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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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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 (n=60) from vervet monkeys fed a uniform diet from birth through young adulthood were collected from archived tissues. 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

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LC-PUFA, docosahexaenoic acid (DHA), in temporal cortex lipids from birth to puberty and then a more gradual decrease though adulthood. This decrease in 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.

Keywords

Docosahexaenoic acid; arachidonic acid; psychiatric and developmental disorders; omega-3 deficiency; brain

1. INTRODUCTION

The brain is highly enriched in lipids and particularly long chain (>20 carbons) polyunsaturated fatty acids (LC-PUFAs). Additionally, it is well established that adequate dietary intake of LC-PUFA is important for proper neural development during prenatal and postnatal periods up to 2 years of age [1–5]. This is especially the case for docosahexaenoic acid (DHA, C22:6 n-3) and arachidonic acid (ARA, C20:4 n-6), the most abundant LC-PUFAs in the brain and retina. The last trimester and particularly the last 5 weeks of pregnancy appear to be a critical period of time for DHA and ARA accretion in the fetal brain [6]. Additionally, DHA and ARA have distinct roles in brain function and a balance of DHA and ARA, and thus specific DHA/ARA ratios, appear to be required for optimal mental health [7].

Numerous studies indicate that DHA 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 [8–24]. Given the symptomatic onset of many of these diseases typically occurs during periods of rapid cortical circuit maturation from early childhood to adulthood, there is a need to better understand alterations in LC-PUFAs within critical regions of the brain during this period of time [25]. Initial studies by Martinez and Mougan demonstrated that DHA increased while ARA remained constant in forebrains of humans up until 7 years of age [26]. Carver and colleagues later showed that there was a bilinear increase in cortical DHA levels in humans, with the latter phase continuing until 18 years of age [27]. In contrast to DHA, ARA and its elongation product, adrenic acid (ADR, C22:4 n-6) decreased during this second phase. DHA in cortex phospholipids (particularly ethanolamine-containing phospholipids) also increases approximately 2 fold from birth to 22 months of age in rhesus monkeys [4]. Together, these studies suggest that there may be an important period of time after early childhood where dietary ingestion and biosynthesis of DHA but not ARA may be particularly important. The objective of the current study was to expand these studies in a non-human primate model where critical environmental variables such as diet could be strictly controlled.

LC-PUFAs are obtained directly from the diet or synthesized in tissues from essential dietary 18 carbon PUFA precursors, such as α-linolenic acid (ALA, C18:3 n-3) and linoleic

acid (LA, C18:2 n-6). For example, ARA is synthesized from LA using alternating desaturation and elongation enzymatic steps [28, 29]. DHA is initially synthesized from dietary ALA utilizing the same desaturation and elongation steps to form C24:6 n-3. Historically, it has been proposed that C24:6 n-3 is then converted to DHA by a peroxisomal β-oxidation step. However, a recent study suggests mammals can also utilize an alternative pathway for DHA biosynthesis where a double bond is introduced at the Δ4 position of docosapentaenoic acid (DPA, C22:5 n-3) to form DHA [29].

Much of the capacity to synthesize LC-PUFAs is thought to reside in the liver. Once formed, LC-PUFAs are released into circulation as complex lipids (phospholipids, lyso-phospholipids, triglycerides, and cholesterol esters) or free fatty acids. These LC-PUFAs can then be specifically transported into tissues such as the brain [30–36]. Additionally, cells within the brain, including astrocytes and some neurons, have been shown to synthesize low levels of ARA and DHA from LA and ALA, respectively, *in vitro* [37].

Significant challenges to a better understanding of LC-PUFA metabolism and accretion after prenatal and early postnatal periods include: 1) the lack of access to human brain tissue from different age groups; 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. Studies in rodent models have attempted to bridge these gaps, but differences in LC-PUFA biosynthesis and metabolism [38] and brain structure between rodent and humans make translation of the findings difficult. The current study was designed to address the question of whether there are temporal changes in LC-PUFA levels in the brain over the early lifespan by focusing on the fatty acid (FA) composition of the temporal lobe cortex verses the liver in the vervet monkey. We measured LC-PUFA from archived liver and brain tissues collected from animals raised on uniform diets from birth to early adulthood. We show that levels of DHA and ratios n-3 and n-6 LC-PUFAs change dramatically within cortical brain tissue of vervet monkeys during the first three years of development.

2. METHODS

2.1 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)* [39]. Animals included in this study were of known-age, US-born, mother-reared and housed in identical indoor-outdoor matrilineal social groups. Archived brain and liver tissue samples were received for 53 and 64 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 ~3 years of age, respectively [39].

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.

2.2 Diets

Animals younger than three months nursed from mothers that consumed the Chow LabDiet 5038 (13.1% calories from fat; LabDiet, St. Louis, MO). 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 and water *ad libitum* until the time of necropsy. Table 2 shows the composition of the LabDiet (manufacturer data) and our analysis of the FA composition of the chow diet measured by gas chromatography/flame ionization detection (GC/FID) of fatty acid methyl esters (FAME) for tissue lipids.

2.3 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*. [40, 41]. 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 FAMEs were identified by their elution times relative to authenticated methylated FA, and quantities were determined by their abundance relative to the added internal standard. On the GC-FID system used for these studies, evaluation of equal weight FAME mixtures produced nearly identical response factors over a range of chain lengths and fatty acid masses indicating that the array of FAMEs generate equivalent peak area at equivalent mass. Approximately 23 and 24 FAs for brain and liver, respectively, were routinely identified and these accounted for ~99% of the FA peaks.

2.4 Calculations and Statistics

Individual FAs were calculated as percent of total FAs from the mass concentration of individual FAs (μmol FA/mg tissue) relative to the total FA concentration within the tissue and are presented as mean ± standard deviation (SD). Linear regression analyses were performed testing differences in mass percent of each FA using SAS and STATA (SAS Institute Inc, Cary NC; STATA version 12.1, College Station, TX). Significance was set at the 0.05 level.

3. RESULTS

The nutrient and FA composition of the chow diets fed to the animals throughout their life span is shown in Table 2. The animals consumed a relatively low fat [13% of energy (en)]

and high carbohydrate (68% en) diet compared to the modern Western diet (MWD; fat, ~35% en and carbohydrates ~50% en). However, composition of PUFAs is similar to that which would be found in a MWD [42, 43]. For example, n-6 and n-3 C18-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.

Table 3 shows the FA composition (expressed as a mean % 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). Major FAs within the cortex were palmitic acid (PA, C16:0), oleic acid (OA, C18:1n-9), arachidonic acid (ARA, C20:4n-6), adrenic acid (ADR, C22:4n-6) and docosahexaenoic acid (DHA, C22:6n-3). There were large age-dependent shifts within individual FAs with PA, ARA and ADR decreasing and OA and DHA increasing with the age of the animals. Overall, ratios of saturated and monounsaturated FA (SFA + MUFA) to PUFA decreased modestly throughout the examined lifespan of the animals, representing a modest overall increase in tissue PUFA concentrations. However, there was a marked (2.8 fold) age-dependent decrease in the overall ratio of n-6 to n-3 PUFAs and a similar reduction n-6 to n-3 LC-PUFAs reflecting a shift in the brain tissue towards n-3 LC-PUFAs.

The levels (expressed as % of total) of the major n-6 and n-3 PUFAs within brain tissue across the different age categories are shown in Figure 1. There was a striking reduction in the proportion of n-6 LC-PUFAs, ARA and ADR concomitantly, with an increase in DHA in the brain tissue during the first 3 years. ARA comprises 13.5% of total FAs at birth, and remains relatively constant ~8% from 3–8 years of age. 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 2 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 approximately 15 μmol/mg tissue in the oldest animals. Moreover, our linear regressions revealed that the rate of DHA accumulation pre-puberty (i.e. age ≤3 years) was 2.32 μmol/mg per year (95% CI: 1.66, 2.98) compared to 0.03 μmol/mg per year (95% CI: −0.35, 0.41) after the age of 3 years (Table 5). 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 the tissue most associated with the biosynthesis of LC-PUFAs that are subsequently

released into circulation. 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), other FA levels remained relatively stable after nursing.

4. 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 [44]. This “DHA accretion spurt” has been associated with a marked increase in Δ-6 desaturase (enzymatic product of *FADS1*) activity in late embryonic and postnatal rodent brain [30, 32, 45]. However, less is known about the accretion of these FAs in the brain during adolescence and throughout adulthood.

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 [46]. These data strongly support the concept that there is a robust accretion of DHA in cortical brain tissue in these animals up until 3 years of age. In captivity, vervet females reach puberty at ~2.5 years of age and achieve full adult size by the age of 4. Male vervets reach puberty at 3 years of age and complete growth by 5 years [39]. 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. 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 pre-formed 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 transporter-specific incorporation rates across the blood-brain barrier (BBB) over time in the primate brain are potential candidates for the marked alterations in the levels and ratios of DHA and ARA. For example, movement of DHA across the blood–brain barrier recently has been shown to require specific transporters, such as Mfsd2a for 2-docosahexaenoyl lysophosphotidylcholine [33–35]

Our findings correspond with previous studies in rhesus monkeys that indicate DHA accumulates in brain tissue during early development [4]. Another study in baboon neonates demonstrated that DHA and ARA are richly distributed into numerous brain gray matter structures, especially brain stem, basal ganglia, limbic regions, thalamus, and midbrain [47]. Interestingly in neonates not receiving DHA, DHA levels are reduced in most of these brain regions. In contrast, ARA levels in all structures were relative resistant to changes in dietary ARA. The observation that DHA levels change and ARA levels remain relatively constant is consistent with data in the current study. Additionally, both studies suggest that levels of the two primary LC-PUFAs, DHA and ARA within the brain, are regulated by distinct mechanisms.

Several lines of evidence indicate that n-3 LC-PUFA deficiencies may play an adverse role in neurodevelopment and childhood behavior [7]. 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 [13, 19–23]. 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 [21]. McNamara and colleagues found that postmortem orbitofrontal cortex from patients with schizophrenia, bipolar disorder and major depressive disorder all had significantly lower amounts of DHA when compared to control subjects [22]. With 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 [24].

Importantly, several studies have also shown that supplementation with n-3 LC-PUFA or combinations of n-3/n-6 PUFAs improve symptoms in ADHD, depression and learning difficulties [15, 24, 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 [15, 24, 50, 51].

Our data are consistent with previous reports that DHA levels increase and n-6 to n-3 LC-PUFA ratios change dramatically in the cortical brain tissue from childhood to adolescence [26, 27]. 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 [7, 12, 52–57]. 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 [58, 59]. 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 [58]. The current data together with an earlier human study [27] 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 and/or transport. 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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Highlights

- Levels of the n-3 long chain polyunsaturated fatty acid (PUFA), docosahexaenoic acid, increased dramatically in the cortex of vervet monkeys from birth to puberty.
- Marked changes in the ratios of n-6 to n-3 PUFAs occurred in the cortex 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 inadequate dietary n-3 PUFAs impacts brain development.

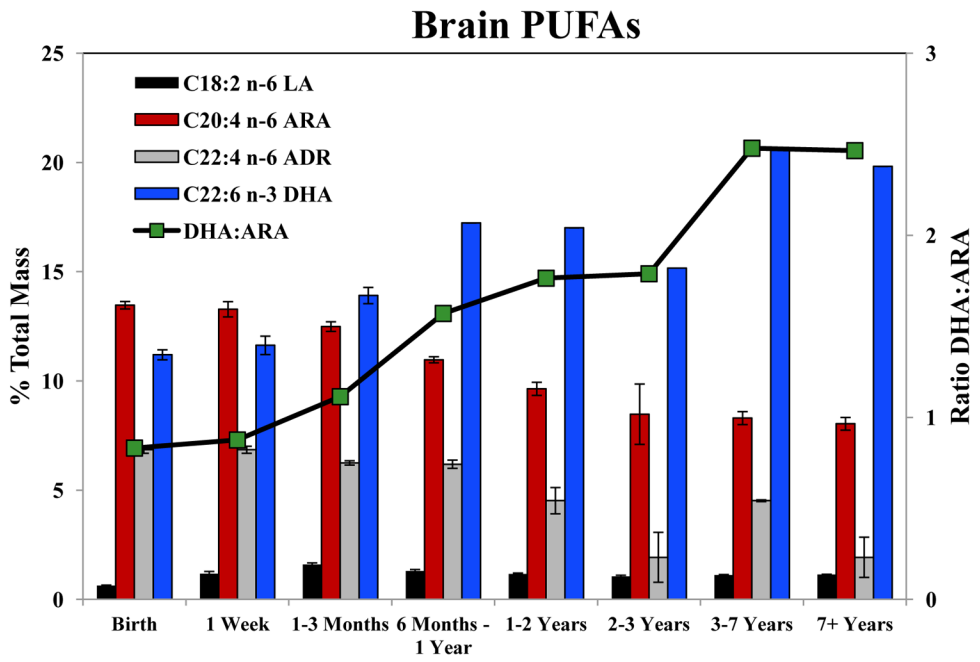

Figure 1. Levels and ratios of select PUFAs in brain tissue

PUFA levels are expressed as % of total mass and the ratio of DHA:ARA in brain tissue as a functions of animal age.

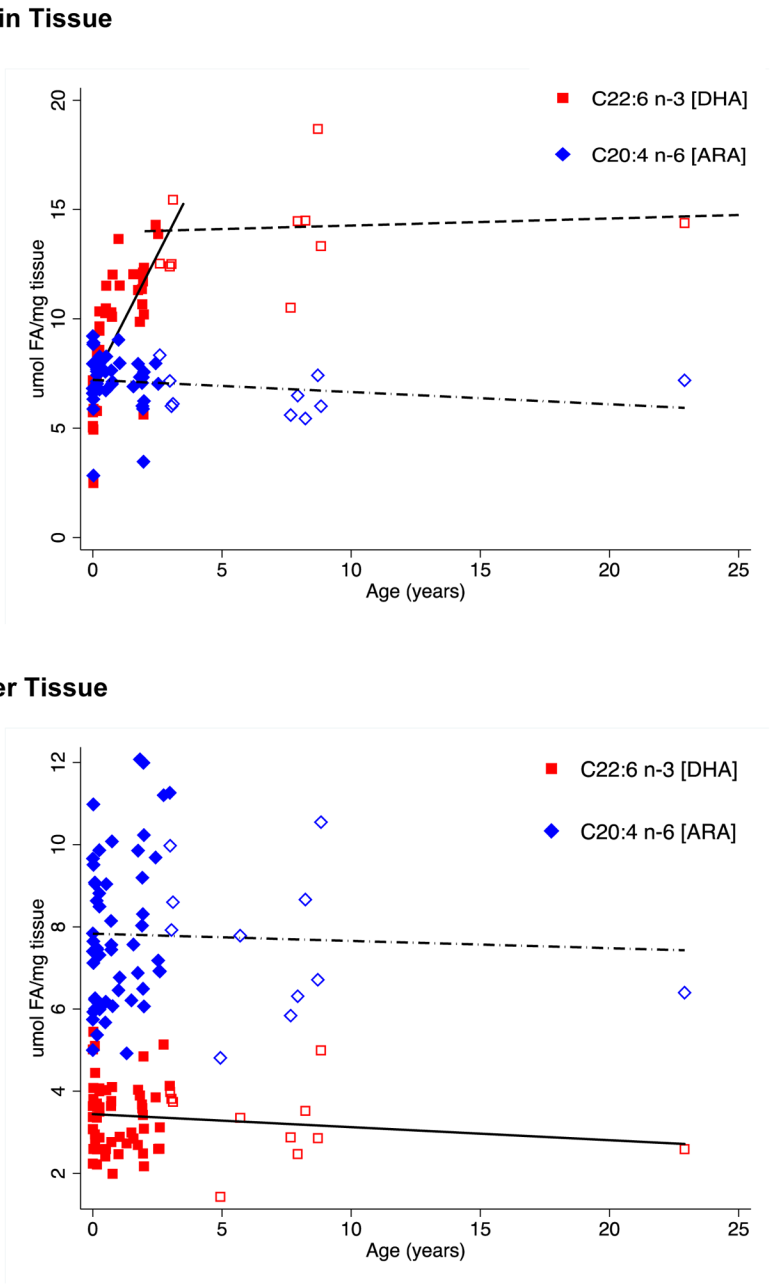

Figure 2. Mass concentration (μmol/mg tissue) of ARA and DHA in (A) brain tissue and (B) liver tissue in Vervet monkeys

Linear regressions are shown for brain (ARA: R^2=0.031, p=0.206; DHA: R^2=0.539, p<0.0001 for Age≤3, *filled markers*; and R^2=0.0071, p=0.843 for Age>3 years, *unfilled markers*) and liver (ARA: R^2=0.0012, p=0.788; DHA: R^2=0.017, p=0.295). Equations for linear regressions are shown in Table 5.

Table 1

Characteristics of the animals and tissues.

Age	# Males	# Females	Mean Age (Days)	Mean Body Weight (kg)	Mean Brain Weight (g)	Mean Liver Weight (g)
Birth	3	2	0.8	0.4	43.5	10.6
~1 week	3	2	7.6	0.3	43.2	10.1
1–3 months	8	7	60.3	0.6	60.2	18.8
6–12 months	5	6	254.0	1.4	69.6	37.3
1–2 years	5	8	654.0	2.5	74.5	56.6
2–3 years	4	3	982.3	3.7	77.1	74.7
3–7 years	1	2	1441.7	4.4	73.6	105.6
7 + years	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	2–3 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 [PA]	**26.5** (0.3)	**25.1** (0.8)	**24.3** (0.4)	**21.9** (0.3)	**21.8** (0.4)	**23.8** (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)	**24.9** (5.2)	**21.2** (0.2)	**21.8** (0.8)	< 0.0001	0.29	< 0.0001
C18:1 n-9 [OA]	**10.3** (0.3)	**11.0** (0.3)	**11.2** (0.3)	**12.3** (0.2)	**13.5** (0.7)	**13.8 (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)	**15.2** (7.6)	**20.6** (1.8)	**19.8** (1.3)	< 0.0001	0.001	< 0.0001

	BREAST FED BRAIN, % Mass				CHOW FED BRAIN, % Mass						
	Age				Age						
	Birth %(SD)	1 Week %(SD)	1–3 Months %(SD)	6 Months-1 Year %(SD)	1–2 Years %(SD)	2–3 Years %(SD)	3–7 Years %(SD)	7+ Years %(SD)	p-value (Δ 0–3 Months)	p-value (Δ ≥6 Months)	p-value (Δ lif
Fatty Acids	**n=5**	**n=4**	**n=14**	**n=6**	**n=10**	**n=4**	**n=2**	**n=5**			
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	< 0.000
n-6/n-3 LC-PUFA	2.1	2.0	1.6	1.1	1.0	1.0	0.7	0.7	< 0.0001	< 0.0001	< 0.000

Cortex samples (n=50) were obtained from the archived Vervet tissues 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 palmitic acid (PA), oleic acid (OA), 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), docosapentaenoic 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 [PA]	**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 [OA]	**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

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	BREAST FED LIVER, % Mass				CHOW FED LIVER, % Mass						
	Age				Age						
	Birth %(SD)	1 Week %(SD)	1–3 Months %(SD)	6 Months-1 Year %(SD)	1–2 Years %(SD)	2–3 Years %(SD)	3–7 Years %(SD)	7+ Years %(SD)	p-value (Δ 0–3 Months)	p-value (Δ ≥6 Months)	p-value (Δ lif
Fatty Acids	**n=5**	**n=5**	**n=15**	**n=8**	**n=12**	**n=7**	**n=3**	**n=5**			
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	< 0.000
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	< 0.000
n-6/n-3 LC-PUFA	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.

Table 5

Linear regressions for DHA and ARA levels in brain and liver tissues.

Fatty Acid (umol/mg)	**Brain Tissue**		**Liver Tissue**
20:4n6 [ARA]	7.21–0.055X		7.83–0.018X
22:6n3 [DHA]	8.81+0.508X***		3.44–0.032X
Fatty Acid (umol/mg)	**Age ≤ 3 years**	**Age > 3 years**	
22:6n3 [DHA]	7.13+2.32X***	13.95+0.03X	

Age is treated as an independent variable in the linear regression. The linear regression for DHA from brain tissue was further divided into subjects with ages ≤ 3 and >3 years, since Vervet monkeys reach puberty at 3 years, and this clearly shows two different rates of DHA accumulation over time as illustrated in Figure 2A.

*** $p<0.0001$)