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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^{☆, ☆☆}

S.H.L. Yi^a, J.A. Kable^b, M.L. Evatt^{c,d}, and R.H. Singh^{a,e,*}

^aEmory University, Nutrition & Health Sciences Program of the Graduate Division of Biological & Biomedical Sciences, Atlanta, GA, United States

^bEmory University, School of Medicine, Department of Pediatrics, United States

^cDepartment of Veterans Affairs Medical Center, Atlanta, GA, United States

^dEmory University School of Medicine, Department of Neurology, United States

^eEmory University School of Medicine, Department of Human Genetics, 2165 N. Decatur Road, Decatur, GA 30033, United States

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).

[☆]*Ethics approval*: Approval was obtained to conduct this study from the Emory University Institutional Review Board. Informed consent was obtained from all participants and a parent and/or guardian if the participant was under age 18 years.

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^{*}Corresponding author at: Emory University School of Medicine, Department of Human Genetics, 2165 N. Decatur Road, Decatur, GA 30033, United States. Tel.: +1 404 778 8519; fax: +1 404 778 8562. rsingh@emory.edu (R.H. Singh).

Keywords

Docosahexaenoic acid; Phenylketonuria; Phenylalanine; Cognitive tests; Protein-restricted diet; Clinical trial; Randomized controlled trial

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), docosa-hexaenoic 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 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.

2.4.1. Blood amino acid profile—Amino acid analyses were performed by Emory Genetics Laboratory's Biochemical Genetics Laboratory (Atlanta, GA). Venous blood was collected into sodium heparin tubes, plasma was deproteinized, and the resulting free amino acid concentrations were measured by quantitative ion-exchange chromatography on a Biochrom 30 Amino Acid Analyzer using lithium buffer [50].

For monthly monitoring or if a participant was unable to provide a venous sample, blood spots from a finger prick were collected on filter paper to quantify whole blood Phe and

tyrosine (Tyr) concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Phe and Tyr were extracted from the blood spots into methanol containing internal standards (stable isotope labeled amino acids). Amino acid analyses were performed using a Micromass Quattro Micro tandem mass spectrometer with a Waters 2795 HPLC system. Amino acids were identified and quantified using NeoLynx software. Concentrations of whole blood Phe analyzed by LC MS/MS are reportedly 19% lower than plasma Phe concentrations analyzed by ion-exchange chromatography [51]; therefore, the blood Phe concentrations obtained by LC MS/MS were multiplied by a factor of 1.19. Amino acid concentrations are reported as $\mu\text{mol/L}$. These values can be converted to mg/dL by dividing by 60.54 [52].

2.4.2. Plasma and RBC total lipid fatty acid profiles—Plasma and RBC total lipid fatty acid profiles were assessed by the Peroxisomal Diseases Laboratory (PDL) at Kennedy Krieger Institute (Baltimore, MD). Venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes, and shipped overnight at room temperature for processing and analysis. Excess plasma and RBCs were stored frozen at -80°C in case a repeat analysis was required. Plasma and RBC C10:0 to C26:0 total lipid fatty acids were quantified by capillary gas chromatography-electron-capture negative-ion mass spectrometry (GC/MS). The method used is modified from the method of Lagerstedt et al. [53]. Plasma and RBC DHA content are presented as percent of total lipids; total lipid fatty acids are presented as $\mu\text{g/mL}$.

2.4.3. Diet assessment—Participants were given instructions and materials for the documentation of dietary intake and portion size estimation. Three-day food records were collected and reviewed with participants by a registered dietitian. Dietary intake data were analyzed using Nutrition Data System for Research software version 2009 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. Average intakes of energy, total protein, medical food protein, fat, carbohydrates, Phe, Tyr, LA, AA, ALA, EPA, and DHA were estimated at baseline and follow up. Intakes of other nutrients with potential cognitive associations were also assessed, including folate, iron, zinc, vitamin B12, and vitamin B6.

2.4.4. Cognitive assessments—Cognitive performance was assessed on tasks drawing upon verbal ability, cognitive processing speed, cognitive inhibition, and cognitive flexibility. Standard scores were calculated from raw scores using normative values included with the testing materials. To minimize scoring errors, each test was scored twice, with the second scoring occurring no sooner than 24 h after the first scoring. The standard scores are age-specific and are based upon the performance of large normative samples which have been standardized to national demographics. Evaluations demonstrate adequate reliability and validity of the tests [54–57]; see Tables 16–19 in Ref. [58] for a summary of these evaluations.

2.4.4.1. Verbal ability: The Peabody Picture Vocabulary Test—Third Edition, Form B (PPVT-III B) is (1) a verbally administered achievement test on standard English vocabulary, and (2) a screening test for verbal ability. The PPVT was individually administered by a

trained tester who asked the participant to match a series of orally presented vocabulary terms with one of four pictures per term. The results from the PPVT are used to describe the sample, to screen for the ability to complete further cognitive testing, and as a proxy for verbal intelligence.

2.4.4.2. Executive functioning skills: Inhibition and cognitive flexibility, two aspects of executive function, were evaluated using the Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference Test (CWIT) individually administered by a trained tester. CWIT evaluates automatic response inhibition and cognitive flexibility through two timed-conditions, inhibition and inhibition/switching, and is based upon the original test developed by Stroop [59]. Performance on the CWIT has been shown to be sensitive to mean lifetime plasma Phe concentrations in children and adolescents treated continuously from soon after birth through at least 14 years [60]. Color naming and word reading are fundamental skills needed to complete the inhibition and inhibition/switching tasks and thus were also evaluated to differentiate poor performance due to deficits in fundamental skills vs. inhibition and/or cognitive flexibility. Test-retest reliability coefficient is reported to be 0.75 for the inhibition task and 0.65 for the inhibition/switching task [55].

2.4.4.3. Cognitive processing speed: Processing speed was assessed using six simple, timed tests; the Decision Speed, Pair Cancellation, Reading Fluency, and Math Fluency tests were from the Woodcock-Johnson III Tests of Cognitive Ability and Achievement (W-J III; Riverside Publishing, Rolling Meadows, IL); and the Color Naming and Word Reading tasks were from the CWIT. Participants were instructed to complete each test as quickly as possible. The scores were calculated using a computer program provided by the test manufacturer and were based upon finishing time, response accuracy, and age.

Due to time constraints, participants who completed baseline evaluation as part of the camp study took the W-J III tests in a group setting led by a licensed psychologist with research assistants recording participant finishing times. The maximum participant to research assistant ratio was 8:1. All other participants took the W-J III tests individually by a trained tester under the supervision of a licensed psychologist. Thirteen participants completed the baseline W-J III in a group setting while 20 completed individually. Mean scores on these tasks did not differ between those who completed the tasks in the group setting vs. individually.

2.4.4.3.1. Decision Speed: The Decision Speed task relies on the ability to connect a concept with a name (test-retest reliability coefficient range: 0.73–0.80 [56]). Participants were instructed to circle two pictures in each row that were conceptually most similar.

2.4.4.3.2. Pair Cancellation: The Pair Cancellation task involves circling every instance of a specific pairing of pictures appearing on a single page (test-retest reliability coefficient range: 0.69–0.84 [56]).

2.4.4.3.3. Reading Fluency: The Reading Fluency task requires reading abilities. Participants were instructed to read a series of statements and circle if the statement was true or false (test-retest reliability coefficient range: 0.80–0.94 [56]).

2.4.4.3.4. Math Fluency: The Math Fluency task is related to math achievement and requires the participant to perform simple arithmetic calculations (addition, subtraction, and multiplication; test–retest reliability coefficient range: 0.89–0.96 [56]).

2.4.4.3.5. Color Naming: The Color Naming task requires the participant to identify verbally a series of colors presented on a page (test–retest reliability coefficient: 0.76 [55]).

2.4.4.3.6. Word Reading: The Word Reading task requires the participant to read aloud a series of color names presented on a page (test–retest reliability coefficient: 0.62 [55]).

2.4.4.3.7. Processing Speed Score: The six processing speed scores were reduced into one processing speed factor score for each time point using principal components analysis with a varimax rotation. The processing speed factors explained 67.9% and 62.0% of the total variance among the baseline ($n=33$) and follow up ($n=27$) variables and had eigenvalues of 4.1 and 3.7, respectively. For analysis, the baseline and follow up processing speed factors were standardized to a z-score.

2.5. Sample size

The target sample size was informed by the following calculations.

2.5.1. Anticipated change in blood DHA concentrations—In a 12-month study of 20 children with PKU in which 10 received DHA supplementation (10 mg/kg day) and 10 received placebo, those supplemented with DHA had a mean 1.2% increase in plasma total lipid DHA and 1.3% increase in RBC total lipid DHA over the control group. At $\beta=0.80$ and $\alpha=0.05$, a sample size of 9 participants and 14 participants in each group was required to see this level of improvement in plasma and RBC total lipid DHA content, respectively [61,62]. A participant loss during follow up of 25% over 4.5 months was expected; therefore, a recruitment goal of at least 35 volunteers was set.

2.5.2. Anticipated change in cognitive outcomes—Based on the results of a study conducted at the baseline of this trial (Ref. [58], Table 26), in order to see a significant change ($\beta=0.80$, $\alpha=0.05$) in a total sample size of 24 participants, the minimum mean changes needed in this study would be about 1 standard deviation of the baseline score for the cognitive tests.

2.6. Randomization: generation

An ACTSI biostatistician provided a computer generated list of randomly assigned treatments to the Emory University Hospital Investigational Drug Services. Assigned treatment was either DHA or placebo supplement. Block randomization was used with a block size of four.

2.7. Allocation concealment

Allocation of treatment was concealed from study investigators and participants through external storage and distribution by the Investigational Drug Services.

2.8. Randomization: implementation

The investigators assessed participant eligibility, discussed the trial, obtained informed consent, and enrolled participants in the trial. The Investigational Drug Services dispensed DHA or placebo supplements according to the computer generated randomization list provided by the biostatistician.

2.9. Blinding

Participants and study personnel were blinded as to which group each participant belonged until recruitment, data collection, cognitive test scoring, laboratory analyses, data entry, and blinded analyses were complete. Only the ACTSI biostatistician and Investigational Drug Services were privy to treatment allocation; however, they did not have contact with study participants. Blinding was maintained by using supplements similar in appearance, weight, and smell.

2.10. Statistical methods

Data were analyzed and reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) statement [63]. A two-tailed P value ≤ 0.05 was considered statistically significant. Statistical analyses were performed using SPSS Statistics 17.0 (SPSS Inc.; Chicago, IL).

2.10.1. Baseline characteristics—To assess similarity of the two groups (DHA vs. placebo), baseline clinical and demographic characteristics were estimated. These characteristics included: plasma amino acid concentrations; diet Phe prescription; plasma and RBC DHA content; dietary intake; BMI; exposure to cigarette smoke; and performance on cognitive tests. Continuous variables are presented as mean (standard deviation), and categorical variables are presented as number (percent). In accordance with CONSORT guidelines, significance testing of baseline differences between the two treatment groups was not conducted [64].

2.10.2. Primary outcome measures—The primary outcome measures of the study consisted of performance on tasks of cognitive processing speed, inhibition, and flexibility after 4.5 months of supplementation. The effect of DHA supplementation on follow up cognitive score was assessed using analysis of covariance (ANCOVA) with the corresponding baseline score as a covariate and treatment group as a fixed factor. Change in plasma Phe concentration and number of days between baseline and follow up were added as covariates to confirm that these findings were not affected by confounding factors. The primary analyses were conducted using intention to treat analysis. The intention to treat analysis included all randomized participants with complete baseline and follow up data.

2.10.3. Secondary outcome measures—The same measures were assessed for the secondary analysis; however, only randomized participants with complete baseline and follow up data who completed the follow up visit within the 4.5 ± 0.5 month after starting supplementation were included. These assessments are referred to as per protocol analysis.

2.10.4. Other analyses—Using ANCOVA, biomarkers of DHA status were compared to evaluate the effectiveness of DHA supplementation. Plasma Phe concentration and diet intake were also assessed to evaluate potential confounding factors.

Compliance to prescribed supplement regime was assessed and defined as taking > 80% of prescribed treatment as indicated by: < 20% of prescribed number of capsules returned, log book entries detailing < 20% missed doses, in the DHA-supplemented group an increase in plasma DHA content of at least 1.2% and an increase in RBC DHA content of at least 1.3%, and in the placebo-supplemented group no such increase.

2.10.5. Adverse events—The proportion of participants reporting any adverse events and adverse events deemed to be related to treatment was calculated for each study group and compared using Fisher's Exact Test.

3. Results

3.1. Flow of participants and protocol deviations

Fig. 1 shows the number of participants who were randomized, completed the follow up visit, and were included in the intention to treat analysis.

3.2. Dates of recruitment and follow up

Participants were recruited from June 2007 to September 2009. Randomization and the start of supplementation occurred within 1 week of the baseline visit for 19 participants and up to 3.5 months after the baseline visit for 14 participants. Twenty participants completed the follow up visit 4.5 (0.5) months after the start of supplementation. Thirteen participants withdrew from the study, and 7 of the 13 completed follow up visits at time points ranging from 1.5 months to 8 months after start of supplementation. Only 1 of the 7 continued taking the supplement until the follow up visit.

3.3. Baseline characteristics

As shown in Table 1, the two participant groups were similar in most clinical and demographic characteristics at baseline. Mean plasma Phe and BMI were clinically different between the two groups; the medians of plasma Phe were still clinically different while the medians of BMI were not clinically different between the two groups.

3.4. Number of participants

Because six participants did not complete a follow up visit, the intention to treat analysis included 27 of the 33 randomized participants. Seven participants were considered protocol violators because they terminated supplementation early and completed the follow up visit outside the 4.5 (0.5) month follow up window. The per protocol analysis included 20 of the 33 randomized participants. Reported adverse events are presented for the 32 randomized participants who took the supplement (Table 2).

3.5. Summary of primary and secondary results

The intention to treat and per protocol analyses failed to show a difference between the follow up cognitive outcomes of the DHA Group and Placebo Group (Tables 3 and 4).

3.6. Other analyses

Mean plasma and RBC DHA content at follow up were significantly higher in the DHA Group compared with the Placebo Group in both the intention to treat and per protocol analyses, controlling for baseline levels ($P<0.001$). The complete fatty acid profiles for the per protocol analysis are shown in Tables 5 and 6. The DHA Group and Placebo Group had similar proportions of compliance to allocated treatment except for expected number of capsules returned (Tables 7 and 8). Mean plasma Phe at follow up did not differ between groups after controlling for baseline concentrations (intention to treat ($n=27$): 861 (608) $\mu\text{mol/L}$ vs. 895 (540) $\mu\text{mol/L}$, $P=0.837$; per protocol ($n=20$): 770 (492) $\mu\text{mol/L}$ vs. 856 (443) $\mu\text{mol/L}$, $P=0.575$). Mean intakes of select nutrients at follow up also did not differ between treatment groups after controlling for baseline intakes (Table 2).

3.7. Adverse events

Most participants reported one or more adverse events, and the proportion of participants reporting any adverse event was similar between the DHA and Placebo Groups (Table 9). The categories of reported adverse events deemed to be related to treatment also did not differ by treatment group (Table 10). No serious adverse event was reported.

4. Discussion and conclusions

This 4.5-month pilot study found no evidence of an effect of DHA supplementation on measures of cognitive processing speed, inhibition, and flexibility in females of reproductive age with PKU; however, because the study was not powered to detect small effect sizes (i.e., less than 1 standard deviation change from baseline), this study is inconclusive regarding the presence or absence of an effect. The data did confirm that DHA supplementation was effective in increasing concentrations of plasma and RBC DHA. In addition, there was no increase in the number of adverse events attributed to treatment in the DHA Group compared with the Placebo Group.

The limited power due to the small sample size in this study requires caution when assessing whether DHA supplementation may have an effect on cognitive processing speed, inhibition, and flexibility. A post hoc sample size calculation utilizing the observations from the current study projected that sample sizes of 853, 426, and 95 in each group would be needed to observe a statistically significant effect of DHA supplementation on cognitive processing speed, inhibition, and flexibility, respectively. Recruitment was conducted through multiple avenues, and participants from other states were included. Because the incidence of PKU is relatively uncommon [6], and participant retention was suboptimal, obtaining an adequate sample size was a challenge. The sample size for this study was comparable to other single-center studies of PKU. Regardless, extra care must be taken in the interpretation of the relationship between DHA supplementation and cognitive outcomes in this study.

One possible reason for a lack of cognitive effect of DHA is appropriateness of the domains being tested. Specifically, cognitive processing speed, inhibition, and flexibility may not be markedly affected by DHA. Recently, other trials have also failed to find an effect of DHA supplementation on measures of processing speed and executive function in children [65], lactating women [66], and older adults [67,68]. In two observational studies, de Groot and colleagues found either no association or instances of negative associations between measures of processing speed, executive function, and DHA status in pregnant and non-pregnant women [69,70].

It is also possible that the cognitive tests relevant to the prefrontal cortex are not sensitive enough to detect changes occurring in the brain related to DHA supplementation. McNamara et al. [71] recently observed increased prefrontal cortex activation in healthy boys while completing a sustained attention task following 8 weeks of DHA supplementation compared with boys taking placebo. Interestingly, although concurrent RBC DHA content was inversely associated with reaction time, performance on the task itself did not differ at follow up between the supplemented and control groups.

Performance on tasks of verbal fluency, memory, and learning, which are associated with the hippocampus and other regions of the brain, may be affected by DHA to a greater extent than cognitive processing speed, inhibition, and flexibility. In a 4-month trial of older women, those who received 800 mg DHA ($n=14$), 12 mg lutein ($n=11$), or 800 mg DHA and 12 mg lutein ($n=14$) performed significantly better on verbal fluency task after 4 months of supplementation compared with those receiving placebo capsules ($n=10$) [68]. The 800 mg DHA plus 12 mg lutein group also showed significant improvements in measures of learning and memory. The intervention showed no effect on performance on the Stroop test performance, which assesses aspects of cognitive processing speed and executive function. The results of Johnson and colleagues confirm our baseline findings that verbal ability was significantly correlated with RBC DHA content after controlling for plasma Phe concentrations [72].

The dosage of DHA used in this and other recent studies assessing cognitive processing speed and/or executive function ranges between approximately 200 and 1000 mg per day. While this dosage may produce changes in other domains, it may be inadequate for measures of cognitive processing speed and executive function. In addition, a longer period of exposure to supplemental DHA and/or exposure during specific stages of the lifecycle may be required.

DHA supplementation was chosen because isotope tracer studies [73] and ALA supplementation trials [74–76] demonstrate inefficient conversion from ALA to DHA in humans. It is also hypothesized that high intake of LA relative to ALA inhibits endogenous synthesis of DHA from ALA due to competition since AA synthesis from LA necessitates the same desaturase and elongase enzymes [77–79]. Improving the dietary LA:ALA ratio in children with PKU, however, has shown either a small change [80] or no change in blood DHA concentrations [45].

Correspondingly, there may be a threshold for neural cell membrane DHA composition above which changes may be seen. Harris and Von Schacky proposed that RBC EPA+DHA content at or above 8% of total lipids were most protective and content at or below 4% were least protective as a risk factor for coronary heart disease mortality [81]. Building upon their proposal, McNamara [82] suggested 1.6% EPA and 7% DHA RBC total lipid content as a protective factor against affective disorders based primarily on observational studies as well as clinical trials. In the present study, participants at baseline presented with means of 0.24 (0.08)% EPA and 2.42 (0.80)% DHA of RBC total lipids ($n=32$). The mean follow up values in the per protocol DHA Group were of 0.34 (0.07)% EPA and 5.97 (1.24)% DHA of RBC total lipids ($n=8$). Perhaps, the threshold of effect was not reached in this study.

Finally, it should be taken into account that this study was conducted with females with PKU between the ages of 12 and 47 years. These results may not extend to individuals outside of this population.

5. Conclusions

The findings of this pilot trial are inconclusive and suggest a large sample size is required to investigate adequately the impact of supplemental DHA on measures of cognitive processing speed and executive function, two domains commonly affected in people with PKU treated early and lifelong, in females of reproductive age with PKU. Due to the small observed effect size and the limited population of people with PKU, further investigation into the effect of DHA on these particular domains would be best suited in a larger population, such as adults without a rare disorder. Further research is needed to clarify which domains are affected by changes in DHA status, and the length and strength of exposure to DHA that is required to see these changes. Future investigations assessing the cognitive effect of DHA should measure cognitive domains more likely to be affected such as verbal ability, memory, and learning.

While the cognitive implications of low blood DHA in adolescents and adults with PKU are still unknown, this study did not find harm with taking 10 mg/kg day DHA for 4.5 months compared with placebo and confirms previous reports that supplementation with pre-formed DHA is effective at increasing biomarkers of DHA. Clinicians should refer to guidelines suggested by national organizations and expert panels if interested in suggesting supplementation to patients. The American Heart Association and the International Society for the Study of Fatty Acids and Lipids [83,84], for example, recommend consumption of two servings of fish per week or 500 mg EPA+DHA per day for the primary prevention of cardiovascular disease. Of relevance to females of reproductive age, expert panels recommend a minimum maternal intake of 200–300 mg pre-formed DHA per day during pregnancy and lactation [85,86].

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Fig. 1.

Flow diagram of a randomized controlled trial of supplemental docosahexaenoic acid on cognitive outcomes in females of reproductive age with phenylketonuria based on the revised template of the CONSORT (Consolidated Standards of Reporting Trials) diagram.

Table 1

Baseline demographic characteristics of females with PKU randomized to receive DHA or placebo supplement.

		DHA Group n=17	Placebo Group n=16
Age, mean (SD), yr		24.4 (10.6)	25.6 (10.7)
Poverty, no. (%) ^a	At or below Threshold	4 (27)	2(14)
Maternal education, no. (%)	HS degree or less	6 (35)	8 (50)
Race/ethnicity, no. (%) ^b	White/Caucasian	15 (88)	15 (94)
Insurance, no. (%)	Yes	17 (100)	14 (88)
Residence, no. (%) ^c	South	14 (82)	13 (81)

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; HS, high school.

^aBased on annual poverty thresholds calculated by the US Census Bureau (DHA Group, n=15; Placebo Group, n=14).

^bParticipants self-identified race/ethnicity as white/Caucasian, black/African-American, or Native American.

^cUS Census regions; other participants from Northeast, West, or Midwest.

Table 2

Baseline health characteristics and cognitive performance of all of females with PKU randomized to receive DHA or placebo supplement.

Baseline characteristics	DHA Group <i>n</i> =17	Placebo Group <i>n</i> =16
Phe prescription, mean (SD), mg/day	411 (133)	343 (105)
% Intake of Phe prescription, mean (SD) ^b	141.0 (84.8)	125.8 (64.5)
Medical food prescription, mean (SD), g pro eq/day ^a	54.4 (10.4)	56.8 (12.4)
% Intake of medical food prescription, mean (SD) ^a	89.8 (23.9)	90.5 (15.4)
Dietary energy, mean (SD), kcal/day ^b	1644 (444)	1616 (441)
Dietary protein, mean (SD), % energy ^b	15.1 (3.9)	15.0 (3.6)
Dietary carbohydrate, mean (SD), % energy ^b	59.2 (5.9)	60.8 (7.3)
Dietary fat, mean (SD), % energy ^b	28.4 (6.9)	26.7 (5.4)
Dietary LA, mean (SD), % energy ^b	7.5 (2.5)	7.7 (3.2)
Dietary ALA, mean (SD), % energy ^b	0.98 (0.44)	0.94 (0.43)
Dietary DHA, mean (SD), g/day ^b	0.002 (0.003)	0.001 (0.004)
Dietary LA:ALA, mean (SD), ratio ^b	8.2 (2.0)	8.6 (2.2)
Dietary Tyr, mean (SD), mg/day ^b	4955 (1535)	5435 (1356)
Dietary folate, mean (SD), µg/day ^b	732 (217)	830 (341)
Dietary iron, mean (SD), mg/day ^b	24.4 (9.6)	27.7 (8.9)
Dietary zinc, mean (SD), mg/day ^b	19.6 (8.9)	23.3 (9.4)
Dietary vitamin B12, mean (SD), µg/day ^b	5.6 (2.3)	7.7 (3.6)
Dietary vitamin B6, mean (SD), mg/day ^b	3.0 (1.3)	3.2 (1.4)
BMI, mean (SD), kg/m ²	25.8 (6.6)	30.4 (9.4)
Plasma Phe, mean (SD), µmol/L	683 (523)	915 (446)
Plasma Tyr, mean (SD), µmol/L	45 (18)	53 (26)
Plasma DHA, mean (SD), % TLFA ^b	1.07 (0.32)	0.97 (0.39)
RBC DHA, mean (SD), % TLFA ^b	2.48 (0.81)	2.34 (0.81)
Smoker, no. (%) ^c	1(6)	1 (6)
Smoker in household, no. (%)	4(25)	4 (27)
Verbal ability, mean (SD), SS	99.7 (12.5)	102.2 (13.6)
Cognitive processing speed, mean (SD), factor score	99.8 (12.5)	100.2 (16.4)
Cognitive inhibition, mean (SD), SS	10.4 (3.8)	9.8 (3.6)
Cognitive switching, mean (SD), SS ^a	10.2 (3.9)	9.9 (3.8)

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; pro eq, protein equivalent; LA, linoleic acid; ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; Tyr, tyrosine; TLFA, total lipid fatty acid; RBC, red blood cell; SS, standard score.

^a DHA Group: medical food prescription, % intake of medical food prescription, *n*=16; cognitive switching, *n*=15.

^b Placebo Group: diet intake, plasma and RBC TLFA, *n*=15.

^cSmoker defined as smoking cigarettes at least once per week.

Table 3

The effect of DHA supplementation on mean follow up cognitive test scores controlling for baseline score in females with PKU: intention-to-treat analysis.

	DHA Group <i>n</i> =12	Placebo Group <i>n</i> =15	ANCOVA ^{ab}		
	Mean (SD) ^c	Mean (SD)	Difference	95% CI	<i>P</i> value
Cognitive processing speed, factor score	98.8 (5.3)	101.0 (5.4)	-2.23	-6.5, 2.0	0.29
Cognitive inhibition, SS	11.3 (1.5)	11.4 (1.5)	-0.05	-1.3, 1.2	0.93
Cognitive flexibility, SS ^d	11.1 (1.4)	10.8 (1.4)	0.28	-0.9, 1.5	0.63

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; ANCOVA, analysis of covariance; SS, standard score.

^a ANCOVA model: follow up score= β_0 + β_1 (baseline score)+ β_2 (treatment group)+error.

^b Statistics are presented for β_2 (treatment group).

^c Mean (SD) adjusted for baseline score.

^d DHA Group: cognitive flexibility, *n*=2 missing.

The effect of DHA supplementation on mean follow up cognitive test scores controlling for baseline score in females with PKU: per protocol analysis.

Table 4

	DHA Group n=9	Placebo Group n=11	ANCOVA ^{ab}		
	Mean (SD) ^c	Mean (SD)	Difference	95% CI	P value
Cognitive processing speed, factor score	101.7 (5.1)	101.6 (5.1)	0.14	-4.7, 5.0	0.95
Cognitive inhibition, SS	11.7 (1.5)	11.4 (1.5)	0.25	-1.2, 1.7	0.72
Cognitive flexibility, SS ^d	11.6 (1.3)	11.1 (1.3)	0.49	-0.9, 1.9	0.45

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; ANCOVA, analysis of covariance; SS, standard score.

^a ANCOVA model: follow up score= β_0 + β_1 (baseline score)+ β_2 (treatment group)+error.

^b Statistics are presented for β_2 (treatment group).

^c Mean and standard deviation adjusted for baseline score.

^d DHA Group: cognitive flexibility, n=2 missing.

Table 5

Baseline and follow up percent plasma total lipid fatty acids of per protocol female participants with PKU randomized to receive DHA or placebo supplement.

Fatty acid ^a	DHA Group ^b		Placebo Group ^c	
	Baseline	Follow up	Baseline	Follow up
C14:0	0.91 (0.69) ^{de}	0.87 (0.37)	0.88 (0.33)	0.86 (0.35)
C16:0	19.76 (2.49)	20.60 (1.00)	21.15 (2.60)	19.59 (1.59)
C18:0	6.96 (1.09)	7.06 (0.60)	6.39 (0.43)	6.54 (1.07)
C22:0	0.83 (0.28)	0.90 (0.21)	0.73 (0.24)	0.78 (0.14)
C24:0	0.69 (0.18)	0.79 (0.20)	0.59 (0.19)	0.68 (0.12)
C16:1(n-9)	0.44 (0.10)	0.42 (0.07)	0.53 (0.15)	0.48 (0.07)
C18:1(n-9)	17.72 (1.94)	16.56 (1.09)	20.00 (2.89)	19.28 (2.16)
C20:3(n-9)	0.07 (0.03)	0.06 (0.02)	0.06 (0.03)	0.06 (0.03)
C24:1(n-9)	0.96 (0.19)	0.99 (0.20)	0.91 (0.35)	0.98 (0.24)
C16:1(n-7)	1.64 (0.84)	1.81 (0.38)	1.95 (0.52)	1.90 (0.55)
C18:1(n-7)	1.51 (0.48)	1.39 (0.18)	1.51 (0.25)	1.56 (0.28)
C18:2(n-6)	31.44 (3.63)	29.86 (1.55)	28.88 (3.46)	31.56 (3.51)
C18:3(n-6)	0.49 (0.22)	0.53 (0.10)	0.43 (0.10)	0.44 (0.19)
C20:3(n-6)	2.04 (0.47)	1.81 (0.27)	1.92 (0.42)	1.86 (0.59)
C20:4(n-6)	6.99 (1.05)	6.38 (1.20)	5.96 (1.97)	5.94 (1.76)
C22:4(n-6)	0.26 (0.06)	0.18 (0.04)	0.24 (0.06)	0.24 (0.05)
C22:5(n-6)	0.21 (0.12)	0.49 (0.09)	0.16 (0.05)	0.18 (0.07)
C18:3(n-3)	0.86 (0.27)	0.89 (0.35)	0.97 (0.36)	0.91 (0.40)
C20:5(n-3)	0.34 (0.10)	0.58 (0.16)	0.36 (0.27)	0.28 (0.15)
C22:5(n-3)	0.45 (0.10)	0.31 (0.07)	0.39 (0.15)	0.41 (0.10)
C22:6(n-3)	1.17 (0.33)	3.14 (0.57)	1.07 (0.41)	0.97 (0.34)
C18:1T SUM	1.03 (0.32)	1.11 (0.52)	1.48 (0.52)	1.26 (0.42)
C18:2T SUM	0.39 (0.12)	0.42 (0.08)	0.54 (0.16)	0.46 (0.08)
Total fatty acids	2486 (691)	2564 (636)	3107 (1216)	2566 (604)

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; TLFA, total lipid fatty acid.

^aSelect fatty acids < 0.5% TLFA are excluded from table.

^bDHA Group: baseline, *n*=9; follow up *n*=7.

^cPlacebo Group: baseline, *n*=10; follow up *n*=11.

^dPlasma % TLFA.

^eMean (SD).

Table 6

Baseline and follow up percent RBC total lipid fatty acids of per protocol female participants with PKU randomized to receive DHA or placebo supplement.

Fatty acid ^a	DHA Group ^b		Placebo Group ^c	
	Baseline	Follow up	Baseline	Follow up
C16:0	18.68 (1.72) ^{de}	18.67 (0.90)	18.48 (1.59)	17.79 (0.79)
C18:0	14.96 (1.49)	14.90 (1.38)	14.58 (1.46)	14.36 (1.05)
C22:0	1.73 (0.28)	1.83 (0.15)	1.83 (0.29)	1.82 (0.26)
C24:0	4.78 (0.75)	5.13 (0.32)	5.03 (0.56)	4.97 (0.70)
C18:1(n-9)	9.67 (1.13)	9.97 (1.11)	9.91 (0.81)	10.44 (0.89)
C20:3(n-9)	0.03 (0.02)	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)
C24:1(n-9)	4.21 (0.59)	4.26 (0.78)	4.09 (0.74)	4.26 (0.69)
C18:1(n-7)	0.83 (0.20)	0.71 (0.08)	0.77 (0.11)	0.80 (0.13)
C18:2(n-6)	9.38 (1.12)	9.63 (1.28)	9.64 (1.26)	10.51 (1.73)
C18:3(n-6)	0.04 (0.01)	0.04 (0.01)	0.05 (0.01)	0.04 (0.01)
C20:3(n-6)	1.72 (0.27)	1.53 (0.17)	2.05 (0.83)	2.01 (0.56)
C20:4(n-6)	12.51 (0.98)	10.99 (1.00)	12.94 (1.69)	12.60 (1.45)
C22:4(n-6)	3.78 (0.61)	2.35 (0.41)	3.62 (0.32)	3.67 (0.52)
C22:5(n-6)	0.71 (0.17)	1.23 (0.21)	0.62 (0.12)	0.65 (0.17)
C24:2	0.95 (0.21)	1.01 (0.31)	0.89 (0.17)	0.98 (0.20)
C18:3(n-3)	0.12 (0.03)	0.13 (0.04)	0.13 (0.04)	0.13 (0.05)
C20:5(n-3)	0.27 (0.10)	0.34 (0.07)	0.27 (0.08)	0.24 (0.10)
C22:5(n-3)	1.98 (0.48)	1.03 (0.20)	1.85 (0.53)	1.87 (0.50)
C22:6(n-3)	2.77 (0.80)	5.82 (1.26)	2.53 (0.90)	2.35 (0.78)
C18:1T SUM	1.09 (0.42)	1.06 (0.56)	1.30 (0.60)	1.21 (0.66)
16:0 DMA	1.78 (0.40)	1.48 (0.27)	1.54 (0.21)	1.61 (0.24)
18:0 DMA	3.21 (0.53)	3.10 (0.21)	3.05 (0.44)	2.99 (0.38)
Total 18:1 DMA	1.12 (0.26)	1.12 (0.24)	1.05 (0.20)	1.06 (0.20)
Total fatty acids	1394 (197)	1488 (182)	1458 (203)	1424 (224)

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; TLFA, total lipid fatty acid; RBC, red blood cell.

^aSelect fatty acids < 0.5% TLFA are excluded from table.

^bDHA Group: baseline, *n*=9; follow up *n*=7.

^cPlacebo Group: baseline, *n*=10; follow up *n*=11.

^dRBC % TLFA.

^eMean (SD).

Table 7

Estimates of participant compliance to treatment allocation in females with PKU: intention-to-treat analysis.

	DHA Group n=11	Placebo Group n=14	Fisher's Exact Test
	No. (%)	No. (%)	P value
Self-reported capsules taken ^a	8 (67)	10 (67)	1.0
Expected capsules returned ^{b,c}	7 (70)	3 (25)	0.08
Plasma DHA ^{d,e}	9 (82)	13 (100)	0.20
RBC DHA ^{e,f}	10 (91)	13 (100)	0.46

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; RBC, red blood cell.

^aExpected capsules taken, self-report: log book reports 80% non-missed doses.

^bExpected 20% of prescribed number of capsules returned.

^cDHA Group: n=10, Placebo Group, n=12.

^dExpected change in plasma DHA: 1.2% in DHA Group and < 1.2% in Placebo Group.

^ePlacebo Group: Plasma DHA, RBC DHA, n=13.

^fExpected change in RBC DHA: 1.3% in DHA Group and < 1.3% in Placebo Group.

Table 8

Estimates of participant compliance to treatment allocation in females with PKU: per protocol analysis.

	DHA Group n=9	Placebo Group n=11	Fisher's Exact Test
	No. (%)	No. (%)	P value
Self-reported capsules taken ^a	8 (89)	10 (91)	1.0
Expected capsules returned ^{bc}	7 (88)	3 (30)	0.03
Plasma DHA ^{de}	8 (89)	10 (100)	0.47
RBC DHA ^{ef}	9 (100)	10 (100)	–

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; RBC, red blood cell.

^a Expected capsules taken, self-report: log book reports 80% non-missed doses.

^b Expected 20% of prescribed number of capsules returned.

^c DHA Group: n=10, Placebo Group, n=12.

^d Expected change in plasma DHA: 1.2% in DHA Group and < 1.2% in Placebo Group.

^e Placebo Group: Plasma DHA, RBC DHA, n=10.

^f Expected change in RBC DHA: 1.3% in DHA Group and < 1.3% in Placebo Group.

Table 9

Summary of reported adverse events by intention-to-treat group in females with PKU.

Adverse event ^a	DHA Group <i>n</i> =17	Placebo Group <i>n</i> =15 ^b	Fisher's Exact Test
	No. (%)	No. (%)	<i>P</i> value
1 or more adverse events	14 (82)	15 (100)	0.23
Angioedema	0 (0)	1 (7)	0.47
Diarrhea	3 (18)	3 (20)	1.0
Fishy eructation	1 (6)	0 (0)	1.0
Nausea	3 (18)	3 (20)	1.0
Vomiting	0 (0)	3 (20)	0.09
Other ^c	13 (76)	14 (93)	0.34

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid.

^a Each participant is counted no more than once per adverse event.

^b Participant who never took the supplement is excluded.

^c Other includes all other reported events, including but not limited to backache, common cold, dysuria, ear infection, headache, nasal congestion, oily skin.

Table 10

Adverse events judged possibly, probably, or definitely associated with supplement in females with PKU.

Adverse event ^{ab}	DHA Group <i>n</i> =17	Placebo Group <i>n</i> =15 ^b	Fisher's Exact Test
	No. (%)	No. (%)	<i>P</i> value
Angioedema	0 (0)	1 (7)	0.47
Diarrhea	2 (12)	2 (13)	1.0
Fishy eructation	1 (6)	0 (0)	1.0
Nausea	1 (6)	3 (20)	0.32
Vomiting	0 (0)	1 (7)	0.47

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid.

^a Each participant is counted no more than once per adverse event.

^b Association determined by investigators.