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Dietary n-6 PUFA deprivation downregulates arachidonate but upregulates docosahexaenoate metabolizing enzymes in rat brain

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Abstract

Background—Dietary n-3 polyunsaturated fatty acid (PUFA) deprivation increases expression of arachidonic acid (AA 20:4n-6)-selective cytosolic phospholipase A₂ (cPLA₂) IVA and cyclooxygenase (COX)-2 in rat brain, while decreasing expression of docosahexaenoic acid (DHA 22:6n-3)-selective calcium-independent iPLA₂ VIA. Assuming that these enzyme changes represented brain homeostatic responses to deprivation, we hypothesized that dietary n-6 PUFA deprivation would produce changes in the opposite directions.

Methods—Brain expression of PUFA-metabolizing enzymes and their transcription factors was quantified in male rats fed an n-6 PUFA adequate or deficient diet for 15 weeks post-weaning.

Results—The deficient compared with adequate diet increased brain mRNA, protein and activity of iPLA₂ VIA and 15-lipoxygenase (LOX), but decreased cPLA₂ IVA and COX-2 expression. The brain protein level of the iPLA₂ transcription factor SREBP-1 was elevated, while protein levels were decreased for AP-2α and NF-κB p65, cPLA₂ and COX-2 transcription factors, respectively.

Conclusions—With dietary n-6 PUFA deprivation, rat brain PUFA metabolizing enzymes and some of their transcription factors change in a way that would homeostatically dampen reductions in brain n-6 PUFA concentrations and metabolism, while n-3 PUFA metabolizing enzyme expression is increased. The changes correspond to reported *in vitro* enzyme selectivities for AA compared with DHA. (198 words)

Keywords

linoleic acid; arachidonic; essential fatty acid; deficient; turnover rate; cyclooxygenase; brain; PUFA; phospholipase; lipoxygenase; SREBP; transcription

INTRODUCTION

The polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are highly enriched in brain [1–4], where they are found

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mainly in the stereospecifically numbered (*sn*)-2 position of membrane phospholipids. *In vitro* studies indicate that DHA and AA can be hydrolyzed selectively from phospholipid by Ca^{2+} -independent phospholipase A₂ (iPLA₂ Type VI) and Ca^{2+} -dependent cytosolic cPLA₂ type IVA, respectively [5–11]. This selectivity is consistent with observations that 15 weeks of dietary n-3 PUFA deprivation in rats increased brain expression (mRNA, protein and/or activity) of cPLA₂ IVA, secretory sPLA₂ type IIA and COX-2 (which is functionally coupled and co-evolved with cPLA₂ [12,13]), while decreasing expression of iPLA₂ VIA and COX-1 [14–16]. The enzyme changes corresponded to reduced DHA metabolic loss from brain (prolonged half life) and a reduced brain DHA concentration, but an increased brain concentration of the AA elongation product, docosapentaenoic acid (DPA, 22:5n-6) [17].

In comparison, the brain AA concentration was decreased and the brain DHA concentration was increased in weaned rats fed an n-6 PUFA deficient diet for 15 weeks [17,18]. Assuming that the enzyme changes in the n-3 PUFA deprived rat (see above) reflected homeostatic dampening of brain DHA loss, we hypothesized that dietary n-6 PUFA deprivation would produce changes in the opposite in direction. Accordingly, in the present study we examined brain expression of PLA₂ and downstream oxidative enzymes (COX-1 and 2, and 5-, 12 - and 15-lipoxygenase (LOX)) involved in PUFA metabolism [19,20], and of some of their transcription factors, in rats fed the n-6 PUFA deficient or adequate diet [18] for 15 weeks after weaning. An abstract of part of this work has been published [21].

MATERIALS AND METHODS

Materials

1-Palmitoyl-2-[1-¹⁴C] arachidonoyl-*sn*-glycerol-3-phosphorylcholine, purchased from PerkinElmer (Boston, MA, USA), had a specific activity of 60 mCi/mmol. 1-Palmitoyl-2-[1-¹⁴C] palmitoyl-*sn*-glycerol-3-phosphorylcholine was purchased from GE Healthcare (Buckinghamshire, UK) and had a specific activity of 53 mCi/mmol. The purity of each exceeded 95%, as determined by thin layer chromatography, scintillation counting and gas chromatography. 1-Palmitoyl-2-arachidonoyl-*sn*-glycerol-3-phosphorylcholine, 1-palmitoyl-2-[1-¹⁴C] palmitoyl-*sn*-glycerol-3-phosphorylcholine and phosphatidylinositol-4, 5-bisphosphate were purchased from Avanti (Alabaster, AL, USA). Protease inhibitor cocktail was purchased from Roche (Indianapolis, IN, USA). Antibodies against group IVA cPLA₂, group IIA sPLA₂, group VIA iPLA₂, COX-1, COX-2, 5-LOX, 12-LOX, 15-LOX, nuclear factor-kappa B (NF-κB) p65, NF-κB p50, activator protein (AP)-2α and AP-2 β were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA), and antibodies against sterol regulatory binding protein (SREBP)-1 and -2 were from Abcam (Cambridge, MA, USA). β-actin antibody was obtained from Sigma-Aldrich (St. Louis, MO, USA) and appropriate horseradish peroxidase-conjugated secondary antibodies were purchased from Cell Signaling (Beverly, MA, 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).

Animals

The protocol was approved by the Animal Care and Use Committee of the *Eunice Kennedy Shriver* 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). 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. The pups were allowed to nurse until 21 days old. Lactating rats had free access to water and

rodent chow formulation NIH-31 18-4, which contained 4% (wt/wt) crude fat (Zeigler Bros., Gardners, PA, USA) and whose fatty acid composition has been reported [18]. Briefly, α -linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3) and DHA contributed 5.1%, 2.0% and 2.3% of total fatty acid, respectively, whereas linoleic acid (18:2n-6) and AA contributed 47.9% and 0.02%, respectively.

After weaning, pups were divided randomly into n-6 PUFA adequate (n = 10) and deficient (n = 10) diet groups as described below. They had free access to food and water, their food being replaced every 2 or 3 days. After 15 weeks on a chosen diet, a rat was asphyxiated by CO₂ inhalation and decapitated. The brain was excised rapidly and frozen in 2-methylbutane with dry ice at -50°C, then stored at -80°C until use. Animals were provided food until sacrifice.

Dietary composition

The n-6 PUFA adequate and deficient diets (Supplementary Table 1) were prepared by Dyets Inc. (Bethlehem, PA, USA), based on the AIN-93G formulation [22, 23], and contained 10% fat [24]. The adequate diet contained hydrogenated coconut oil (6 g/100 g diet), safflower oil (3.23 g/100 g) and flaxseed oil (0.77 g/100 g) (Supplementary Table 1) [17, 25, 26]. The deficient diet contained hydrogenated coconut oil (8.73 g/100 g), flaxseed oil (0.77 g/100 g), and olive oil (0.5 g/100 g), but no safflower oil (Supplementary Table 1).

Fatty acid concentrations ($\mu\text{mol/g}$ food or percent of total fatty acid) of the two diets have been reported [24] and are shown in Table 1. The n-6 PUFA adequate diet contained LA at 52.1 $\mu\text{mol/g}$ diet (27.6% of total fatty acid), whereas the deficient diet contained LA at 4.2 $\mu\text{mol/g}$ (2.3% of total fatty acid), 10% of the suggested minimum requirement for rodents (42.8 $\mu\text{mol/g}$) [27]. Both diets contained α -LNA 8.5–8.9 $\mu\text{mol/g}$ (4.5–4.8% of total fatty acid), close to the minimum requirement for dietary n-3 PUFA adequacy in rodents [28,29], and oleic acid (18:1n-9) at 13.6–14.4 $\mu\text{mol/g}$ (7.3–7.7 % of total fatty acids). Other n-3 and n-6 PUFAs were absent from both diets.

Preparation of cytoplasmic and nuclear extracts for Western blotting

Cytoplasmic and nuclear proteins were prepared with a compartmental protein extraction kit (Chemicon, Temecula, CA, USA), according to the manufacturer's protocol. Prepared fractions were kept at -80 °C until used for Western blotting (see below). Protein concentrations of cytoplasmic and nuclear fractions were measured by the Bradford assay (Bio-Rad) [30]. Expression of group IVA cPLA₂, group IIA sPLA₂, group VIA iPLA₂, COX-1, COX-2, 5-LOX, 12-LOX and 15-LOX was determined in the cytoplasmic fraction, whereas expression of NF- κ B p65, NF- κ B p50, AP-2 α , AP-2 β , SREBP-1, SREBP-2, and lamin B was determined in the nuclear fraction.

Western blot analysis

Proteins from the cytoplasmic (50 μg) and nuclear (50 μg) extracts were separated on 4–20% SDS-polyacrylamide gels (PAGE) (Bio-Rad). Following SDS-PAGE, the proteins were transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). Protein blots were incubated overnight at 4 °C in TBS buffer containing 5% nonfat dried milk and 0.1% Tween-20, with specific primary antibodies (1:1000 dilution): group IVA cPLA₂, group IIA sPLA₂, group VIA iPLA₂, COX-1, COX-2, 5-LOX, 12-LOX, 15-LOX, NF- κ B p65, NF- κ B p50, AP-2 α , AP-2 β , SREBP-1, SREBP-2, β -actin and lamin B. Protein blots were incubated with appropriate HRP-conjugated secondary antibodies (Cell Signaling, Beverly, MA, USA) and visualized using a chemiluminescence reaction (Pierce, Rockford, IL, USA) on BioMax X-ray film (Eastman Kodak, Rochester, NY, USA). Optical densities of immunoblot bands were measured with Alpha Innotech Software (Alpha

Innotech, San Leandro, CA, USA) and were normalized to the optical density of β -actin (Sigma-Aldrich) to correct for unequal loading. All experiments were carried out with 10 independent samples per group. Values are expressed as percent of control.

RNA isolation and real time RT-PCR

Total RNA was isolated from brain using commercial kits (RNeasy Lipid Tissue Kit; Qiagen, Valencia, CA). cDNA was prepared from total RNA using a high-capacity cDNA Archive Kit (Qiagen). mRNA levels of cPLA₂ IVA (Rn 00591916_m1), sPLA₂ IIA (Rn 00580999_m1), iPLA₂ VIA (Rn 01504424_m1), COX-1 (Rn 00566881_m1), COX-2 (Rn 00568225_m1), 5-LOX (Rn 00563172_m1), 12-LOX (Rn 01461082_m1), and 15-LOX (Rn 00696151_m1), were measured by real time quantitative RT-PCR, using the ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). The fold change in gene expression was determined by the $\Delta\Delta C_T$ method [31]. Data are expressed as the relative level of the target gene in the n-6 PUFA deficient group normalized to the endogenous control (β -globulin, Rn_00560865_m1) and relative to the level in the n-6 PUFA adequate diet group (calibrator). All experiments were carried out in triplicate with 10 independent samples per group.

Phospholipase A₂ activities

We used a radioactivity method designed by the Dennis Group to analyze cPLA₂ and iPLA₂ activities [32–34]. A commercial kit (Cayman, Ann Arbor, MI, USA) was used to determine sPLA₂ activity.

Sample preparation—Brain tissue was homogenized with 3 vol of homogenization buffer (10 mM HEPES, pH 7.5, containing 1 mM EDTA, 0.34 μ M sucrose and protease inhibitor cocktail (Roche, Indianapolis, IN)), using a glass homogenizer. The homogenized sample was centrifuged at 100,000 g for 1 h at 4 °C, and the supernatant was used for all PLA₂ enzyme activity analysis. Supernatants were kept at –80 °C until use. The protein concentration was analyzed by the Bradford assay (Bio-Rad).

Enzyme assay with radioisotope method—The final incubation volume was 0.5 ml. To measure cPLA₂ activity, the cytosolic fraction (0.3 mg protein in one assay) was mixed with 100 mM HEPES, pH 7.5 containing 80 μ M Ca²⁺, 2 mM dithiothreitol, 0.1 mg/ml fatty acid-free bovine serum albumin in 450 μ l. Fifty μ l of substrate solution contained 100 μ M 1-palmitoyl-2-arachidonoyl-*sn*-glycerol-3-phosphorylcholine and phosphatidylinositol 4,5-bisphosphate (97:3) (containing approximately 100,000 dpm of 1-palmitoyl-2-[1-¹⁴C] arachidonoyl-*sn*-glycerol-3-phosphorylcholine in one assay) in 400 μ M triton X-100 to start the enzyme reaction. To measure iPLA₂ activity, the cytosolic fraction (0.3 mg protein in one assay) was mixed with 100 mM HEPES, pH 7.5, 5 mM EDTA, 2 mM dithiothreitol, and 1 mM ATP in 450 μ l. 50 μ l substrate mixture of 100 μ M 1-palmitoyl-2-palmitoyl-*sn*-glycerol-3-phosphorylcholine (containing approximately 100,000 dpm of 1-palmitoyl-2-[1-¹⁴C] palmitoyl-*sn*-glycerol-3-phosphorylcholine) in 400 μ M Triton X-100 was added to start the enzyme reaction.

Substrate preparation for radioisotope method—Substrates for the iPLA₂ and cPLA₂ activity analyses described above were prepared daily. Appropriate amounts of cold and radiolabeled phospholipids were added to an appropriate amount of triton X-100, and the mixture was dried with nitrogen gas. Water was added to the residues to give a 10 $\times$ lipid mixture (1 mM phospholipid, 1,000,000 dpm, and 4 mM Triton X-100), which was mixed vigorously.

Enzyme assay—Routinely, the cytosolic fraction (0.3 mg in one assay) was mixed in 450 μ l assay mixture, and 50 μ l substrate mixture was added to start the enzyme reaction. The reaction mixture was incubated for 30 min at 40 °C, and then 2.5 ml of Dole reagent (2-propanol, heptane: 0.5M H₂SO₄, 400:100:20, vol/vol/vol) was added to stop the reaction. One and a half ml of heptane and 1.5 ml H₂O were added to the mixture, followed by vortexing and centrifugation. The upper phase (about 2 ml) was transferred to a tube and 200 mg of silicic acid (200–400 mesh) was added, followed by vortexing and centrifugation. The supernatant (1.5 ml) was transferred to a scintillation vial, and scintillation cocktail was added (Ready Safe™ plus 1 % glacial acetic acid). Radioactivity of released unesterified fatty acid from substrate was counted as described above. iPLA₂ and cPLA₂ activities were expressed as the release rate of fatty acid from phospholipids.

sPLA₂ activity—sPLA₂ activity was measured using an appropriate assay kit (Cayman), according to the manufacturer's instructions.

Statistical analysis

Data are presented as means $\pm$ SD (n = 10 for each group). An unpaired Student's t-test was used to compare means, taking p < 0.05 as the cut off for statistical significance.

RESULTS

Effects of n-6 PUFA deprivation on body and brain weight

Dietary n-6 PUFA deprivation for 15 weeks did not affect body weight. Mean body weight at 21 days was 29 ± 2 g and 30 ± 2 g in the diet-adequate and -deficient groups, respectively (p = 0.16). After 15 weeks on the diet, weight equaled 379 ± 20 g and 380 ± 16 g in the adequate and deficient groups, respectively (p = 0.87), and brain weight equaled 1.9 ± 0.1 g and 1.9 ± 0.1 g, respectively (p = 0.40).

Effects of n-6 PUFA on phospholipase A₂ expression

Brain iPLA₂ activity was increased significantly (62%, p < 0.01) in rats fed the n-6 PUFA deficient compared with adequate diet (Fig. 1C). The increase was accompanied by a 54% increase in iPLA₂ VIA protein (Fig. 1B, p < 0.05) and a 50% increase in iPLA₂ VIA mRNA (p < 0.01) (Fig. 1A). In contrast, cPLA₂ activity was decreased significantly in the n-6 PUFA deprived rats (−25%, p < 0.05) (Fig. 1F), as was cPLA₂ IVA protein (−32%, p < 0.05) (Fig. 1E) and mRNA (−24%, p < 0.05) (Fig. 1D). The deficient diet did not change brain sPLA₂ activity, protein or mRNA (Figs. 1G–1I).

Protein levels of transcription factors

We determined whether the observed changes in iPLA₂ and cPLA₂ mRNA with n-3 PUFA deprivation corresponded to changes in expression of some of their transcription factors. n-6 PUFA deprivation significantly increased the protein level of the iPLA₂ transcription factor, SREBP-1, by 90% (p < 0.05), while decreasing protein levels of the cPLA₂ transcription factors, AP-2 α (−33%, p < 0.01) and NF- κ B p65 (−29%, p < 0.05) (Figs. 2C and 2E). Deprivation did not alter brain protein levels of SREBP-2, AP-2 β or NF- κ B p50 (Figs. 2B, 2D and 2F).

mRNA and protein levels of COX-1 and COX-2

COX-2 mRNA was decreased significantly (−23%, p < 0.05) (Fig. 3A) in the n-6 PUFA deprived rats compared with adequate rats, as was COX-2 protein (−32%, p < 0.05) (Fig. 3B). The deficient diet did not change COX-1 or mRNA significantly (Figs. 3C and D).

mRNA and protein levels of 5-LOX, 12-LOX and 15-LOX

Brain 15-LOX mRNA was increased significantly (33%) in rats fed the n-6 PUFA deficient compared with adequate diet ($p < 0.05$) (Fig. 4C), and 15-LOX protein was increased as well (45%, $p < 0.05$) (Fig. 4F). The deficient diet did not change mRNA or protein levels of 5-LOX or 12-LOX (Fig. 4).

DISCUSSION

We quantified expression (mRNA, protein and/or activity) of enzymes regulating brain AA and/or DHA metabolism, and protein levels of some of their transcription factors, in rats fed an n-6 PUFA deficient or adequate diet for 15 weeks after weaning. Dietary n-6 PUFA deprivation increased brain mRNA and protein of iPLA₂ VIA, iPLA₂ activity, and the protein level of one of its transcription factors, SREBP-1, while decreasing mRNA and protein of cPLA₂ IVA, cPLA₂ activity, and protein levels of two cPLA₂ transcription factors, AP-2 α and NF- κ B p65. In addition, n-6 PUFA deprivation decreased brain COX-2 expression, which can be regulated by AP-2 α and NF- κ B p65 [35,36], while increasing brain 15-LOX expression. The deficient diet did not affect brain expression of 5- or 12-LOX, sPLA₂ or COX-1.

Consistent with the reported *in vitro* selectivity of cPLA₂ for AA and of iPLA₂ for DHA hydrolysis from phospholipid [5–11], and with reported functional coupling of cPLA₂ and COX-2 *in vitro* [12], the changes in these enzymes and their transcription factors with dietary n-6 PUFA deprivation are accompanied by a decreased brain AA concentration and an increased brain DHA concentration [18] and increased DHA turnover in brain phospholipids (M. Igarashi, unpublished observations). Since 15-LOX expression also was elevated by deprivation, conversion of DHA to metabolites such as anti-neuroinflammatory neuroprotectin D1 by 15-LOX [37] may have been increased as well, but this remains to be confirmed. 15-LOX and iPLA₂ are reported to be functionally coupled in macrophages [38].

Some of the enzyme changes following n-6 PUFA deprivation were opposite in direction from changes caused by dietary n-3 PUFA deprivation, which increased cPLA₂, sPLA₂, COX-2 and 12-LOX expression while reducing iPLA₂ and COX-1 expression [14]. The different directional effects of n-6 PUFA compared with n-3 PUFA deprivation imply underlying molecular mechanisms that tend to maintain a homeostatic n-3/n-6 PUFA concentration balance for optimal brain function [39,40]. The n-3 PUFA deprivation for 15 weeks increases aggression and depression test scores in rats [25], but it remains to see whether n-6 PUFA deprivation produces opposite effects.

The increased brain iPLA₂ VIA mRNA caused by n-6 PUFA deprivation is consistent with the increased protein level of SREBP-1, an iPLA₂ transcription factor, whereas the reduced protein levels of AP-2 α and NF- κ B p65, which control cPLA₂ IVA and/or COX-2 transcription, may account for the lower mRNA levels of these enzymes [35,36,41,42]. PUFAs also are reported to directly influence gene expression in various tissues including brain [43–47]. Thus, DHA inhibits binding activity and nuclear translocation of NF- κ B [48–51], so that its increased concentration in the brain of the n-6 PUFA deprived rats may explain why NF- κ B was downregulated. The reduced brain cPLA₂ IVA and COX-2 levels in the n-6 PUFA deprived rats, and the associated increased iPLA₂ VIA and 15-LOX expression and the increased DHA concentration [18,52], may increase resistance to neuroinflammation, which is characterized by increased brain expression of cPLA₂ IVA, sPLA₂ IIA and COX-2 and increased AA metabolism [53,54].

In summary, 15 weeks of dietary n-6 PUFA deprivation after weaning produced changes in rat brain enzyme expression consistent with downregulated AA but upregulated DHA

metabolism, and with reported *in vitro* PLA₂ enzyme selectivities for AA compared with DHA. Expression of DHA-selective iPLA₂ VIA and of 15-LOX was upregulated, whereas expression of AA-selective cPLA₂ IVA and of COX-2 was downregulated, consistent reported enzyme coupling. Increased SREBP-1 protein could have contributed to the increased iPLA₂ VIA mRNA, whereas decreased NF-κB 65 and AP-1α could have contributed to the decreased cPLA₂ IVA and COX-1 mRNA. Many of the changes were opposite in direction from changes produced by dietary n-3 PUFA deprivation, consistent with independent regulation of brain AA and DHA metabolizing enzymes [40]. The enzyme changes in each condition would tend to maintain homeostasis of brain AA and DHA function metabolism *in vivo*, in the face of dietary stress.

- Dietary n-6 PUFA deprivation downregulated cPLA₂ and COX-2 in rat brain.
- Dietary n-6 PUFA deprivation upregulated iPLA₂ and 15-LOX in rat brain.
- These changes are opposite to n-3 PUFA deprivation in rat brain.
- These changes would tend to maintain homeostasis of brain AA and DHA function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AA	arachidonic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
PUFA	polyunsaturated fatty acid
cPLA₂	cytosolic phospholipase A ₂
sPLA₂	secretory PLA ₂
iPLA₂	Ca ²⁺ -independent PLA ₂
COX	cyclooxygenase
SREBP	sterol regulatory element binding protein
LOX	lipoxygenase
NF-κB	nuclear factor-κB
AP	activator protein
sn	stereospecifically numbered

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Figure 1. Expression of brain PLA₂ enzymes with n-6 PUFA deprivation
mRNA, protein levels and activities of iPLA₂ (A-C), cPLA₂ (D-F) and sPLA₂ (G-I). mRNA data are expressed as the relative level of the PLA₂ normalized to the endogenous control (β-globulin) using the $\Delta\Delta C_T$ method. Protein levels are ratios of optical density of PLA₂ to β-actin, expressed as percent of control. Values are mean ± SD (n = 10 for both groups). *p < 0.05, **p < 0.01. A, adequate; D, deficient.

Figure 2. Expression of brain transcription factors

Protein levels of SREBP-1, SREBP-2, AP-2α, AP-2β, NF-κB p65 and NF-κB p50 (A to F in order). Lamin B antibody was used as the nuclear protein control. The protein level is the ratio of optical density of transcription factor to β-actin, expressed as percent of control. Values are mean ± SD (n = 10 for both groups). *p < 0.05, **p < 0.01. A, adequate; D, deficient.

Figure 3. Brain expression of cyclooxygenases

mRNA and protein levels of COX-2 (A and B) and COX-1 (C and D). mRNA data are expressed as relative level of PLA₂ normalized to endogenous control (β -globulin) using the $\Delta\Delta C_T$ method. The protein level is ratio of optical density of COX to β -actin, expressed as percent of control. Values are mean $\pm$ SD (n = 10 for both groups). *p < 0.05. A, adequate; D, deficient.

Figure 4. Brain expression of lipoxygenases

mRNA and protein levels of 5-LOX (A and D), 12-LOX (B and E) and 15-LOX (C and F). mRNA data are expressed as the relative level of PLA₂ normalized to the endogenous control (β-globulin) using the $\Delta\Delta C_T$ method. Protein level is ratio of optical density of PLA₂ to β-actin, expressed as percent of control. Values are mean $\pm$ SD (n = 10 for both groups). *p < 0.05. A, adequate; D, deficient.

Table 1

Fatty acid composition of n-6 PUFA adequate and deficient diets

Fatty acid	n-6 PUFA Adequate Diet		n-6 PUFA Deficient Diet	
	$\mu\text{mol/g food}$	% of total fatty acid	$\mu\text{mol/g food}$	% of total fatty acid
12:0	54.6 ± 3.3	29.0	81.1 ± 20.7	43.8
14:0	23.5 ± 1.4	12.5	34.6 ± 9.0	18.7
14:1n-5	0.06 ± 0.01	0.03	0.06 ± 0.02	0.03
16:0	18.2 ± 1.0	9.7	20.6 ± 5.3	11.1
16:1n-7	0.08 ± 0.01	0.04	0.10 ± 0.03	0.1
18:0	17.1 ± 1.0	9.0	22.0 ± 5.8	11.9
18:1n-9	14.4 ± 0.8	7.7	13.6 ± 3.5	7.3
18:2n-6	52.1 ± 7.6	27.6	4.2 ± 1.1	2.3
18:3n-3	8.5 ± 0.5	4.5	8.9 ± 2.4	4.8
Saturated	113.5 ± 6.6	60.1	158.2 ± 40.8	85.5
Monounsaturated	14.6 ± 0.8	7.7	13.7 ± 3.5	7.4
n-6 PUFA	52.1 ± 7.6	27.6	4.2 ± 1.1	2.3
n-3 PUFA	8.5 ± 0.5	4.5	8.9 ± 2.4	4.8
n-6/n-3	6.1		0.5	

Values are mean $\pm$ SD (n = 3)