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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

Diana M. Lindquist [a], Ruth H. Asch [b], Jennifer D. Schurdak [b], Robert K. McNamara [b, *]

[a] Imaging Research Center, Department of Radiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA
[b] Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA

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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 7 T Bruker Biospec system. Compared with controls, brain DHA levels were significantly lower in adult rats fed the DEF diet (−17%, $p \leq 0.0001$) and significantly higher in rats fed the FO diet (+14%, $p \leq 0.0001$). 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 phosphodiesters. 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.

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; Meltzer, 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., phosphodiesters, 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.,

* Corresponding author. Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, 260 Stetson Street, Cincinnati, OH 45219-0516, USA.
E-mail address: robert.mcnamara@uc.edu (R.K. McNamara).

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2012; Kitajka et al., 2004; Ximenes da Silva et al., 2002). Meta-analyses indicate that bipolar disorder (McNamara and 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 non-fat 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. ^{31}P 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 7 T 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 x 10 mm) (Fig. 1C and D) was shimmed prior to acquiring proton data i.e., total creatine (Cr) (PRESS, TR 2.5s, TE 20 ms) followed by ^{31}P data (ISIS, TR 6s). Spectra were analyzed using jMRUI (Stefan et al., 2009). A representative ^{31}P MRS spectrum acquired from rat brain at 7 T 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 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

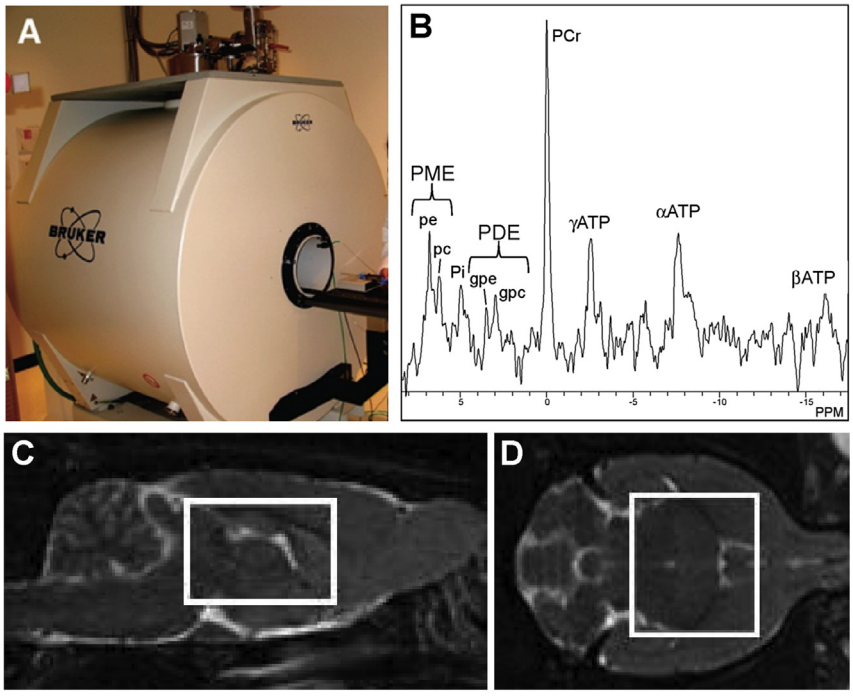

Fig. 1. The 7T Bruker Biospec Imaging System (**A**), a representative ^{31}P MRS spectrum from a control rat (**B**), and voxel placement in sagittal (**C**) and horizontal (**D**) orientations. ATP, adenosine triphosphate (α, β, γ phosphates); PME, phosphomonoesters; PDE, phosphodiesters; Pi, inorganic phosphate; PCr, phosphocreatine; PE, phosphoethanolamine; PC, phosphocholine; GPE, glycerophosphoethanolamine; GPC, glycerolphosphocholine. X-axis in (**B**) is parts per million (ppm).