Prostaglandins, Leukotrienes and Essential Fatty Acids 88 (2013) 79–85

Contents lists available at SciVerse ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Translational studies on regulation of brain docosahexaenoic acid (DHA) metabolism *in vivo*

Stanley I. Rapoport *

Brain Physiology and Metabolism Section, National Institute on Aging, National Institutes of Health, Building 9, Room 1S128 Bethesda, MD 20892, USA

A B S T R A C T

One goal in the field of brain polyunsaturated fatty acid (PUFA) metabolism is to translate the many studies that have been conducted *in vitro* and in animal models to the clinical setting. Doing so should elucidate the role of PUFAs in the human brain, and effects of diet, drugs, disease and genetics on this role. This review discusses new *in vivo* radiotracer kinetic and neuroimaging techniques that allow us to do this, with a focus on docosahexaenoic acid (DHA). We illustrate how brain PUFA metabolism is influenced by graded reductions in dietary n-3 PUFA content in unanesthetized rats. We also show how kinetic tracer techniques in rodents have helped to identify mechanisms of action of mood stabilizers used in bipolar disorder, how DHA participates in neurotransmission, and how brain DHA metabolism is regulated by calcium-independent iPLA$_2$β. In humans, regional rates of brain DHA metabolism can be quantitatively imaged with positron emission tomography following intravenous injection of [1-^{11}C]DHA.

1. Introduction

A challenge in the field of brain polyunsaturated fatty acid (PUFA) metabolism is to translate results from studies that have been conducted in the test tube, in cells *in vitro* and in animal models, to the clinical setting. Doing so may clarify human brain function involving lipid metabolism, and help to identify effects of diet, drugs, disease and genetics on this function. Sophisticated lipidomic analytical methods have been developed in pre-clinical models and applied to human body tissues (plasma, cerebrospinal fluid, or postmortem brain) in the identification of novel biological mediators and their receptor targets of therapeutic relevance [1,2]. Genetic studies also have identified defects in PUFA metabolizing enzymes that underlie some human brain diseases [3–5].

Additionally, *in vivo* brain imaging and kinetic techniques have been elaborated in rodent and primate studies, and can be translated to address clinically relevant questions. In this paper, I discuss briefly these *in vivo* approaches and their potential applications, particularly with regard to docosahexaenoic acid (DHA, 22:6n-3). DHA is found in high concentrations in the stereospecifically numbered (*sn*)-2 position of brain membrane phospholipids and is critical for maintaining normal brain structure, function and metabolism. DHA influences brain signal transduction, gene transcription, and membrane stability, and is a precursor for neuroprotectins, resolvins and other antiinflammatory products [6–8].

2. Dietary effects on brain α-LNA, DHA and DPAn-6

2.1. Clinical evidence

The brain concentration of DHA depends on the dietary n-3 PUFA content and on hepatic synthesis and secretion of DHA from its circulating shorter-chain nutritionally essential precursor, α-linolenic acid (α-LNA, 18:3n-3), as well as from circulating eicosapentaenoic acid (EPA, 20:5n-3) [9–11]. Epidemiological studies have suggested that a low dietary DHA+EPA intake, due to low intake of fish or fish products, is associated with a number of human neuropsychiatric diseases, including Alzheimer disease, bipolar disorder and depression, and that dietary EPA and/or DHA supplementation may be helpful in some of these conditions. Studies also have reported reduced DHA levels in blood or postmortem brain tissue in Alzheimer disease, bipolar disorder and other brain disorders [12–15]. Supporting these clinical results are rodent studies, involving dietary n-3 PUFA depletion for as long as 3 generations but as short as 15 weeks in a single generation, which indicate that n-3 PUFA dietary deficiency can disturb brain function and behavior [16,17]. However, some of the clinical observations have not been confirmed [18–21], and the

Abbreviations: DHA, Docosahexaenoic acid; AA, Arachidonic acid; LA, Linoleic acid; α-LNA, α-linolenic acid; DPA, Docosapentaenoic acid; EDP, Epoxy-docosapentaenoic acid; EPA, Eicosapentaenoic acid; PLA$_2$, Phospholipase A$_2$; cPLA$_2$, Cytosolic PLA$_2$; sPLA$_2$, Secretory PLA$_2$; iPLA$_2$, Calcium-independent PLA$_2$; NMDA, N-methyl-D-aspartate; PUFA, Polyunsaturated fatty acid; PET, Positron emission tomography

* Tel.: +1 301 496 1765; fax: +1 301 402 0074.
E-mail address: sir@helix.nih.gov

0952-3278/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.plefa.2012.05.003

deprivation studies in rodents may have been too extreme to be clinically relevant.

2.2. Graded dietary n-3 PUFA reductions in rats

To more closely consider effects of dietary n-3 PUFA deficiency on brain function and metabolism, we determined plasma, liver and brain PUFA concentrations and brain and liver expression of PUFA-metabolizing enzymes in rats, in relation to graded dietary n-3 PUFA reductions for 15 weeks after weaning at 21 days. We chose a reference diet containing 4.6% (of total fatty acid) α-LNA, free of DHA or arachidonic acid (AA, 20:4n-6) [22]. This diet is nutritionally "adequate" for maintaining normal body organ function and DHA metabolism in rats [23,24].

Compared with their concentrations in rats fed the n-3 PUFA "adequate" diet, plasma unesterified and esterified α-LNA and DHA concentrations fell progressively with graded reductions in dietary α-LNA (Fig. 1). The threshold (indicated as arrows in Fig. 1) that produced a statistically significant reductions in unesterified plasma α-LNA or DHA concentration compared to control concentration was 2.8% dietary α-LNA, whereas the threshold for significant reductions in total (mainly esterified) plasma α-LNA and DHA concentrations was 1.7% dietary α-LNA. Plasma levels of unesterified and total AA were not changed significantly by any dietary α-LNA reduction, whereas the plasma esterified concentration of docosapentaenoic acid (DPA 20:5n-6) was elevated at and below 1.7% dietary α-LNA, while unesterified plasma DPAn-6 was first elevated at 0.8% α-LNA. These DPA changes reflected increased liver synthesis and secretion (Fig. 2) [25].

Brain DHA concentration remained unchanged down to 1.7% dietary α-LNA (Fig. 2), at which point activity of DHA-selective calcium independent phospholipase A_2 (iPLA$_2$ VIA(β)) was down-regulated [22] and activity of calcium dependent cPLA$_2$ IVA was upregulated. Since brain and liver AA concentrations were unaffected at all dietary α-LNA levels, cPLA$_2$ IVA may hydrolyze DPAn-6 as well as AA from brain phospholipid. In any case, homeostatic mechanisms maintained a normal brain DHA concentration down to 1.7% dietary α-LNA, despite reduced plasma unesterified and esterified DHA concentrations. Brain DHA fell significantly only when it was displaced by circulating liver-derived DPAn-6.

2.3. Translational relevance

The observations indicate that the brain DHA level is related nonlinearly to plasma esterified and unesterified DHA concentrations. Establishing the exact relations could help to use plasma DHA concentrations as "biomarkers" of the brain DHA level and metabolism [26]. Reports that overall mortality or mortality from any cause does not differ significantly between vegetarians and omnivores, despite a 50% lower blood DHA concentration in vegetarians [27,28], support determining the relation of plasma DHA to brain DHA concentration and metabolism in humans. This might be accomplished with positron emission tomography (PET) (see below).

3. Measuring *in vivo* brain DHA kinetics

3.1. New kinetic paradigms

Despite the unchanged brain DHA concentration at 1.7% dietary α-LNA in the face of reduced plasma DHA, rates of brain DHA metabolism and of formation of downstream DHA metabolites [29] may have been reduced, since they are reduced in rats fed a 0.2% α-LNA diet (see below) [30]. This could be tested as a function of dietary content by applying kinetic methods in unanesthetized animals and in humans. These methods include: (1) quantitation of DHPUFA turnover due to deacylation–reacylation in brain phospholipids (Fig. 3) [31,32]; (2) quantitation of DHPUFA incorporation from plasma into different brain lipid compartments, including the acyl-CoA pool, individual phospholipids, triacylglycerol and cholesteryl esters; (3) quantitative imaging of DHPUFA incorporation into different brain regions [33–36]. Some of these methods, particularly those involving neuroimaging, have been translated with the help of PET for human studies (see below) [37–39].

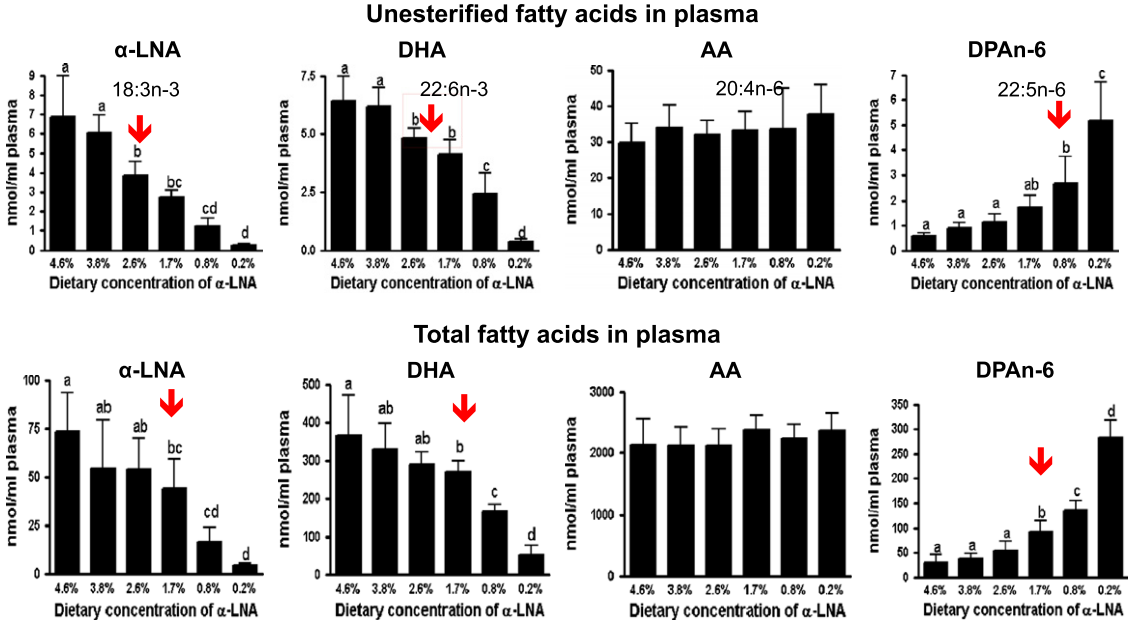

Fig. 1. *Plasma unesterified and total fatty acid concentrations in rats fed different α-LNA containing diets for 15 weeks.* Values are mean ± SD (n=6 per group). Superscripts show significant differences at $p < 0.05$. Arrows indicate threshold difference from control (4.6% α-LNA). From [22].