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A randomized, placebo-controlled, double-blind trial of supplemental docosahexaenoic acid on cognitive processing speed and executive function in females of reproductive age with phenylketonuria: A pilot study ☆, ☆☆

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ABSTRACT

Low blood docosahexaenoic acid (DHA) is reported in patients with phenylketonuria (PKU); however, the functional implications in adolescents and adults are unknown. This pilot study investigated the effect of supplemental DHA on cognitive performance in 33 females with PKU ages 12–47 years. Participants were randomly assigned to receive DHA (10 mg/kg/day) or placebo for 4.5 months. Performance on cognitive processing speed and executive functioning tasks was evaluated at baseline and follow up. Intention-to-treat and per protocol analyses were performed. At follow up, biomarkers of DHA status were significantly higher in the DHA-supplemented group. Performance on the cognitive tasks and reported treatment-related adverse events did not differ. While no evidence of cognitive effect was seen, a larger sample size is needed to be conclusive, which may not be feasible in this population. Supplementation was a safe and effective way to increase biomarkers of DHA status (www.clinicaltrials.gov; Identifier: NCT00892554).

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is a genetic disorder detected through newborn screening in the US, and is most commonly caused by a deficiency in the enzyme phenylalanine hydroxylase (PAH). When diagnosed and treated soon after birth, associated developmental delays and behavioral disturbances can be prevented [1,2]. The diet treatment for PKU requires restricted phenylalanine (Phe) intake which relies on food choices that are low in protein (e.g., measured amounts of fruits, vegetables, and grains) and avoidance of foods high in protein (e.g., meats, eggs, dairy products, beans, and nuts). Nutrient needs are largely met through an amino acid-based synthetic medical food, which provides approximately 50–80% of protein intake [3–5]. Although some medical foods contain the essential fatty acids alpha-linolenic acid (ALA) and linoleic acid (LA), most do not contain the preformed fatty acids eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or arachidonic acid (AA) that are typically found in certain high protein foods.

Currently, lifelong diet treatment for PKU is recommended [6] to prevent cognitive, neurological, and psychiatric declines [7–12]. Despite successful prevention of major developmental delay, adolescents and adults treated early for PKU reportedly still display minor cognitive deficits in domains including processing speed, executive function (inhibition), attention, and overall IQ [13–18]. A 2007 meta-analysis identified cognitive processing speed and cognitive inhibition, an aspect of executive function, as the domains having the largest effect size in adolescents and adults who were treated early and continuously for PKU

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compared with controls without PKU [18]. These deficits have been shown to resolve with improved plasma Phe control; however, improved nutrition may also optimize cognitive performance. Individuals treated early in life for PKU show on average lower plasma and RBC percentage of DHA compared with controls without PKU [19–22]. Accordingly, it has been proposed that inadequate DHA concentrations in neural lipids may be related to cognitive deficits in people treated early for PKU [22–24].

DHA is a major fatty acid in the brain [25,26] and its presence in the cell membrane affects multiple membrane properties including degree of membrane disorder [27], lateral membrane compressibility [28], and formation and fusion of synaptic vesicles [29]. DHA is a precursor to the bioactive molecules neuroprotectin D1 and resolvins [30,31]. Increased brain concentrations of nitric oxide synthetase, dopamine, serotonin, brain-derived neurotrophic factor have been shown in DHA-supplemented animals [32,33]. DHA appears to regulate neuronal apoptosis [31,34] and n-3 fatty acids may regulate neurogenesis [35,36] in adults. Because DHA has multiple potential short-term and long-term effects on neuronal composition, chemistry, and activities, there is much interest in the functional implications of inadequacy of DHA in the diet, blood, and brain.

Interest in the relationship between cognition and DHA was sparked following animal studies showing the impact of omega-3 fatty acid deficiency on learning ability and attention [37–41]. Animal studies continue to reveal positive effects of DHA adequacy and supplementation on behavior and cognitive performance [42]; however, human studies have not reached a consensus on the effect of DHA status on cognitive performance in infants, children, adults, or older adults [43]. PKU offers a unique model in which to learn potential cognitive effects of increasing dietary and biomarker levels of DHA, since, in this population, there is no expected intake of preformed DHA and subtle deficits in specific cognitive domains exist. More practically, it is currently unclear whether DHA should be supplemented as part of the diet treatment for PKU. Clinicians, patients and their families, and manufacturers of medical foods and low protein foods in the PKU community need a strong evidence base for optimizing diet treatment options.

Previous studies in children with PKU have shown improved plasma and red blood cell (RBC) DHA content after supplementation with 10–15 mg/kg day preformed DHA for 3–12 months [44–47]. Correspondingly, infants and children with PKU have shown small but significant improvements in visual function and motor skills after 3–12 months of supplementation compared with controls [44,46–48].

Previous studies have not investigated the effect of DHA supplementation on cognitive status in adolescents and adults with PKU. In the present study, we investigated in a randomized controlled trial whether females of reproductive age supplemented with DHA-rich oil 4.5 months would exhibit better performance on tests of cognitive processing speed, inhibition, and flexibility than those supplemented with placebo oils (www.clinicaltrials.gov; Identifier: NCT00892554).

2. Participants and methods

2.1. Study participants

Eligible participants were females with PKU and aged at least 12 years. Those who were pregnant, currently taking supplemental DHA, or scored less than 2 standard deviations below average on a standardized verbal ability task were ineligible for the trial. Volunteers were recruited primarily from an Atlanta-based metabolic clinic and an Atlanta-based metabolic camp. Recruitment was also conducted at regional and national meetings for individuals with PKU and clinicians treating individuals with PKU. Online recruitment tools included a study website and registration on clinicaltrials.gov. Approval was obtained to conduct this study from the Emory University Institutional Review Board. Participants, and a parent and/or guardian if the participant was under 18 years, gave informed consent to participate in research in accordance with Emory University policies and the Code of Federal Regulations, Title 45 (Public Welfare), Part 46 (Protection of Human Subjects).

Baseline and end of study assessments were performed at the Emory University Clinical Interaction Site (CIS) of the Atlanta Clinical & Translational Science Institute (ACTSI; previously known as the General Clinical Research Center (GCRC)). The primary data collector traveled to a location closer to the participant to complete data collection with one participant at baseline and four at follow up.

At baseline, each participant received a container to store their study log book, monthly food records and filter paper supplies, measuring cups and spoons, a ruler, a pen, and the supplements. The study log book included study contact information, the participant's supplement prescription (number of capsules to take per day), medication logs, illness logs, a supplement calendar log, and food record instructions. A website was created for participants in the study as an additional way to access study information.

To monitor compliance to the study protocol and changes in health status during the study, participants were asked to submit blood spotted on a filter paper to assess blood Phe status and a three-day food record every month, and a completed study log book, unused supplements, and supplement bottles at the end of the study. Participants were provided with shipping materials and postage and reminded of each submission by telephone call or electronic mail.

2.2. Intervention

Participants were randomized to receive either a DHA supplement or placebo orally at a dose of 10 mg/kg/day for 4.5 months. This dosage is based on previous methods which resulted in increased plasma and RBC DHA content (measured as a percentage of total lipid fatty acids) in children with PKU [44,45]. The study length of 4.5 months was chosen because cognitive effects of DHA have been shown in children with PKU after 3 months of supplementation [46,47]. DHA was provided in microalgae oil (''DHASCO-S'') capsules. Each DHASCO-S capsule contained approximately 200 mg DHA and is described in detail elsewhere [49]. The placebo oil was a mixture of soy and corn oils and was provided in capsules of matching size, weight, color, and flavor to the DHASCO-S capsules. The capsules were provided by Martek Biosciences Corporation (Columbia, MD, USA).

2.3. Objective

The primary objective of this pilot study was to investigate whether performance on tests of cognitive processing speed, inhibition, and flexibility would improve after supplementation with DHA.

2.4. Outcomes

Performance on tasks of cognitive processing speed, inhibition, and flexibility at follow up were the primary outcome measures. Biomarkers of DHA, plasma Phe, and estimated diet intake at follow up, compliance to assigned treatment, and adverse events were also assessed.