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Regulation of rat brain polyunsaturated fatty acid (PUFA) metabolism during graded dietary n-3 PUFA deprivation

Hyung-Wook Kim *, Jagadeesh S. Rao, Stanley I. Rapoport, Miki Igarashi [1]

Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Bldg. 9, Room 1S126, Bethesda, MD 20892, USA

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ABSTRACT

Knowing threshold changes in brain lipids and lipid enzymes during dietary n-3 polyunsaturated fatty acid deprivation may elucidate dietary regulation of brain lipid metabolism. To determine thresholds, rats were fed for 15 weeks DHA-free diets having graded reductions of α-linolenic acid (α-LNA). Compared with control diet (4.6% α-LNA), plasma DHA fell significantly at 1.7% dietary α-LNA while brain DHA remained unchanged down to 0.8% α-LNA, when plasma and brain docosapentaenoic acid (DPAn-6) were increased and DHA-selective $iPLA_2$ and COX-1 activities were downregulated. Brain AA was unchanged by deprivation, but AA selective-$cPLA_2$, $sPLA_2$ and COX-2 activities were increased at or below 0.8% dietary α-LNA, possibly in response to elevated brain DPAn-6. In summary, homeostatic mechanisms appear to maintain a control brain DHA concentration down to 0.8% dietary DHA despite reduced plasma DHA, when DPAn-6 replaces DHA. At extreme deprivation, decreased brain $iPLA_2$ and COX-1 activities may reduce brain DHA loss.

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1. Introduction

The brain is enriched in docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6), which are critical for its normal structure and function [1–3]. In vertebrates, these polyunsaturated fatty acids (PUFAs) cannot be synthesized *de novo* from 2-carbon fragments, but can be elongated in liver (minimally in brain) from their respective shorter-chain PUFA precursors, α-linolenic acid (α-LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6) [4–7]. In humans, a low dietary n-3 PUFA intake or a low plasma DHA concentration has been correlated with increased risk for neuropsychiatric and/or neurodegenerative diseases [8,9]. Dietary n-3 PUFA supplementation may be beneficial in these conditions [8,10].

Multiple animal studies have been conducted to understand how dietary-derived n-3 PUFAs influence body integrity and metabolism. For example, in rats fed a DHA-free diet containing α-LNA at 4.6% total fatty acid, brain, heart and liver DHA concentrations are sufficient to maintain organ function, so this diet is considered n-3 PUFA "adequate" [2,11]. In contrast, in rats fed a DHA-free diet containing 0.2% α-LNA, brain DHA concentrations are reduced, behavior is disturbed and brain derived neurotrophic factor (BDNF) is reduced compared with the 4.6% α-LNA diet, so this diet is considered n-3 PUFA "inadequate" or "deficient" [2,12,13]. Brain changes in rats fed this deficient diet include a prolonged DHA half-life; an increased concentration of docosapentaenoic acid (DPAn-6, 22:5n-6), an AA elongation product; reduced expression of enzymes that regulate DHA metabolism, Ca^{2+}-independent phospholipase A_2 ($iPLA_2$ Type VI, $iPLA_2\beta$) [14–17] and cyclooxygenase (COX)-1 [18,19]; and increased expression of enzymes that regulate AA metabolism, cytosolic $cPLA_2$ Type IV, secretory $sPLA_2$ Type II and COX-2 [14,20].

The brain lipid and enzyme changes in animals exposed to dietary n-3 PUFA deprivation, noted above and reported elsewhere [3,21,22], may not be clinically relevant because deprivation was too severe and prolonged, sometimes spanning several generations. This severity also limits the ability to identify causes and effects. To overcome these limitations, in the present study we exposed rats after weaning to 15 weeks of graded reductions in dietary n-3 PUFA content below the 4.6% α-LNA "adequate" level, and estimated when statistically significant changes in different lipid parameters first appeared (thresholds) in plasma, brain and liver.

2. Materials and methods

2.1. Materials

1-Palmitoyl-2-[1-^{14}C] arachidonoyl-*sn*-glycerol-3-phosphorylcholine was purchased from PerkinElmer (Boston, MA, USA) and

Abbreviations: AA, arachidonic acid; BDNF, brain derived neurotrophic factor; COX, cyclooxygenase; DHA, docosahexaenoic acid; DPAn-6, docosapentaenoic acid; LA, linoleic acid; α–LNA, α-linolenic acid; LOX, lipoxygenase; $cPLA_2$, cytosolic phospholipase A_2; $sPLA_2$, secretory PLA_2; $iPLA_2$, Ca^{2+}-independent PLA_2; PUFA, polyunsaturated fatty acid; *sn*, stereospecifically numbered

* Corresponding author. Current address: University of Washington, Seattle, Department of Environmental and Occupational Health Science, 1705 Pacific St., Seattle, WA 98195, BOX 357234, USA. Tel.: +1 206 616 3634; fax: +1 206 685 3990.
E-mail address: peterkhw@gmail.com (H.-W. Kim).
[1] Current address: University of California, Irvine, Neurobiology and Behavior, 2205 McGaugh Hall, Irvine, CA 92697-4550, USA.

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had a specific activity of 60 mCi/mmol. 1-Palmitoyl-2-[1-^{14}C] palmitoyl-*sn*-glycerol-3-phosphorylcholine was purchased from GE Healthcare (Buckinghamshire, UK) and had specific activity of 53 mCi/mmol. The purity of each was $>95\%$, as determined by TLC, scintillation counting and GC. 1-Palmitoyl-2-arachidonoyl-*sn*-glycerol-3-phosphorylcholine, 1-palmitoyl-2-[1-^{14}C] palmitoyl-*sn*-glycerol-3-phosphorylcholine and phosphatidylinositol 4, 5-bisphosphate were obtained from Avanti (Alabaster, AL, USA), protease inhibitor cocktail from Roche (Indianapolis, IN, USA). A high capacity cDNA reverse transcription kit, Taqman® gene expression master mix, and specific primers for real time RT-PCR were purchased from Applied Biosystems (Foster City, CA, USA).

2.2. Animals

Fischer-344 (CDF) male rat pups (19 days old) and their surrogate mothers, purchased from Charles River Laboratories (Portage, MI, USA), were housed in an animal facility with regulated temperature, humidity, and a 12 h light/12 h dark cycle. Lactating rats had free access to water and rodent chow formulation NIH-31 18-4 (Zeigler Bros, Gardners, PA, USA), which contained 4% (wt/wt) crude fat and (as percent total fatty acid) α-LNA (5.1%), eicosapentaenoic acid (20:5n-3) (2.0%), DHA (2.3%), LA (47.9%) and AA (0.02%) [23].

After nursing for 21 days, the pups were divided randomly into six groups and placed on a predetermined diet. They had free access to food and water, and their food was replaced every 2 or 3 days. After 15 weeks on a given diet, they were asphyxiated by CO_2 inhalation and decapitated. The brains and liver were excised rapidly and frozen in 2-methylbutane cooled by dry ice to $-50\,^{\circ}C$, then stored at $-80\,^{\circ}C$ until used. Blood was collected from the abdominal aorta with EDTA, and centrifuged at 1500 rpm for 5 min. Plasma was removed and stored at 80 °C until assayed. The protocol was approved by the Animal Care and Use Committee of the *Eunice Kennedy Schriver* National Institute of Child Health and Human Development, and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23).

2.3. Graded n-3 PUFA diets

The different n-3 PUFA diets, prepared by Dyets Inc. (Bethlehem, PA, USA), were based on the AIN-93G formulation [24,25]. Each diet contained 10% crude fat, but a different amount of flaxseed oil. Fatty acid composition of each diet (mol% and μmol/g diet) is shown in Table 1. The n-3 PUFA "adequate" diet contained 6.7 μmol/g α-LNA (4.6% of total fatty acid) [2]. The extreme "deficient" diet contained 0.21 μmol/g α-LNA (0.2% total fatty acid). The less deficient diets contained α-LNA at 3.8%, 2.6%, 1.7% or 0.8% of total fatty acid. Other n-3 PUFAs were absent from all diets. Each diet contained 40 μmol/g LA (23–24% total fatty acid).

2.4. Lipid extraction and methylation

Methods of lipid extraction and methylation have been described [6,19]. Total lipids from brain, liver and plasma were extracted by the Folch procedure [26], and fatty acids were transmethylated with 1% H_2SO_4–methanol for 3 h at 70 °C. Appropriate quantities of di-17:0 PC for total fatty acid analysis and of unesterified 17:0 for unesterified fatty acids were added as internal standards before transmethylation to fatty acid methyl esters.

2.5. Gas chromatography

Fatty acid methyl esters from brain and liver (nmol/g wet wt) and from plasma (nmol/ml plasma) were quantified with a gas chromatograph (6890N, Agilent Technologies, Palo Alto, CA, USA) equipped

Table 1
Composition of graded n-3 PUFA diets.

Ingredient	4.6% ALA diet	3.8% ALA diet	2.6% ALA diet	1.7% ALA diet	0.8% ALA diet	0.2% ALA diet
			g/100 g food			
Carbohydrate	60	60	60	60	60	60
Protein	20	20	20	20	20	20
Fat	10	10	10	10	10	10
Hydrogenated coconut oil	6.00	6.12	6.25	6.37	6.50	6.62
Safflower oil	3.23	3.26	3.29	3.32	3.35	33.8
Flaxseed oil	0.77	0.62	0.46	0.31	0.15	0
Other ingredient	10	10	10	10	10	10
Fatty acid composition (%)			**% of total fatty acid concentration**			
12:0	28.8	30.3	30.7	31.6	32.4	34.0
14:0	12.5	12.9	13.1	13.3	14.0	14.6
14:1n-5	0.04	0.04	0.03	0.04	0.05	0.05
16:0	9.5	9.6	9.6	9.5	9.8	9.9
16:1n-7	0.04	0.04	0.04	0.06	0.06	0.13
18:0	8.0	7.9	8.1	8.3	9.3	8.0
18:1n-9	8.4	7.7	8.0	7.6	7.1	5.9
18:2n-6	27.9	27.7	27.9	27.9	27.5	27.2
18:3n-3	4.6	3.8	2.6	1.7	0.8	0.2
Total saturated	58.8	60.7	61.5	62.7	64.5	66.5
Total monounsaturated	8.5	7.8	8.0	7.7	7.2	6.1
n-6 PUFA	27.9	27.7	27.9	27.9	27.5	27.2
n-3 PUFA	4.6	3.8	2.6	1.7	0.8	0.2
n-6/n-3	4.2	5.1	7.0	11.8	23.4	132.9
Fatty acid content[a]			**μmol/g diet**			
18:2n-6	40.5 ± 3.6	40.6 ± 1.8	44.1 ± 3.0	38.1 ± 0.8	41.1 ± 5.0	35.8 ± 2.7
18:3n-3	6.7 ± 0.3	5.5 ± 0.6	4.0 ± 0.6	2.4 ± 0.1	1.2 ± 0.2	0.21 ± 0.03

[a] Data represent averages or means ± SD of 3 analyses.