essential for proper plasma membrane function, energy production by β-oxidation and precursors of bioactive lipids essential for immunity and inflammation. Omega-3 (n-3) and omega-6 (n-6) LC-PUFAs also play a critical role in proper development and function of the brain (1-5). The brain is particularly rich in LC-PUFAs as compared to other mammalian tissues; brain dry matter is about 50% lipid (6), and docosahexaenoic acid (DHA, C22:6 n-3) is the most abundant n-3 LC-PUFA in the brain and retina. Additionally, DHA is selectively incorporated into the synaptic membranes of neurons (7).

The importance of adequate n-3 LC-PUFA intake for proper neural development during fetal and postnatal period is well established (3-5, 8-10). More recent studies suggest that n-3 LC-PUFA deficiency and altered ratios of n-6 to n-3 LC-PUFAs are associated with attention-deficit/hyperactivity (ADHD), schizophrenia, autism spectrum (ASD) and major depressive disorders (MDD) in children, adolescents and young adults and impact cognitive loss and associated brain disorders (such as Alzheimer's disease) in older adults (11-17). The n-6 LC-PUFA, arachidonic acid (ARA, C20:4 n-6) is also a major LC-PUFA found in the brain and its metabolic products are crucial to orchestrating cell signaling in both immunity and inflammation (18). In humans, ARA constitutes 8-15% of the total FAs within inflammatory and neural cellular lipids (6, 19).

LC-PUFAs cannot be synthesized *de novo*, but must be obtained preformed from the diet or synthesized from essential dietary 18 carbon (18C-) PUFA precursors, such as α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6). The pathways and attendant enzymes are presented in Figure 1. ARA is synthesized from LA using three (2 desaturation and 1 elongation) enzymatic steps (20). The primary n-3 LC-PUFA found in the brain, DHA, is synthesized from dietary ALA through seven (3 desaturation, 3 elongation and 1 β-oxidation) enzymatic steps. The first three enzymatic steps that form ARA and eicosapentaenoic acid (EPA, 20:5n-3) from LA and ALA, respectively, are carried out by the same three enzymes. EPA is then further converted to DHA utilizing additional biosynthetic steps (2 elongations, 1 desaturation and 1 β-oxidation).

Recent studies suggest that the efficiency of some of these steps (particularly the desaturation steps) is highly impacted by variants in genes (such as *FADS1* and *FADS2*) that encode for these enzymes (21-24). In mammals, much of the capacity to synthesize LC-PUFAs is thought to reside in liver and adipose tissues (25). However, astrocytes have been demonstrated to synthesize the DHA necessary for neuronal survival and differentiation (26-28). In addition, LC-PUFAs are moved from circulation across the blood brain barrier (29, 30).

The aforementioned studies reveal that the balance of dietary intake and biosynthesis of LC-PUFAs together with the movement of LC-PUFAs across the BBB are all critical to human health, especially the development and maintenance of brain function. While it is well established that there is a marked accretion in DHA and ARA within the brain early in development (31, 32), much less is known about levels and the relationship of n-3 and n-6 LC-PUFA throughout the life span. However, the symptomatic onset of several psychiatric illnesses such as ADHD, ASD, schizophrenia and mood disorders occurs from adolescence to early adulthood and many of these disorders have been linked to n-3 deficiency (33). These studies raise the important question of whether childhood/early adolescence is a particularly vulnerable time with regard LC-PUFA homeostasis in the brain.

Significant challenges to understanding the role of LC-PUFA metabolism during this period of time include: 1) the lack of access to human brain tissue from this age group; 2) genetic differences in the capacity of different human populations to synthesize tissue LC-PUFAs; and 3) variance in human diets that impact tissue levels of LC-PUFAs. Animal models have attempted to bridge these gaps, but differences (between animal and human models) in LC-PUFA biosynthesis and metabolism (34) and brain structure make translation of the findings difficult. The current study was designed to fill this gap by focusing on the FA composition of the temporal lobe cortex and liver in nonhuman primates raised on uniform diets from birth to early adulthood.

METHODS

Animals and Tissues.

The Vervet Research Colony at Wake Forest Primate Center is a multigenerational pedigreed colony of vervets/African green monkeys (*Chlorocebus aethiops sabaeus)* (35). Animals included in this study were known-age, US-born, mother-reared and housed in identical indoor-outdoor matrilineal social groups. Archived brain and liver tissue samples were received for 50 and 62 vervets, respectively. Brain tissues were retrieved from the cortex of the inferior temporal lobe. The characteristics of animals used in this study are shown in Table 1. Tissue samples were organized into nine age groups ranging from less than one day to 8.8 years (Table 1). Vervet females and males reach puberty at 2.5 and 4 years, respectively (35).

Tissues were collected during experimental necropsies that were part of a separate study. Animals were anesthetized with ketamine (10-20 mg/kg, i.m.) and then administered sodium pentobarbital (60-100 mg/kg i.v.) to a deep plane of anesthesia. The chest was opened, a 14G needle was inserted into the left ventricle, a 1 cm incision was made in the inferior vena cava and the vasculature was flushed with cold saline until outflow was clear (~5-10 minutes). The brain was removed from the skull, weighed and hemisected. A section of the temporal lobe from the left hemisphere was frozen in liquid nitrogen and transferred to a -80°C freezer for long-term storage. An aliquot of liver was also collected, frozen in liquid nitrogen and stored at -80°C.

Diets.

Animals younger than three months nursed from mothers that also consumed the Chow LabDiet 5038. It is possible that these young animals consumed chow in addition to mother's milk, although this was not actively observed by caretakers. Animals above the age of three months received Chow LabDiet 5038 (13.1% calories from fat; LabDiet, St. Louis, MO) and water *ad libitum* until the time of necropsy. Table 2 shows the FA composition of the chow diet analyzed by gas chromatography/flame ionization detection (GC/FID) of fatty acid methyl esters (FAME) for tissue lipids.

Fatty Acid Analysis.

The FA within total lipids was analyzed after saponification to account for esterified and nonesterified FAs in postmortem tissues. Chow diet, brain (temporal lobe) and liver tissue FAs were measured as FAME by GC/FID. FAME were prepared following a modification of the protocol by Metcalfe *et al*. (36, 37). Briefly, tissue samples were homogenized at 100 mg tissue/mL in distilled water. Triheptadecanoin (100 μg; a triglyceride of C17:0; NuChek Prep, Elysian MN, USA,) was added to homogenates as an internal standard and the mixture exposed to boron trifluoride to form fatty acid methyl esters. FAME were separated using an Agilent J&W DB-23 column (30 m × 0.25 mm ID, film thickness 0.25 μm) on an HP 5890 GC with a flame ionization detector. The FAs were identified by their elution times relative to authenticated FA standards, and quantities were determined by their abundance relative to the added internal standard. Approximately 23 and 24 FAs for brain and liver, respectively, were routinely identified and these accounted for ~99% of the FA peaks.

Calculations and Statistics.

In some instances, individual FAs were calculated as percent of total FAs from the concentration of individual FAs (μmol FA/mg tissue) relative to the total FA concentration within the tissue and are presented as mean ± standard error of the mean (±SEM). Linear regression analyses were performed, testing differences in mass percent of each FA using SAS (SAS Institute Inc, Cary NC). In figures 3A and 3B, the data are presented as the concentrations of LC-PUFAs in the tissue.

RESULTS

Table 2 shows the nutrient and FA composition of the chow diets fed to the animals throughout their life span. The animals consumed a relatively low fat (13% energy) and high carbohydrate (68% energy) diet compared to the modern Western diet (MWD). However, composition of PUFAs is similar to that which would be found in a MWD (38, 39). For example, n-6 and n-3 18C-PUFAs represented >97% of the total PUFA in the diet; consequently, the diets contained low concentrations of n-3 and n-6, preformed LC-PUFAs such as ARA and DHA. Additionally, the ratio of n-6 to n-3 PUFAs of 12.3 was consistent with that observed in the MWD.

The FA composition (expressed as a mean % of mass of total FAs) of the cortex of the temporal lobe from vervet monkeys ranging from birth to 3 months (during nursing) and 1 to >7 years old (on a chow diet) is shown in Table 3. Major FAs within the cortex were palmitic acid (PA, 16:0), oleic acid (OA, 18:1n-9), arachidonic acid (ARA, 20:4n-6), adrenic acid (ADR, 22:4n-6) and docosahexaenoic acid (DHA, 22:6n-3). There were large age-dependent shifts within individual FAs with PA, ARA and ADR decreasing and OA and DHA increasing. Overall, ratios of saturated FA (SFA) + MUFA to PUFA decreased modestly throughout the examined lifespan of the animals. There was a marked (2.8 fold) decrease in the overall ratio of n-6 to n-3 PUFAs and a similar reduction n-6 to n-3 LC-PUFAs reflecting the fact that the majority of PUFAs in this tissue were LC-PUFAs.

Figure 2 shows the levels (expressed as % of total) of the major n-6 and n-3 PUFAs within different age categories. There was a striking reduction in the proportion of n-6 LC-PUFAs, ARA and ADR concomitantly with an increase in DHA. ARA comprises 13.5% of total FAs at birth, and remains relatively constant ~8% from 3-8 years of age. Similarly, the elongation product of ARA, ADR, decreased from 6.9% to 1.9% from the youngest to the oldest age group. In contrast, the major n-3 LC-PUFA, DHA represented 11.2% of FAs at birth, increased to 20.6 % in the 3-7 year group, and remained at that level in older animals. The major PUFAs in the chow diets of the animals, LA and ALA represented small proportions of the total FAs within the cortex at all ages (Figure 2 and Table 3).

While these data showed marked alterations in the proportion of LC-PUFAs when expressed as a % of total FAs within the tissues, it was unclear whether these changes actually represented alterations in concentrations of both DHA and ARA within the cortex. Consequently, μmol quantities of these LC-PUFAs were determined and standardized to mg of cortical tissue.

Figure 3 shows the concentrations of DHA and ARA in the cortex of individual animals. These data clearly point out that DHA accumulates in the cortex of these animals throughout their early lifespan with approximately 5 μmol/mg tissue at birth and increasing 3-fold to approximately15 μmol/mg tissue in the oldest animals. There were no statistically significant changes in the concentration of ARA, remaining at 6-8 μmol/mg tissue during the animals' entire lifespan.

The FA composition across the life span was next examined in the liver (Table 4). The liver is an important target as it is the tissue most associated with LC-PUFA biosynthesis. As expected, LC-PUFAs comprised a much lower percentage of total FAs within the liver. In contrast, the liver contained much higher levels of 18C-PUFAs. The FA composition of the liver (after nursing) mirrored the content of dietary FAs (Tables 2 and 4). There was a consistent increase in all PUFAs between birth and 1 week in conjunction with nursing. This is reflected by a 1.9-, 1.8- and 1.7- fold increase in ARA, DHA and LA, respectively. With the exception of stearic acid (1.9-fold increase), levels remained relatively stable after nursing.

DISCUSSION

It has long been recognized that the ingestion of LC-PUFAs such as DHA, especially prenatally during the third trimester of pregnancy and postnatal through the first two years of life, is critical to proper brain and eye development and function in humans. It is during this period of time that PUFAs such as DHA and ARA accumulate in the central nervous system (40). This "DHA accretion spurt" has been associated with a marked increase in Δ-6 desaturase (enzymatic product of *FADS1*; Figure 1) activity in late embryonic and postnatal rodent brain (41, 42). However, less is known about the accretion of these FAs in the brain during adolescence and throughout adulthood.

In 2001, Carver and colleagues first showed that there was a bilinear increase in cortical DHA in humans with the latter phase continuing until 18 years of age (43). In contrast to DHA, ARA and its elongation product ADR decreased during this second phase. This study suggested that there may be a second period of vulnerability to LC-PUFA deficiency where biosynthesis and dietary ingestion of LC-PUFAs may be particularly important. However to our knowledge, this study has not been replicated. Evidence for a second phase of accretion would provide important context to the accumulating data which indicate that levels and ratios of LC-PUFA are associated with child and adolescent, psychiatric and developmental disorders (44-46).

The current study has addressed the dynamics of LC-PUFA levels in cortical brain and liver tissues in nonhuman primates (*i.e*. vervet monkeys) from birth through early adulthood. This animal model offered significant advantages including the capacity to control diet and other environmental factors, to limit genetic factors that could influence LC-PUFA metabolism, and the ability to sample tissues (*i.e.* liver) that could impact brain FA levels throughout the animals' early lifespan (47). These data strongly support the concept that there is a robust accretion of DHA in cortical brain tissue throughout adolescence into early adulthood in these animals. During the same period of time, concentrations of ARA and ADR remained relatively constant. These changes result in marked alterations in the ratios of n-3 to n-6 LC-PUFA found in the cortex at different points in time. In contrast, the PUFA composition of the liver closely represented the PUFA levels in the diets of the animals. LA was the

primary PUFA in liver tissue at all ages, and LC-PUFAs represented small proportions of the total PUFAs. Importantly, there was no evidence for large age-dependent changes in LC-PUFA levels or ratios in the liver.

These data raise the important question of the source of DHA that accumulates in the brain. Clearly, there are only small quantities of preformed DHA in the diet and the analysis of the PUFA composition of the liver suggests that the biosynthetic capacity of the liver to produce LC-PUFAs is not responsible for the changes in DHA observed in the brain. Brain specific mechanism(s) such as changes in LC-PUFA biosynthesis or the incorporation rates across the BBB over time in the primate brain are potential candidates for the marked alterations in the levels and ratios of DHA and ARA.

Several lines of evidence indicate that n-3 LC-PUFA deficiencies may play an adverse role in neurodevelopment and childhood behavior (33). Numerous studies report associations between reduced DHA and/or altered ratios of n-6 to n-3 ratios in both peripheral blood components as well as postmortem brain tissue and psychiatric illnesses, mood and developmental disorders and dementia (11-16). Depressive disorders are perhaps the most studied with regard to n-3 LC-PUFA composition. A recent meta-analysis of 14 studies concluded that patients with depression had significantly lower n-3 LC-PUFAs in blood compartments than control subjects (14). McNamara and colleagues found that postmortem orbitofrontal cortex from patients with schizophrenia, bipolar disorder and MDD all had significantly lower amounts of DHA when compared to control subjects (15). In regard to developmental disorders such as ADHD in childhood/adolescence, a recent meta-analysis of nine studies (n=586) found significantly lower blood levels of n-3 LC-PUFAs in ADHD children versus controls and concluded that n-3 LC-PUFAs are reduced in children with ADHD (17).

Importantly, several studies have shown that supplementation with n-3 LC-PUFA or combinations of n-3/n-6 PUFAs improve symptoms in ADHD, depression and learning difficulties (17, 44, 48-51). For example, Amminger and colleagues demonstrated the potential for n-3 LC-PUFAs to prevent adolescents at high risk for psychosis from transitioning to a disorder (48). A randomized, double blind, placebo-controlled study with n-3 LC-PUFAs in children showed the experimental group to be

superior to placebo on several depression scale measures (49). Recent meta-analyses suggest improvement of composite ADHD symptoms in n-3 LC-PUFA treatment groups as compared to placebo controls (17, 44, 50, 51).

Our data are consistent with Carver and colleagues report that DHA levels increase and n-6 to n-3 LC-PUFA ratios change dramatically in the cortical brain tissue from childhood to adolescence (43). If levels of these FAs are critical to basic neurobiology such as the neuronal growth, survival, synaptogenesis and neurotransmitter release as has been reported, then a disruption in the rapidly changing milieu of LC-PUFA homeostasis could have important biological and clinical effects (33, 52-58). The incidences of several childhood psychiatric and developmental disorders including depression, ADHD and ASD have increased over the past two decades. Such increases suggest that environmental factors (such as diet) maybe playing an important role. Perhaps the largest change as a result of the MWD is the dramatic elevation in the dietary n-6 PUFAs in only 50 years (59, 60). This increase has altered the balance of PUFAs entering the biosynthetic pathway leading to reductions in DHA and alterations in n-6 to n-3 PUFA ratios in human tissues such as the brain (59). The current data together with an earlier human study (43) reveal that concentrations of DHA rapidly accumulate (~3-fold) in the cortex of the brain from childhood through early adulthood suggesting this may be a critical period of n-3 LC-PUFA biosynthesis. Consequently, this time period may represent an important opportunity to alter the intake of PUFAs (either from the diet or utilizing supplements) in a fashion that will have meaningful impact on the incidence and severity of child/adolescent psychiatric and developmental disorders.

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Table 1. Characteristics of the animals and tissues.

Age in Days	# Males	# Females	Mean Age (Days)	Mean Body Weight (kg)	Mean Brain Weight (g)	Mean Liver Weight (g)
0-1	3	2	0.8	0.4	43.5	10.6
7-9	3	2	7.6	0.3	43.2	10.1
29-95	8	7	60.3	0.6	60.2	18.8
176-379	5	6	254.0	1.4	69.6	37.3
477-722	5	8	654.0	2.5	74.5	56.6
887-1132	4	3	982.3	3.7	77.1	74.7
2082-2894	1	2	1441.7	4.4	73.6	105.6
3003-3223	3	2	3018.8	2.5	71.0	58.6

Table 2. Composition of the chow diet. This Labdiet 5038 (LabDiet, St. Louis, MO) was consumed by all animals after cessation of nursing. Data in the diet composition section of this table were obtained from the nutritional facts published by LabDiet (http://www.labdiet.com/Products/StandardDiets/Primates/index.htm). Fatty acid composition was analyzed by GC/FID as described in the methods section.

Diet Composition	
Protein (%)	18.2
Carbohydrates (%)	68.7
Cholesterol (ppm)	75.0
Fat (%)	13.1
Fatty Acid	**% of Total Fatty Acids**
C14:0 [myristolate]	1.0
C16:0 [palmitic]	19.8
C16:1 [palmitoleic]	1.4
C18:0 [stearic]	7.9
C18:1 n-9 [oleic]	28.5
C18:1 n-11	2.3
C18:2 n-6 [LA]	35.2
C18:3 n-3 [ALA]	2.3
C20:1 n-9	0.6
C20:2 n-6	0.3
C20:4 n-6 [ARA]	0.1
C20:5 n-3 [EPA]	0.3
C22:6 n-3 [DHA]	0.2
SFA + MUFA/PUFA	**1.6**
n-6/n-3 PUFA	**12.3**

Table 3. Fatty acids in brain tissue of different age groups

	BREAST FED BRAIN, % Mass			CHOW FED BRAIN, % Mass							
	Age			Age							
Fatty Acids	Birth %(SD) n=5	1 Week %(SD) n=4	1-3 Months %(SD) n=14	6 Months- 1 Year %(SD) n=6	1-2 Years %(SD) n=10	Years %(SD) n=4	3-7 Years %(SD) n=2	7+ Years %(SD) n=5	p-value (Δ 0-3 Months)	p-value (Δ ≥6 Months)	p-value (Δ lifespan)
C14:0	**1.0** (0.2)	**0.8** (0.1)	**0.5** (0.2)	**0.3** (0.2)	**0.3** (0.0)	**0.3** (0.0)	**0.3** (0.0)	**0.3** (0.0)	< 0.0001	0.077	< 0.0001
C16:0	**26.5** (0.3)	**25.1** (0.8)	**24.3** (0.4)	**21.9** (0.3)	**21.8** (0.4)	(3.6)	**22.3** (0.6)	**21.5** (0.5)	< 0.0001	0.28	< 0.0001
C16:1	**1.2** (0.1)	**0.9** (0.2)	**0.5** (0.1)	**0.4** (0.1)	**0.4** (0.0)	**0.4** (0.0)	**0.4** (0.0)	**0.5** (0.0)	< 0.0001	< 0.0001	< 0.0001
C18:0	**20.3** (0.2)	**21.0** (0.7)	**21.1** (0.2)	**22.2** (0.3)	**22.2** (0.5)	(5.2)	**21.2** (0.2)	**21.8** (0.8)	< 0.0001	0.29	< 0.0001
C18:1 n-9	**10.3** (0.3)	**11.0** (0.3)	**11.2** (0.3)	**12.3** (0.2)	**13.5** (0.7)	**(2.6)**	**13.5** (0.8)	**14.4** (0.8)	< 0.0001	0.002	< 0.0001
C18:1 n-7	**3.7** (0.2)	**3.5** (0.0)	**3.4** (0.1)	**3.6** (0.0)	**4.1** (0.4)	**4.4** (0.9)	**4.7** (0.1)	**4.9** (0.3)	0.0007	< 0.0001	< 0.0001
C18:2 n-6 [LA]	**0.6** (0.1)	**1.2** (0.3)	**1.6** (0.4)	**1.3** (0.1)	**1.2** (0.1)	**1.1** (0.1)	**1.1** (0.0)	**1.1** (0.0)	< 0.0001	0.14	0.087
C18:3 n-6 [GLA]	**0.2** (0.0)	**0.1** (0.1)	**0.2** (0.0)	**0.1** (0.0)	**0.1** (0.1)	**0.1** (0.1)	**0.1** (0.1)	**0.1** (0.0)	0.50	0.45	0.15
C18:3 n-3 [ALA]	**0.1** (0.2)	**0.0** (0.0)	**0.0** (0.0)	-	-	-	-	-	0.13	-	0.16
C18:4 n-3 [SDA]	-	-	-	-	-	-	-	-	-	-	-
C20:0	-	-	-	-	-	-	-	-	-	-	-
C20:1 n-9	**0.2** (0.0)	**0.2** (0.0)	**0.2** (0.0)	**0.3** (0.0)	**0.5** (0.1)	**0.3** (0.1)	**0.5** (0.1)	**0.6** (0.1)	0.003	0.013	< 0.0001
C20:2 n-6	**0.5** (0.1)	**0.4** (0.0)	**0.3** (0.1)	**0.3** (0.0)	**0.3** (0.0)	**0.2** (0.0)	**0.3** (0.0)	**0.3** (0.0)	< 0.0001	0.26	< 0.0001
C20:3 n-6 [DGLA]	**1.1** (0.1)	**1.3** (0.0)	**1.4** (0.1)	**1.0** (0.2)	**1.1** (0.1)	**0.9** (0.2)	**0.9** (0.1)	**0.9** (0.1)	< 0.0001	0.15	0.001
C20:4 n-6 [ARA]	**13.5** (0.4)	**13.3** (0.6)	**12.5** (0.9)	**11.0** (0.3)	**9.6** (0.9)	**8.5** (3.1)	**8.3** (0.4)	**8.0** (0.6)	0.0004	< 0.0001	0.0002
C20:4 n-3	-	-	-	-	-	-	-	-	-	-	-
C20:5 n-3 [EPA]	-	-	-	-	-	-	-	-	-	-	-
C22:0	-	-	-	-	-	-	-	-	-	-	-
C22:1 n-9	-	-	-	-	-	-	-	-	-	-	-
C22:4 n-6 [ADR]	**6.9** (0.4)	**6.9** (0.2)	**6.3** (0.4)	**6.2** (0.3)	**4.5** (0.6)	**1.9** (2.9)	**4.5** (0.6)	**1.9** (2.7)	0.006	0.004	< 0.0001
C22:5 n-6	**2.6** (0.4)	**2.5** (0.5)	**2.2** (0.3)	**1.5** (0.4)	**2.9** (0.5)	**3.9** (2.6)	**1.0** (0.1)	**3.3** (2.1)	0.039	0.13	0.29
C22:5 n-3 [DPA]	**0.3** (0.1)	**0.3** (0.1)	**0.4** (0.1)	**0.4** (0.0)	**0.3** (0.1)	**0.3** (0.1)	**0.3** (0.0)	**0.3** (0.0)	< 0.0001	0.53	0.72
C22:6 n-3 [DHA]	**11.2** (0.5)	**11.6** (0.4)	**13.9** (1.8)	**17.2** (0.7)	**17.0** (1.3)	(7.6)	**20.6** (1.8)	**19.8** (1.3)	< 0.0001	0.001	< 0.0001
C24:1 n-9	**0.0** (0.0)	**0.04** (0.1)	**0.0** (0.0)	-	-	-	-	-	0.62	-	0.21
(SFA+MUFA)/PUFA	1.8	1.8	1.7	2.2	2.3	2.9	2.2	2.3	< 0.0001	0.001	0.28
Total n-6/n-3	2.2	2.1	1.7	1.2	1.1	1.1	0.8	0.8	< 0.0001	< 0.0001	< 0.0001
LC-PUFA n-6/LC-PUFA n-3	2.1	2.0	1.6	1.1	1.0	1.0	0.7	0.7	< 0.0001	< 0.0001	< 0.0001

Cortex samples (n=50) were obtained from the tissue bank and analyzed for total fatty acid content as described in Methods. Data are expressed as **mean % mass**, (SD) of total fatty acids. Linear regression p-values are listed for the time span of both nursing and chow feeding, in addition to the entire lifespan. Ratios representing fatty acid intake and metabolism are also included. Fatty acids of interest included linoleic acid (LA), Gamma -Linoleic Acid (GLA), Alpha-Linolenic Acid (ALA), Stearodonic Acid (SDA), Dihomo-Gamma-Linolenic Acid (DGLA), Arachidonic Acid (ARA), Eicosapentaenoic Acid (EPA), Adrenic Acid (ADR), Docsapentaenoic Acid (DPA), Docosahexaenoic Acid (DHA).

Table 4. Fatty acids (expressed as % of total) in liver tissue in different age groups

	BREAST FED LIVER, % Mass			CHOW FED LIVER, % Mass							
	Age			Age							
Fatty Acids	**Birth** **%**(SD) n=5	**1 Week** **%**(SD) n=5	**1-3 Months** **%**(SD) n=15	**6 Months- 1 Year** %(SD) n=8	**1-2 Years** **%**(SD) n=12	**2-3 Years** %(SD) n=7	**3-7 Years** **%**(SD) n=3	**7+ Years** **%**(SD) n=5	**p-value (Δ 0-3 Months)**	**p-value (Δ ≥6 Months)**	**p-value (Δ lifespan)**
C14:0	**0.7** (0.2)	**0.3** (0.1)	**0.3** (0.1)	**0.3** (0.1)	**0.3** (0.1)	**0.3** (0.1)	**0.4** (0.1)	**0.4** (0.1)	< 0.0001	0.00009	0.018
C16:0	**30.5** (1.8)	**21.6** (1.2)	**20.3** (1.4)	**18.1** (0.7)	**18.7** (0.9)	**20.4** (1.3)	**22.4** (2.8)	**24.6** (1.5)	< 0.0001	< 0.0001	0.0002
C16:1	**4.8** (1.0)	**0.6** (0.1)	**0.4** (0.2)	**0.5** (0.1)	**0.5** (0.1)	**0.6** (0.1)	**0.7** (0.2)	**0.7** (0.2)	< 0.0001	0.0003	< 0.0001
C18:0	**10.2** (1.2)	**18.2** (0.8)	**19.8** (1.9)	**21.3** (1.1)	**21.1** (0.5)	**20.0** (2.9)	**19.1** (2.7)	**20.2** (0.5)	< 0.0001	0.058	< 0.0001
C18:1 n-9	**23.4** (1.7)	**10.1** (1.8)	**8.5** (1.4)	**8.1** (1.2)	**9.5** (1.1)	**9.7** (0.9)	**10.2** (2.3)	**8.8** (1.0)	< 0.0001	0.066	< 0.0001
C18:1 n-7	**3.6** (0.6)	**2.5** (0.3)	**2.1** (0.6)	**2.1** (0.2)	**2.0** (0.3)	**2.1** (0.5)	**2.4** (0.3)	**2.3** (0.3)	< 0.0001	0.35	< 0.0001
C18:2 n-6 [LA]	**12.3** (2.0)	**17.7** (1.5)	**20.8** (1.0)	**23.6** (1.5)	**23.1** (1.1)	**22.7** (1.2)	**21.0** (2.5)	**21.7** (1.1)	< 0.0001	0.003	< 0.0001
C18:3 n-6 [GLA]	**0.2** (0.1)	**0.3** (0.1)	**0.2** (0.1)	**0.2** (0.1)	**0.2** (0.1)	**0.2** (0.1)	**0.2** (0.0)	**0.2** (0.1)	0.17	0.47	0.41
C18:3 n-3 [ALA]	**0.23** (0.1)	**0.2** (0.1)	**0.3**(0.3)	**0.3** (0.1)	**0.3** (0.1)	**0.3** (0.3)	**0.4** (0.1)	**0.3** (0.1)	0.89	0.69	0.15
C18:4 n-3 [SDA]	-	-	-	-	-	-	-	-	-	-	-
C20:0	-	-	-	-	-	-	-	-	-	-	-
C20:1 n-9	**0.1** (0.0)	**0.1** (0.0)	**0.2** (0.1)	**0.3** (0.1)	**0.3** (0.1)	**0.5** (0.3)	**0.5** (0.2)	**0.3** (0.1)	0.13	0.43	< 0.0001
C20:2 n-6	**0.4** (0.2)	**0.7** (0.1)	**0.8** (0.2)	**1.3** (0.2)	**1.2** (0.2)	**1.2** (0.1)	**1.0** (0.4)	**1.2** (0.1)	< 0.0001	0.12	< 0.0001
C20:3 n-6 [DGLA]	**1.2** (0.3)	**4.5** (0.8)	**3.6** (0.8)	**3.6** (1.0)	**4.1** (1.1)	**3.5** (0.6)	**3.6** (0.6)	**2.6** (1.3)	0.003	0.14	0.070
C20:4 n-6 [ARA]	**7.1** (1.1)	**13.5** (1.6)	**13.1** (0.7)	**12.2** (1.3)	**11.2** (0.7)	**10.9** (1.0)	**10.3** (0.2)	**9.5** (0.5)	0.25	0.15	0.42
C20:4 n-3	**0.03** (0.0)	**0.04** (0.0)	**0.1** (0.2)	**0.0** (0.1)	**0.1** (0.1)	**0.1** (0.1)	**0.1** (0.1)	**0.1** (0.1)	< 0.0001	< 0.0001	0.27
C20:5 n-3 [EPA]	**0.1** (0.1)	**0.2** (0.1)	**0.2** (0.2)	**0.4** (0.0)	**0.3** (0.1)	**0.3** (0.1)	**0.2** (0.0)	**0.3** (0.2)	0.036	0.030	< 0.0001
C22:0	-	-	-	-	-	-	-	-	-	-	-
C22:1 n-9	-	-	-	-	-	-	-	-	-	-	-
C22:4 n-6 [ADR]	**0.4** (0.1)	**0.6** (0.2)	**0.6** (0.1)	**0.6** (0.1)	**0.6** (0.1)	**0.7** (0.1)	**0.7** (0.1)	**0.8** (0.2)	0.016	0.022	< 0.0001
C22:5 n-6	**0.6** (0.1)	**1.1** (0.3)	**0.7** (0.2)	**0.5** (0.1)	**0.5** (0.1)	**0.5** (0.1)	**0.5** (0.1)	**0.4** (0.1)	0.70	0.38	< 0.0001
C22:5 n-3 [DPA]	**0.5** (0.1)	**1** (0.2)	**1.4** (0.3)	**1.3** (0.13	**1.0** (0.1)	**1.2** (0.5)	**1.3** (0.1)	**1.1** (0.1)	< 0.0001	0.64	0.011
C22:6 n-3 [DHA]	**3.7** (0.7)	**6.9** (1.2)	**6.7** (1.3)	**5.4** (1.0)	**4.8** (0.6)	**4.7** (0.5)	**5.0** (0.2)	**4.5** (0.7)	0.009	0.005	0.040
C24:1 n-9	**0.0** (0.0)	**0.0** (0.0)	**0.01** (0.0)	-	-	-	-	-	0.76	-	0.51
(SFA+MUFA)/PUFA	2.8	1.1	1.1	1.0	1.1	1.2	1.3	1.3	< 0.0001	< 0.0001	< 0.0001
Total n-6/n-3	5.0	4.7	4.6	5.7	6.3	6.0	5.3	5.8	0.50	0.96	< 0.0001
LC-PUFA n-6/LC-PUFA n-3	2.2	2.4	2.2	2.4	2.6	2.5	2.3	2.2	0.81	0.54	0.015

Liver samples (n=60) were obtained from the tissue bank and analyzed for total fatty acid content as described in Methods. Data are expressed as **mean %mass**, (SD) of total fatty acids. Linear regression p-values are listed for the time span of both nursing and chow feeding, in addition to the entire lifespan. Ratios representing fatty acid intake and metabolism are also included. Abbreviations as in Table 3.

Figure 1. Polyunsaturated Fatty Acid Biosynthesis. Biosynthetic pathway leading to long-chain polyunsaturated fatty acids (LC-PUFAs).

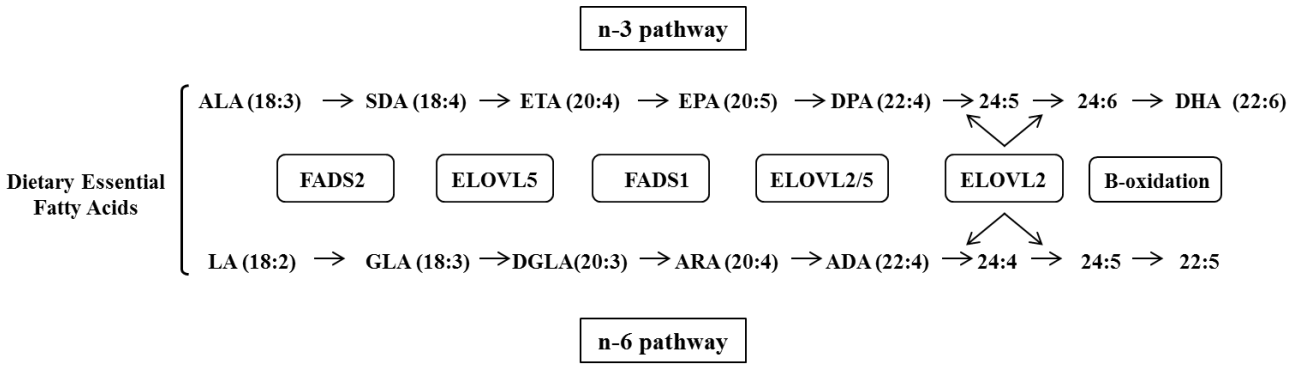

Figure 2. Levels and ratios of select PUFAs in brain tissue. PUFA levels are expressed as % of total mass and the ratio of DHA:ARA in brain tissue as a functions of animal age.

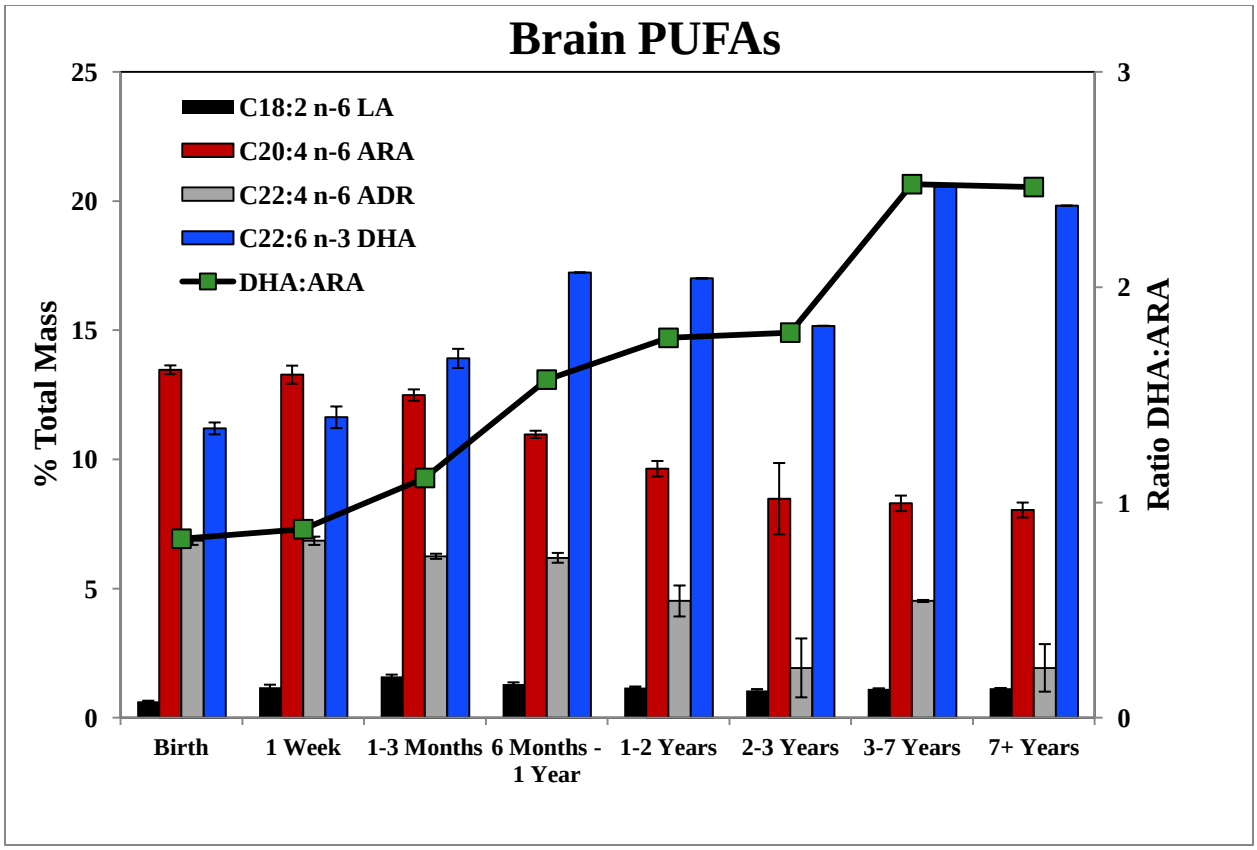

Figure 3. Mass concentration (µmol/ mg tissue) of ARA and DHA in (A) brain tissue and (B) liver tissue in individual animals. Linear regressions are shown for brain (ARA: R^2=0.027, p=0.238; DHA:

R^2=0.712, p<0.0001) and liver (ARA: R^2=0.014, p=0.357; DHA: R^2=0.026, p=0.197).