Perinatal n-3 fatty acid deficiency selectively reduces *myo*-inositol levels in the adult rat PFC: an in vivo ^{1}H-MRS study[S]

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Abstract To investigate the effects of omega-3 fatty acid deficiency on phosphatidylinositol signaling in brain, *myo*-inositol (mI) concentrations were determined in the prefrontal cortex (PFC) of omega-3 fatty acid deficient rats by in vivo proton magnetic resonance spectroscopy (^{1}H-MRS). To generate graded deficits in PFC docosahexaenoic acid (22:6*n*-3) (DHA) composition, perinatal and postweaning α-linolenic acid (18:3*n*-3) (ALA) deficiency models were used. Adult male rats were scanned in a 7T Bruker Biospec system and a ^{1}H-MRS spectrum acquired from the bilateral medial PFC. Rats were then challenged with SKF83959, a selective agonist at phosphoinositide (PI)-coupled dopamine D_1 receptors. Postmortem PFC fatty acid composition was determined by gas chromatography. Relative to controls, PFC DHA composition was significantly reduced in adult postweaning (−27%) and perinatal (−65%) ALA-deficiency groups. Basal PFC mI concentrations were significantly reduced in the perinatal deficiency group (−21%, $P = 0.001$), but not in the postweaning deficiency group (−1%, $P = 0.86$). Among all rats, DHA composition was positively correlated with mI concentrations and the mI/creatine (Cr) ratio. SKF83959 challenge significantly increased mI concentrations only in the perinatal deficiency group (+16%, $P = 0.02$). These data demonstrate that perinatal deficits in cortical DHA accrual significantly and selectively reduce mI concentrations and augment receptor-generated mI synthesis.—McNamara, R. K., J. Able, R. Jandacek, T. Rider, P. Tso, and D. M. Lindquist. **Perinatal n-3 fatty acid deficiency selectively reduces *myo*-inositol levels in the adult rat PFC: an in vivo ^{1}H-MRS study. *J. Lipid Res.* 2009.** 50: **405–411.**

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Mammalian brain lipids are comprised of a ratio of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. The principle omega-3 polyunsaturated fatty acid found in brain is docosahexaenoic acid (22:6*n*-3) (DHA), which comprises approximately 15–20 percent of total fatty acid composition (1). DHA is predominantly esterified into the *sn*-2 position of phosphatidylethanolamine and phosphatidylserine phospholipids (2), and is mobilized by phospholipase A_2 isoforms (3). Within brain tissues, DHA preferentially accumulates in neuronal growth cones, synaptosomes, astrocytes, microsomal, and mitochondrial membranes (4, 5). Because mammals are incapable of synthesizing *n*-3 fatty acids de novo, they are entirely dependent on dietary sources to procure and maintain adequate peripheral and central tissue composition. The dietary *n*-3 fatty acid precursor alpha-linolenic acid (18:3*n*-3) (ALA) is converted to DHA following a series of microsomal desaturation-elongation reactions mediated predominantly by the liver, and the final synthesis of DHA requires additional metabolism within peroxisomes (6). Perinatal or postweaning dietary ALA deficiency significantly reduces cortical DHA composition in the adult rat brain (7, 8). Moreover, perinatal ALA deficiency is associated with abnormalities in multiple neurotransmitter systems including dopamine and serotonin (9), and neurocognitive impairments and elevations in behavioral indices of depression, anxiety, and aggression (10).

Abbreviations: AA, arachidonic acid (20:4n-6); ALA, alpha-linolenic acid (18:3*n*-3); Cho, choline; Cr, creatine; DHA, docosahexaenoic acid (22:6*n*-3); DPA, docosapentaenoic acid (22:5*n*-6); Glx, glutamine and glutamate; ^{1}H-MRS, proton magnetic resonance spectroscopy; MARCKS, myristoylated alanine-rich C kinase substrate; mI, *myo*-inositol; NAA, *N*-acetyl aspartate; PFC, prefrontal cortex; PI, phosphoinositide; PLC, phospholipase C; Tau, taurine.

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One potential mechanism by which DHA deficiency may adversely affect cortical function is by altering the biophysical properties of neuronal membranes and interdependent second messenger systems. For example, prior studies have found that perinatal omega-3 fatty acid deficiency is associated with significant reductions in neuronal membrane phosphatidylserine content (11) and a significant reduction in membrane-associated myristoylated alanine-rich C kinase substrate (MARCKS) protein expression (12). Importantly, electrostatic binding of MARCKS with membrane phosphatidylserine serves to inhibit phospholipase C (PLC)-mediated phosphatidylinositol (PI) hydrolysis by sequestering phosphatidylinositol 4,5-bisphosphate in lateral membrane domains (13). These findings suggest that reductions in membrane phosphatidylserine content, and associated reductions in MARCKS membrane binding, would dysregulate PI second messenger signaling activity in the DHA-deficient rat brain. Although prior studies have found that increasing omega-3 fatty acid intake down-regulates basal and stimulated PI hydrolysis in peripheral tissues (14–16), there is currently nothing about the effects of DHA deficiency on PI signaling in brain.

In the present study, we determined the effects of brain DHA deficits on concentrations of *myo*-inositol (mI), a principle product of PI hydrolysis as well as a precursor for PI synthesis, in the PFC of *n*-3 fatty acid deficient rats by in vivo proton magnetic resonance spectroscopy (^{1}H-MRS). To generate graded deficits in PFC DHA composition, we employed perinatal and postweaning ALA deficiency models. To determine the effects of receptor-generated PI hydrolysis in the PFC in vivo, MRS acquisitions were also obtained following acute treatment with SKF83959, a selective agonist at PI-coupled dopamine D_1 receptors (17). Based on the evidence reviewed above, our principle hypothesis was that brain DHA deficits would significantly augment receptor-generated mI synthesis.

MATERIALS AND METHODS

Diets and animals

Diets were either ALA-fortified (ALA+, TD.04285) or ALA-free (ALA−, TD.04286) (Harlan-TEKLAD, Madison, WI). Both diets were matched for nonfat nutrients [Casein (vitamin-free) 200 g/kg, L-cystine 3 g/kg, sucrose 270 g/kg, dextrose monohydrate 99.5 g/kg, corn starch 200 g/kg, maltodextrin 60 g/kg, cellulose 50 g/kg, mineral mixture AIMN-93G-MX 35 g/kg, vitamin mixture AIN-93-VX 10 g/kg, choline (Cho) bitartrate 2.5 g/kg, TBHQ (antioxidant) 0.02 g/kg]. ALA+ and ALA− diets contained *n*-3 fatty acid-free hydrogenated coconut (45 g/kg) and safflower (19 g/kg) oils, and the ALA+ diet additionally contained ALA-containing flaxseed oil (6 g/kg). Analysis of diet fatty acid composition by gas chromatography confirmed that the ALA− diet did not contain ALA, but was similar to the ALA+ diet in saturated (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0), monounsaturated (18:1*n*-9, oleic acid), and *n*-6 fatty acid (18:2*n*-6, linoleic acid) composition, and that ALA represented 4.6% of total fatty acid composition in the ALA+ diet. Neither diet contained preformed DHA or arachidonic acid (20:4n-6) (AA), or their intermediate precursors (see supplementary Table I).

Male Long-Evans hooded rats (Harlan Farms, Indianapolis, IN) were bred in house. For perinatal ALA deficiency, female Long-Evans hooded rats were fed the ALA− diet beginning 1 month prior to mating, and male offspring were maintained on the ALA− diet postweaning (P21) to adulthood (P90). For postweaning ALA deficiency, male offspring of female rats maintained on the ALA+ diet were switched to the ALA− diet postweaning (P21) to adulthood (P90). Controls were born to females maintained on the ALA+ diet, and received the ALA+ diet postweaning (P21) to adulthood (P90). Following weaning, male rats were housed two per cage with food and water available ad libtum and maintained under standard vivarium conditions on a 12:12 h light/dark cycle. All experimental procedures were approved by the University of Cincinnati and Children's Hospital Institutional Animal Care and Use Committees, and adhere to the guidelines set by the National Institutes of Health.

^{1}H-MRS

Adult (P90) male rats were anesthetized with 1.5% isoflurane in air, positioned supine with their teeth in a bite bar, and scanned in a 7T Bruker Biospec system (Bruker BioSpin, Ettlingen, Germany) (**Fig. 1A**). Respiration was monitored and body temperature was maintained at 36–38°C using an animal monitoring system (SAI Inc., Stony Brook, NY). The head was centered inside a 38 mm Litz coil (Doty Scientific, Inc., Columbia, SC), and a set of localizers from each orthogonal plane were collected. Following acquisition of these localizers, RARE images (effective TE 45 ms, TR 3300 ms, RARE factor 16, matrix 256 × 256, FOV 35 mm, 20 slices in the axial direction, and 10 slices in the sagittal direction) were collected for voxel placement. A single voxel was placed in the bilateral medial PFC (Fig. 1C, D). The voxel was shimmed using FASTMAP to an average line width of 10.5 Hz (18). Water suppressed data were acquired using CHESS water suppression followed by PRESS localization with a TE of 20 ms, TR of 2,500 ms, and 128 averages. A representative MRS spectrum acquired from the PFC of a control rat is illustrated in Fig. 1B. Unsuppressed data were acquired by turning the CHESS RF pulses off, decreasing the receive gain, and acquiring four averages. The unsuppressed data were used to phase- and eddy-current-correct the suppressed data. Once the spectra were phased, they were imported into LCModel for quantitation (19). The resulting values were corrected for differences in the number of averages and the receiver gain between suppressed and unsuppressed data, but were not corrected for metabolite relaxation times, to obtain concentration estimates in institutional units (IU).

Following baseline scans, SKF83959 (3-methyl-6-chloro-7,8-hydroxy-1-[3-methylphenyl]2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide, supplied by the NIMH Chemical Synthesis and Drug Supply Program) was injected via an indwelling subcutaneous catheter at a dose of 1.0 mg/ml/kg. SKF83959 was chosen because it is a selective agonist at PI-coupled dopamine D_1 receptors (17), which are highly concentrated in the rat medial PFC (20), and prior studies have found that dopamine D_1 receptor binding is not significantly altered in perinatal DHA-deficient rat brain (9). MRS acquisitions were obtained 90 min following SKF83959 administration to allow drug absorption and brain penetration, and we have previously found that this dose induces a stable and protracted (>2 h) elevation in locomotor activity in rats following acute administration (21). Immediately following scanning, rats were sacrificed by decapitation, brains removed, weighed, and immediately immersed in ice-cold 0.9% NaCl for 2 min. The brain was then dissected on ice with a tissue chopper to uniformly isolate the PFC, from which the olfactory tubercle and residual striatal tissue were removed. Tissues were individually placed in cryotubes, flash frozen in liquid nitrogen, and stored at −80°C.

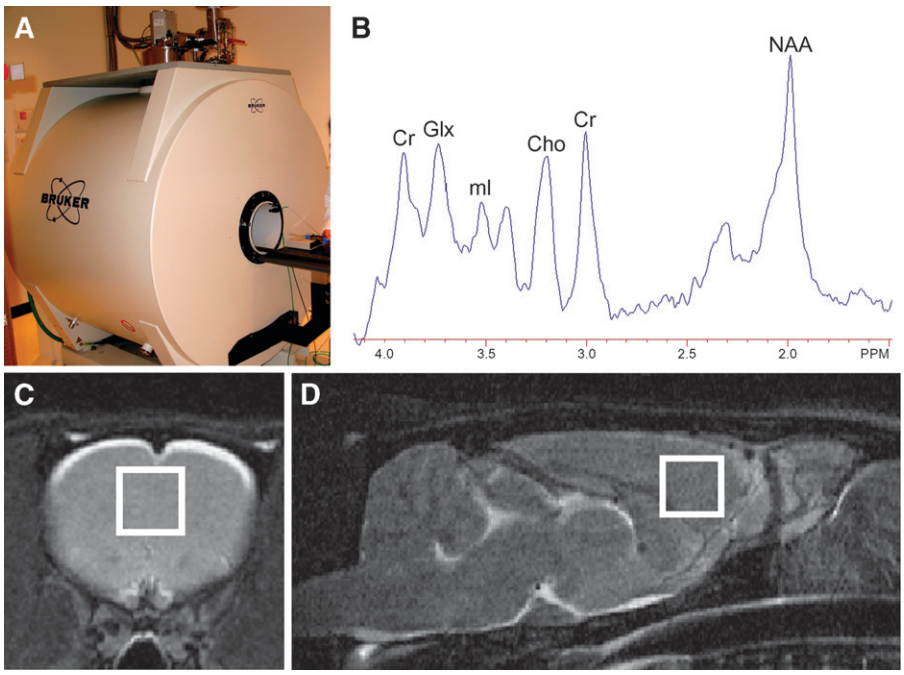

Fig. 1. The 7T Bruker Biospec system (A). A representative proton magnetic resonance spectroscopy (^{1}H-MRS) spectrum from a control rat prefrontal cortex (PFC) (B). Localization of the single voxel in the bilateral medial PFC in the axial (C) and sagital (D) planes. Note that the unlabed peak to the right of mI is taurine. Cho, choline; Cr, creatine; Glx, glutamate and glutamine; mI, *myo*-inositol; NAA, *N*-acetyl aspartate. X-axis in B is ppm.

Gas chromatography

Total (triglyceride, phospholipid, and cholesteryl ester) fatty acid composition was determined using the saponification and methylation methods originally described by Mecalfe, Schmitz, and Pelka (22). We have previously demonstrated that this method yields the same fatty acid composition as Folch (chloroform/methanol) extraction (23). PFC samples (mean weight: 135 ± 6.4 mg wet weight) were placed in a 20 ml glass vial into which 4 ml of 0.5N methanolic sodium hydroxide was added, and the sample heated at 80°C for 5 min. Following a 10 min cooling period, 3 ml of boron trifluoride in methanol was added to methylate the sample. After an additional 5 minutes of heating in the water bath (80°C), the sample vial was allowed to cool, and 2 ml of a saturated solution (6.2 M) of sodium chloride and 10 ml of hexane was added. The samples were then mixed by vortex for 1 minute. The hexane fraction was then transferred into a 20 ml vial containing 10 mg of sodium sulfate to dry the sample. The hexane solution was then removed for gas chromatography analysis. Samples were analyzed with a Shimadzu GC-2014 equipped with an auto-injector (Shimadzu Scientific Instruments Inc., Columbia MD). The column was a DB-23 (123–2,332): 30 m (length), internal diameter. (mm) 0.32 wide bore, film thickness of 0.25 μM (J and W Scientific, Folsom, CA). The gas chromatography conditions were: column temperature ramping by holding at 120°C for 1 minute followed by an increase of 5°C/min from 120–240°C. The temperature of the injector and flame ionization detector was 250°C. A split (8:1) injection mode was used. The carrier gas was helium with a column flow rate of 2.5 ml/min. Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap, PA). Analysis of fatty acid methyl esters is based on areas calculated with Shimadzu Class VP 4.3 software, and fatty acid composition data expressed as mg fatty acid/100 mg fatty acids (% TTL fatty acids). All samples were processed by a technician that was blind to treatment.

Statistical analysis

Group differences in brain fatty acid composition, basal metabolite concentrations, brain weight, and body weight were determined with a one-way ANOVA. Effects of SKF83959 were analyzed with a two-way ANOVA with diet group (control, postweaning, perinatal) and treatment (baseline, postinjection) as the main factors. Pairwise comparisons were made with unpaired *t*-tests (2-tailed, $\alpha = 0.05$). Parametric linear regression analyses were used to determine the relationship between PFC fatty acid composition and metabolite concentrations. In cases of statistical significance, effect size was calculated using Cohen's *d*, with small, medium, and large effect sizes being equivalent to *d*-values of 0.30, 0.50, and 0.80, respectively. All statistical analyses were performed with GB-STAT software (Dynamic Microsystems, Inc., Silver Springs, MD).

RESULTS

Body and brain weights

Prior to scanning, mean body weights for control (n = 14, 419 ± 11 g), postweaning (n = 13, 390 ± 6.5 g), and perinatal (n = 8, 393 ± 11 g) groups did not differ significantly, $F(2,33) = 3.0$, $P = 0.06$. At the time of sacrifice, mean brain weights (wet) for control (1.99 ± 0.04 g), postweaning (1.95 ± 0.04 g), and perinatal (2.00 ± 0.02 g) groups did not differ significantly, $F(2,35) = 1.10$, $P = 0.344$.

PFC fatty acid composition

Group differences in the compositions of the main fatty acids are presented in **Table 1**. There was a significant main effect for DHA (22:6*n*-3) composition, $F(2,34) = 687$, $P \leq 0.0001$. Relative to controls, DHA composition was significantly reduced in postweaning ($-27 \pm 1.0\%$, $P \leq 0.0001$) and perinatal ($-65 \pm 1.8\%$, $P \leq 0.0001$) groups, and DHA composition in the perinatal group was significantly lower than the postweaning group ($-53 \pm 0.6\%$, $P \leq 0.0001$). There was a significant main effect for AA composition, $F(2,34) = 4.39$, $P = 0.021$. Relative to controls, AA composition was significantly greater in the postweaning ($P \leq 0.01$) and perinatal ($P \leq 0.05$) groups. There was a significant main effect for the AA/DHA ratio, $F(2,34) = 256$, $P \leq 0.0001$. Relative to controls, the AA/DHA ratio was significantly greater in the postweaning ($P \leq 0.0001$) and perinatal ($P \leq 0.0001$) groups, and the AA/DHA ratio in the perinatal group was significantly greater than the postweaning group ($P \leq 0.0001$). There was a significant main effect for docosapentaenoic acid (22:5*n*-6) (DPA) composition, $F(2,34) = 848$, $P \leq 0.0001$. Relative to controls, DPA composition was significantly greater in the postweaning ($P \leq 0.0001$) and perinatal ($P \leq 0.0001$) groups, and DPA composition in the perinatal group was significantly greater than the postweaning group ($P \leq 0.0001$). There was a significant main effect for adrenic acid (22:4*n*-6) composition, $F(2,34) = 37.1$, $P \leq 0.0001$. Relative to controls, adrenic acid composition was significantly greater in the postweaning ($P \leq 0.0001$) and perinatal ($P \leq 0.0001$) groups. There was a significant main effect for linoleic acid (18:2*n*-6) composition, $F(2,34) = 24.5$, $P \leq 0.0001$. Relative to controls, linoleic acid composition was significantly lower in the postweaning ($P \leq 0.0001$) and perinatal ($P \leq 0.0001$) groups. There was a significant main effect for oleic acid (18:1*n*-9) composition, $F(2,34) = 12.4$, $P = 0.0001$. Relative to controls, oleic acid composition was significantly lower in the postweaning ($P = 0.0009$) and perinatal ($P = 0.0006$) groups. There was a significant main effect for *cis*-vaccenic acid (18:1*n*-7) composition, $F(2,34) = 10.4$, $P = 0.0003$. Relative to controls, *cis*-vaccenic acid composition was significantly greater in the postweaning ($P = 0.006$) and perinatal ($P = 0.0001$) groups. There no significant main effects for palmitic acid (16:0), $F(2,34) = 2.4$, $P = 0.11$, or stearic acid (18:0), $F(2,34) = 1.2$, $P = 0.30$.

TABLE 1. Prefrontal cortex (PFC) fatty acid composition

	Control	Postweaning	Perinatal
16:0	22.1 ± 0.4	22.3 ± 0.3	22.3 ± 0.3
18:0	19.3 ± 0.5	19.5 ± 0.4	19.3 ± 0.3
18:1n-9	14.6 ± 0.5	13.9 ± 0.4[a]	13.7 ± 0.3[a]
18:1n-7	3.2 ± 0.1	3.3 ± 0.1[a]	3.4 ± 0.1[b]
18:2n-6	0.5 ± 0.0	0.4 ± 0.0[b]	0.4 ± 0.0[b]
20:4n-6	12.1 ± 0.2	12.4 ± 0.3[a]	12.4 ± 0.3[c]
22:4n-6	3.1 ± 0.1	3.3 ± 0.1[b]	3.4 ± 0.1[b]
22:5n-6	0.4 ± 0.0	4.2 ± 0.3[b]	11.4 ± 1.2[b,d]
18:3n-3	ND	ND	ND
22:6n-3	18.1 ± 0.6	13.3 ± 0.6[b]	6.3 ± 0.9[b,d]
20:4n-6/22:6n-3	0.7 ± 0.0	0.9 ± 0.0[b]	2.0 ± 0.3[b,d]

Values are means ± SD (mg/100 mg fatty acids). ND, not detectable. Minor saturated and monounsaturated fatty acids accounting for less than 7 mg/100 mg fatty acids are not presented.
[a] $P < 0.01$ vs. controls.
[b] $P < 0.0001$ vs. controls.
[c] $P < 0.05$ vs. controls.
[d] $P < 0.001$ vs. postweaning.

^{1}H-MRS

Baseline. The main effect of group was not significant for PFC concentrations of *N*-acetyl aspartate (NAA), $F(2,33) = 1.0$, $p = P = 0.37$, creatine (Cr), $F(2,32) = 1.3$, $P = 0.29$, choline (Cho), $F(2,31) = 0.22$, $P = 0.81$, taurine (Tau), $F(2,32) = 1.7$, $P = 0.21$, or the sum of glutamine and glutamate (Glx), $F(2,32) = 1.2$, $P = 0.32$ (**Fig. 2**). The main effect was significant for PFC mI concentrations, $F(2,29) = 5.6$, $P = 0.009$. Relative to controls, PFC mI concentrations were significantly lower in the perinatal group (-21%, $P = 0.001$, $d = 2.1$) but not in the postweaning group (-1%, $P = 0.86$, $d = 0.08$), and PFC mI concentrations were significantly lower in the perinatal group relative to the postweaning group (-20%, $P = 0.01$, $d = 1.3$) (**Fig. 3A**). For the mI/Cr ratio, which is commonly used in human ^{1}H-

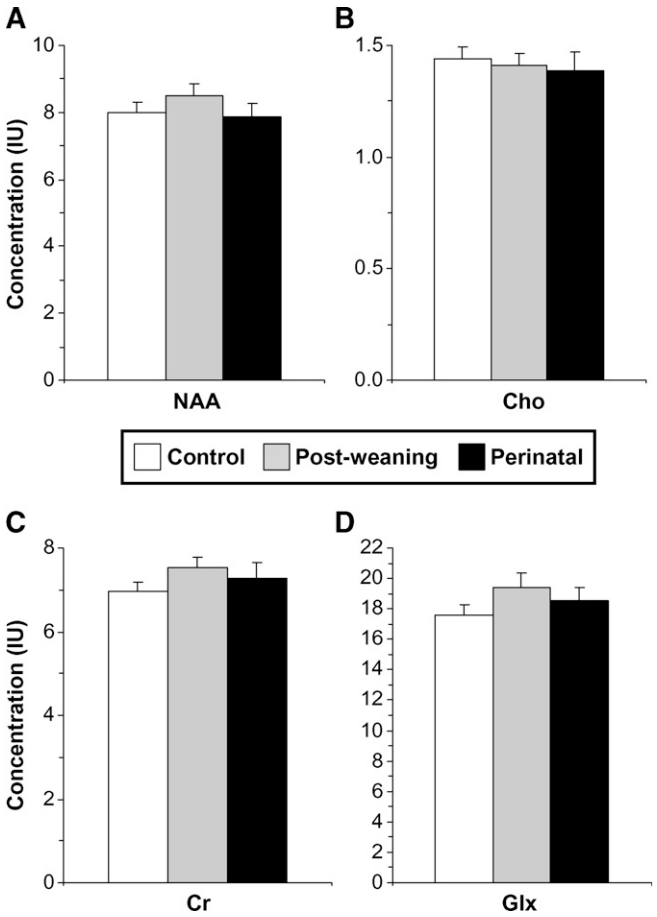

Fig. 2. Baseline concentrations of NAA (A), Ch (B), Cr (C), and Glx (D) in the PFC of control and postweaning or perinatal docosahexaenoic acid (22:6*n*-3) (DHA)-deficient rats. Metabolite concentration (IU) data are expressed as group mean ± SEM.

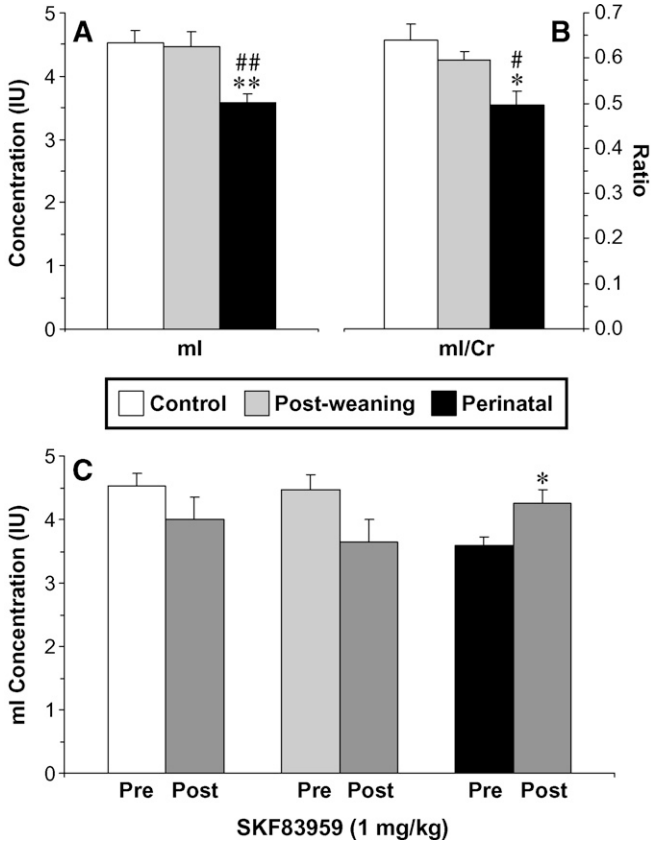

Fig. 3. Baseline mI concentrations (A) and the mI/Cr ratio (B) in the PFC of control and postweaning or perinatal DHA-deficient rats. Note that PFC mI concentrations are significantly lower in the PFC of perinatal DHA-deficient rats. C: Effects of SKF83959 (1.0 mg/kg) treatment on PFC mI concentrations in control and postweaning or perinatal DHA-deficient rats. mI concentration (IU) and mI/Cr ratio data are expressed as group mean ± SEM. * $P \leq 0.05$, ** $P \leq 0.01$ compared with controls; # $P \leq 0.05$, ## $P \leq 0.01$ compared with postweaning rats.

MRS studies, the main effect of group was significant, $F(2,28) = 5.5$, $P = 0.009$. Relative to controls, the mI/Cr ratio was significantly lower in the perinatal group (−22%, $P = 0.01$, $d = 1.6$) but not in the postweaning group (−7%, $P = 0.24$), and the mI/Cr ratio was significantly lower in the perinatal group relative to the postweaning group (−16%, $P = 0.013$, $d = 1.3$) (Fig. 3B). Among all rats, basal mI concentrations were positively correlated with DHA composition ($r = +0.52$, $P = 0.004$), and inversely correlated with DPA composition ($r = -0.52$, $P = 0.001$) and the AA/DHA ratio ($r = -0.56$, $P = 0.001$). Similarly, the mI/Cr ratio was positively correlated with DHA composition ($r = +0.88$, $P \leq 0.0001$), and inversely correlated with DPA composition ($r = -0.85$, $P \leq 0.0001$) and the AA/DHA ratio ($r = -0.71$, $P \leq 0.0001$).

SKF83959 challenge. Following the SKF83959 challenge, the two-way ANOVA found that the diet x treatment interaction was significant, $F(2,48) = 4.0$, $P = 0.025$, and the main effect of diet, $F(2,48) = 0.8$, $P = 0.47$, and treatment, $F(1,48) = 1.1$, $P = 0.30$, were not significant. Relative to basal mI concentrations, a significant increase in mI concentrations was found in the perinatal deficiency group (+16%, $P = 0.02$, $d = 1.4$), whereas trends toward reduced mI concentrations were found in controls (−12%, $P = 0.24$), and the postweaning deficiency group (−19%, $P = 0.053$) (Fig. 3C).

DISCUSSION

This study determined the effects of graded PFC DHA deficits on PFC mI concentrations by in vivo ^{1}H-MRS. A principle finding is that perinatal ALA deficiency resulting in large PFC DHA deficits (−65%) was associated with significantly lower basal mI concentrations in the adult rat PFC (−21%). Postweaning ALA deficiency resulting in smaller PFC DHA deficits (−27%) did not alter PFC mI concentrations. Among all rats, PFC mI concentrations were positively correlated with PFC DHA composition. This effect was selective for mI because other principle brain metabolites (NAA, Tau, Cr, Cho, Glx) were not significantly altered in DHA-deficient rats. Consistent with our hypothesis, treatment with SKF83959, a selective agonist at PI-linked dopamine D_1-like receptors, significantly increased PFC mI concentrations in the perinatal-deficiency group and produced trends toward reduced mI concentrations in control and the postweaning deficiency groups. These results extend prior findings that increasing omega-3 fatty acid intake down-regulates PI signaling in peripheral tissues (14–16) and demonstrate that perinatal, but not postnatal, deficits in cortical DHA accrual are associated with significant and selective reductions in mI concentrations, and augmented receptor-generated mI synthesis in the adult rat PFC.

Several findings suggest that the basal mI deficits observed in the PFC of perinatal DHA-deficient rats may be due to impairments in astrocyte maturation and associated deficits in mI osmotic regulation. First, because mI concentrations are several-fold greater in astroctyes than neurons (24, 25), the astrocyte mI pool acquired at 7T is likely the major contributor to the mI peak. This is supported by prior studies finding that treatment with D-amphetamine increases mI concentrations in rat cerebrospinal fluid (26), but does not significantly alter mI concentrations in ex vivo rat frontal cortex by ^{1}H-MRS (27). Second, NAA concentrations were not reduced in the PFC of perinatal deficient rats, suggesting that neuronal metabolism/integrity was not significantly impaired. Third, rat astrocyte ontogenesis occurs predominantly during prenatal development (28), and mI concentrations were reduced by perinatal, but not postweaning, ALA-deficiency (present results). Furthermore, DHA is required for the normal growth and maturation of cortical astrocytes in culture (29). Fourth, the astrocyte-specific glucose transporter (45 kDa GLUT1), but not the neuronal-specific glucose transporter (GLUT3), is significantly reduced in the cortex of DHA-deficient rats (30). Although these and the present results are consistent with impairments in astrocyte maturation and function, additional

studies will be required to fully elucidate the mechanisms mediating lower basal mI concentrations in perinatal DHA-deficient rat PFC.

Based on prior studies finding that DHA deficits alter the biophysical properties of neuronal membranes and membrane-binding proteins that regulate PLC-mediated PI hydrolysis, it was hypothesized that brain DHA deficiency would significantly augment receptor-generated PI hydrolysis and associated increases in mI content. In the present study, this was supported by the finding that perinatal DHA-deficient rats exhibited a significant increase in mI concentrations following acute treatment with SKF83959, a selective agonist at PI-coupled dopamine D_1 receptors (17). In contrast, postweaning and control rats exhibited trends toward reduced mI concentrations. One interpretation of these results is that astrocyte mI stores decrease in the PFC of control and postweaning DHA-deficient rats in support of PI resynthesis, whereas the reduced basal astrocyte mI stores in perinatal DHA-deficient rats are elevated above a lower basal mI background in response to de novo mI synthesis. Additionally, these data demonstrate that reduced basal mI concentrations in the PFC of perinatal DHA-deficient rats are partially normalized with SKF83959 treatment, suggesting that low basal mI concentrations may reflect lower de novo mI synthesis. It is relevant, therefore, that mI is a carbohydrate that is directly metabolized from glucose via 1L-mI 1-phosphate synthase, and prior studies have observed significant reductions in glucose transporter expression (30) and glucose uptake (31) in the frontal cortex of perinatal DHA-deficient rats.

The present findings may take on additional significance in view of clinical data demonstrating that patients with major affective disorders exhibit significant DHA deficits (23, 32) and reduced mI concentrations (33) in postmortem frontal cortex. Patients with affective disorders also exhibit mI deficits in cerebrospinal fluid (34). Although results from in vivo ^{1}H-MRS studies have been varied with regard to mI levels, potentially due to medication effects (35), some studies have observed reduced basal mI concentrations in the frontal cortex of patients with major depression (36–38). Furthermore, it may be relevant that patients with affective disorders also exhibit glial cell pathology and reductions in the expression of astrocyte-specific markers in postmortem frontal cortex (39).

In summary, the present results demonstrate that ALA deficiency resulting in large brain DHA deficits during perinatal brain development is associated with significant and selective reductions in basal mI concentrations in the PFC. In contrast, basal mI concentrations were not altered in the PFC of postweaning ALA-deficient rats, suggesting a potential developmental window of vulnerability. We additionally provide evidence that perinatal ALA deficiency is associated with augmented receptor-generated PI hydrolysis. Future studies will be required to elucidate the mechanisms mediating reduced mI content in the DHA-deficient rat PFC and to determine whether this reduction in PFC mI content is revered following normalization of PFC DHA composition.

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