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Effects of Dietary-Induced Alterations in Rat Brain Docosahexaenoic Acid Accrual on Phospholipid Metabolism and Mitochondrial Bioenergetics: An *in vivo* ^{31}P MRS study

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Abstract

Evidence from ^{31}P magnetic resonance spectroscopy (^{31}P MRS) studies suggest that different psychiatric disorders, which typically emerge during adolescence and young adulthood, are associated with abnormalities in mitochondrial bioenergetics and membrane phospholipid metabolism. These disorders are also associated with deficits in omega-3 polyunsaturated fatty acids (*n*-3 PUFA), including docosahexaenoic acid (DHA) which accumulates in mitochondrial and synaptic membranes. The present study investigated the effects of dietary-induced alterations in brain DHA accrual during adolescence on phospholipid metabolism and bioenergetics in the adult rat brain using ^{31}P MRS. During the peri-adolescent period (P21–P90), male rats were fed a diet with no *n*-3 fatty acids (Deficient, DEF, *n*=20), a diet fortified with preformed DHA (fish oil, FO, *n*=20), or a control diet fortified with alpha-linolenic acid (18:3 *n*-3, *n*=20). On P90, ^{31}P MRS was performed under isoflurane anesthetic using a 7T Bruker Biospec system. Compared with controls, brain DHA levels were significantly lower in adult rats fed the DEF diet (–17%, *p* 0.0001) and significantly higher in rats fed the FO diet (+14%, *p* 0.0001). There were no significant group differences for indices of bioenergetics, including adenosine triphosphate and phosphocreatine levels, or indices of membrane phospholipid metabolism including phosphomonoesters and phosphodiester. Therefore, the present ^{31}P MRS data suggest that rat brain DHA levels are not a significant predictor of mitochondrial bioenergetics or membrane phospholipid metabolism.

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Keywords

Omega-3 fatty acids; Docosahexaenoic acid (DHA); ATP; Brain; Phosphomonoester; Phosphocreatine; Rat

1. Introduction

Emerging evidence suggests that mitochondrial dysfunction and membrane phospholipid abnormalities are associated with the pathophysiology of different psychiatric disorders, including bipolar disorder, depression, and schizophrenia (Horrobin et al., 1994; Melzer, 1991; Rezin et al., 2009; Scaini et al., 2016; Schaeffer et al., 2012). Supporting evidence has been provided by studies using phosphorous magnetic resonance spectroscopy (^{31}P MRS), which measures indices of membrane phospholipid turnover and mitochondrial bioenergetics. Indices of membrane phospholipid turnover include phospholipid anabolites (i.e., phosphomonoesters, PME) and catabolites (i.e., phosphodiesteres, PDE), and a reduction in the PME:PDE ratio is thought to reflect a decrease in the synthesis and/or an increase in the breakdown of membrane phospholipids. Indices of mitochondrial bioenergetics include high-energy phosphates, including phosphocreatine (PCr) and adenosine triphosphate (ATP). While ^{31}P MRS studies have reported abnormalities in phospholipid metabolism and/or mitochondrial bioenergetics in patients with bipolar disorder (Yildiz et al., 2001), major depressive disorder (MDD) (Kato et al., 1992; Volz et al., 1998), and schizophrenia (Yuksel et al., 2015), the underlying risk factors remain poorly understood.

One potential risk factor is a deficiency in the omega-3 polyunsaturated fatty acid (*n*-3 PUFA) docosahexaenoic acid (DHA). DHA is highly concentrated in brain and preferentially accumulates in gray matter mitochondrial and synaptosomal membranes (Suzuki et al., 1997). Evidence from postmortem rat brain studies suggest that DHA modulates the activity of enzymes involved in membrane phospholipid metabolism (Rao et al., 2007) and mitochondrial ATP generation (Afshordel et al., 2015; Harbeby et al., 2012; Kitajka et al., 2004; Ximenes da Silva et al., 2002). Meta-analyses indicate that bipolar disorder (McNamara & Welge, 2016), schizophrenia (van der Kemp et al., 2012), and MDD (Lin et al., 2010) are associated with lower erythrocyte phospholipid membrane DHA levels, and preliminary clinical ^{31}P MRS evidence suggests that erythrocyte membrane DHA levels were correlated with membrane phospholipid metabolism (Richardson et al., 2001). However, the relationships among brain DHA membrane levels, phospholipid metabolism, and mitochondrial bioenergetics have not been systematically evaluated by ^{31}P MRS.

The objective of the present study was to use ^{31}P MRS to investigate the effects of dietary-induced alterations in rat brain DHA levels on phospholipid metabolism and bioenergetics *in vivo*. Based on the translational evidence reviewed above, our primary hypothesis was that brain DHA levels would be positively associated with indices of mitochondrial bioenergetics and membrane phospholipid metabolism.

2. Materials and methods

2.1. Animals and diets

Post-weaning (P20) male Long-Evans hooded rats from different nulliparous dams were purchased from Harlan Farms, Indianapolis, IN, and randomized to one of three diets (n=20/diet group) upon arrival at P21 until young adulthood (P90). Control (CON) rats were maintained on an α -linolenic acid (ALA, 18:3*n*-3)-fortified diet (TD.04285, Harlan-TEKLAD, Madison, WI). Deficient (DEF) rats were maintained on the ALA-free diet (ALA-, TD.04286), and *n*-3 PUFA enriched rats were maintained on a diet containing 1.1% fish oil in place of ALA (FO, TD.110837, Harlan-TEKLAD, Madison, WI). Diets were closely matched for all nonfat nutrients and fatty acid composition with the exception of ALA (18:3*n*-3), which was absent from the DEF and FO diets, and EPA and DHA which were present in the FO diet but not the CON and DEF diets (Supplemental Table 1). Rats were pair-housed with food and water available *ad libitum*, and were 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.

2.2. ³¹P MRS

Adult (P90) rats from each diet group were anesthetized with 2.5–3.5% isoflurane in air, positioned supine with their teeth in a bite bar, and the head centered in a dual-tuned proton/phosphorus 38 mm Litz coil (Doty Scientific, Inc., Columbia, SC). Respiration was monitored and body temperature was maintained at 36–38°C using an animal monitoring system (SAI Inc., Stony Brook, NY). The coil and animal were positioned in a 7T Bruker Biospec system (Bruker BioSpin, Ettlingen, Germany) (Fig. 1A). After acquiring a set of localizers from each orthogonal plane to use for voxel placement, a brain voxel (7 mm × 7 mm × 10 mm) (Fig. 1C,D) was shimmed prior to acquiring proton data i.e., total creatine (Cr) (PRESS, TR 2.5s, TE 20 ms) followed by ³¹P data (ISIS, TR 6s). Spectra were analyzed using jMRUI (Stefen et al., 2009). A representative ³¹P MRS spectrum acquired from rat brain at 7T is illustrated (Fig. 1B). Primary measures of interest were ATP (α , β , γ phosphates), PCr, inorganic phosphate (Pi), PME, and PDE. Data are expressed in institutional units (IU). Immediately following scanning, isoflurane-anesthetized rats were sacrificed by decapitation. The brain region encompassed by the voxel (Fig. 1C) was isolated for fatty acid analysis.

2.3. Gas chromatography

Fatty acid composition was determined with a Shimadzu GC-2014 (Shimadzu Scientific Instruments Inc., Columbia MD) using procedures described in detail previously (McNamara et al., 2009). Fatty acid composition data are expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). Primary measures of interest were DHA, arachidonic acid (AA), and the AA/DHA ratio.

2.4. Statistical analyses

Group differences in neurochemical concentrations and fatty acid composition data were determined with a one-way ANOVA. Post hoc comparisons were made with unpaired *t*-tests (2-tailed, $\alpha=0.05$). Linear regression analyses were used to determine the relationship between brain fatty acid composition and neurochemical concentrations. Statistical analyses were performed with GB-STAT software (Dynamic Microsystems, Inc., Silver Springs MD).

3. Results

3.1. Fatty acid composition

Compared with controls, brain DHA levels were significantly lower in adult rats fed the DEF diet (-17% , $p = 0.0001$) and significantly higher in rats fed the FO diet ($+14\%$, $p = 0.0001$)(Fig. 1A), brain arachidonic acid (AA) levels were significantly greater in adult rats fed the DEF diet ($+20\%$, $p = 0.0001$) but not rats fed the FO diet ($+2\%$, $p=0.67$)(Fig. 1B). Compared with controls, the AA/DHA ratio was significantly greater in adult rats fed the DEF diet ($+35\%$, $p = 0.0001$) and significantly lower in rats fed the FO diet (-12% , $p=0.0002$) (Fig. 1C).

3.2. ^{31}P MRS

There were no significant group differences for indices of mitochondrial bioenergetics, including ATP, PCr, and PCr/Pi ratio, or indices of phospholipid metabolism including PME, PDE, and the PDE/PME ratio (Table 1). Among all rats ($n=60$), brain DHA levels were not significantly correlated with any measure.

4. Discussion

This study used ^{31}P MRS to evaluate whether alterations in brain DHA accrual during adolescence would impact indices of mitochondrial bioenergetics and membrane phospholipid synthesis in the adult rat brain. Compared with controls, brain DHA levels were significantly lower in adult rats fed the DEF diet and significantly higher in rats fed the FO diet. However, we did not observe significant group differences for indices of bioenergetics, including ATP and PCr, or indices of membrane phospholipid metabolism including PME and PDE. Therefore, the present ^{31}P MRS data do not support the hypotheses that brain DHA levels are positively associated with indices of mitochondrial bioenergetics and membrane phospholipid metabolism.

Several prior postmortem rat brain studies have observed a positive relationship between brain DHA levels and enzymes involved in membrane phospholipid metabolism (Rao et al., 2007) and mitochondrial ATP generation (Afshordel et al., 2015; Harbeby et al., 2012; Kitajka et al., 2004; Ximenes da Silva et al., 2002). This raises the possibility that the isoflurane anesthetic used in the present study suppressed indices of bioenergetics and membrane phospholipid turnover, thereby limiting our ability to detect differences across diet groups. However, a recent ^{31}P magnetization transfer study found that ATP and PCr concentrations in rat brain remain stable in response to increasing isoflurane concentrations (Bresnen & Duong, 2015). Nevertheless, the use isoflurane anesthetic must be viewed as a

study limitation. Additionally, it is notable that the rats used in this study were housed in cages for the majority of time, and therefore did not experience significant cognitive stimulation or physical activity. Therefore, it remains possible that group differences in mitochondrial bioenergetics and membrane phospholipid metabolism may become apparent under conditions requiring greater neuronal activation.

Another potential limitation of the present study is the size of the voxel which did not encompass a single brain region, and potential regional signal differences may have altered the total signal. However, prior studies have found that reductions in cytochrome oxidase, a mitochondrial respiratory enzyme, were observed across different brain regions in *n*-3 deficient rats (Ximenes da Silva et al., 2002), and the up-regulation of genes encoding for subunits of cytochrome oxidase and ATP synthases were detected in whole brain of rats fed high *n*-3 PUFA diets (Kitajka et al., 2004). While the latter findings would suggest that we would be able to detect changes within our multi-region voxel, another study found that genes involved in mitochondrial ATP generation were up-regulated in the hippocampal CA1 region, but not fronto-parietal cortex, of rats fed high *n*-3 PUFA diets (Harbeby et al., 2012). It is also possible that different brain regions may accrue and lose DHA at different rates in response to dietary manipulations. Therefore, brain regional heterogeneity should also be viewed as a study limitation.

The present preclinical ³¹P MRS findings do not support an association among brain DHA levels, membrane phospholipid metabolism, and mitochondrial bioenergetics. However noted limitations associated with this study and evidence from postmortem rat brain studies which do support a role of DHA in these processes encourage additional research using alternative approaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The 7T Bruker Biospec Imaging System (A), a representative ^{31}P MRS spectrum from a control rat (B), and voxel placement in sagittal (C) and horizontal (D) orientations. ATP, adenosine triphosphate (α , β , γ phosphates); PME, phosphomonoesters; PDE, phosphodiester; Pi, inorganic phosphate; PCr, phosphocreatine; PE, phosphoethanolamine; PC, phosphocholine; GPE, glycerophosphoethanolamine; GPC, glycerolphosphocholine. X-axis in (B) is parts per million (ppm)

Figure 2.

DHA (A) and arachidonic acid (AA)(B) compositions, and the AA/DHA ratio (C) in the voxel region of adult rats maintained on the control diet (CON, ALA-fortified), *n*-3-free diet (DEF), and fish oil-fortified diet (FO). Values are group mean composition $\pm$ S.E.M.

*** P 0.0001 vs. CON, ### P 0.0001 vs. DEF.

Table 1**³¹P MRS Neurochemical Levels**

Neurochemical ¹	CON (n=20)	DEF (n=20)	FO (n=20)	P-value ²
PME	4.7 ± 0.5	4.6 ± 0.8	4.4 ± 1.0	0.55
PDE	1.8 ± 0.7	1.5 ± 0.5	1.5 ± 0.5	0.11
PDE/PME	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.18
PCr	6.8 ± 0.8	6.8 ± 0.8	6.7 ± 0.7	0.96
Pi	3.1 ± 0.5	3.2 ± 0.6	3.4 ± 0.7	0.52
PCr/Pi	2.2 ± 0.4	2.3 ± 0.7	2.1 ± 0.4	0.70
Cr ³	4.3 ± 0.3	4.1 ± 0.5	4.3 ± 0.3	0.21
Cr+PCr	11.2 ± 1.1	10.9 ± 1.0	11.1 ± 0.9	0.68
PCr/Cr	1.6 ± 0.2	1.7 ± 0.3	1.6 ± 0.2	0.26
αATP	3.1 ± 0.5	3.1 ± 0.6	3.0 ± 0.5	0.78
βATP	1.7 ± 0.4	1.8 ± 0.4	1.9 ± 0.5	0.48
γATP	2.5 ± 0.4	2.5 ± 0.4	2.5 ± 0.3	0.93
PCr/γATP	2.8 ± 0.2	2.7 ± 0.3	2.7 ± 0.2	0.62
γATP/Pi	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.81
pH	7.1 ± 0.1	7.1 ± 0.0	7.1 ± 0.0	0.49

¹Values are mean concentration estimate (IU) or ratio ± S.D.

²One-way ANOVA

³Acquired by ¹H MRS